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WARNING
If the SPOT RT camera is moved from one location to another location, the user is strongly advised to protect the camera and the power supply to avoid the entry of any small object or liquids into the camera casing through the camera's ventilation openings.

Failure to do so may result in damage to the camera and may pose a danger to the user.

User Guide to the SPOT RT Camera

About This Manual

Introduction

The manual contains instructions for the SPOT RT line of cameras (B/W, Color Mosaic, 3-Shot Color and Slider) and version 4.0 of the accompanying SPOT software. An on-line version of this manual in PDF (portable document format) is also included on the CD that contains the SPOT software. Refer to the **Installation Guidelines** chapter for more information on accessing and using the on-line manual.

Document Conventions

The following is a list of the textual conventions used in this manual:

Notes	*Note: This is an example of a note. Notes appear within the text to indicate information for further consideration or reference.*
Cautions	**CAUTION!** **This is an example of a cautionary note. Cautionary notes appear to indicate that you should carefully consider the implications of an action.**
Warnings	**☀WARNING☀** **THIS IS AN EXAMPLE OF A WARNING. WARNINGS INDICATE THAT THE ACTION YOU ARE TAKING COULD EITHER CAUSE INJURY TO YOURSELF OR COULD HARM YOUR SYSTEM**
RT B/W	*RT B/W* RT B/W notes indicate specific instructions for users with the monochrome version of the SPOT RT camera. In most cases, these notes indicate that a color related option is unavailable.
RT 3-Shot	*RT 3-Shot* RT 3-Shot notes indicate specific instructions for users with the 3-Shot version of the SPOT RT camera.
RT Color Mosaic	*RT Color* RT Color notes indicate specific instructions for users with the Color Mosaic version of the SPOT RT camera.
RT Slider	*RT Slider* RT Slider notes indicate specific instructions for users with the Slider version of the SPOT RT camera.
Software Development	Software Development informational notes indicate special circumstances related to the current release of the SPOT software. These notes also describe how to obtain free updates from Diagnostic Instruments.

Organization of the Manual

The *User Guide to the SPOT RT Camera* is structured as shown in the table below. In addition to the chapter names and descriptions, the table indicates whether the chapter applies to the Basic program mode, the Advanced program mode, or both:

Chapter	Description	Basic or Advanced
About This Manual	Describes document conventions, manual structure.	Both
Installation Guidelines	Describes hardware/software installation.	Both
Quick Start	Steps for immediately capturing your first image in the Basic Program Mode.	Basic
Ch. 1 – New Features	Describes the software enhancements for version 4.0 (Windows and Mac editions) of the SPOT software.	Both
Ch.2 – Basic and Advanced Program Modes	Compares the features of the Basic and Advanced program modes.	Both
Ch. 3 – The Basic Program Mode	Details the SPOT Basic program mode, which is designed for beginning users who want to start taking pictures immediately without having to make a lot of processing decisions.	Basic
Ch. 4 – The Advanced Program Mode	Describes the SPOT Advanced program environment, including basic file commands and preliminary setup instructions.	Advanced
Ch. 5 – Preparing to Take a Picture	Describes the different procedures involved in setting up the software for image captures.	Advanced
Ch. 6 – Using Live Images	Describes SPOT software's real-time imaging functionality.	Advanced
Ch. 7 – Taking a Picture	Describes the image capture process.	Advanced
Ch. 8 – Viewing, Editing, and Annotating Images	Describes the rich set of image editing and customization features in the SPOT software.	Advanced
Ch. 9 – Using SPOT Databases	Describes the procedures for creating and working with SPOT image databases.	Advanced
This functionality is not yet available for Mac users. Refer to the Free Software Updates *section below for details on obtaining updated versions of the software.*		
Ch. 10 – Reporting	Describes the Advanced mode reporting functionality in the SPOT software.	Advanced
Ch. 11 – Printing Images	Describes the procedures for printing images from the SPOT program.	Both
Chapter 12 – Taskbars	Describes how to develop and utilize the floating taskbar feature	Advanced
Chapter 13 – Macros	Describes the Macro Scripting feature including how to develop and implement Macro programs to automate your work	Advanced
Ch. 14 – Using Other Windows Imaging Applications in Advanced Mode	Describes how to capture images (via an Advanced Mode TWAIN interface) with the SPOT camera using other Windows -based imaging programs such as Photoshop or Image-Pro Plus.	Advanced

(cont.)

Chapter	Description	Basic or Advanced
Ch. 15 – Using Other Windows Imaging Applications in Basic Mode	Describes how to capture images (via a Basic Mode TWAIN interface) with the SPOT camera using other Windows -based imaging programs such as Photoshop or Image-Pro Plus.	Basic
Ch. 16– Using Other Mac Imaging Applications	Describes how to capture images with the SPOT camera using other Mac imaging programs such as Photoshop or Image-Pro Plus.	Advanced
Ch. 17– Using Other Mac Imaging Applications in Basic Mode	Describes how to capture images (via a Basic Mode 3rd party interface) with the SPOT camera using other Mac imaging programs such as Photoshop or Image-Pro Plus.	Basic
Appendix A: Electronic Imaging Theory and SPOT Cameras	An introduction to the theory behind SPOT cameras.	Both
Appendix B: Specifications	Technical specifications for the SPOT hardware and software.	Both
Appendix C: Keyboard and Toolbar Button Commands	A table of menu commands and their keystroke and button equivalents.	Both
Appendix D: Converting Previous Version SPOT Databases	Instructions for performing a one-time conversion of SPOT databases from earlier versions of the software.	Advanced
Appendix E: Third Party Software	A list of imaging software systems and their levels of compatibility with SPOT Enhanced, RT, and Insight cameras.	Both
Appendix F: Enhanced, RT Cameras and Polarized Light Microscopy	A discussion of how to set up your Enhanced or RT camera for the correct rotational orientation when using polarizers in your microscopy technique.	Both

Free Software Updates

Registered SPOT camera owners can download free software updates via the Internet. When you register your product, Diagnostic Instruments supplies you with the user name and password needed to download the latest release of the software, as well as PDF user guides, software developer kits, and SPOT third party DLLs.   To register, contact Diagnostic Instruments at 1 (586) 731-6000, or you can register online at www.diaginc.com/camreg.htm.   You should periodically check Diagnostic Instruments' web site **(www.diaginc.com/software)** for the latest release.

To download files, follow these steps:

1. Access the software download page on Diagnostics Instruments' website – **www.diaginc.com/software**.

2. Click on the link for your software/operating system. You are prompted for a user name and password.

3. Enter your **User Name** and **Password**. If you have not yet registered your product, contact Diagnostic Instruments at 1 (586) 731-6000 or register online and obtain the user name and password.

4. Click **OK**.

Installation Guidelines

QUICK REFERENCE:

- Confirm the minimal hardware requirements for using the SPOT RT camera… pp. 8-10

- Connect the SPOT RT camera to your microscope… p. 15

- Use multiple SPOT cameras with a single computer… p. 15

- Access the on-line PDF documentation provided on the SPOT software CD… p. 15

Introduction

The following sections detail the system and installation guidelines for using the SPOT RT camera and version 4.0 of the SPOT software. They are as follows:

- System Requirements
- What is in the Box
- Installing the Software
- Installing the Hardware
- Multiple Ca mera Installations
- Using SPOT On -line Documentation

WARNING

If the SPOT RT camera is moved from one location to another location, the user is strongly advised to protect the camera and the power supply to avoid the entry of any small object or liquids into the camera casing through the camera's ventilation openings.

Failure to do so may result in damage to the camera and may pose a danger to the user.

System Requirements

The following sections detail the minimum hardware and software requirements needed for the SPOT RT camera and associated software. These requirements are valid at the time of your camera purchase and may change with future development of our camera and software products as well as the computers to which they interface. For optimal system suggestions, please consult our website at www.diaginc.comfor the most timely information.

> ✎ *Note: For the most up-to-date suggestions on an optimal computer system configuration, please consult our website at www.diaginc.com*

Equipment Standards - Windows

	Minimum
Processor	Pentium, 400 MHz
Video Card	A card that supports 24 bit, true color at the desired resolution with a refresh rate above 70 Hz.
	With some video cards, you might experience system crashes or display problems in live image mode; you might also experience display problems when zooming in on captured images. To correct these problems, lower the Graphics Acceleration setting in Windows, as described in the "Video Card Related Problems" section below.
RAM	128 Mb
PCI Card Slot	One available 1/2 length PCI slot
PCMCIA Cardbus Adapter with Optional Magma Adapter (for laptop)	One available PCMCIA Cardbus slot
Monitor	Any color monitor with at least 800 x 600 pixel resolution at 24 bit RGB, and a refresh rate over 70 Hz.
Operating System	Windows 95/98/NT/2000/ME/XP
	For Multiple SPOT camera users - Windows 95, 98, and NT are not supported for the Ins ight FireWire Camera interface
Printer	Driver required for reports feature
Recommended Web Browser	Internet Explorer 4.0 or greater

Equipment Standards - Mac

Minimum	
Processor	400 Mhz G3 PowerPC (**Mac G5 users, see note in PCI below**)
Video Memory/ Card	Enough video memory on either the mother board or a plug in video card to display millions of colors at the desired resolution, and with a refresh rate above 70 Hz
RAM	256 Mb
PCI	One full length PCI slot

Mac G5 Users – Please Note.

Current models of the RT Camera utilize a PCI plug-in card that is not compatible with G5 computers. Please do not attempt to use this PCI card with the G5.

Monitor	Any color monitor with at least 800 x 600 pixel resolution at 24 bit RGB, and a refresh rate over 70 Hz.
Operating System	Mac OS10.1 or greater
Printer	Driver required for reports feature
Image Editing Software (optional)	The SPOT 3rd Party Interface, which is included with Mac software, is a stand-alone mini-application designed for Mac users who want to use the SPOT camera to capture images, and then edit the images in another imaging application. Some commonly used third party applications include:

- Adobe Photoshop
- Image Pro Plus
- Color It

The image editing software used with the SPOT 3rd Party Interface does not need to be TWAIN compliant. Refer to Ch. 17 – Using Other Mac Imaging Applications for more information.

Equipment Standards - General

Additional Hardware and Software

Coupler   The ***RT Color Mosaic and Monochrome*** cameras require couplers designed to fit a camera with a C mount.

The ***RT 3-Shot and RT Slider*** cameras require an F mount coupler.

> **A variety of couplers customized for your combination of Microscope and SPOT camera are available from Diagnostic Instruments. You may consult your dealer for assistance in matching the correct coupler to your imaging microscope system, or contact Diagnostic Instruments directly for assistance from our expert Technical Support staff.**

Lens   C- Mount lens for 1" format CCD

Light Source   Because the SPOT RT camera is capable of extremely short exposure times, light sources whose intensity varies with the waveform of the 60 Hz line supply should be avoided. Problem light sources include fluorescent lamps, 120 volt incandescent bulbs, and 6 volt 20 watt halogen lamps. These light sources cause the Live Image window to flicker and result in incorrect brightness and color balance readings in captured images. We have found that the low voltage halogen/xenon lamps used on most microscopes work well, as do the mercury burners used on fluorescence illuminators.

If your work requires you to use a light source that causes problems, you can use the Minimum Exposure option on the Image Setup window to avoid flicker and give you correct brightness and color balance readings. Refer to Ch. 5 – Preparing to Take a Picture for more information.

What is in the Box

In addition to the manual, the shipping box should contain the following items:

- Camera head with a dust cap
- Power supply (with cable to camera)
- Power cord for the power supply
- PCI interface board
- 25 pin cable to connect the power supply to the camera (6 feet long)
- 50 pin cable to connect the interface board to the camera (10 feet long)
- Grounding wrist strap
- One CD-R that contains:
  - The software required to operate the SPOT camera.
  - A PDF (portable document format) version of the user manual, User Guide to the SPOT RT Camera.
  - The Adobe Acrobat Reader software that is required to read and/or print the user's manual. Refer to "Using SPOT On-line Documentation" below for instructions on installing the Reader on your computer.
- A registration card (you may also register online at www.diaginc.com/camreg.htm)
- 2 year limited warranty, 5 year limited warranty on desiccation.

Installing the Software

The CD provided with your camera contains the SPOT 4.0 software, which can be used with:

- Windows 95/98/NT/2000/ME/XP
- Mac OS10.1 or higher

> *Note: Although the SPOT software can be installed either before or after installing the PCI card, Diagnostic Instruments recommends installing the software first.*

> *Note: In some instances, Diagnostic Instruments has found that SPOT users with the* **Windows 2000** *operating system will get a* **No response from camera…** *message when trying to either capture an image or view a live image. This is due to incorrect BIOS settings. For information on correcting this problem, refer to* Appendix D: Selected Error Messages.

Windows Installation

1. Ensure that all other application programs are closed. Windows NT , 2000, and XP Users should also ensure that they are logged on with administrator privileges.

2. Insert the disk into your CD drive. The SPOT InstallShield Wizard automatically runs, and the Setup window appears.

3. Click on **Next** at the Readme Information window. The **User Information** window appears. Enter your name and company name.

4. Click on **Next**. The **Choose Destination Location** window appears. Either press the Browse button to specify the destination directory, or click on Next to accept the default directory, **C:\SpotCam**.

5. The **Select Program Folder** window appears. Click on **Next**. The Start Copying Files window displays the settings that you selected. Either click on Ne xt to accept the settings, or click on Back to return to the previous screens and revise the settings.

6. After you have accepted the settings, the program files are copied over, and the installation program prompts you with the message **Will there be a SPOT camera connected this computer?**

7. Select **Yes**. The installation program runs and looks for a camera. If you select No, the program still installs but will not look for a camera.

8. When the installation is complete you may be requested to re-start your computer.

9. You should see the following two desktop shortcuts.

Use the SPOT Advanced program mode if you are an experienced user, or you are a new user who wants to explore the broad range of functionality and choices offered by the program.

Use the SPOT Basic program mode if you are a new user who wants to immediately begin taking high quality pictures without having to make a lot of processing decisions.

To replace a shortcut that was deleted from the desktop, follow these steps:

a) Right click on the desktop.

b) Select **New > Shortcut**. The Create Shortcut window appears.

c) Enter the information in the **Command line** box (this presumes that you selected the default Spotcam directory in step 4 above).

   ♦ For the **Advanced** program, enter **C:\Spotcam\Spot32.exe -a**

   ♦ For the **Basic** program, enter **C:\Spotcam\Spot32.exe** .

   If you found the file by browsing, you will see only one **Spot32.exe** program. If the shortcut is for the Advanced program, you will have to modify the command line, as described above.

d) Click on **Next**.

e) Name the shortcut.

f) Click on **Finish**. The new shortcut appears on the desktop.

Video Card Related Problems (Windows)

Diagnostic Instruments has found that when the graphics acceleration is set too high on certain video cards, the SPOT program can crash when using the Live Image feature or the Live Image can be corrupted. If you experience this problem, change the Graphics Acceleration in Windows to a lower setting. Follow these steps:

1. From the **Start** menu button, select **Settings > Control Panel**.
2. Double click on the **Display** icon,
3. Click on the **Settings** tab.
4. Click on the **Advanced** button.
5. Click on the **Performance** tab.
6. Change the **Hardware Acceleration** setting by moving the slider to a lower setting. With some cards, you might have to move the slider to the lowest setting in order for live imaging to work.
7. Click on **Apply**.
8. Click on **OK**.
9. Click on **Close**.

Mac Installation

The CD disk provided with your camera contains the SPOT software, which can be used with Mac OS10.1 or higher. *Ensure that you have an administrator password* **BEFORE BEGINNING THE INSTALLATION.**

To install the SPOT software on a Mac, follow the steps listed below.

1. Insert the disk into your CD drive.
2. Double click on the installer icon.
3. Enter the Administrator User Name and Password
4. Click on **Continue**. The Spot Installer window appears.
5. Select the installation location for the program.
6. Click on **Install**. The Installing progress indicator appears.
7. When the installation is complete, you may be notified that the computer will restart.

Installing the Hardware

There are three simple steps to installing the hardware:

- Install the PCI card
- Connect the camera to the microscope
- Connect the PCI cable and power supply cable to the camera and to the appropriate computer or electrical interface

---

Note: Although the SPOT software can be installed either before or after installing the PCI cable, Diagnostic Instruments recommends installing the software first.

☙❈**WARNING**❧❈

This (the SPOT camera) is a Class A Product. In a domestic environment this product may cause radio interference in which case the user may be required to take adequate measures.

Installing the PCI Card

To install the PCI plug-in card (board), follow these steps:

CAUTION!

If you are unfamiliar with the procedures for installing plug in boards, refer the installation to your MIS department or to a qualified computer technician.

1. Turn off the computer, and let it sit for a few minutes to let the power supply drain.

2. Unplug all cables, and remove the power cord from the back of the computer.

☙❈**WARNING**❧❈

TO AVOID ELECTRICAL SHOCK, ALWAYS REMOVE THE POWER CORD AND ALL EXTERNAL CABLES, AND WAIT A FEW MINUTES FOR THE POWER SUPPLY TO DRAIN BEFORE OPENING THE CASE.

3. Place the computer on a clean, uncluttered surface, and remove the cover.

4. Position the computer so that the motherboard is facing you.

☙❈**WARNING**❧❈

DO NOT HANDLE THE PCI INTERFACE BOARD OR TOUCH COMPONENTS INSIDE THE COMPUTER WITHOUT USING THE ENCLOSED GROUNDING WRIST STRAP.

5. To discharge any static charge that might have built up on your body, touch your finger to the metal case of the computer's power supply.

6. Stick the copper foil end of the grounding wrist strap to the metal case of the computer's power supply.

7. Loop the other end of the strap around your wrist by pressing the sticky side against the strap, keeping the dark strip against your skin.

8. Use your computer's manual to identify the location of the PCI slot(s) on the motherboard.

9. Remove the metal cover plate adjacent to an available PCI slot. Save the screw.

> ✐ *Note: PC users should avoid installing the PCI card in the shared slot (i.e., the PCI slot next to the first ISA slot.*

10. Carefully insert the PCI bus interface board into the PCI slot.

11. Secure the metal bracket back into place with the screw from step nine.

12. Put the cover back on the computer.

13. Reboot the computer.

Connecting the Camera to the Microscope and the Computer

- The *RT Color Mosaic* and *RT B/W* model cameras require C mount couplers. The *RT 3-Shot and RT-Slider* cameras require an F mount coupler. Consult your authorized SPOT dealer or call Diagnostic Instruments for assistance in selecting the appropriate coupler for your Camera/Microscope combination.

To attach the **camera to the microscope**, follow these steps:

1. Attach the bottom clamp to the coupler.
2. Screw the RT camera into the coupler/bottom clamp until it is tight.
3. Insert the camera/coupler/bottom clamp combination into the microscope's photoport or phototube.
4. Tighten the thumbscrew on the microscope's phototube (or the coupler's bottom clamp) to lock the combination to the microscope.

To connect the **camera to your computer and power supply**, follow these steps:

1. Attach one end of the of the 50 pin (10 ft.) PCI cable to the PCI Card port on the computer (see PCI card installation below), and attach the other end to the camera head.
2. Attach the power supply cable 25 pin (6 ft.) to the camera head and the other end to the power supply.
3. Plug one end of the power cord into the power supply and the other end into a surge protector.

Multiple Camera Installations

More than one SPOT camera can be operated from a single computer, provided adequate PCI and/or FireWire ports are available. Any combination of SPOT Enhanced, RT, and/or Insight cameras can be used as long as your hardware meets the minimum requirements for every SPOT camera installed.

Using the SPOT On-line Documentation

To use the on-line documentation that accompanies your SPOT camera, you must first install the Acrobat Reader software that is included on the CD. This software enables you to read and/or print the Portable Document Format (PDF) version of the user guide that is on your CD.

Quick Start

Taking Your First Picture

The following sections list the basic steps for setting up your microscope and camera to immediately begin capturing images in the **Basic** program mode.

The picture taking process consists of two multi-part steps:

1. Preparing the microscope, specimen, and camera.
2. Capturing the image.

These steps assume that you have already installed the SPOT software and connected the camera to your computer. If you have not done so, refer back to the previous part of this manual, **Installation Guidelines**.

Preparing the Microscope and Camera

1. Select the specimen that you want to photograph, and set up the microscope for Koehler illumination.
2. Ensure that the specimen is in focus.
3. Using a daylight filter (5500° K), set the lamp voltage to the photo setting (or the rated lamp voltage).

Keep an assortment of ND (neutral density) filters on hand to use for images that are too bright (i.e., you receive the **Exposure time too short for Camera** error message).

Capturing the Image

To capture an image, follow these steps:

1. Open the SPOT Basic Program Mode.

From the desktop click on the SPOT Basic icon.

**2. View a real-time preview of your image capture.**

Click on the Live toolbar button. The Live Image window appears. As the camera computes the optimal auto-exposure sequence, the title bar displays **Computing Exposure**. When the program finishes the exposure computation sequence, the title bar displays **Running**, as shown at right

3. Use the microscope's controls to focus and frame the image as needed.
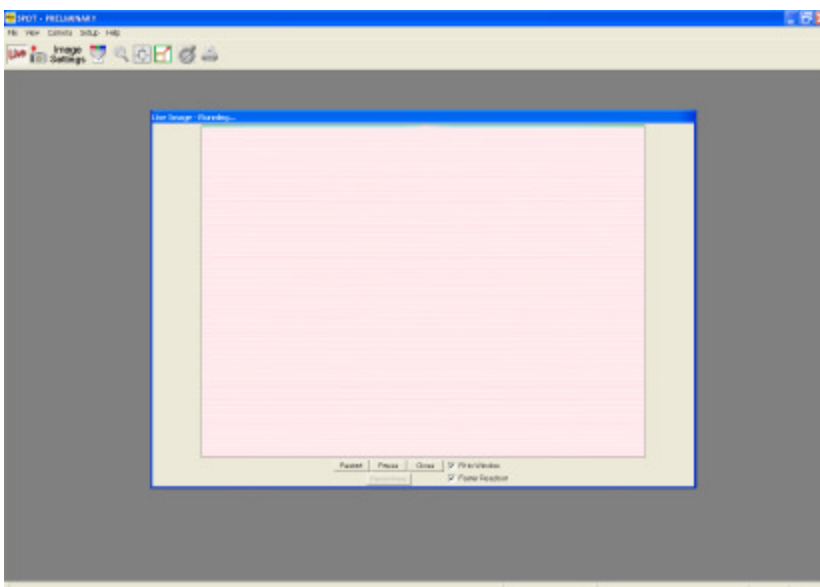
4. **Perform a White Balance on the image.**

    a) Click on the White Balance toolbar button. The live image display pauses, and the Compute White Balance dialog prompts you with a number of options to calibrate the white balance.

    b) Follow one of the instructions, based upon your application.

    c) Click on **Begin**. The Compute White Balance dialog displays **Computing Exposure**, and the Live Image window restarts and computes the new exposure settings.
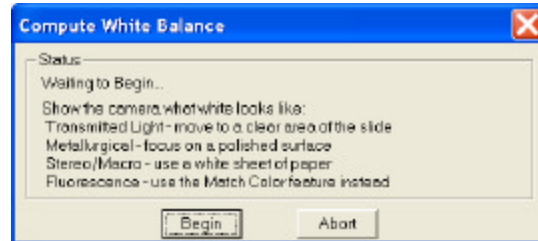
5. **Click on the Image Settings toolbar button.**

    The Image Settings window appears. Ensure that the settings are as follows**:**

    Main Window:

    - **Profile** – Default
    - **Exposure** - Auto
    - **Image Type** – (your type of microscopy)
    - **Brightness** – 1.00
    - **Quality/Speed** – Quality (left-most position)
    - **Gamma** – 1.00
    - **Chip Imaging Area** – Full Chip
    - **Live Image Auto Brightness** - Selected
    - **Flatfield** – unselected
    - **Color Enhancement** – selected

    More Image Settings:

    - **Flip** (Image) – as needed
    - **Rotate 90°** – as needed
    - **Minimum Exposure** – unselected
    - **Live Image Maximum** – default (10 sec)
    - **Filter Color – RGB for color images**
    - **Binning** – none
    - **Display Images in Grayscale** – not selected
    - **Noise Filter** – selected

    The suggested settings are designed to give you quality image captures in most, but not all cases. If your work involves fluorescence techniques, for example, you will need to adjust the Image Type, brightness, gamma and Quality/Speed settings accordingly. For more details on selecting Image Settings, refer to Ch. 3 – The Basic Program Mode.

6. **Click on the Get Image toolbar button.**

    The Get Image dialog box displays the status of the capture as it is downloaded to your screen.

    Because Get Image uses the values from the live image view, the image capture skips the initial exposure
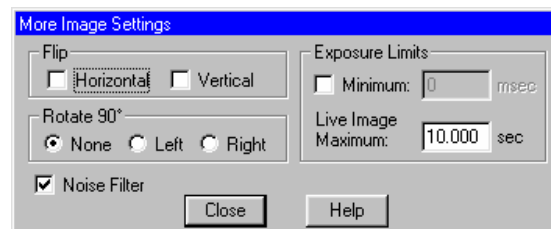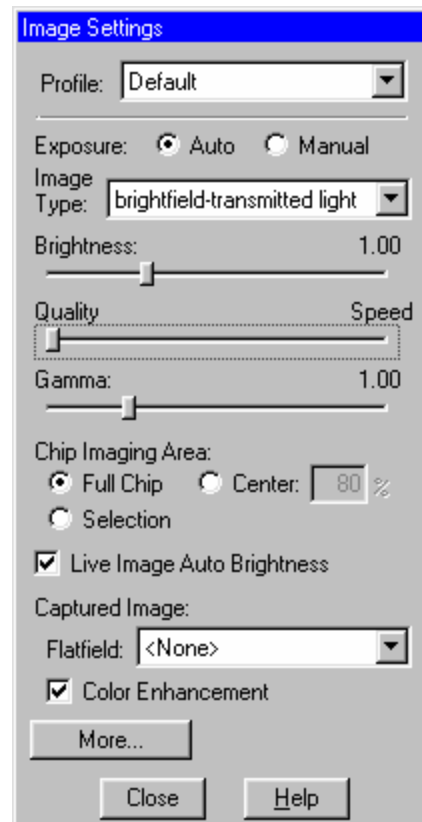
Ch. 1 - New Features

Version 4.0 of the SPOT software includes the following new features:

Feature	Description	Find in Chapter(s)
Annotation	Title and time options have been added to the annotation options. An auto-number text object has also been added.	8
Bias Frame & Thermal Frame Subtract	Bias Frame subtraction and Thermal Frame subtraction have been added. As part of this change, Background Subtraction has been removed.	5,7
Database Export	A database export feature has now been added to the SPOT Windows (PC) version.	9
Embedded Variables	The ability to use embedded variables in static text and runtime text has been added for reports.	10, 13
Image Acquisition Settings	The image acquisition settings save/display feature has been added.	8
Macros	Automated "Macro" subroutines can now be written, saved, and implemented saving the user time and effort.	13
Median Filter	Median filter image processing has been added to the other filter modes.	8
On the Fly hardware selection	The ability to change the interface board/camera selection while the application is running has been added. The application program will update the image setup to accommodate the new camera.	4
Shutter Sound	An option for an audible "shutter click" has been added. The sound indicates when an image has been captured.	3
Spot Metering	A spot metering feature has been added. Now the user has the option of metering off of the entire image (normal) for automatic exposure or defining the region of interest in which to meter the automatic exposure.	5,7
Taskbars	A "floating" taskbar feature has now been added to the SPOT advanced mode.	12

Ch. 2 – Basic and Advanced Program Modes

Introduction

With the SPOT software you can work in either a Basic or an Advanced program mode.

- The **Basic** program mode is a simplified version of the SPOT program that enables new users to quickly start capturing images without having to go through all of the decision making processes associated with the Advanced version of the program. The Basic program mode is also ideal for users who simply need "snapshots" of their specimens. Refer to Ch. 3 – The Basic Program Mode for more details.

- The **Advanced** program mode is an enhanced version of the SPOT software. It is designed for more advanced users who want both greater control over the way images are captured and edited, as well as access to non-image capture functions such as database image archiving and reporting. Refer to Chapters 4 – 13 for more information on working in the Advanced program mode.

When you install the SPOT software, the installation process adds the Basic and Advanced program modes to your menu structure as well as two icons to the desktop. The following section briefly compares the two program modes.

Basic vs. Advanced Features

The Basic SPOT program includes only basic image capture and editing functionality. The following table compares the general features of the Basic and Advanced SPOT programs.

Features	Advanced	Basic
automated post-processing adjustments	X	X
calibration and measurement functions	X	X
customizable (user-defined) image setups	Extensive	Simple
customizable printing	X	X
database (Windows only)	X	
exposure adjustment	X	X
image annotation	X	X
image editing	Extensive	Simple
live image preview	X	X
image editing in multiple color spaces	X	X
Macro scripting	X	
pre-defined image setups	X	X
recall of exposure values used in previous images	X	
reporting	Extensive	Simple
sequential imaging	X	X
taskbars	X	

Ch. 3 – The Basic Program Mode

Introduction: Using the Basic Program

The Basic SPOT program allows you to quickly and conveniently capture and edit images, without having to make decisions related to the numerous processing and editing options available in the Advanced program.

This chapter is divided into the following sections:

- Navigating in Basic Program Mode
- Preparing to Take a Picture
- Taking a Picture
- Viewing, Editing, and Annotating Images
- Creating Reports
- Printing Options

Navigating in Basic Program Mode

The following sections are an introduction to the SPOT Basic mode operating environment. They include:

- The Viewing and Editing Window - The primary window for all image capture and editing functions.

- Opening and Saving Files - Standard file save and open features, as well as those that are unique to SPOT.

- Setup - Initial settings to customize your software.

The Viewing and Editing Window

When you open or capture an image, it appears in the main viewing and editing window. All of the image save, view, edit, and annotation functions are accessed from this window, which consists of three areas:

- The Menu Bar
- The Toolbar
- The Status Bar

The Menu Bar

The Menu Bar in the Basic program varies according to whether an image is open.

If you have not yet either captured a new image or opened an existing image, the following menus are available:	If you have either captured a new image or opened an existing image, the following menus are available:
File	File
View	Edit
Camera	View
Setup	Camera
Help	Setup
	Window
	Help

- The **File** menu contains file open and save options, which for the most part, are identical to the standard file open and save options. Refer to Opening and Saving Files (below) for details. In addition, the File menu contains the report creation and viewing options. Refer to the Basic Mode Reporting section of this chapter for more details.

- The **Camera** menu contains initialization, image capture, white balance, flatfield correction, and live imaging options.

- The **View** menu contains different options for viewing captured images. Refer to Viewing, Editing, and Annotating Images (below) for details. View selections are limited to "Full Screen" when an image has not yet been captured.

- The **Setup** menu contains options used to define operating preferences. Refer to Setup (below) for details.

- The **Edit** menu contains image-editing options.

- The **Window** menu contains standard Windows display options. Refer to Viewing, Editing, and Annotating Images (below) for details.

- The **Help** menu contains information about the SPOT software and enables you to access the on-line help system.

The Toolbar

The Toolbar appears underneath the Menu Bar and contains all of the image capture, editing, and annotation functions in the Basic program. The following diagram illustrates the Basic program Toolbar, as it appears for an open image:

Figure 3- 1: Basic Mode Tool bar

The Status Bar

The status bar at the bottom of your screen displays information about the on- screen image. The following is an illustration of the status bar, as it appears for an open image:
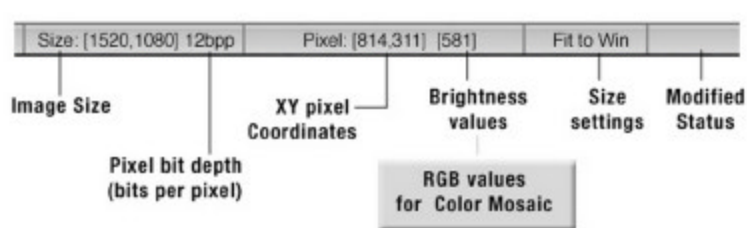

Figure 3- 2: Basic Mode Status Bar

Each of the above call-outs is described in the following list:

- **Image size** – (Columns, rows) in pixels [e.g., (1520, 1080)].

- **Pixel bit depth** (bits per pixel) - The pixel bit depth at which the image was captured. This value reflects the pixel bit depth for the image setup that was used to capture the image. All images captured in Basic mode are 24 bpp.

- **X Y pixel coordinates** - As you move your cursor over an image, the status bar displays the x y coordinates of the cursor in pixels.

- **Brightness values** - As you move your cursor over an image, the view status bar indicates the brightness values of the pixel coordinates. The status bar displays separate red, green, and blue brightness values for each part of the image.

- **Size Settings** - In the illustration above, the view status bar displays **Fit to Win** (Fit to Window), the default image capture setting.
  - If the Fit to Window option has been deselected, this area displays the word **Normal**.
  - If you are in zoom mode, this area displays the magnification level (e.g., **Mag:x1**, **Mag:x1/4** etc…).

- **Modified Status** - The section to the right of the size settings remains blank until you modify an image. Once an image has been modified from the original status, the word **Modified** appears here.

-

> *Note: When the camera is on, but no image has been captured or loaded from an image file, only a limited number of toolbar icons will be functional.*

> *Note: When the camera is off or not connected, only five toolbar icons will be present on the toolbar.*

Opening and Saving Files

All of the file open and save options in the Basic SPOT program are accessed from the File menu. These options are described below.

> *Note: Standard Windows functions are not described in detail in this manual. For more detail on these functions, refer to the Windows on-line help.*

Open Image File	Open image files of a specified format (i.e., JPEG, TIFF, BMP, etc.). In addition to using this menu option, you can open images by dragging an image file from the Windows 98SE/2000/XP Desktop or Explorer onto the SPOT program window.
Show Image File Thumbnails	Display thumbnail previews of specified files and open images from the preview screen. To show file thumbnails, follow these steps: 1. From the File menu, select the Show Image File Thumbnails option. The Thumbnail File Spec window displays all of the image files in the directory. 2. Navigate to the directory for the image files that you want to display. 3. Use the **Files of type** drop down box, to specify the file type.

Show Image File Thumbnails (cont.)

4. Select the file or files for which you want to display thumbnails. If you do not select a file or files, the program displays thumbnails for all files of the specified type in the directory.

5. Click on Open. The Thumbnails preview window appears. For each thumbnail, you can:
   – *Double-click* (Mac and Windows) on a thumbnail to open an image for viewing or modification.
   – *Right-click* **(Windows)/[⌘] Click (Mac)** on a thumbnail to display a list of options. The right-click menu enables you to perform a variety of actions directly from the thumbnail window:
      ♦ **Select Image.**
      ♦ **Open Image.**
      ♦ **Delete Image.**
      ♦ **Print Image.**
      ♦ **Move/Rename Image File**
      ♦ **Copy Image**
      ♦ **Add Image to Image Sequence** (see below)
      ♦ **Add Image to Report** (see below)
      ♦ **Select All Images** in the thumbnail view. When you use this option, the right-click menu expands to include the above options for multiple images

   Click and drag with the mouse to select two or more images.

Open Image Sequence File

Open an image sequence file. An image sequence file is a single TIFF format file that contains multiple images.

- Refer to **Taking a Picture** section of this chapter for information on capturing an image sequence file.

- Refer to the Viewing, Editing, and Annotating Images for information on viewing and editing image sequences.

New Image Sequence

Create a new image sequence file. This option enables you to specify the first image in a new image sequence. This image can come from an open image or an image on disk (from file). Subsequent images can be added to the sequence in one of two ways:

- Via the **Add Image to Image Sequence** File menu option (see below). This option allows you either to add an open, non-sequence image to a sequence or to create a new image sequence from the image.

- Via the **Sequence** menu options. Here you can add any image to any point in a sequence. Refer to **Working With Image Sequences** in Ch. 8 for more details.

New Report

Create a new Basic Mode report. Refer to the **Creating Reports** section of this chapter for more details.

Open Report

Open a Basic Mode report. Refer to the **Creating Reports** section of this chapter for more details.

Reload

Reload the image from the hard drive (or other media). All changes made since opening the image file are lost. This option appears only when a saved image is open.

Save

Save a previously unsaved image to new file, or update the changes to an existing file. This option appears only when an image is open.

Save As

Save an image to a new file and/or file location. This option appears only when an image is open.

Save All

Save all open image files. The program prompts you for the name and file location of each image.

Close

Close an image. The program prompts you to specify a location to save the file. This option appears only when an image is open.

Move/ Rename

Move and/or rename an image file. This option differs from the Save As option in that it *does not* retain a copy of the original file in the original location: using Move/Rename is comparable to cutting and pasting a file.

Delete

Delete an open image. The program prompts you to verify the deletion.

Export Movie File (Windows)

Export a SPOT image sequence file to AVI format. Follow these steps:

1. Open a SPOT image sequence. Refer to Ch. 7 – Taking a Picture, for details on capturing image sequences.

2. Select **File>Export Movie File**. The **Export Movie File** appears.

3. Enter the **File Name** for the avi file.

4. Specify the location where you want to save the avi file.

5. Specify which images in the sequence that you want to **Export**. Select either:

    • **All Images** – the avi file will contain all of the images in the sequence.

    • **Specified Images** - the avi file will contain only the images that you specify (i.e., below the radio button).

6. Specify the **Frame Rate** in frames per second.

7. Specify the **Compression Settings**. When you click on the **Compression Settings** button, the **Video Compression** dialog displays the compression application(s) available on your computer. Because each computer will have different installed applications, the Video Compression options will vary.

8. After you have entered all of the desired settings, click on **Save**. The program displays a status message as it exports the file.

Add Image to Image Sequence

Add an open, non-sequence image to an open image sequence, or create a new image sequence from an open image. To use this option, the non-sequence image must be the active window and the image sequence must be open in the background.

When you click on **Add Image to Image Sequence**, SPOT prompts you to specify either the **Image Sequence** or a new image sequence. If more than one image sequence is open, select the sequence from the drop-down list.

Add Image to Report

Add a selected open image to an open SPOT report. When you select this option, SPOT prompts you to select from a list of reports. If you have not defined a report, SPOT automatically creates a new report for you and inserts the image as a **Static Image** object. Refer to the **Creating Reports** section of this chapter for more details.

Print

Print an open image. Refer to Chapter 11 – Printing Images for information on standard Windows and SPOT custom printing features.

Printer Setup

Define the page setup parameters. Refer to Ch. 11 – Printing Images for information on SPOT's custom printing features.

Exit (Windows) Quit (Mac)

Exit the SPOT program.

Setup

The Basic program mode Setup menu options are as follows:

- Show Image Settings
- Calibration Setup
- Image Auto-Save
- Select Device
- Preferences

Show Image Settings

Use this option to display the **Image Settings** window. In the SPOT Basic Program Mode, the **Image Settings** window is used to define the settings for both the live image view and the final image capture. This means that you can use the Image Settings window in one of two ways:

- As a stand-alone dialog to pre-define the settings that the SPOT camera uses for capture images.

- In conjunction with the Live Image window. When using the Image Settings dialog to define the live image view, the live image settings also serve as the image capture settings. In other words, the live image view can be used as a guide to how the final image will appear.

Refer to the **Preparing to Take a Picture** section below for a description of the options on the Image Settings window.

Calibration Setups

With the SPOT program, you can use a stage micrometer to calibrate each objective on your microscope. Once calibrated, you can use the SPOT program to perform basic measurements on an image.

- *For details on calibrating objectives...*
  refer to the **Calibration Setups** section of Ch. 4.

- *For details on adding measurements and/or calibration marks to your images...*
  refer to the **Annotation – Calibration and Measurement Marks** section of Ch. 8.

Image Auto-Save

Use the **Image Auto-Save** feature to automatically save images as part of the capture process. To enable SPOT's auto-save feature, select **Setup>Image Auto-Save**, and check the **Auto-Save Images** box, as shown at right. For details on auto-saving images, refer to the **Image Auto-Save** section of Ch. 4.

Select Device

If you have multiple cameras connected to your computer, you can select the camera to use for your imaging using this window.

Preferences

Use the Preferences option to specify:
- Size of the onscreen toolbar
- Sequential imaging memory settings
- Thumbnail appearance.
- Live image viewing settings
- Captured Image Bit Depth and Fit to window view settings
- File type extension setting (*Macs only*).

Note: In SPOT 4.0, the Preferences window appearance has changed. Preferences are now subdivided into multiple windows.

To open the Preferences window, select **Preferences** from the Setup menu. Each preference is discussed below.

Toolbar

Toolbar
Allows the user to specify whether the onscreen toolbar icons in the SPOT program should be displayed in large or small format.

Image Sequences

Memory Usage Limit
Specify how much memory is allocated for image acquisition. Note that the entry parameters are system dependent.

Cache Images During Play
When "playing" an image sequence (see Ch. 8), the SPOT program allows you to select how it loads the displayed images:

- *If **Cache Images During Play** is selected…* The program pre-loads as many images from the sequence as your computer's RAM allows. This enables a faster display of the image sequence. But, if you have a sequence with many images, the size of the combined images could exceed the RAM, and in this case, the SPOT program will load as much of the sequence as possible, play that part of the sequence, and then pause to load the remaining images.

- *If **Cache Images During Play** is not selected…* The program loads each image in the sequence individually. In cases where you have a large sequence with many files, this allows you to work without being restricted by RAM limitations. The display of images will be somewhat slower than a display where images are cached

Thumbnails

Specify the size of thumbnails for the Show Image File Thumbnails option

Select from one of the following options:
- **Small (60 x 60 pixels)**
- **Medium (90 x 90 pixels)**
- **Large (120 x 120 pixels)**
- **User Defined**
  Minimum width and height = 20 pixels
  Maximum width and height = 200 pixels

Specify the resolution of image thumbnails (Show Image File Thumbnails option)

Select from one of the following options:
- **Small (54 pixels)**
- **Medium (84 pixels)**
- **Large (114 pixels)**

- **User Defined**
  Minimum resolution = 16 pixels
  Maximum resolution = 200 pixels

Live Images

Always Show Full Chip in Live Image
Ensures that you will always see the Full Chip view when viewing a live image. Keep in mind that the selection that you make here determines how the live image displays when viewing a region of the chip. Refer to the **Chip Imaging Area** section for details.

Miscellaneous

Specify whether images should open in Fit to Window mode.
The Fit to Window option, a program default, automatically sizes images to fit the available screen space when you open or capture an image. Thus, you can immediately see the entire image without having to zoom out. This is especially useful with smaller monitors. Open Images in Fit to Window Mode is checked as a default. If you do not want images to open in the Fit to Window mode, deselect the box.

Auto-save Image File Name Suffix Number.
This feature allows you to specify a suffix number after the file name in order to keep track of the sequence of images captured.

Specify the pixel bit depth for captured images.
The Captured Image Bit Depth option enables you to specify a different image bit depth when saving an image captured in the SPOT program

- **8 Bits per Channel** – The 8 bits per channel option provides 256 gray levels per color:
  - With RT B/W cameras, captured images are 8 bits per pixel monochrome.
  - With RT Color cameras, captured images are 24 bits per pixel RGB color.

  This option results in smaller, more manageable image sizes, and is best for images that do not require extensive modification.

- **12 Bits per Channel** – The 12 Bits per channel option provides 4,096 gray levels per color.
  - With RT B/W cameras, captured images are 12 bits per pixel monochrome.
  - With RT Color cameras, captured images are 36 bits per pixel RGB color.

  This option reduces banding problems that can result with modified images, but also results in larger file sizes.

  When saving 12 bit per channel images, SPOT prompts you to select either 12 bits per channel or 16 bits per channel. If you use Adobe's Photoshop for image editing, you should select the 16 bit per channel option because Photoshop cannot read 12 bit per channel images.

Note: The SPOT RT camera captures all images using 10 bits per channel (color). The SPOT software immediately converts the captured image to either 8 bits per channel (color) or 12 bits per channel (color), depending on the **Captured Image Bit Depth** *that you select.*

Specify whether you would like to utilize a shutter sound on image capture.
The Make Shutter Sound option, when checked, will provide a "click" sound during the camera exposure sequence, confirming that the image capture is complete. If the image capture is relatively quick, you will hear a single click. If the exposure is longer (i.e. greater than one second) you may hear a "beginning exposure" click and an "ending exposure" click.

Monitor Color Filter Position.
If checked, the software will monitor which position the color changing filter is in.

The Monitor Camera Color Filter Position box is checked as a default. If this option is left checked, the SPOT program displays error messages when the sensors in the camera head detect that the color filter is not in the correct position. This option **does not** monitor the position of the filter slider in the Live Image window. In Basic mode, the program will give you an error message if you are capturing an image and the sliding color filter is in the **B/W** position.

Note: Monitor Color Filter Position will only appear when you are using a RT Slider camera.

Add Extensions to File Names on Save (Mac)

When selected, the **Add Extensions to File Names on Save** option automatically appends the file type extension (e.g., JPG, tiff, bmp, etc…) to your image file. This enables cross platform file sharing, because Windows based programs require a file type extension, while Mac based programs do not. Once the file type extension is added, Windows based programs can recognize the Mac file.

Preparing to Take a Picture

The **Image Settings** dialog is the central point for defining both live image and image capture settings. The Image Settings window enables you to optimize the live image view, and then capture an image using these settings. To open the Image Settings dialog, either click on the **Image Settings** toolbar button, or select **Show Image Settings** from the Setup menu. Each image capture/live image view option is discussed in the sections below.

Profile

The **Profile** option allows you to create and re-use sets of live image/image capture settings. When you make changes to any of the live image/image capture settings, these changes can be saved to a Profile – either the Default or one that you have defined. For example, if you gamma adjust a live image and then decide that the result is ideal for the type of specimen you are working with, you could create a new Profile with the gamma and exposure values and name it for that type of specimen – i.e., "Diseased Cells."
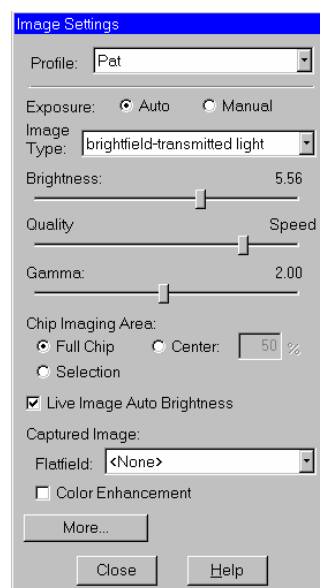
When you close the Image Settings window, the new settings will be preserved until you close the SPOT software program or until you open the Image Settings window again and reset the values (in the same session). If you want to save the settings to the profile of interest, modify the settings, then right click on the Profile name (Windows) and choose save from the menu options that are listed below. Mac users should hold the Control key while mouse clicking on the Profile name.

To add, edit, or delete Profiles, **right-click** on the Profile box. Right clicking gives the following options:

- **Add –** Add a new Profile. When you add a new Profile, the program prompts you to name it.
- **Save –** Save a Profile that you have modified.
- **Rename –** Rename an existing Profile.
- **Delete –** Delete a Profile. This option appears only if there is more than one Profile.

Exposure: Auto or Manual

The **Exposure** option gives you the choice of having the camera calculate exposure time (**Auto**), or setting the exposure on your own (**Manual**).   The exposure options will vary according to whether you select **Auto** or **Manual**, as illustrated below. When you enter one of the above exposure settings, note that it applies **both** to the live image that you are viewing and the captured image.

Auto Exposure options

Image Type

An Image Type tells the SPOT program how to expose and process image captures. Each of the 11 available Image Types (see below) corresponds to a microscopy technique: select the Image Type that matches the technique you are using. The camera will capture the image accordingly. The table below describes typical situations for each image type.

Image Type	Description

Brightfield-reflected light	Polished metallurgical specimens and semi-conductors using a microscope with a vertical illuminator.
Brightfield-transmitted light	Stained specimens on glass slides using transmitted light microscopes.
Darkfield-reflected light	Bright areas of specimens being viewed with darkfield, reflected light techniques.
Darkfield-transmitted light	Bright specimens on dark backgrounds using transmitted light darkfield technique.
Fluorescence	Fluorescence specimens not fluorescing in a single red, green, or blue primary color
Nomarski DIC/Hoffman	Specimens being viewed with either Nomarski DIC or Hoffman techniques.
Phase contrast	Specimens being viewed using the phase contrast technique.
Polarized light	Specimens being viewed with polarized light techniques.
Stereo/macro-metallic glints	Metallic specimens with intense glints.
Stereo/macro–no glints	Matte specimens without bright glints.
Stereo/macro–shiny glints	Shiny specimens with bright glints.

Brightness

Enter a **Brightness** value between **.24** and **34**.
A brightness value of 1, the default value, gives you the correct image brightness for most images. The Brightness control allows you to overexpose or underexpose (darken or lighten) the image area. In many cases, an image has varying levels of brightness; to see a specific area better you might want to lighten or darken the image. When using the Brightness control, the SPOT camera uses an algorithm that combines gain and exposure time to provide the fastest possible image update rate (shutter speed) in live mode:

- When overexposing images to make them brighter (i.e., entering values greater than one), the camera first selects the highest possible gain before lengthening the exposure time. Because gain also brightens an image, less exposure time is needed.

- When underexposing images to make them darker (i.e., entering values less then one) the camera uses the lowest possible exposure time before lowering the gain.

Quality/Speed (Auto)

Use the slider to balance speed and quality when previewing dim images.

- Moving the slider toward the **Quality** side increases image quality and decreases frame rates.
- Moving the slider toward the **Speed** side increases frame rates and decreases image quality.

The Quality/Speed measurement corresponds to the Auto-Gain limit setting that allows you to set an upper limit on the gain options that will be available during the Auto-Exposure process.

- When the slider is at the left-most point, the Auto-Gain Limit gives you higher quality pictures, but lengthier exposure times.

- When the slider is at the right-most point, the Auto-Gain Limit allows the camera to select from all available gain options. This shortens exposure times, but decreases image quality.

Refer to the "Auto-Gain Limit" section in Ch. 5 – Preparing to Take a Picture for a detailed explanation of gain and exposure.

Gamma (Auto)

Enter a **Gamma** value between **.1** and **4**.
Gamma adjustment smoothly lightens darker areas of an image without burning out bright areas or lightening black areas. This is especially useful for capturing/editing images with a wide range of brightness values (dynamic range) such as fluorescence specimens or macroscopic objects with glints, (e.g., printed circuitry).

- Values between **1** and **4** lighten the darker areas of the image.
- Values between **.1** and **1** darken the mid-tones of the image.

Gamma adjustments on live images are made in the RGB color space, the only color space where adjustments can be made quickly enough to keep up with live imaging. Refer to Ch. 5 – Preparing to Take a Picture for more details on gamma adjustment and color spaces.

Manual Exposure Options

Exposure Time(s)

Use the slider-bar to specify an exposure time (or times) between **.04 ms** and **526.746 seconds**.
When you specify an exposure time, the camera skips the automated exposure sequence that takes place when you open the live image view. This is very useful in cases where exposure times are lengthy, as with fluorescence samples.

Gain

Enter the precise gain that the camera will use for an image capture. Use the slider-bar to select one of the gain options: **1**, **2**, **4**, **8**, or **16**. The gain value displays to the right of the slider-bar. Refer to the "Auto-Gain Limit " and "Gain" sections of Ch. 5 – Preparing to Take a Picture for details on gain and exposure.

Gamma

The manual Gamma adjustment is identical to the auto Gamma adjustment.

Chip Imaging Area

SPOT allows you to use any rectangular portion of the CCD chip for image capture. Using a portion of the chip is helpful in cases where you want to:

- Keep file sizes small.
- Shorten download times.
- Avoid areas of the image with optical problems (e.g., vignette areas).

There are three image area options:

- **Full Chip** – Capture the entire area of the CCD chip.

- **Center** – Capture a rectangular part of the CCD chip's area that has the same height - to - width ratio as the full chip, and is centered on the center of the full chip. The percentage that you enter is the percentage of full chip width that the new area's width will be.

    To capture a centered proportion of an image, follow these steps:
    a) Check the Center option.
    b) Enter the percentage of the image that you want to capture. The minimum percentage is 10 and the maximum is 100.
    c) Capture the image.

- **Selection** –Capture a specific part of the CCD chip.

    a) Check the **Selection** option.
    b) Close the Image Settings window and open the Live Image dialog (click on the **Live** toolbar button).
    c) Use the mouse to select a region for capture. Click and drag to define the selection.
    d) Capture the image.

When you are viewing a live image **Selection**, how this selection is displayed, depends on whether the **Open Images in Fit to Window Mode** and **Always Show Full Chip in Live Image** preferences (see the **Preferences** section above) are checked. The following table illustrates the possibilities:

Fit to Window Checked?	Always Show Full Chip in Live Image Checked?	Selection Display	Live or Captured Image
YES	YES	When you select a portion of the chip, the selection is outlined with a **yellow rectangle**.	LIVE
YES	NO	When you select a portion of the chip the cursor is a **zoom tool**. To zoom in on a portion of a live or captured image, click and drag over the area that you want to zoom in on. To return to the non-zoomed image, click on the **Full Chip** option.	BOTH
NO	YES	When you select a portion of the chip, the selection is outlined with a yellow rectangle. However, because **Fit to Window** is not checked, you will not see the entire chip in the window.	LIVE
NO	NO	When you select a portion of the image, the cursor is a cropping tool. If you select a portion of an image, the program displays only the selected area and discards the rest of the image. To return to the non-cropped image, click on the **Full Chip** option.	BOTH

Live Image Auto-Brightness

Available only when you are using **Auto** exposure and viewing a live image, the **Live Image Auto-Brightness** option enables you to maintain a consistent brightness level for changing specimens.

Flatfield

The Flatfield option is used to correct for display problems associated with uneven intensity or coloration in illumination, or to correct for artifacts (e.g., dust) in the optical system.

> *Note: Flatfield correction with the SPOT camera refers only to the correction of uneven lighting. It should not be confused with the correction of optical field flatness.*

Flatfield correction works by taking a picture of the brightfield background without the specimen in the field of view. This image lets the SPOT software know, on a pixel-by pixel basis, which areas in the field of view are receiving less illumination than the brightest areas. When taking a picture that contains a specimen, the software uses this information to accurately increase the brightness of each pixel in the dimly illuminated areas. The resulting image gives the appearance of an even illumination.

The Flatfield correction process involves two images - the flatfield image and the final image. To use Flatfield Correct, follow these steps:

1. Ensure that the specimen is under the desired objective and in focus.

2. From the Camera menu, select Get Flatfield Image. The Get Flatfield dialog box appears.

3. Remove the slide from the field of view.

4. Click on Begin. When the program finishes computing the exposure times and acquiring the flatfield file, the program prompts you to name and save the file.

5. Name the Flatfield file. Because flatfield image files are different for each objective, you might want to name the file according to the objective's magnification (e.g., 10x).

6. Click on OK. If you do not specify a directory path, the program automatically saves the flatfield file:
   – In Windows, the file is saved to the **c:\SpotCam\Fltflds** directory
   – In the Mac OS, the file is saved to **System Folder>Preferences>SPOTPrefs>Flatfields**.

> *Note: The default file location varies, depending on where you installed the SPOT program.*

7. Move the specimen back into the field of view.

8. Click on the **Live** toolbar button.

9. Open the **Image Settings** window, and select the Flatfield box. The name of the flatfield file that you defined in step four should appear in the drop-down list of flatfield files.

10. Perform the image capture. SPOT automatically uses the flatfield image to correct the capture.

Refer to the Taking a Picture section of this chapter for more details.

Color Enhancement (Captured Images)

The Color Enhancement option optimizes image captures by closely matching the final capture to the image as seen through the microscope. To optimize the final image, the SPOT program processes the raw image data to produce a final image that matches the view seen through the microscope.

If you want an image capture to match the live image view, you will want to leave Color Enhancement off. Color Enhancement applies only to captured images.

More (Additional Orientation and Exposure Settings)

When you click on **More**, the program displays a dialog of additional orientation and exposure settings, as shown at right:

> ✎ *Note: The screen capture at right is from an RT 3-Shot and an RT Slider – the Filter Color and Display Images in Grayscale options do not appear with other RT models. The RT Color Mosaic will not show the binning option.*
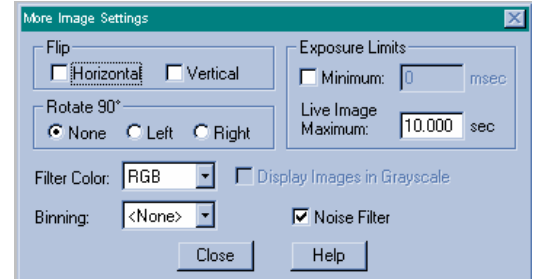
Flip

Specify whether images will be flipped during the image capture/live image viewing process.

- Select **Horizontal** to flip the image from left to right.
- Select **Vertical** to flip the image from top to bottom.
- Select both options to flip the image from left to right and from top to bottom.

Rotate 90°

Specify whether images will be rotated during the image capture/live image viewing process.

- Select **None** if you do not want the image to be rotated.
- Select **Right** to rotate the image clockwise 90 degrees.
- Select **Left** to rotate the image counterclockwise 90 degrees.

Exposure Limits - Minimum

Because SPOT RT cameras are capable of extremely short exposure times, light sources with intensities that vary with the waveform of the 60 Hz line supply (e.g., fluorescent lamps, 120 volt incandescent bulbs, and 6 volt 20 watt halogen lamps) can cause the Live Image window to flicker and result in incorrect brightness and color balance readings in captured images. The **Minimum Exposure** option allows you to correct for these problems by limiting the camera to a minimum exposure time that is greater than the pulse interval of the light source. Consider the illustration to the right. In this case, the pulse interval of
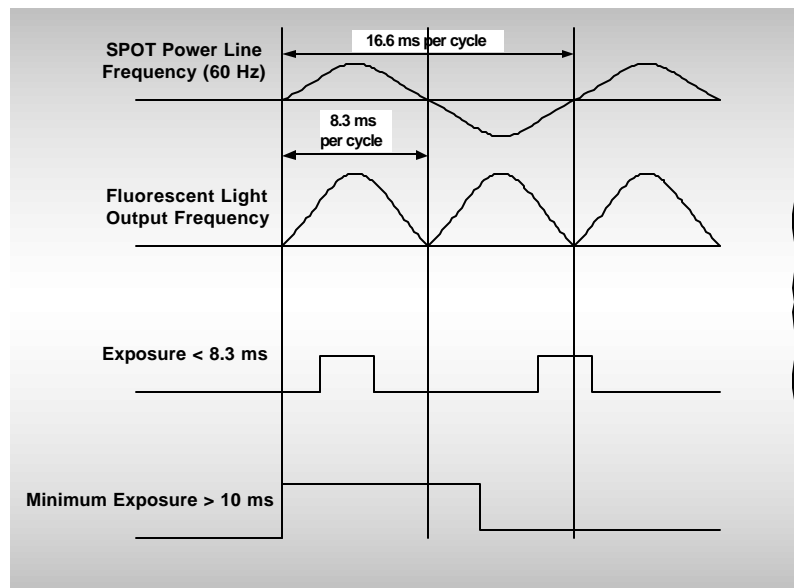

Figure 3- 3: Setting a Minimum Exposure to Accommodate Varied Light Sources (Basic Mode)

the fluorescent light source is 8.3 ms. Entering a Minimum Exposure of 25 ms ensures that the camera's exposure time is greater than the pulse interval of the light source and that any error is minimized. The greater the value above 25 ms, the more the error is reduced. Alternatively, you can enter any multiple of 8.333 ms. To enter a minimum exposure, check the **Minimum** box, and enter a value in milliseconds.

Exposure Limits – Live Image Maximum

The Live Image Maximum option helps you to avoid unnecessarily long exposures when viewing a live image. Consider the following situation: When you change from a lower resolution objective to a higher resolution objective, the camera detects a brief interval of blackness. When the Auto-Brightness option (see above) is enabled, it compensates by modifying the exposure time to maintain a constant brightness level. This results in you having to wait for the camera to compute a lengthy and incorrect exposure for the black interval. Using the **Live Image Maximum** option, you can limit this exposure time. For most cases, Diagnostic Instruments recommends a value of 0.5 seconds. Similarly, the Live Image Maximum option can also be used in cases where the microscope's light path selector is incorrectly set.

Filter Color (RT 3-Shot, RT Slider)

The Filter Color option specifies the color or colors that will be exposed in the live image preview/image capture.

- **Red** – Preview/capture a monochrome preview of your specimen using the red state of the LCD filter.

- **Green** – Preview/capture a monochrome preview of your specimen using the green state of the LCD filter.

- **Blue** - Preview/capture a monochrome preview of your specimen using the blue state of the LCD filter.

- **Clear** – Preview/capture a monochrome preview of your specimen using the clear state of the LCD filter. Selecting the Clear option does not provide the entirely unfiltered image that is possible with the RT Slider camera. Although all three colors are transmitted, the polarizing filter, which is part of the liquid crystal filter, and the IR filter remain in the light path, blocking some of the light. When using the Live Image window to focus or position dim images, RT Slider users can slide the filter out to provide higher frame rates and less noisy images.

- **None (RT Slider)** – Preview capture a completely unfiltered image.

RT Slider	RT Slider users can select **RGB** you to preview/capture live images in full color. If the filter slider on the camera is moved to the **B/W** position, the Filter Color setting should likewise be changed to one of the monochrome options (e.g., Clear, Red, Green, or Blue). The **Monitor Camera Color Filter Position** preference monitors the color filter position only for image captures– it does not monitor color filter position when viewing live images.
RT Slider	Selecting a Filter Color option *does not* change the position of the filter: this must be done by physically sliding the filter to the desired position. If the Filter Color setting does not match the filter's position, SPOT prompts you with an error message. *But, this error message appears only y if the* **Monitor Camera Color Filter Position** *option on the* **Preferences** *window (see above) is selected.*

Display Images in Grayscale (RT 3-Shot, RT Slider)

To counter the difficulty of viewing, focusing, and adjusting monochrome images in live mode, the **Display Images in Grayscale** option enables you to view a monochrome image in grayscale, as opposed to with the red, green, or blue filter. This option applies only when the **Filter Color** (see above) is set to Red, Green, or Blue.

Binning (RT 3-Shot, RT Slider, RT Monochrome)

Binning combines adjacent pixels on the CCD chip, thereby reducing the number of pixels used in the image capture. Binning is useful when you have dim images and want to increase sensitivity and reduce download time. This gives you higher fra me rates and less noisy images. But, as binning level increases, image resolution decreases. All of these factors should be considered when you select a binning option.

There are four binning options:
- None..................1 pixel = 1 pixel
- 2 x 2 ....4 pixels = 1 pixel
- 3 x 3 ....9 pixels = 1 pixel
- 4 x 4 ....16 pixels = 1 pixel

Noise Filter

In addition to the noise suppression from the physical cooling of the RT camera, the Noise Filter also corrects for the effects of noise. When capturing dim images with lengthy exposure times, the noise filter acts to eliminate "hot pixels." The Noise Filter checks each pixel on the CCD chip as described below:

1. The program compares the value of each red pixel with the values of the eight neighboring red pixels.

2. If none of the eight neighboring red pixels has a value within ±15 % (i.e., the threshold value that the SPOT program uses) of the value for the red pixel being checked, the value for the red pixel being checked is replaced with the average value of the eight neighboring red pixels.

3. The program repeats this procedure for the values of the green and blue pixels being checked.

To activate the Noise Filter, check the Noise Filter box. The program runs the noise filter each time an image is captured.

Taking a Picture

The actual capture of a digital image (taking the picture) is similar to traditional photographic techniques. In digital photography, however, it is possible to continually capture, refine, and recapture images until you have an optimal representation: the first image that you capture might be only one of several that you use to produce a publishable product. A sample image capture process, from start to finish might proceed as follows:

1. Adjust the Preferences as needed (see above).

2. Open the Live Image window.

3. Frame and focus the image as needed.

4. Click on the Image Settings toolbar button to access the Image Settings window and adjust the live image view settings.

5. Click on the Get Image toolbar button to capture the final image.

In the Basic program mode, image captures settings and live image view settings are defined from the same source - the **Image Settings** window. This gives you the convenience of being able to view a live image, adjust the settings in real-time, and then capture the image with the live image view settings.

> ✎ *Note: Except for the* **Captured Image Bit Depth***, which is defined on the* **Preferences** *window, all image capture/live image settings are defined from the Image Settings window.*

Initialize Camera

If you turned on the SPOT power supply after you opened the program, you need to initialize the camera in order to activate all of the capture and editing options. To do so, select the Initialize option from the Camera menu.

Viewing a Live Image

The Live Image option enables you to see a real time preview of your specimen/image capture. For instance, you can use the Live Image window to:

- Scan the image for an area of interest.

- Zoom in on an area of interest.

- Focus on an area of interest.

- Frame an area of interest.

- Define image capture settings – see **Preparing to Take a Picture** above for more details
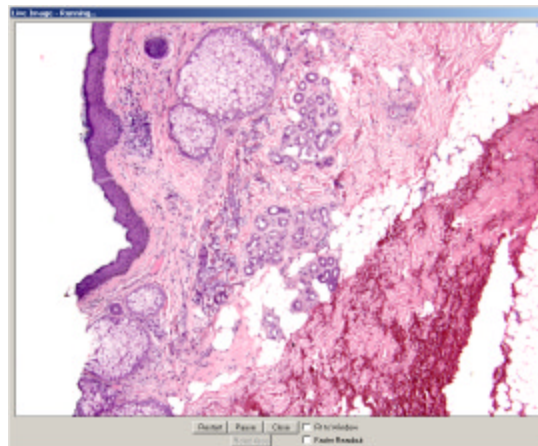
> *Note: With some video cards, you might experience system crashes or display problems when working with live images. To correct these problems, lower the Graphics Acceleration setting in Windows, as described in the* Video Card Related Problems *section of the* Installation Guidelines *chapter.*

To open the Live Image window, either click on the Live Image toolbar button, or select the Live Image option from the Camera menu.

> *Note: Occasionally, when viewing a dim live image, you might notice that the right half of the image is brighter or dimmer than the left half. To display images in real time, the camera (except for the RT-SE6) uses two separate amplifiers to simultaneously read the right and left halves of the CCD chip. With some images, particularly dimmer samples, the brightness of the two halves may not match. This results in a "split" appearance. To avoid this problem with captured images, the camera reads the whole CCD through a single amplifier. A "split" Live Image view is **not** indicative of what your image capture will look like. Refer to* Appendix A: Electronic Imaging Theory and SPOT Cameras *for more details.*

- The title bar at the top of the Live Image window displays the status of the live image view - **Running, Computing Exposure**, or **Paused**. Double-click on the title bar to "roll up" the live image display. This pauses the live image display. Double-click again to display the whole window and resume the live image display.

- When the **Fit to Window** option is checked, the live image window cursor becomes a **zoom tool**. To zoom in on a portion of a live image, click and drag over the area that you want to zoom in on. To return to the non-zoomed image, click on the **Reset Area** button on the bottom of the window.

- When the **Fit to Window** option is not checked, the cursor is a **cropping tool**. If you select a portion of an image, the program displays only the selected area and discards the rest of the image. To return to the non-cropped image, click on the **Reset Area** button on the bottom of the window.

> *Note: Due to a flaw in the Windows operating system, monitors that are set to higher resolutions cannot zoom to very high magnification levels. If zoomed in to a high magnification, the monitor displays white instead of the zoomed image.*

- The **Full Screen** option (accessed via the View menu or [F 7]) can be used with live images. To access live image viewing options in the full screen view, right click on the live image.
  - To exit the full screen view, either press [F 7], or move the cursor to the top of the screen and click on the **Exit Full Screen Mode** button that appears.

- There are six **view/exposure options** at the bottom of the Live Image window:

Restart	Resample the brightness of the image, compute new exposure times, and restart the live image display. This is useful for updating exposure times when specimen brightness has changed.
Pause/Resume	Freeze the live image window. This is useful when you are changing a specimen and want to preserve the last view. In addition, to use the Transfer option (see below), you must first Pause the live image view. Click on Resume to restart the live image view
Close	Close the Live Image window.
Fit to Window	Size images to automatically fit the resizable display window.
Reset Area	Return to the non-zoomed view of the live image.
Faster Readout	Faster Readout enables you to "speed up" the live image display (higher frame rates) in cases where you are willing to sacrifice some resolution for a higher frame rate.

Computing White Balance Values (RT Color Mosaic, RT Color 3 Shot, and RT Slider)

White balance is the ratio of red, green, and blue gains necessary to achieve the proper color rendition for an image. Many factors impact the coloration of an image capture, including lamp voltage, the coloration of the glass used in the slide, and the coloration of the glass used in the lenses of the microscope's objectives. To minimize the impact of these factors, use the Compute White Balance feature. When you perform a white balance calculation, SPOT samples the color of the light from your light source after it has passed through the slide and the objective, and then calculates the red, green, and blue gains needed to produce images where the white areas are white and the color areas are true.

You should perform a new white balance calculation when:

- Starting a new image capture session.
- Changing the lamp voltage.
- Switching to a different objective on the microscope.

To compute new white balance values, follow these steps:

1. Set the illumination and the objective that you will use for the image capture.

2. If you have not done so, select your **Image Type** (see above). Each Image Type has its own set of white balance values. Any white balance values that you compute for an image type remain linked to that image type until you compute new values. When selecting a new image type, remember that you will be using the white balance values linked to that image type.

3. Click on the **White Balance** button.

4. Show the camera a sample of white light. This step varies according to microscopy technique and Image Type (sample), as shown in the table that follows:

5.

Image Type	White Balance Technique
Brightfield-transmitted light	Position the slide so that the specimen is not in the field of view, but light is going through the slide near the specimen.
Darkfield-transmitted light	Perform the white balance using the brightfield mode on your microscope. See Brightfield – transmitted light above.
Fluorescence	Use the Match Color feature
Nomarski DIC/Hoffman	**For Nomarski DIC:** Adjust the Nomarski prism until the image is in either the "brightfield" or the "gray scale" v iew. **For Hoffman:** Perform the white balance directly on the Hoffman image.
Phase contrast	Position the slide so that the specimen is not in the field of view, but light is going through the slide near the specimen.
Polarized light	Pull all polarizers and compensators out of the light path and then position the slide so that the specimen is not in the field of view, but light is going through the slide near the specimen.
Brightfield-reflected light	Replace your sample with a mirror.
Darkfield-reflected light	Perform the white balance using the brightfield mode on your microscope. See Brightfield – reflected light above.
Stereo/Macro (all three options)	Using an EPI-illuminator (shining a light on the top of your specimen from off to one side), replace your sample with a white sheet of paper.

Click on **Begin** to start the calculation. The camera computes the white balance values, and they are automatically saved to the image settings for the image type that you selected.

Capturing the Final Image

Get Image captures an image according to the Image Settings that you defined (see previous sections). To capture an image, click on the Get Image button; select Get Image from the Camera menu, or press **[F 9]**.

The Get Image status dialog box appears. Status messages display as the camera computes the gain and exposure times. If the live image window is open, the image capture uses the gain and exposure times calculated for the live image and skips the exposure computation (step 1 below). The status messages are as follows:

1. **Computing Exposure** – The camera is sampling brightness levels in the image in order to determine proper gain and exposure time.

2. **Exposing** – A countdown clock displays when the exposure will last longer than 2 seconds. This informs the user that the electronic shutter is open and counts down the minutes: seconds remaining until it closes.

3. **Getting Image** – The camera is downloading the image to the computer.

(For the Color 3-shot and Slider versions, steps 2 and 3 will occur from one to three times, depending upon which color filters have been selected in the image set-ups)

4. **Processing** – The camera is preparing to display the image.

The newly captured image appears on screen. If the live image screen is open, the captured image will appear behind the live image screen. To view it, simply close the live image window.

Get Sequential Images

The Get Sequential Images option allows you to capture a sequence of images. SPOT's sequential image capture process enables you to save an image sequence in two different ways:

- You can save a sequence as a group of individual image files.

- You can save a sequence as a single (TIF format), "sequence image file" that is composed of multiple images. Refer to the Viewing, Editing, and Annotating Images section for details on viewing and modifying image sequence files.

Refer to Ch. 7 – Taking a Picture for details on capturing sequential images.

Viewing, Editing, and Annotating Images

After capturing an image, you can adjust viewing options, and edit/annotate the captured image.

View

All of the image viewing options are accessible from two menus:

- **View**– Contains options specifically designed for viewing microscopic specimens
- **Window** – Contains standard Windows options that enable you to work with and view multiple images.

> *Note: The Window menu options include Cascade, Arrange Horizontally, Arrange Vertically, Arrange Icons, and Close All. For descriptions of these features, refer to the standard Windows help.*

To help you view and analyze your image captures, the View menu contains seven options – **Fit to Window**, **Set Zoom Mode, Zoom Level, Show Grid Lines**, **Show Pan Window**, **Show Image Date/Time**, and **Full Screen**.

> *Note: The Show Grid Lines option is available only when you zoom in on an image.*

Fit to Window

Fit to Window allows you to view the whole image, regardless of the window size. Typically, reducing the size of a window also cuts off part of the image. The Fit to Window option solves this problem by resizing the image so that it is always the same size as the window.

Set Zoom Mode

1. Either select Zoom Mode from the View menu, or click on the Zoom toolbar button:

2. The cursor changes to a magnifying glass, and the Zoom toolbar button becomes active. The precise reference point for the zoom location is indicated by the white dot in the center of the magnifying glass. The row and column numbers at the bottom of the screen indicate the exact position of the white dot as you move the magnifying glass over the image.

3. Use the mouse to zoom in or zoom out:

    a) Position the mouse over the center of the area that you want to zoom in on.

    b) Either left click to zoom in 2x, or right click to zoom out 2x.

    c) To return to the normal view mode, de-select the Zoom option.

The Zoom feature works in conjunction with the pan window (see **Show Pan Window** below), which allows you to see where you are in relation to the larger image while zoomed in.

> *Note: All of the standard save, view, and edit options, with three exceptions –* **Add Calibration Mark***,* **Add Measurement***, and* **Annotate** *– can be used while the program is in zoom mode. If you need to annotate a zoomed image, use the* **Zoom Level** *option (see below).*

> *Note: With some video cards, you might experience display problems when zooming in on captured images. To correct these problems, lower the Graphics Acceleration setting in Windows, as described in the* Video Card Related Problems *section of the* Installation Guidelines *chapter.*

Zoom Level

The **Zoom Level** option, like the **Zoom Mode** option enables you to view zoomed images. However, unlike the Zoom Mode option, the Zoom Level feature gives you the option to add annotations, including text, calibration marks, and measurements, to the zoomed image. To use the Zoom Level option, select **Zoom Level** from the View menu and select a percentage option from the list.

Like the Zoom Mode option, the Zoom Level option can be used in conjunction with the pan window (see **Show Pan Window** below), which allows you to see where you are in relation to the larger image while zoomed in.

Note: With some video cards, you might experience display problems when zooming in on captured images. To correct these problems, lower the Graphics Acceleration setting in Windows, as described in the Video Card Related Problems *section of the* Installation Guidelines *chapter.*

Show Grid Lines

Show Grid Lines clearly defines pixel borders, which are often invisible in low contrast areas of an image. Clearly defined borders help in positioning the cursor at an exact row and column location (i.e., using the readouts at the bottom of the screen), which then allows you to view the brightness value for the individual pixel.

To show the grid lines on an image, follow these steps:

1. Position the magnifying glass in the center of the area that you want to zoom in on.
2. Zoom to either 8x or 16x. If you are using the **Zoom Level** option, select a percentage of 800 or greater.
3. From the View menu, select the Show Grid Lines option. Grid lines appear around each pixel.

Show Pan Window

Show Pan Window enables you to navigate around an image while zoomed in at high magnifications. When you select the Show Pan Window option, the program highlights your location in relation to the whole image, and, by dragging the highlighted area around the pan window; you can explore the image, piece by piece. Without the Show Pan Window option, the process of moving to a new point while in high magnification would involve several steps:

1. Zooming in to the first area of interest.
2. Zooming out to determine where you are.
3. Determining a new area of interest.
4. Re-zooming in to the new area of interest.

The pan window replaces this tedious process. When you select Show Pan Window, a smaller version of the image appears with a color negative over the zoomed area of the image.

To use the pan window, follow these steps:

1. Either select Show Pan Window from the View menu, or click on the Show Pan Window toolbar button.

   Note: The pan window can be opened at any level of magnification, however the panning "window within the window" feature is only functional when the level of magnification exceeds the "fit to window" size for the captured image.
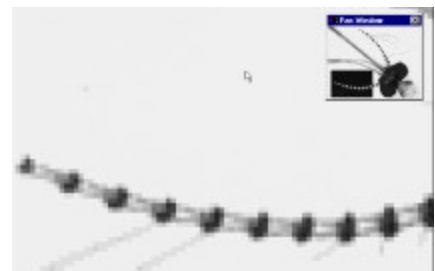
The pan window appears, superimposed upon

the image.

2. Position the cursor on the image in the pan window: it changes from a magnifying glass to a four-arrow pointer.

3. To move to a different area of the image, either left click on the rectangle and drag it to the new location, or left click on the new location.

Image Date/Time

The Image Date/Time feature pulls up the date and time associated with the capture of an image. When you capture an image, the SPOT software remembers the date and time of the image capture. When you save the image, even if this is many hours later, the program sets the **Created** date and time of the image file to the date and time of the original image capture.

> ✎ *Note: The date/time feature is synchronized to the date and time setting in your computer..*

When image files are transferred over networks, the **Created** date and time is sometimes reset to the date and time of the transfer, making it an unreliable indicator of the image capture time. Because TIFF and JPEG format images have tags both for the Created date and the original capture date and time, network transfers do not affect them. If reliable image capture date and time information is important to your work, you should save images in either TIFF or JPEG format.

When you select the Image Date/Time option from the View menu, a dialog box displays the date and time associated with the capture.

- For non-TIFF, non-JPEG format image files, this is the **Created** date/time, as explained above.

- For TIFF and JPEG format image files, this is the TIFF/JPEG tag date and time.

- For SPOT Database images (PCs only) this is the original date and time that was stored in the database file.

Full Screen

View a full screen version of an image – i.e., without the SPOT menus or toolbars or the Windows navigation bar. This option allows you to utilize the entire window for previewing images. Press **[F 7]** to view a full screen image or click on the **Full Screen** toolbar button. Press **[F 7]** again to exit the full screen view. Alternatively, Windows users can exit the full screen view by moving the cursor to the top of the window and clicking on the **Exit Full Screen Mode** button that appears.

While working in Full Screen mode, Windows users can access SPOT menu options using standard keystroke combinations, as illustrated in the table below:

[Alt] + **A**	=	Attributes (Report mode)	**[Alt]** + **H**	=	Help
[Alt] + **C**	=	Camera	**[Alt]** + **O**	=	Object (Report mode)
[Alt] + **D**	=	Database (Advanced mode)	**[Alt]** + **Q**	=	Sequence
[Alt] + **E**	=	Edit	**[Alt]** + **S**	=	Setup
[Alt] + **F**	=	File	**[Alt]** + **V**	=	View
			[Alt] + **W**	=	Window

Edit

All of the editing options in the Basic program mode are accessed via the Edit menu. There are five editing categories in the **Basic** program mode:

- Basic editing

- Image position
- Image quality
- Calibration and Measurement
- Annotation
- Memos

The options associated with each category are described below. In some cases, where the options are identical to those in the Advanced program mode, the manual refers you to the appropriate chapter and section.

Basic Editing

The Edit menu contains several functions that are common to most operating systems.

Undo Undo the last edit that you made. The Undo option only allows for one level of edits: in other words, you can only undo the immediately preceding edit. To Undo an action, select Undo from the Edit menu.

Cut (Mac) Cut text fields within the SPOT program.

Copy to Clipboard (PC)/Copy (Mac) Copy all or part of an image onto the clipboard for insertion into another program. These options are identical to the Windows **[Ctrl C]** command and the Mac [⌘ **C]** command.

1. Press and hold the left mouse button (PC)/mouse button (Mac).
2. Drag the cursor over the image until the dotted rectangle covers the area of the image that you want to copy. If you copy without "cutting out" a specific portion of the image, SPOT selects the entire image.
3. Either select the Copy to Clipboard/Copy option from the Edit menu, or press **[Ctrl C]** (PC)/[⌘ **C]** (Mac). The image is now ready to be pasted into another application.
4. Open the application that you want to paste the image into, and select from the available pasting options.

Paste (Mac) Paste text fields in the SPOT program. The Paste field is grayed when editing images.

Clear (Mac) Clear a text field in the SPOT program. The Clear field is grayed when editing images.

Select All (Mac) Select an image for copying into another application.

Image Position

From the Edit menu, you can:
- Reposition images
- Match the on-screen orientation of a specimen to the microscopic view
- Crop an image to better reflect the area of interest

Rotate Right Rotate an image clockwise in 90-degree increments.

Rotate Left Rotate an image counter-clockwise in 90-degree increments.

Flip Horizontal Flip an image from right to left

Flip Vertical Flip an image from top to bottom.

Crop   Cut out a portion of an image. To crop an image, follow these steps:

1.   Press and hold the left mouse button.

2.   Drag the cursor over the image until the dotted rectangle covers the area of the image that you want to crop.

3.   From the Edit menu, select Crop. The Crop Area window displays the pixel coordinates of the rectangle that you selected.

From this window you can fine-tune the crop area by modifying the pixel coordinates. If you select the Crop option directly from the menu (i.e., without specifying a crop area), the Crop Area window displays the values for the entire image.

4.   Click on OK to crop the image. The cropped image appears on-screen.

5.   When you crop an unsaved image, the program automatically discards the area of the image outside the crop. To give yourself the opportunity to change the crop area, you should save the image before you crop it.

Image Quality (Adjust Image)

The Adjust Image options allow you to correct images that are overly bright or dark, or to modify areas of interest that are too light or too dark to see important details.   To access these editing options, select **Adjust Image** from the Edit menu. The Adjust Image window appears, as shown at right. Adjust images either by entering a value in the box or by moving the slider bar.
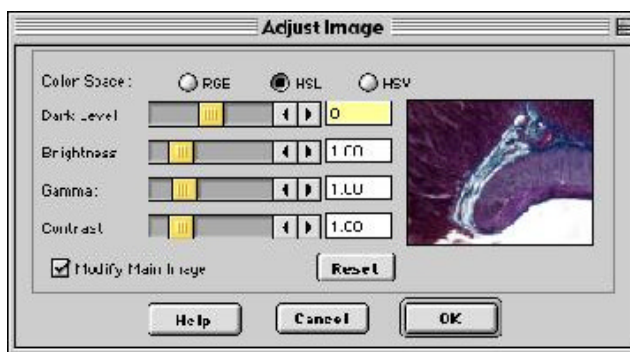
The Adjust Image window provides three adjustment parameters:

- **Color Space** – This option enables you to select the color space that is best suited to the type of image that you are editing:

    - **RGB** (Red Green Blue) – Use for monochrome images

    - **HSL** (Hue Saturation Luminance) – Use for all color images except fluorescence

    - **HSV** (Hue Saturation Value) – Use for fluorescence images

    Refer to the **Image Editing and the Concept of Color Space** section of Ch. 8 for more detailed descriptions of the three color spaces.

- **Modify Main Image -** When this box is checked (the default), both the thumbnail image and the main image change as you are adjusting the image. If you want the main image to change after you have made the adjustments – and thereby speed the editing process - deselect the Modify Main Image box.

- **Reset** – Click on the Reset button to return the image to its pre-adjustment appearance.

The following sections describe the effect of each of the four adjustments.

Dark Level   The Dark Level adjustment lightens or darkens an entire image by adding the value that you specify to all of the pixels in the image.

To adjust Dark Level, enter a number between -100 and 100.
- Numbers between zero and –100 darken the image.
- Numbers between zero and 100 lighten the image.

Brightness   The Brightness adjustment lightens or darkens the bright areas in an image. For example, if you used the Dark Level adjustment to modify an image, but the new bright areas are either too dark or too light to see the details, you can you use the Brightness feature to correct this. Because the Brightness adjustment is *multiplicative* (as opposed to the Dark Level adjustment which is *additive*), it multiplies the pixel values in the image by the value that you specify.   A multiplicative process affects bright areas more than dark areas. Before adjustment an image's Brightness value is always one.

To adjust Brightness, enter a number between 0 and 5.

- Numbers between zero and one darken the image.
- Numbers between one and five lighten the image.

Gamma   The Gamma adjustment lightens the darker (mid-tone) areas of an image without burning out bright areas or lightening black areas. This is useful when modifying images with a wide *dynamic range*, (i.e., the range of brightness values recorded by the CCD chip. Before adjustment, the Gamma value is always one.

To adjust Gamma, enter a number between .1 and 4.

- Numbers above one lighten the mid-tone areas in the image.
- Numbers below one darken the mid-tone areas in the image.

Contrast   The Contrast adjustment exaggerates or subdues the difference between the bright and dark areas in an image. Before adjustment, the Contrast value is always one.

To adjust Contrast, enter a number between 0.10 and 5.

- Numbers above one exaggerate the contrast between bright and dark areas.
- Numbers below one subdue the contrast between bright and dark areas.

Match Color

The Match Color feature is typically used in two situations:

- You want to change the colors of an image on the screen to match the way that you think the specimen looks.

- You want to set the white balance for pictures taken with a microscopic technique that never has white in its images. A prime example of this situation is fluorescence microscopy.

To use the Match Color feature, follow these steps:

1. From the Edit menu, select **Match Color.** The cursor becomes an eyedropper.

2. Use the mouse to move the eyedropper to the pixel location of the color that you want to match. Refer to the status bar at the bottom of the window to ensure that each of the R, G, and B values are less than 255 (8 bit) and 4095 (12 bit).

3. Click on the pixel.   The Match Color window appears.
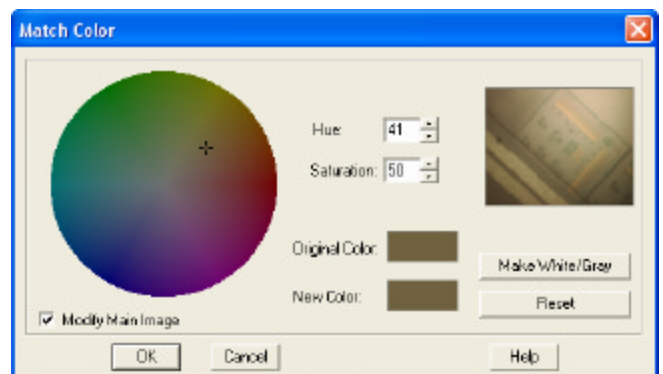
Note: The Set White Balance button appears only for image captures that have not yet been saved and closed.

The window displays information about the pixel that you selected:

– **Hue** – The actual color, as distinguished from others in the color spectrum. Hue is derived from a color wheel and is expressed in the color's angular location (e.g., 0 to 360 degrees).

– **Saturation** - The proportion of perceived pure hue in the color. Saturation (in the SPOT software) is measured on a numerical scale of 0 to 100, where zero equals pure gray and 100 equals pure hue (e.g., "day glow").

– **Original Color** – A sample "swatch" of the color that you selected. The cross hairs on the color wheel (e.g., in the center of the window) indicate the relative position of the selected color.

– The **Modify Main Image** box is checked as a default.

4. Set the new color. This can be done in one of two ways, depending on whether the image should contain white:

– If you are trying to make the selected pixel in your image appear as white (or gray if the pixel is darker), click on the **Make White/Gray** button.

– If you are working with an image that does not have any white, modify the Hue and Saturation values so that the **New Color** "swatch" shows up as the color that you want. You can do this either by entering specific Hue and Saturation values, or dragging the cross hairs on the color wheel to the desired location.

Duplicate Image

Click on the Duplicate Image option to create a copy of the active (on-screen) image.

Calibration and Measurement

The **Add Calibration Mark** and **Add Measurement** options enable you to add calibration and measurement references to SPOT images. Refer to the **Annotation – Calibration and Measurement Marks** section of Ch. 8 for detailed steps.

Annotate

Image annotation is useful when you want to prepare captures for formal presentation, or inclusion in an article or reference work. Using SPOT's image annotation features, you can:

• Add text to an image.
• Use an arrow, line, rectangle, or ellipse to emphasize a particular area in an image.
• Add a graphic stamp such as a company logo to an image.
• Add freehand lines to an image.

To annotate an image, select Annotation from the Edit menu. Several changes take place in the SPOT image-editing environment:

• The **Edit** menu options change to reflect annotation editing.
Refer to **Editing Annotation Objects** (Ch. 8) for descriptions of each option.

• The **Object** menu appears with options for inserting drawing objects, text, images, and image related information onto an image. Cursor functionality in SPOT reports varies according to which **Object** menu option is selected.

– If **None** is selected, the cursor is an editing tool for selecting, sizing, and positioning objects. When creating new reports, **None** is the default option.

– If a report object is selected, the cursor changes to a cross-hair icon for placing new report objects on the grid. Refer to **Adding Annotation Objects to SPOT Images** (Ch. 8) for descriptions of each object.

- The **View** menu changes to reflect annotation mode:

    – The two zoom options and the Show Grid Lines options are no longer displayed..

    – The **Show Tools** option is present – this option displays the Tools dialog box, which contains shortcuts for inserting annotation-drawing objects.
    Refer to **Adding Annotation Objects to SPOT Images** (Ch. 8) for more details on using the Tools dialog box.

- The **Attributes** menu appears with options for specifying annotation qualities.
  Refer to **Defining Annotation Attributes** (Ch. 8) for descriptions of each option.

- The **Done!** menu (Windows)/ **Mode** menu (Mac) allows you to switch back to the image capture/edit mode.

    – **Windows** – The Done! menu has only one option, **End Annotation** Mode. When you select this option the SPOT program prompts you to merge the changes into the image.

    – **Mac** – The Mode menu has only one option, **Exit Annotation Mode**. When you select this option, the SPOT program prompts you to merge the changes into the image.

CAUTION!

If you select Yes after clicking on Done! The annotations become a permanent part of the image. If you want to preserve the original image, save the original image to a file. After annotating it, use Save As to save the annotated version to a different file.

Memos

In the SPOT software, you can attach a memo to TIFF or JPEG format image files.   To use the Memo option, follow these steps.

1. From the Edit menu, select Memo.   The Image Memo window appears for you to enter a new memo or edit an existing memo.
2. Enter the message.
3. Click on OK to attach the memo to the image.

Note: If you attach a memo to an image that is not in either TIFF or JPEG format and then attempt to save the memo (i.e., when you close the image file), the SPOT program displays a warning message that asks you if you want to save the image without the memo. If you want to retain the memo, click on Cancel and use the **Save As** *option to save the image in either TIFF or JPEG format.*

Working with Image Sequences

Image sequence files can be edited in the following ways, as described below:

- The **Sequence** menu – Rearrange the order of a sequence, add and/or remove images to and from the sequence, extract images from the sequence, and play the sequence. Refer to the **Sequence Menu Options** section of Ch.8 – Viewing, Editing, and Annotating Images for details.

- The **Sequence Controls (Image Sequence)** dialog – Set view options and "play" the sequence. Refer to the **Image Sequence Controls** section of Ch.8 – Viewing, Editing, and Annotating Images for details.

Creating a Report

Using the Basic mode's built-in reporting functionality, you can collect, organize, and present information related to your image captures. This chapter discusses the process of creating and printing reports. It is divided into the following sections:

- **Creating a New Report**
- **Working in Report Mode** – general reporting features and functionality
- **Defining View Options** – the view of the report definition screen
- **Defining Report Attributes** – the style and appearance of the report
- **Defining Report Objects** – the text, field, and image information that appears in the report
- **Editing Report Objects** – edit options for the report objects
- **Saving a Report**
- **Printing and Distributing Reports**

Creating a New Report

To create a new SPOT report, select **New Report** from the File menu. The following changes take place in the main window:

- The report definition window appears as a separate window. If an image or image(s) were open, they remain open. When you move between an image window and the report definition window, the menu options change accordingly.

- The **Object** menu appears. Use these options to specify the fields, text, and images that appear in the report.

- The **Attributes** menu appears. Use these options to define the appearance and style of the report.

- The **View** menu options change to reflect report definition function.

- The **Edit** menu options change to reflect report-editing functions. These options are available only after one or more report objects have been added to the New Report window.

Working in Report Mode

The report mode contains functionality that differs from the image capture and editing functionality. The following is a brief overview of these features.

Cursor Functionality

Cursor functionality in SPOT reports varies according to which **Object** menu option is selected.

- If **None** is selected, the cursor is an editing tool for selecting, sizing, and positioning objects. When creating new reports, **None** is the default option.

- If a report object is selected, the cursor changes to a cross-hair icon for placing new report objects to the grid.

Sizing and Positioning Report Objects

When sizing and positioning a report object in the report window, you must first select the object by single clicking on it. When an object has been selected, handles appear at the corners; for line objects, the handles appear at each end. You can select multiple report objects by holding the mouse button down and dragging the cursor around both objects. Handles appear around both objects. When selected, report objects can be sized or positioned as needed.

- To size objects, move the cursor either to a point on the object's border or to one of the handles. The cursor changes to a two pointed arrow
    - To resize one dimension of a box, position the cursor on a vertical or horizontal line; click and drag the side to the new size.
    - To proportionally resize a box, position the cursor on a handle; click and drag the box to the new size.
- To position objects, move the cursor over the selected object. The cursor changes to a four-point arrow. Click and drag the object to a new location. In addition, if an object is selected, you can use the arrow keys to fine-tune its position.

> ✎ *Note: When multiple objects are selected, they can be sized and positioned as a single object.*

Using Right/Ctrl-click and Double-click Functionality

When creating a report, you can use the right-click and double-click mouse functions as editing shortcuts.

- Double clicking on a report object enables you to edit the object's attributes – e.g., the thickness of a line.

- Right clicking (Windows)/[Ctrl] clicking (Mac) on a report object calls an object specific edit menu that contains both general editing options and attribute editing options specific to that object. Right clicking on an Image object, for example, gives you a list of editing options, as shown at right.

- Right clicking (Windows)\[Ctrl] clicking (Mac) on the report grid gives you three options: **Select All** objects, Paste an object that you copied or cut; or **Delete All** of the objects on the grid.
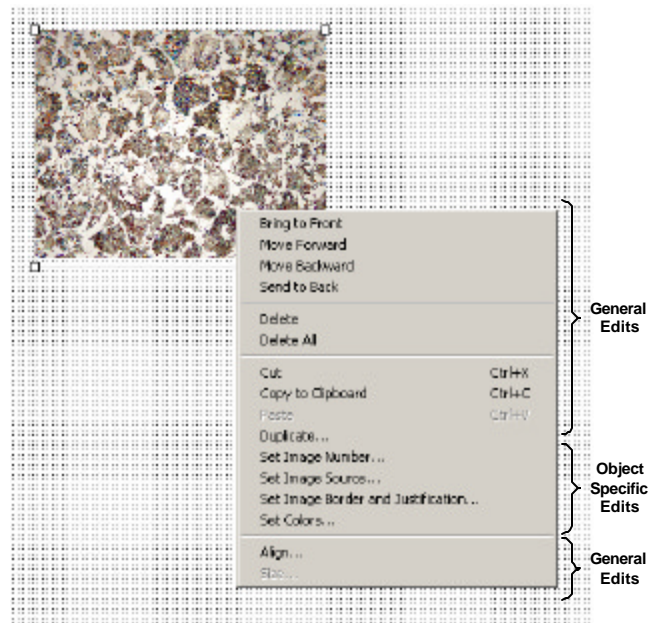
Refer to the **Defining Report Attributes** and **Editing Report Objects** sections for more details.

Defining View Options

The **View** menu provides you with nine options:

Zoom
Zoom in or out to six different sizes. From the View menu, select Zoom and enter the desired percentage.

First Page
Go to the first page in the report.

Previous Page
Go back one page in the report.

Next Page
Go forward one page in the report.

Last Page
Go to the last page in the report.

Set Display Grid Size
The report definition window includes an adjustable grid (i.e., the dots on the screen) to assist you in placing and aligning report objects on the report page. By default, the grid is turned on. To turn off or modify the grid, follow these steps:

1. From the View menu, select Set Display Grid Size.

   – To turn off the display grid, deselect the Show Grid box.
   – To modify the spacing follow steps two through four.

2. Select either inches or millimeters.

3. Enter the spacing for the display grid. The number that you enter represents the space in inches or millimeters between the dots on the grid.

   – If the measurement is **inches**, enter a number between **.04** and **10**.
   – If the measurement is in **millimeters**, enter a number between **1** and **25**.

4. Click on OK. The display grid appears.

Set Object Grid Size
In addition to the display grid, you can define a second grid specific to report objects. The object grid controls the movement of objects as you work with them on the display grid. When you select a report object and move it, the object automatically "snaps" to the nearest line in the object grid, allowing you to more precisely align and arrange the objects on your report page.

1. From the View menu, select Set Object Grid Size.
2. If desired, check the Snap to Grid option.

3. Define the spacing for the object grid. In many cases, the object grid and the display grid will have the same spacing. In other cases, you might not want the display grid to be as finely spaced as the object grid (i.e., so the dots do not interfere with the view of the report definition window).

   – If the measurement is **inches**, enter a number between **.04** and **10**.
   – If the measurement is in **millimeters**, enter a number between **1** and **25**.

   The default spacing value for the Object Grid is the last entered value.

4. Click on OK.

Show Tools

Click on the Show Tools option to display a floating palette of navigation buttons and report objects, as shown. Refer to the **Defining Report Objects** section of this chapter for more details.

Full Screen

- . This option allows you to use the entire screen to view a live image, without the SPOT menus/toolbar and the Windows navigation bar.

Horizontal Line · Vertical Line · object select mode (None) · Arrow · Rounded Box · Ellipse · Box · Text · Freehand · Image · Polygon · Polyline · First Page · Last Page · Previous Page · Next Page

Defining Report Attributes

All of the general style and appearance options (report attributes) can be accessed from the **Attributes** menu. Report attributes can be defined for an entire report or on an object-by-object basis (see the Defining Report Objects section below).

- When a report object is selected, the attribute is defined for the selected object(s) only.

- When nothing is selected, the attribute is defined for all future, relevant objects.

The following sections detail each of the report attributes and the report objects that they affect.

Colors

Define the foreground and background colors for all objects. Follow these steps:

1. From the Attributes menu, select Colors.

2. Define the **Text/Lines** color. This is the color of the text, the line, or the border around a text/image box. The default foreground color is black. To select a color click on Color.

3. The next window varies, depending on whether you are using a Windows or a Mac system.
   - Windows allows you to select from a palette of colors or enter specific HSL or RGB color values.
   - Mac OS allows you to select from a palette of colors, enter specific RGB, CMYK, HLS, or HSV values, and enter Internet specific colors.

   Define the **Background/Fill** color. This is the color that appears behind text and in boxes. Lines and images do not display a background color.

Line Thickness

Determine line thickness for the following report objects: **Line**, **Horizontal Line**, **Vertical Line**, **Arrow, Box**, **Rounded Box**, **Ellipse**, **Freehand**, **Polygon**, and **Polyline**.

1. From the Attributes menu, select Line Thickness.

2. Enter a Thickness value between **.05** and **10** points.

3. Click on OK.

✎ *Note: In order to define a background/fill color for a Box or a Rounded Box, a fill must be selected. Similarly, in order to define a background/fill color for a text box, the Text Style must be set to Opaque.*

Image Border and Justification

Determine the line thickness for image borders and define the justification of the image within the display box. This option applies only to the **Image** report object.

1. To create a border around the image, click on the Border box, and enter a **Thickness** value between **.05** and **10**.
2. Select the **Justification** for the image. This value is the justification of the image within its display box, not the justification on the report page.
3. Click on OK.

Arrow Size and Direction

Specify the size of the arrow head and define the direction that the arrow points.

1. To specify the size of the arrow head, enter a value between **1** and **10**.
2. Specify the direction of the arrow:
   – To draw an arrow starting tail first and finishing with the point (head), select **Tail to Head**.
   – To draw an arrow starting point (head) first and finishing with the tail, select **Head to Tail**.
3. Click on OK.

Box/Ellipse Fill
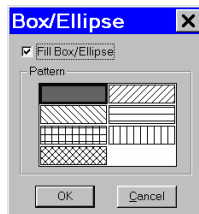
Define the appearance of the Box, Rounded Box, and Ellipse objects.

1. Specify whether you want a fill for the object.
   – If you do not want the box to be filled (i.e., you want a transparent box), click on OK.
   – If you want to specify a fill pattern for the box, check the Fill Box/Ellipse. The **Pattern** options appear.
2. Specify a pattern by clicking on one of the seven options.
3. Click on OK.

Text Font
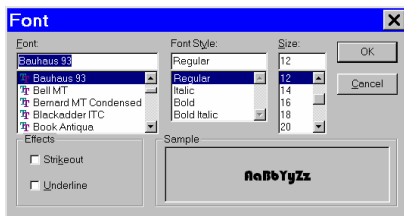
Define the appearance of the text for the **Text**, **Date**, and **Time** objects.

1. Select a **Font**, a **Font Style**, and a point **Size**. The Sample box displays a preview of the font.
2. Define any Strikeout or Underline effects.
3. Click on OK.
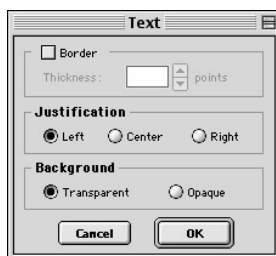
Text Border, Justification, and Background

Define the appearance of the text boxes for the **Text**, **Date**, and **Time** objects

1. Specify whether the text box should have a border. To add a border, click on the **Border** box, and enter a Thickness value between **.05** and **10**.
2. Define the **Justification** of the text. This justification applies to the position of the text in the box, not the position of the text on the page.
3. Define the Background. Select either **Transparent** or **Opaque**.
4. Click on OK.

Defining Report Objects

All of the components of your report, including the formatting, text, images, and fields are known as objects. Using the objects, you can specify the information that your report will contain. Before discussing the individual report objects, review the following general procedure for inserting an object into a report:

1. From the Object menu, select the type of item (i.e., Lines, Connected Lines, etc…) that you want to add to the image. The cursor's functionality varies according to whether a report object type (i.e., line, text) is selected:.
    –   If a report object type is selected, the cursor changers to a cross-hair icon that is used to add new report objects to the grid.
    –   If **None** is selected, the cursor is a an editing tool that is used to select and position objects.
2. Use the mouse to drag the cross hair to the location where you want to insert the object.
3. Left-click at the insertion point.
    –   If you selected a line or a box, hold down the mouse button and drag the line/box to the desired size.
    –   If you selected any of the other report objects, the object will appear and you will be prompted to enter the associated information (e.g., static text, the location of an image file, the database field, etc…).
4. Once it appears on screen, you can resize, reposition, and edit an object as needed.
    –   **Resize** - Select (click on) the object and drag the handles in the direction of the resize.
    –   **Reposition** – Left click and drag the object the new area.
    –   **Edit** – Select the object and choose an option from either the Edit menu or the Attributes menu. You can also edit an object by **right clicking** (Windows)/**[Ctrl] clicking** (Mac) on it.

The following table describes the Basic program mode report objects:

None	Select **None** when you want to use the cursor to edit and position the objects.
Lines	**Unconstrained Line** – Add a straight-line annotation to the report.
	Horizontal Line - Add a horizontal line annotation to the report.
	Vertical Line – Add a vertical line annotation to the report.
	Arrow - Add an arrow to the report. Either select Arrow from the Object menu, or click on the Tools box button. Left click on the report and drag toward the area that the arrowhead will point to.
Connected Lines	**Polygon** – Add a closed polygon to the report. Either select Connected Lines>Polygon from the Object menu, or click on the Tools box button. Left click and release, and then drag the mouse to create a side. Left click again to start a new side. Double click to finish the polygon. The program automatically adds any additional lines needed to close the figure.
	Polyline – Add an open polygon to the report. Either select Connected Lines>Polyline from the Object menu, or click on the Tools box button. Left click and release, and then drag the mouse to create a side. Left click again to start a new side. Double click to finish the polyline.
	Freehand - Add a freehand line to the report.

Shapes

Rectangular Box – Add a square cornered box to the report.

Rounded Box – Add a round cornered box to the report.

Ellipse - Add an elliptical object to the report. Ellipses are drawn inside of the rectangular area that you define. Ellipses touch the top, bottom, right and left sides of this rectangle.

Either select Shapes>Ellipse from the Object menu, or click on the Tools box button. To draw an ellipse, left click at the point where you want to position one vertex of the rectangle and drag the cursor to the point of the opposite vertex.

Image

Add an image to the report.

1.   From the Object menu, select Image.

2.   Left click at the point on the display grid where you want to insert the Image box, and holding down the mouse button, drag the cursor to create the desired shape and size for the image (it can also be resized/re-positioned at a later point). When you release the mouse button, the Image dialog box appears.

3.   Specify the file source for the image:

   –   **Currently Open Image** – Add a currently open image to the report. If you have one or more open images, SPOT lists the file name(s) and path(s) in the drop-down box. Select from the list.

   –   **Image File –** Add an image from disk to the report. Specify the file name and location either by entering the path in the Name box, or clicking on the selection box to the right.

4.   Click on OK. The image appears on your report grid.
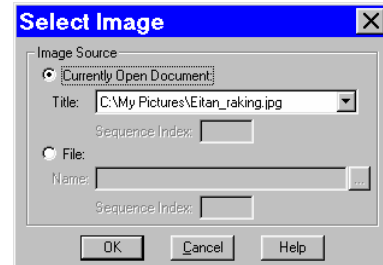
Text

Text

Add text to the report.

Date

Add a date text box to the report. The current date is the default.

Time

Add a time text box to the report. The current time is the default.

User Name

Add a user name to the report. The user currently logged on to the computer appears as the default.

Editing Report Objects

Once you have created a report, you can edit the report objects as needed. There are three basic ways to edit a report object:

•   **Use the Attributes menu** - The Attributes menu, as noted in the Defining Report Attributes section of this chapter, allows you to either set the attributes for adding specific types of objects, or to select existing objects and modify them as needed. Refer to the Defining Report Attributes section (above) for more details.

•   **Use the Edit menu** - The Edit menu contains general editing features such as Undo, Copy, Cut, Send to Back, etc…

•   **Right-click (Windows)/[Ctrl] click (Mac) on the report object –** Right/[Ctrl] clicking on a report object brings up a customized editing menu that contains both general editing commands and the attributes that can be edited for that object. Refer to the Working in the Report Mode section for more information on the right click function.

The Edit Menu

The Edit menu provides general editing features for arranging, copying, and sizing report objects. Edit menu options are active only when a report object is selected.

Undo Undo the last action. The Undo feature allows you to undo up to ten actions.

Bring to Front Bring a selected object in front of all other objects.

Move Forward Bring a selected object one layer forward.

Move Backward Send the selected object one layer back.

Send to Back Send a selected object behind all other objects.

Select All Select all of the objects in the report.

Unselect All Removes the affect of select all or multiple highlights.

Delete Delete the selected object(s).

Delete All Delete all of the objects in the report.

Copy to Clipboard Copy the selected object(s) to the Windows clipboard. You can then paste the object(s) into:

- A new SPOT report
- A different location in the same report

This feature is particularly useful when you are creating a new report and want to use objects from an existing report.

Cut Cut the selected object(s) for pasting into a new report or to a new location in the existing report.

Paste Paste the object that you cut or copied to its new location.

Duplicate Create additional copies of a report object. To duplicate a report object, follow these steps:

1. Select the object(s) that you want to duplicate.

2. Select Duplicate from the Edit menu. The program prompts you to enter the number of duplicate copies.

3. Enter the number of copies, and click on OK.

4. The duplicate object(s) appear at the top of report window.

Set Position, Alignment, and Size The **Set Position**, **Alignment**, and **Size** option allows you to specify precise sizing and position values for single or multiple selected report and annotation objects.

The **Position** options enable you to:

- Align top/bottom edges of multiple objects.

- Align left/right edges of multiple objects.

- Align the centers of multiple objects.

- Center an object(s) on the page.

- Enter exact values in inches or millimeters to position the object.

The **Size** options enable you to enter:

- Exact width/height in inches or millimeters for one or more objects.

- Set the width/height dimension for multiple objects the to smallest or largest object's value in inches or millimeters.

Add Page   Add a page to your report. When you add a page, the program prompts you with the Add Page dialog box:

1. Specify whether the page that you want to add will be inserted Before or After a specified page.

2. Specify the Page Number that the additional page will precede or follow.

3. Click on OK.

SPOT reports can have a maximum of ten pages.

Delete Page   Delete a page from your report. When you select the Delete Page option, the program prompts you to specify the page number.

Add Images   Add a static image to your report.

Saving Reports

1. To save a newly created report, select the **Save** option from the File menu.

2. When saving a new report (**.rpb** file type), SPOT prompts you with the default file location – i.e., **C:SpotCam\Reports** folder (Windows) or **Users\Shared\SPOT Files\Reports** (Mac) . Either save to the default location or specify a new location. If you plan to save many reports without compressing the image files, you should save them to a Jaz drive or to a network drive.

> ✎ *Note: Mac users will see the **.rpb** file type extension only if the **Add Extensions to File Names on Save** option is selected on the Preferences window*.
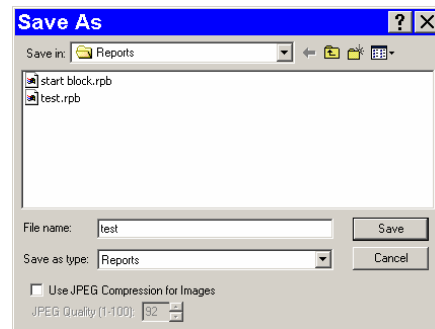
3. Enter a file name for the report.

4. Define the Compression for the image(s) in the report by selecting from None, JPEG and JPEG 2000. The **JPEG 2000 Compression** box is checked as a default. If you are using JPEG 2000 Compression, select either lossless or lossy from the checkbox associated with JPEG 2000.

   − A value of from 1 to 1000 must be entered in the value box is lossy is selected.

   To turn on JPEG compression, select that box. If you are using JPEG Compression, enter a number between one and 100.

   − A value of 1 is the most compressed. File size and image resolution are decreased to the maximum extent.

   − A value of 100 is non-compressed. File size remains large and the image resolution remains high.

5. Click on Save. You can now use the SPOT program to open and view the report at a later time.

Printing and Distributing Reports

To print a report, follow these steps:

1. Open the report.

2. Select the printer. If you have Adobe Acrobat, you can create a PDF version of your report by selecting **Acrobat PDF Writer** as the printer of choice: select the Printer from the **Page Setup** dialog – i.e., File >**Printer Setup>Printer**. PDF files can be distributed to anyone with the Acrobat Reader software, which, as described below, is available from Adobe as a free download.

3. Click on Properties to define the printer's properties, if needed.

4. Specify the page range if you are not printing an entire report.

5. Specify the number of copies.

6. Click on OK.
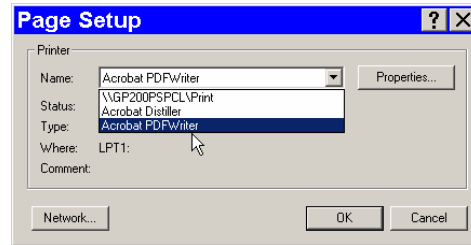
If you intend to distribute SPOT image reports to people who do not have the SPOT program, we recommend that you purchase Adobe Acrobat. Using Acrobat, you can convert the SPOT report (.rpb) files to PDF files, which can be distributed to users who do not have the SPOT software. They can view the reports with the free Acrobat Reader software, which is included on the SPOT software CD. The latest version of the Acrobat Reader can also be downloaded from the Adobe's web site www.Adobe.com.

Image Printing

Image printing features in the SPOT basic program and the SPOT advanced program are identical. Refer to Ch. 11 – Printing Images for details.

Ch. 4 - The Advanced Program Mode

QUICK REFERENCE:

➲ Display thumbnails of selected images… p. 67

➲ Access the Image Setup window to define image capture settings… p. 70

➲ Use a stage micrometer with the SPOT camera/ software to calibrate the microscope's objectives… p. 70

➲ Define color palettes that can be applied to monochrome images… p. 71

➲ Select which camera to use or switch to without closing the SPOT program… p. 73

➲ Change the appearance of the toolbar… p. 74

➲ Specify image sequencing preferences…p. 74

➲ Specify thumbnail display preferences… p. 74

Introduction

The **Advanced Program Mode** is an enhanced version of the SPOT software. It is designed for more advanced users who want greater control over the way images are captured and edited, and who want to make use of the image archiving database, the report generator, and the calibration mark imprinting and measuring functions. Additionally, the 4.0 version includes Macro Scripting allowing the user to automate image capture and post-processing procedures. Furthermore, a new Taskbar feature has been added allowing the user to customize an onscreen taskbar with one touch initiation of virtually any function found in the SPOT Advanced Software program.

This chapter is an introduction to the operating environment of the Advanced SPOT program mode. Detailed discussions of the features of this mode are found in chapters 5-13. This introduction is divided into three sections:

- **The Viewing and Editing Window** - Describes the primary window for all image capture and editing functions.

- **Working With Image Files** - Discusses file save and open features.

- **Setup** - Describes the initial Setup options.

The Viewing and Editing Window

When you open or capture an image, it appears in the main viewing and editing window. All of the image save, view, edit, and annotation functions are accessed from this window, which consists of three areas:

- Menus
- The Toolbar
- The Status Bar

Menus

The menu bar in the Advanced program varies according to whether an image is open. The table below briefly describes each menu, indicates whether an image must be open in order to see it, and provides a reference to the chapter that discusses the menu options in more detail:

Menu	Functions	Image Must be Open?	Ch. Ref.
File	Open, save, and print files. Create and run reports.	No	4, 10
Database	Create, access, and modify SPOT image databases.	No	9
Edit	Edit and annotate images.	Yes	8
Sequence	Viewing/play options for image sequences.	Yes (image sequence file)	8
View	Viewing options for captured images	Yes	4
Camera	Initialize the camera, view live preview images, capture images, and perform white balances.	No	6, 7
Setup	Define image setups, image capture preferences, calibration setups, and Taskbars.	No	5, 12
Macro	Create, Edit, and Play automated image acquisition routines	No	13
Window	Standard Windows display options.	Yes	4
Help	Information about the SPOT software and an on-line help system.	No	

The Toolbar

The Toolbar provides shortcut buttons to frequently used menu items. Dragging the cursor over the Toolbar displays information about each button.

- The name of the button (and the associated menu option) displays on the toolbar.
- The function that the button performs displays on the left of the status bar.

Refer to Appendix C: Keyboard and Toolbar Button Commands for a complete listing of command equivalents in the SPOT program.

Like the menus, the Toolbar options vary according to whether an image is open, and what you are doing. The toolbar displays the options necessary in each of the following situations:

- When you first open the SPOT program…
- After you have captured or opened an image…

When You First Open the SPOT Program…

When you first open the program, the toolbar displays only the image capture options:

Toolbar Button	Description
Live Image	Show/hide live image window
Get Image (exposure from setup)	Take a picture
Get Image (last used exposure)	Take a picture using the last used exposure
Compute White Balance Values	Compute RGB white-balance ratios
Full Screen	Toggle full screen mode
Search Database	Search the database

(The Search Database option is active only when a database is open.)

RT B/W	If you are using an RT B/W camera, the Compute White Balance Values option is inactive.

Refer to Ch. 6 – Working with Live Images, Ch. 7 - Taking a Picture, and Ch. 8 - Viewing, Editing, and Annotating Images for more details.

After You Have Captured or Opened an Image…

After you have either captured a new image or opened an existing image, the toolbar **expands** to include the following options:

Toolbar Button	Description
Zoom	Turn on/off zoom mode
Show Pan Window	Show/hide the pan window (useful for navigating when zoomed)
Save As	Save the image to disk with a new name and/or type
Save to Database (Windows)	Save the image to the database (*this option is active only when a database is open)*
Reload	Reload the image from disk

	Print	Print the image

Refer to Ch. 8 – Viewing, Editing, and Annotating Images and Ch. 11 - Printing Images, for more details.

The Status Bar

The status bar, which appears at the bottom of your screen, displays information about the image that is on screen. The following is an illustration of the view status bar, as it appears for an open image:
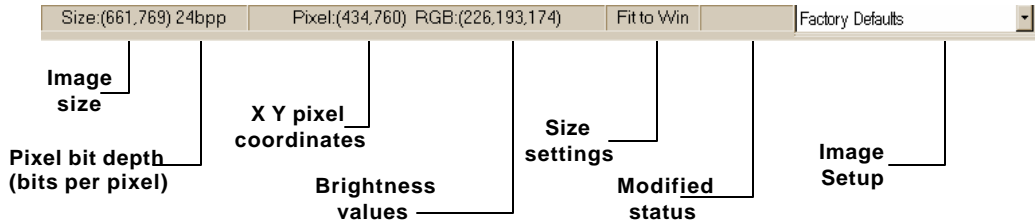
Figure 4- 1: Advanced Mode Status Bar

Note: The status bar displays values for captured images, not for live images

Each of the call-outs is described below.

- **Image size** – The number of columns by the number of rows of pixels in the image.

- **Pixel bit depth (bits per pixel - bpp)** - The pixel bit depth of the image. There are four options:
  - 8 bpp (monochrome) – RT B/W, Slider (B/W mode)
  - 12 bpp (monochrome) – RT B/W, Slider (B/W mode)
  - 24 bpp (RGB color) – RT Color Mosaic & 3-Shot, Slider (Color mode)
  - 36 bpp (RGB color) - RT Color Mosaic & 3-Shot, Slider (Color mode)

  For a detailed discussion of pixel bit depth, refer to Ch. 5 - Preparing to Take a Picture.

- **X Y pixel coordinates** - As you move your cursor over an image, the status bar displays the x y coordinates of the cursor in pixels. For example, the values for the upper left corner pixel would be (0,0).

- **Brightness values** - As you move your cursor over an image, the status bar indicates the pixel brightness values at the indicated x y pixel coordinates. The values depend on the pixel bit depth (see Ch. 5 – Preparing to Take a Picture) for the image. For example, if a color image is open, the status bar displays separate red, green, and blue brightness values [e.g., RGB: (226, 193, 174)]. If a monochrome image is open, the status bar displays only one brightness value [e.g., (176)].

- **Size Settings** - In the illustration above, the view status bar displays **Fit to Win** (Fit to Window), the default image capture setting.
  - If the Fit to Window option has been deselected, this area displays the word **Normal**.
  - If you are in zoom mode, this area displays the magnification level (e.g., 100%, 200%…)

- **Modified Status** - If you open an image file or take a picture, the section to the right of the magnification setting will read **"New"** until you modify that image. Once that image has been modified, the word **Modified** appears here. Use the Undo command to return to the New state.

- **Image Setup** - The far right end of the view status bar displays the currently active Image Setup (e.g., Factory Defaults). This is the image setup that the program uses to capture images. From this part of the view status bar, you can:
  - Change the currently active image setup.
  - Modify an existing image setup.
  - Add a new image setup to the list.

Refer to Ch. 5 - Preparing to Take a Picture, for more information on image setups.

Working With Image Files

The File menu contains the following options:

Open Image File	Open image files of a specified format (i.e., JPEG, GIF, BMP, etc.). In addition to using this menu option, you can open images by dragging the image file from the Windows Explorer onto the SPOT program window.
Show Image File Thumbnails	Display thumbnail previews of specified files. From the thumbnail display window, there are a variety of options. To view image file thumbnails, follow these steps:

1. From the File menu, select the Show Image File Thumbnails option. The Thumbnail File Spec window displays all of the image files in the directory.
2. Navigate to the directory for the image files that you want to display.
3. Use the **Files of type** drop down box, to specify the file type.
4. Select the file or files for which you want to display thumbnails. If you do not select a file or files, the program displays thumbnails for all files of the specified type in the directory.
5. Click on Open. The Thumbnails preview window appears. For each thumbnail, you can:
   - *Double-click* (Mac and Windows) on a thumbnail to open an image for viewing or modification.
   - *Right-click* **(Windows)/[Ctrl] Click (Mac)** on a thumbnail to display a list of options. The right-click menu enables you to perform a variety of actions directly from the thumbnail window:
     - **Select Image.**
     - **Open Image.**
     - **Delete Image.**
     - **Print image.**
     - **Move/Rename Image File**
     - **Copy Image File**
     - **Add Image to Image sequence** (see below)
     - **Add Image to Report Template** (see below)
     - **Select All Images** in the thumbnail view. When you use this option, the right-click menu expands to include the above options for multiple images
6. Click and drag with the mouse to select two or more images.

Open Image Sequence File   Open an image sequence file. An image sequence file is a single TIFF format file that contains multiple images.

- Refer to Ch. 7 – Taking a Picture for information on capturing an image sequence file.

- Refer to Ch. 8 – Viewing, Editing, and Annotating Images for information on viewing and editing image sequences.

Open Database Image   Open images from a SPOT database. The database must be open in order to use this feature. Refer to Ch. 9 – Using SPOT Databases for details on working with databases and database images.

New Image Sequence   Create a new image sequence file. This option enables you to specify the first image in a new image sequence. This image can come from an open image, an image on disk (from file), or a SPOT database. Subsequent images can be added to the sequence in one of two ways:

- Via the **Add Image to Image Sequence** File menu option (see below). This option allows you either to add an open, non-sequence image to a sequence or to create a new image sequence from the image.

- Via the **Sequence** menu options. Here you can add any image to any point in a sequence. Refer to **Working With Image Sequences** in Ch. 8 for more details.

New Report   Create a new Advanced mode report. Refer to Ch. 10 – Reporting for details.

Open Report   Open an Advanced mode report. Refer to Ch. 10 – Reporting for details.

Run Report   Run a report. Reports can be run in three ways - previewed, printed, or saved to file. Refer to Ch. 10 – Reporting for details.

Reload   Reload the image from the hard drive (or other media). All changes made since opening the image file are lost. This option displays only when a saved image is open.

Save   Save a previously unsaved image to new file, or update the changes to an existing file. This option appears only when an image is open.

Save As   Save an image to a new file and/or file location. This option appears only when an image is open.

Save to Database   Save an image to a database file. This option appears only when a database is open.

Save All   Save all open image files. The program prompts you for a name, file location, and image file format for each image.

Close   Close an image. The program prompts you to specify a location to save the file. This option appears only when an image is open.

Move/Rename   Move and/or rename an image file. This option differs from the Save As option in that it *does not* retain a copy of the original file in the original location: using Move/Rename is comparable to cutting and pasting a file.

Delete   Delete an open image. The program prompts you to verify the deletion.

Export Movie File (Windows)	Export a SPOT image sequence file to AVI format. Follow these steps:

1. Open a SPOT image sequence. Refer to Ch. 7 – Taking a Picture, for details on capturing image sequences.

2. Select **File>Export Movie File**. The **Export Movie File** appears.

3. Enter the **File Name** for the avi file.

4. Specify the location where you want to save the avi file.

5. Specify which images in the sequence that you want to **Export**. Select either:

    - **All Images** – the avi file will contain all of the images in the sequence.

    - **Specified Images** - the avi file will contain only the images that you specify (i.e., below the radio button).

6. Specify the **Frame Rate** in frames per second.

7. Specify the **Compression Settings**. When you click on the **Compression Settings** button, the **Video Compression** dialog displays the compression application(s) available on your computer. Because each computer will have different installed applications, the Video Compression options will vary.

8. After you have entered all of the desired settings, click on **Save**. The program displays a status message as it exports the file.

Add Image to Image Sequence	Add an open, non-sequence image to an open image sequence, or create a new image sequence from an open image. To use this option, the non-sequence image must be the active window and the image sequence must be open in the background.

When you click on **Add Image to Image Sequence**, SPOT prompts you to specify either the **Image Sequence** or a new image sequence. If more than one image sequence is open, select the sequence from the drop-down list.

Add Image to Report Template	Add a selected open image to an open SPOT report template. When you select this option, SPOT prompts you to select from a list of report templates. If you have not defined a report template, SPOT automatically creates a new report for you and inserts the image as a **Static Image** object. Refer to Ch. 10 – Reporting for details on working with SPOT reports.
Print	Print an open image. Refer to Ch. 11 – Printing Images for details.
Print Setup	Define the page setup parameters. Refer to Ch. 11 - Printing Images for details.
Exit (Windows) Quit (Mac)	Exit the SPOT program.

Setup

The Setup menu options allow you to define operational standards used for image capture and editing. Refer to the sections below for descriptions of each option.

Image Setups

In the SPOT software, an image setup serves two purposes:

- It defines the way a picture will be taken.
- It defines the way the program will automatically modify a picture after it is taken.

Pre-configured image setups for standard microscope techniques (e.g., brightfield, fluorescence, etc.) help novice users to take good pictures the first time. Experienced users can modify these pre-configured setups as needed. Refer to Ch. 5 - Preparing to Take a Picture for details.

Calibration Setups

With the SPOT program, you can use a stage micrometer to calibrate each objective on your microscope. Once calibrated, you can use the SPOT program to perform basic measurements on an image. Refer to Ch. 8, Viewing, Editing, and Annotating Images for more information on using the measurement functions.

To calibrate an objective, follow these steps:

1. Place the stage micrometer under the objective.

2. Adjust the microscope to display as much of the stage micrometer as possible. You can use the Live Image option, as described in Ch. 6 – Using Live Images, to help position the micrometer

3. Focus on it. Use the Live Image option.

4. Capture an image of the stage micrometer. Refer to Ch. 7 – Taking a Picture for details.

5. From the Setup menu, select Calibration Setups. The Calibration Setups window appears (see right).

6. Click on Add. The Calibration Setup window appears.

7. Enter the name of the calibration setup. Typically this corresponds to the magnification of the objective (e.g., 4x).

8. Using the mouse, draw the calibration line along the length of the stage micrometer image. When you draw the line, keep these guidelines in mind:

   - Keep the line as straight as possible.
   - Make the line as long as possible.
   - Use the largest unit of measurement that the micrometer allows for.
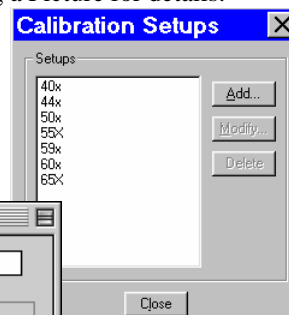
To draw the line, follow these steps:

   a.   Position the crosshair cursor over the stage micrometer mark that represents the start point of your calibration line. In the illustration below, this is the 20 mm mark.

   b.   Left click on the mouse.

   c.   Position the crosshair cursor at the stage micrometer mark that represents the end point of your calibration line. In the illustration below, this is the 23 mm mark.

   d.   Left click on the mouse again. The length of the line in pixels appears on the Calibration Setup window. To re-draw a line, double click anywhere on the image. This clears the previously drawn calibration line from the screen.

9. Enter the length and units of the line that you drew (e.g., 3 mm).

10. Click on OK. The 4x objective is now calibrated to perform measurements. Repeat steps one through ten for each objective.

Palettes

The Palettes option on the Setup menu allows you to define new palettes and edit existing palettes. SPOT color palettes allow you to apply specific hue and saturation values to monochrome (8 bit or 12 bit) images. Palettes are useful in fluorescence applications where a sample is fluorescing at a single wavelength, and you want the acquired image to have the correct color. For such a case, you could create a palette to represent the color of the fluorescent dye and then apply that palette to the captured image.   There are two ways to apply a palette to an image:

- You can associate a palette with an Image Setup, as discussed in Ch. 5 - Preparing to Take a Picture.

- You can apply a palette to a captured image by selecting the **Set Palette** option from the **Edit** menu. Refer to Ch. 8 – Viewing, Editing, and Annotating Images for more details.

To define a new palette, follow these steps:

1. From the **Setup** menu, select **Palettes**.

2. Click on **Add**. The Color Palette dialog appears as shown here:

3. Either use the **Wavelength or Hue** and **Saturation** selection lists or move the cross-hairs on the color wheel to enter the values

   –   **Wavelength** allows the user to specify the exact wavelength ranging in value from 400nm to 700 nm. Wavelengths will automatically correspond to various Hues at full (100%) saturation.

   –   **Hue** is the designation for the actual color, as distinguished from others in the color spectrum. Hue is derived from the color wheel and is expressed in the color's angular location (in degrees) on the wheel. (e.g., 0° to 360°).

   –   **Saturation** is the proportion of perceived pure hue in the color. Saturation (in the SPOT software) is measured on a numerical scale of 0 to 100, where zero is pure gray and 100 is pure hue (e.g., day glow).

4. Click on **OK**.

Image Auto-Save (Windows)

Use the **Image Auto-Save** feature to automatically save images as part of the capture process. To enable SPOT's auto-save feature, select **Setup>Image Auto-Save**, and check the **Auto-Save Images** box, as shown at right. You can then specify the auto-save options, as described in the table below.

File Name Prefix	This prefix appears before the number of each image capture in the sequence. To enter the File Name Prefix, you can:

- Type only the name (e.g., **Cell**) in the box. The program automatically saves the image to the last used directory. If you specify the prefix only, you cannot see exactly where the files are being saved. Thus, to avoid having to search for your files, you should verify the location of the last save.

- Type the full path and file name (e.g., **C:\Spotcam\T-cells**) in the box to specify the location of the image. When entering the file path and name, you can also specify a new directory simply by entering it in the path (e.g., C:\Spotcam**Cells**\T-Cells).

- Click on the browse button to select a new file location and file name (prefix). All of the image files are saved with the prefix that you enter. Thus, if your Suffix Begin Number (see below) is one, the file name for the image would be **T-Cells0001**.

Format	Select the file format for the sequence of images.

- For individual files, there are six options: **Windows Bitmap, JPEG, Mac PICT**, **TIFF Uncompressed TIFF JPEG**, or **JPEG2000**.

- For image sequences there are two options: **TIFF Uncompressed** or **TIFF JPEG**.

Suffix Begin Number	Select the beginning number for image auto-save. The Begin Number automatically defaults to the first number that follows the previous sequence of images. So, if you captured a sequence of 10 images, 11 would appear as the Begin Number when you next opened the Get Sequential Images window.

The program prefaces numbers with enough zeros to create a four character suffix. For example, if you have 35 images, the first image suffix will be **0001** and the last image suffix will be **0035**. This enables Windows to correctly order images, so that Growth_Sequence0002 appears before Growth_Sequence1000, and not the other way around.

JPEG Quality (1-100)	JPEG quality is the level of compression for JPEG images: this option is available only when JPEG or TIFF JPEG is selected as the image file format. Enter an integer between 1 and 100 to set the JPEG quality for images. A value of one is the most compressed (and lowest image quality), while a value of 100 is the least compressed (and highest image quality).
Compression (JPEG2000)	When the JPEG2000 file format is selected, the Image Auto-Save Setup dialog displays compression options:

- **Lossless** – Information is not lost when the image is compressed.

- **Lossy –** Information is lost according to the ratio that you enter: the higher the ratio that you enter, the smaller the file size and the lower the quality of the image.

Save Thumbnails	Save thumbnails of each image capture. This option is available only when JPEG, TIFF JPEG, or TIFF Uncompressed is selected as the image file format.
File Name Conflict Action	Determine how the program will respond if it finds an image file with the same name as an image from the current session. Select one of the following options:

- **Overwrite Existing File** – The program overwrites the existing file with the new image capture.

- **Display Warning** – The program displays a warning message indicating that a file of the same name already exists, and stops the image capture.

Image Display	Determine how image captures will be displayed. Select one of the following options:

- **Display Each Image in a New Window** – The program opens a window for each new image capture. This option works well if you plan to capture a limited number of images in the session. If you plan to capture many images, however, displaying numerous windows uses up memory and could interfere with use of other programs.

- **Display Only Current Image –** The program sequentially displays each image in the same window. Each image displays only until the next image is captured. This is useful when you have numerous captures where you do not want to be concerned with running out of memory.

- **Don't Display Images –** The program automatically saves all of the image files to the specified location without displaying them.

Taskbar

The taskbar option, when selected, will open a taskbar development window, allowing the user to add, modify, or delete taskbar buttons from the floating taskbar. Please see Chapter 12 for a detailed discussion regarding the development and use of the timesaving Taskbar feature.

Select Device

Selecting this option from the dropdown menu will allow the user to switch between SPOT cameras running on the same computer. When an alternate device is selected, for example from an Insight camera to an RT camera, the menu and toolbars will adjust to acknowledge the appropriate options and settings for the new device. The SPOT program, however, will not shut down. This on the fly device switching capability can be very convenient for the user with the need for multiple cameras during the day.

Preferences

Use the Preferences option to specify:
- Size of the onscreen toolbar
- Sequential imaging memory settings
- Thumbnail appearance.
- Live image viewing settings
- Fit to Window view settings
- File type extension setting (*Macs only*).

📝 *Note: In SPOT 4.0, the Preferences window appearance has changed. Preferences are now subdivided into multiple windows.*

To open the Preferences window, select **Preferences** from the Setup menu. Each preference is discussed below.

Toolbar

Toolbar
Allows the user to specify whether the onscreen toolbar icons in the SPOT program should be displayed in large or small format.

Image Sequences

Memory Usage Limit
Specify how much memory is allocated for image acquisition. Note that the entry parameters are system dependent.

Cache Images During Play

When "playing" an image sequence (see Ch. 8), the SPOT program allows you to select how it loads the displayed images:

- *If **Cache Images During Play** is selected…* The program pre-loads as many images from the sequence as your computer's RAM allows. This enables a faster display of the image sequence. But, if you have a sequence with many images, the size of the combined images could exceed the RAM, and in this case, the SPOT program will load as much of the sequence as possible, play that part of the sequence, and then pause to load the remaining images.

- *If **Cache Images During Play** is not selected…* The program loads each image in the sequence individually. In cases where you have a large sequence with many files, this allows you to work without being restricted by RAM limitations. The display of images will be somewhat slower than a display where images are cached

Thumbnails

Specify the size of thumbnails for the Show Image File Thumbnails option

Select from one of the following options:
- **Small (60 x 60 pixels)**
- **Medium (90 x 90 pixels)**
- **Large (120 x 120 pixels)**
- **User Defined**
  Minimum width and height = 20 pixels
  Maximum width and height = 200 pixels

Specify the resolution of image thumbnails (Show Image File Thumbnails option)

Select from one of the following options:
- **Small (54 pixels)**
- **Medium (84 pixels)**
- **Large (114 pixels)**

- **User Defined**
  Minimum resolution = 16 pixels
  Maximum resolution = 200 pixels

Live Images

Always Show Full Chip in Live Image ensures that you will always see the Full Chip view when viewing a live image. Keep in mind that the selection that you make here determines how the live image displays when viewing a region of the chip. Refer to the **Chip Imaging Area** section for details.

Close Window on Snap will close the Live Image window when you select Snap to capture an image. Otherwise, the Live Image window will remain open with the captured image(s) below it.

Maximum Exposure

The Maximum Exposure option helps you to avoid unnecessarily long exposures when viewing a live image. Consider the following situation: When you change from a lower resolution objective to a higher resolution objective, the camera detects a brief interval of blackness. When the Auto-Brightness option on the Live Image Controls dialog (see Ch. 6) is enabled, it compensates by modifying the exposure time to maintain a constant brightness level. This results in you having to wait for the camera to compute a lengthy and incorrect exposure for the black interval. Using the **Maximum Exposure** option, you can limit this exposure time. For most cases, Diagnostic Instruments recommends a value of 0.5 seconds.

Similarly, the Live Image Maximum option can also be used in cases where the microscope's light path selector is incorrectly set.

Do Post Processing On

Specify whether to apply the **Post Processing** options for the current Image Setup to images

captured from the live image view.

- **Trans ferred Images**
  - *If the **Transferred Images** box is selected*, the Transfer option applies the **Noise Filter**, **Color Enhancement**, and **Image Size Double** options as specified for the current Image Setup.
  - *If the **Transferred Images** box is unselected*, the **Transfer** option ignores the Post Processing options for the current Image Setup, with one exception: if the **Chip Defect Correction** option is selected for the current image setup (Enhanced and RT cameras only) , the corrections will appear in the image capture, regardless of the Transferred Images preference.

- **Snapped Images**
  - *If the **Snapped Images** box is selected*, the Snap option uses all of the Post Processing options from the current Image Setup, except for Background Subtraction and Flatfield Correction.

*If the **Snapped Images** box is unselected*, the Snap option ignores the Post Processing options for the current Image Setup, with one exception: if the **Chip Defect Correction** option is selected for the current image setup (RT and Enhanced cameras only), the corrections will appear in the image capture, regardless of the Snapped Images preference.

Miscellaneous

Specify whether images should open in Fit to Window mode.
The Fit to Window option, a program default, automatically sizes images to fit the available screen space when you open or capture an image. Thus, you can immediately see the entire image without having to zoom out. This is especially useful with smaller monitors. Open Images in Fit to Window Mode is checked as a default.   If you do not want images to open in the Fit to Window mode, deselect the box.

Auto-save Image File Name Suffix Number.
This feature allows you to specify a suffix number after the file name in order to keep track of the sequence of images captured.

Specify whether you would like to utilize a shutter sound on image capture.
The Make Shutter Sound option, when checked, will provide a "click" sound during the camera exposure sequence, confirming that the image capture is complete. If the image capture is relatively quick, you will hear a single click. If the exposure is longer (i.e. greater than one second) you may hear both a begin click and an end click.

Specify whether you would like to monitor the color filter position (RT Slider).
The Monitor Camera Color Filter Position box is checked as a default. If this option is left checked, the SPOT program displays error messages when the sensors in the camera head detect that the color filter is not in the correct position. This option **does not** monitor the position of the filter slider in Live Image mode. In Basic mode, the program will give you an error message if you are capturing an image and the sliding color filter is in the **B/W** position.

Add Extensions to File Names on Save (Mac)

When selected, the **Add Extensions to File Names on Save** option automatically appends the file type extension (e.g., JPG, tiff, bmp, etc…) to your image file. This enables cross platform file sharing, because Windows based programs require a file type extension, while Mac based programs do not. Once the file type extension is added, Windows based programs can recognize the Mac file.

Ch. 5 - Preparing to Take a Picture

Introduction: What is an Image Setup?

An image setup serves two crucial purposes:

- It defines the way a picture will be taken.
- It defines the way a picture will be modified after it is taken.

Image setups are defined to optimize the picture taking process for different types of images or microscopy techniques. They can be created, saved, and recalled as needed.

Accessing the Image Setup Window

To define a new image setup or to edit an existing setup, follow these steps:

1. From the Setup menu, select the Image Setups option. The Image Setups window appears. Either double-click on a setup to open it, or select one of the following options:

   – **Add** - To create a new image setup, click on Add. The New Image Setup dialog box appears with a list of templates (image types) that serve as the basis for the new image setup.

   – **Modify** - To edit an existing image setup, select a setup from the list, and click on Modify. The Image Setup window appears.

   – **Delete** - To delete an image setup, select a setup from the list and click on Delete.

   – **Current** – To make a setup the active setup, select the setup from the list and click on Current.

   – **Close** - To exit the dialog box, click on Close.
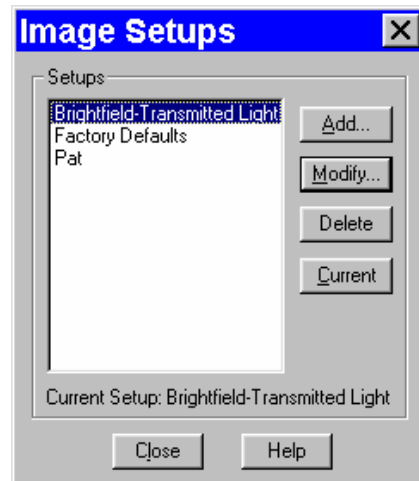
Shortcuts: You can quickly access the Image Setup window by right clicking the drop-down box in the bottom right corner of your screen. The box displays the name of the current (active) Image Setup.

   – To **change the current (active) image setup**, left click on the tab and select the new Image Setup.

   – To **define a new image setup** or to **modify an existing image setup**, right click on the box, and select Add.

   – To **modify the current image setup**, right click on the box and select modify.

   – To **access the current image setup**, press **[F12]**.

If you select the Add option (i.e. to create a new image setup), the New Image Setup window appears. The pre-defined templates (image types) are indicated by brackets < >, while user-defined setups appear without brackets:

RT B/W	RT B/W and Color Mosaic camera users will not see the fluorescence – red, fluorescence – green, and fluorescence – blue Image Type options.
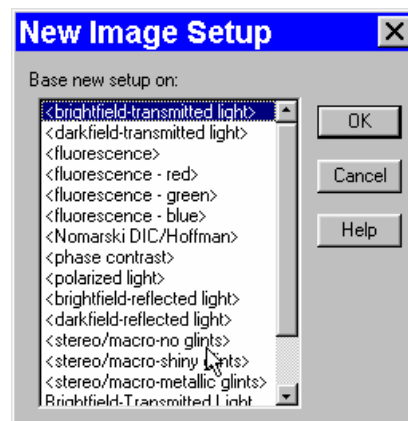
1. **Select an image type from the list.**
   The 14 pre-defined image types (in addition to the Factory Defaults setting) correspond to commonly used microscopy techniques. To define a new image setup, you must also select an image type as a template for defining the new setup. The image type provides a baseline set of values for that setup, according to the microscopy technique you are using. The following table describes SPOT's pre-defined image types:

Image Type	Description
Brightfield-transmitted light	Stained specimens on glass slides using transmitted light microscopes.
Darkfield-transmitted light	Bright specimens on dark backgrounds using transmitted light darkfield technique.
Fluorescence	Fluorescence specimens not fluorescing in a single red, green, or blue primary color. The camera exposes images using all three colors.
Fluorescence – red	Fluorescence specimens fluorescing in red. Exposure time is reduced because only one color is exposed.
Fluorescence – green	Fluorescence specimens fluorescing in green. Exposure time is reduced because only one color is exposed.
Fluorescence – blue	Fluorescence specimens fluorescing in blue. Exposure time is reduced because only one color is exposed.
Nomarski DIC/Hoffman	Specimens being viewed using Nomarski DIC or Hoffman techniques.
Phase contrast	Specimens being viewed using the phase contrast technique.
Polarized light	Specimens being viewed with a polarized light microscope.
Brightfield-reflected light	Polished metallurgical specimens and semi-conductors using a microscope with a vertical illuminator.
Darkfield-reflected light	Bright areas of specimens being viewed with darkfield, reflected light techniques.
Stereo/macro–no glints	Matte specimens without bright glints.
Stereo/macro–shiny glints	Shiny specimens with bright glints.
Stereo/macro-metallic glints	Metallic specimens with intense glints.

RT Slider	When the liquid crystal color filter has been moved out of the light path, RT slider-type users may notice a diffuse bright area on non-fluorescence image captures. This is due to infrared light that is transmitted when the filter is out of the light path. Our infrared filter is bonded to the liquid crystal color filter: when the color filter is moved out of the light path, the infrared filter is also moved. To correct this problem, you should use an external infrared filter.

2. **Click on OK.**
   The Image Setup window appears.
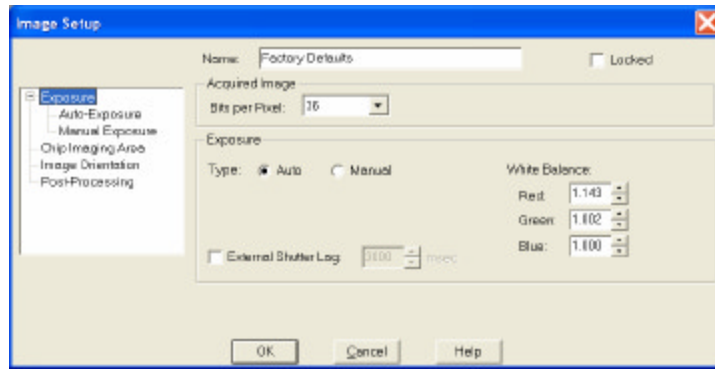
Image Setup Options

The Image Setup window now consists of six options, each with a different window. Each window (above) has a menu on the left that allows you to navigate to any of the six windows. The right side of the window includes options specific to that window.

General
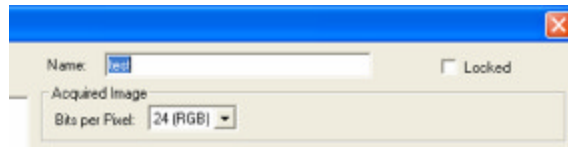
You will note that the header provides a consistent reminder of the following information, no matter which of the six windows you are in.

- Name
- Locked
- Acquired Image - Bits per Pixel

Name

Use this box to enter the name of a new image setup, or to modify the name of an existing setup. If you modify the name of an existing setup, the program prompts you (when you click OK) to save this as a new setup or overwrite the original setup.

Locked

The Locked option allows you freeze the settings for an Image Setup that you have defined. This is useful in cases where multiple users have access to the program and you want to preserve the settings that you defined.

To lock an Image Setup, click on the Locked box. When another user opens the Image Setup, the settings are unavailable for entry until the Locked box is unchecked. The Locked box along with the appearance of the window serve as a message that the settings should not be modified.

Acquired Image

The Acquired Image section options enable you to define the characteristics of captured images. This includes:

- Bits per Pixel
- Palette (RT B/W only)

Bits per Pixel

Bits per pixel (or *Pixel Bit Depth)* is the number of *bits per pixel* (bpp) that the camera uses to create an image. The SPOT program provides different options, depending on which RT camera model you are using.

- **RT Color - 24 bpp** and **36 bpp**.
- **RT B/***W* **- 8 bpp** and **12 bpp**.
- **RT 3-Shot/Slider – 8 bpp, 12 bpp, 24 bpp,** and **36 bpp**.

> ✏️   *Note: RT Color users can use the* Change Pixel Bit Depth *option to convert the pixel bit depth for a captured image to a monochrome setting.*

For RT Color and 3-Shot users, the 24 bpp RGB color setting is the most common selection because it is an industry standard color format that is used by Windows and most printers. Most monitors cannot display more than 24 bpp. Select a Pixel Bit Depth by clicking on the Bits per Pixel drop-down box. In addition to specifying the bits per pixel of the image, your selection determines the Gain and Gain Limit choices (see appropriate sections in this chapter) as illustrated in the table below:

Bits Per Pixel	Camera	Available Gain/Gain Limit Options
8	B/W, 3-Shot	1, 2, 4, 8, 16
12	B/W 3-Shot	1, 2, 4, 8
24	Color, 3-Shot	1, 2, 4, 8, 16
36	Color 3-Shot	1,2, 4, 8

The following table lists the available image depth options and describes some of the potential uses, advantages, and disadvantages associated with each setting.

Pixel Bit Depth Settings	Characteristics
8 bpp (monochrome) *RT B/W* *RT 3-Shot*	The 8 bit setting is often used in cases where 256 gray levels are adequate. The lower number of bits per pixel results in smaller, more manageable file sizes.
12 bpp (monochrome) *RT B/W* *RT 3-Shot*	The greater number of bits per pixel in the 12 bit setting provides users with 4096 gray levels. This provides a more accurate means of measuring brightness in a monochrome image, and helps to reduce *banding* problems that often appear when an image is modified.
24 bpp (RGB color) *RT Color* *RT 3-Shot*	The 24 bit setting provides 8 bits per color, per pixel (i.e., 8 x 3 = 24). As noted, 24 bpp is the most common choice because it is an industry standard in terms of printing, the Windows operating system, and monitor display.
36 bpp (RGB color) *RT Color* *RT 3-Shot*	The 36 bit setting provides 12 bits per color, per pixel (i.e., 12 x 3 = 36). 36 bpp is used in cases where images are heavily modified. The 36 bit setting provides users with 4096 brightness levels per color and thereby reduces banding problems when images are stretched.

Palette (B/W; 3-Shot)

SPOT color palettes allow you to apply specific hue and saturation values to monochrome (8 bit or 12 bit) images. Palettes are useful in fluorescence applications where a sample is fluorescing at a single wavelength, and you want the acquired image to have the correct color. For such a case, you could create a palette to represent the color of the fluorescent dye and then apply that palette to the captured image. The **Palette** option on the Image Setup window allows you to automatically apply a palette to a monochrome image capture.

✎ *Note: Applying a color palette to an image capture does not affect image data.*

To apply a palette, select one from the drop down list. There are four pre-defined palettes: **Red**, **Green**, **Blue**, and **Grayscale**. Because the **Palettes** option applies only to monochrome images, it is available only when either an 8 bpp or 12 bpp Pixel Bit Depth is selected. You can define custom palettes using the **Palettes** option on the **Setup** men. Refer to Ch. 4 – The Advanced Program Mode for more information.

✎ *Note: The palette option will not appear when you have a color mosaic camera model.*

Exposure Window

The Exposure window selections tell the camera what settings to use when taking a picture. Carefully considered definition of these settings helps to ensure that your images come out as planned. The Exposure options vary according to two variables:

- The RT camera model that you have – Color, B/W, or 3-Shot.
- The Bits per pixel setting

Each Exposure option is described below. If an option is unique to a specific camera, this is indicated parenthetically.
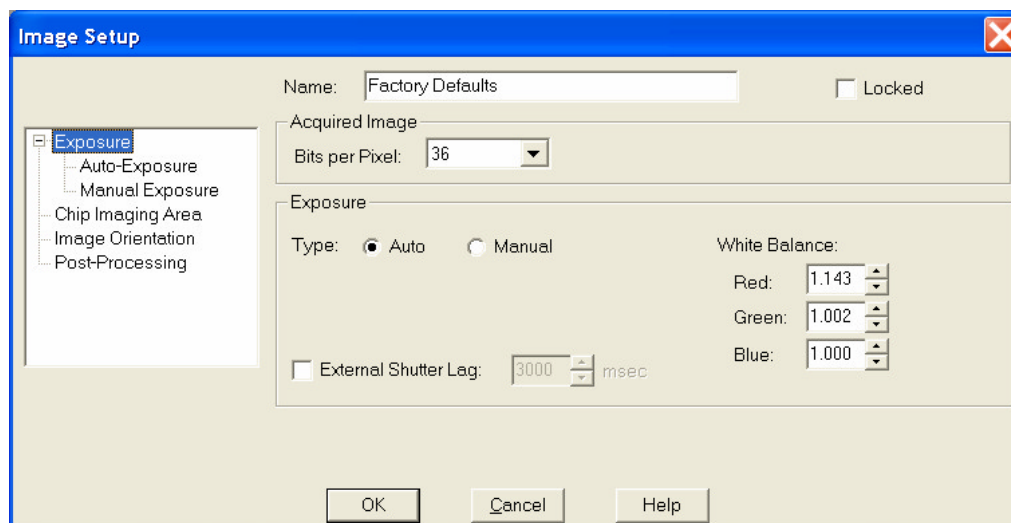
✎ *Note: **Please note** that this window is where you select between allowing the program to determine the exposure automatically (Autoexposure) or setting it yourself (Manual). The subwindows of Auto-Exposure and Manual Exposure are where you set the details of either protocol.*

Type

Auto

When you select **Auto**, the camera samples light levels in the image plane (as the initial part of the image capture) and uses the values to calculate the final exposure time.

Manual

When you select **Manual**, you specify the exposure times. This can be done either by entering a value or values, using the **Compute Exposure** option, or by the **Recall Previous Exposure** option.

The sections that follow detail all of the Exposure options. If an option is available only for specific RT camera models, only at certain pixel bit depths, or only for either automated or user-defined exposure, this is indicated parenthetically next to the heading.

RT KE Color Mosaic	RT KE Color Mosaic camera users will not see the Use Filter, Filter Color, Use: Red Green Blue, or Color Order options.

Use Filter (RT Slider; 8 and 12 bit mono)

The Use Color Filter option specifies the position of the color filter for image captures, as well as the color that will be exposed in the image capture. The Use Filter option does not change the position of the filter: this must be done by physically sliding the filter to the desired position. If the Use Color Filter setting does not match the filter's position, SPOT prompts you with an error message. However, this error message appears *only* if the Monitor Camera Color Filter Position option on the Preferences window (see Ch. 4) is selected.

To specify the Use Filter settings, follow these steps:

1. Ensure that the **Bits per Pixel** is set to either 8 bpp or 12 bpp.

2. Specify the position of the color filter:
   - To specify that the sliding color filter will be in the light path, ensure that the Use Color Filter box is selected. Go to step three.
   - To specify that the sliding color filter will be out of the light path, deselect the Use Color Filter box. Go to step four.

3. Specify the color that will be used.

4. Ensure that the color filter is in the correct position.
   - If you are using the filter, the RT slider knob should be in the **COLOR** position.
   - If you are capturing an unfiltered image, the RT slider knob should be in the **B/W** position.

Filter Color (RT Color; 8 and 12 bit mono)

Filter Color specifies the color of the filter that will be used when taking a monochrome picture.

To ensure a high image quality, SPOT users should select the color that is closest to the color of the specimen. For example, if you are looking at a single stained fluorescence, selecting the color filter that most closely matches the color of the stain shortens the time it takes to acquire the image. In addition, SPOT RT cameras feature a second generation liquid crystal filter that allows all three colors (e.g., red, green, and blue) to be transmitted. This is the **Clear** option.

> *Note: Selecting the Clear option does not provide the entirely unfiltered image that is possible with the RT Slider camera. Although all three colors are transmitted, the polarizing filter, which is part of the liquid crystal filter, and the IR filter remain in the light path, blocking some of the light. When using the Live Image window to focus or position dim images, RT Slider users can slide the filter out to provide higher frame rates and less noisy images.*

To select a the filter color for capturing a monochrome image, follow these steps:

1. Ensure that the Pixel Bit Depth is set to either 8 (mono) or 12 (mono).
2. Click on either the Auto-Exposure or the User-Defined Exposure option.
3. Select one of the following options:
    - Red
    - Green
    - Blue
    - Clear

Use: Red Green Blue (3-Shot; 24 and 36 bit RGB)

Use: Red Green Blue specifies the colors that will be exposed. For most situations, you should select all three colors. However, in situations where you know that your specimen lacks one or two of the colors, you can avoid the additional exposure and download time by "turning off" (deselecting) the missing colors. For example, if you have a pumpkin colored fluorescence sample with no blue in it, you can deselect the blue value. The camera only exposes the red and green values.

> *Note: When you add a new 24 bpp or 36 bpp image setup, all three colors are selected as the default.*

To set the color filters for capturing a color (RGB) image, follow these steps:

1. Ensure that the Pixel Bit Depth is set to either 24 (color) or 36 (color).

2. Click on either the Auto-Exposure or the User-Defined Exposure option.

3. Deselect one or two of the color option boxes, or leave all three checked.
    - Red
    - Green
    - Blue

    To save the image setup, at least one color must be selected.

> *RT Slider – If capturing an unfiltered image, it does not matter which color you select.*

To recall the exposure time and gain from the previously captured image, follow these steps:

1. From the Camera menu, select the Recall Previous Exposure option. The Exposure window displays the exposure time and gain used for the previously captured image

2. Select the setup that you want to save the exposure settings to, and click on OK. The settings are saved to the image setup that you specify.

> *Note: When you save the previously used exposure value to an image setup, it automatically changes the setup from Auto – Exposure to User Defined.*

Binning (B/W, 3-Shot, Slider)

Binning is a pre-exposure option that tells the camera to combine adjacent pixels on the CCD chip. The camera treats each set of combined pixels as one large pixel. The large pixels accumulate electrons faster and are therefore more sensitive to light. Binning has three effects on image processing:

- Light sensitivity is increased.
- Download time is decreased.
- Resolution is decreased.

Binning is particularly helpful when you want to drastically reduce exposure and download times for dim images and you don't mind a decreased resolution. This gives you higher frame rates and less noisy images. The SPOT camera offers four binning options, as illustrated below.

Binning Options	Combined Pixels on the CCD Chip
None	
2 x 2 **(4 pixels = 1)**	
3 x 3 **(9 pixels = 1)**	
4 x4 **(16 pixels =1)**	

Figure 5- 1: Binning Options

Color Order (3-Shot; 24 and 36 bit RGB)

In addition to allowing you to specify specific exposure colors, the SPOT camera allows you to specify the order in which the colors are exposed. This feature is particularly useful for triple stained fluorescence samples, where one color dye might fade before the others. For example, if a green dye fades first, you can set green to be the first exposure, thereby catching the green parts of the sample before they fade. To set the Color Order for an image capture, follow these steps:

1. Select the color or colors that you want to expose for the picture.
2. Click on the Color Order scroll bar.
3. Select the Color Order that you want to use.

Note: The default Color Order setting for a full color image is RGB.

External Shutter Lag

When using an external shutter - to shutter a fluorescence illuminator, for example - there is a delay between the application of voltage and the shutter opening to 100 %, as is the case with any mechanical shutter (see the graph at right). This delay between the application of voltage and the full opening of the shutter is referred to as the *external shutter lag*. SPOT RT cameras feature electronic shutters that open almost instantaneously. When using an external shutter with a SPOT RT camera, then, the SPOT shutter needs to be delayed until the mechanical shutter is fully open. To avoid triggering the electronic shutter before the external shutter has had a chance to open fully, check the **External Shutter Lag** box, and enter the external shutter lag time value in milliseconds. To determine the correct value, refer to the specifications guide that accompanies the external shutter.
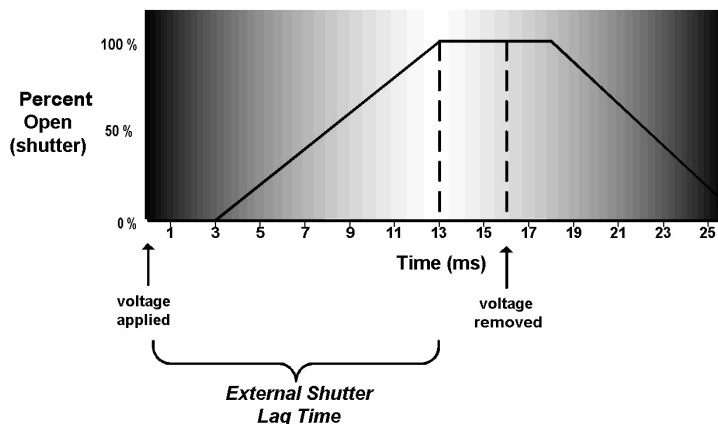
Figure 5- 2: External Shutter Lag Time

Auto-Exposure Window

The Auto-exposure window indicates and allows the user to modify further settings that will be used when auto-exposure imaging is selected in the Exposure window.
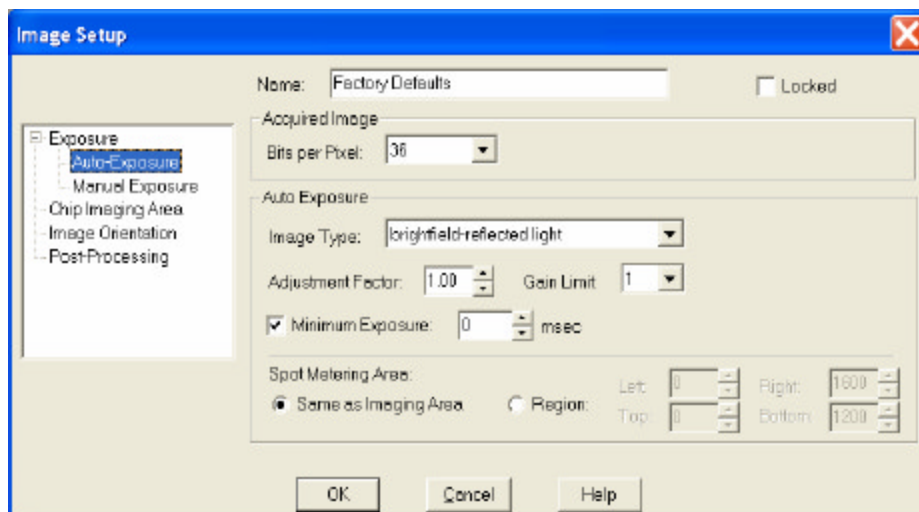
Image Type

When using Auto-Exposure, the SPOT software provides 11 image type settings that correspond to various types of microscopy. The image type that you enter when creating a new setup appears on the Image Setup window. You can enter a new Image Type from the Image Setup window. Refer to the Accessing the Image Setup Window section of this chapter for descriptions of the different image types.

Adjustment Factor

Use the Adjustment Factor option to modify the automated exposure settings determined by the SPOT camera. The Adjustment Factor defaults to a setting of 1 (i.e., 1x the exposure times determined by the Auto-Exposure setting). The Adjustment Factor is used to deliberately over-expose or under-expose an image.

- If an image that you captured (using the default Auto-Exposure settings) comes out too bright or too dark, you can re-capture the image using a different Adjustment Factor to compensate for the excess brightness or darkness.

- If you use the SPOT camera to take pictures of printed circuit boards or other metallic objects, the Adjustment Factor allows you to over-expose the image in order to burn out the glints that appear when capturing images of metallic objects. Over-exposing the glints is necessary to correctly expose the non-glinted areas.

To set the adjustment factor, enter a number between .25 and 32.

- Values **greater than 1** *overexpose* the image.
- Values **less than 1** *underexpose* the image.

.

CAUTION!

To avoid blooming problems in cases where you have entered an Adjustment Factor of 2 or higher, ensure that the Gain Limit is set to 16.

Gain Limit

Normally an auto exposure routine in a digital camera sets the exposure time so that the brightest pixel on the CCD chip is completely "filled" with electrons, or is "full well". This simple technique works well for bright images, but as an image gets dimmer, exposure times can get uncomfortably long. With the SPOT camera you can shorten exposure times by filling the brightest pixel on the CCD to less than full well, and then using this lower level as *full scale*. The inverse fraction of full well that the brightest pixel on the CCD is filled to is known as the *gain*. For example, filling a pixel to half of full well cuts the exposure time in half. Since the exposure time is cut in half, we call this a gain of 2.

The **Gain Limit** feature allows you to set an upper limit on the gain options that will be available during the Auto-Exposure process.

🖉 *Note: If you select 12 or 36 bpp as the pixel bit depth, Gain values/Gain Limits of 8 and 16 are not available.*

For example, with a Gain Limit of 16 the camera can "choose" from the full range of gain options. However, if you find that gains of 8 or 16 are too noisy for your images, you can prevent the camera from selecting these options by setting the Gain Limit to four. When the Gain Limit option is set to four, the camera is limited to the lower three gain options: 1, 2, or 4. The following table illustrates the five levels of gain, the fraction of full well that the brightest pixel is filled to for each level, and a sample exposure time for each level:

Gain	Fraction of Full Well	Exposure Time (sample)
1	1	80 seconds
2	½	40 seconds
4	¼	20 seconds
8	1/8	10 seconds
16	1/16	5 seconds

Table 5- 1: Gain Settings

Note: If you select 36 bpp as the pixel bit depth, Gain values/Gain Limits of 16 are not available.

Higher gain levels for dim images significantly reduce exposure time. For each doubling of gain number, exposure time is halved. Thus, an image with an exposure time of 5 minutes at full scale (a gain of 1) would take only 1.25 minutes at a gain of 4. Unfortunately, background noise increases proportionally to the gain. Thus, the shortened exposure time would result in an image with four times the amount of background noise.

The following table illustrates the relationship between gain, exposure time, and background noise:

Gain Limit	Possible Auto – Gain Settings	Exposure Time (dim images)	Background Noise (dim images)
1	1	Longest	Lowest
2	1, 2	↑	↓
4	1, 2, 4	↑	↓
8	1, 2, 4, 8	↑	↓
16	1, 2, 4, 8, 16	Shortest	Highest

Table 5- 2: Relationship Between Gain, Exposure Time and Background Noise

To set the Gain Limit, follow these steps:

1. Ensure that the camera is set to use the Auto-Exposure option.
2. Click on the Gain Limit select list, and select the number that you want to limit the gain to.

For more information on the concept of gain, refer to Appendix A: Electronic Imaging Theory and the SPOT RT Camera.

Minimum Exposure

Although the auto-exposure feature will select the best-calculated exposure time for you, you may find it preferable to set a minimum exposure time to allow certain areas of the image to be brought out. In those cases, the user can select the Minimum Exposure checkbox in the Auto-exposure window. The value box next to the checkbox must then be filled in with a value in the range from 0 to 1000 milliseconds.

Because SPOT RT cameras are capable of extremely short exposure times, light sources with intensities that vary with the waveform of the 60 Hz line supply (e.g., fluorescent lamps 120 volt incandescent bulbs, and 6 volt 20 watt halogen lamps) can cause the Live Image window to flicker and result in incorrect brightness and color balance readings in captured images. The Minimum Exposure option allows you to correct for these problems by limiting the camera to a minimumexposure time that is greater than the pulse interval of the light source. Consider the illustration to the right. In this case, the

Figure 5- 3: Setting a Minimum Exposure to Accommodate Varied Light Sources (Advanced Mode)

> ✎ *Note: For countries with 50 Mhz power line frequency, a minimum exposure of 15 ms is recommended.*

pulse interval of the fluorescent light source is 8.3 ms. Entering a Minimum Value of 10 ms ensures that the camera's exposure time is greater than the interval between the pulses of the light source. To enter a minimum exposure, ensure that you have selected the **Auto-Exposure** option, check the Minimum Exposure box, and enter a value in milliseconds.

White Balance (RT Color mode)

The exposure window also indicates the white balance settings currently associated with the open image set-up. These values from Red, Green and Blue can be modified by using the increase/decrease arrow keys next to the value or by directly typing in the new value.

White balance is the ratio of red, green, and blue necessary to achieve the proper color rendition for an image. Many factors impact the coloration of an image capture, including lamp voltage, the coloration of the glass used in the slide, and the coloration of the glass used in the lenses of the microscope's objectives. To minimize the impact of these factors, use the Compute White Balance feature. When you perform a white balance calculation, SPOT samples the color of the light from your light source after it has passed through the slide and the objective, and then calculates the exposure values needed to produce images where the white areas are white and the color areas are true.

You should perform a new white balance calculation when:

- Starting a new image capture session.
- Changing the lamp voltage.
- Switching to a different objective on the microscope.

When you open an image setup, the Image Setup screen displays the white balance values that were last saved to the Image Setup. If you capture an image without computing a new white balance, SPOT uses these default values.

To compute new white balance values for an image setup, follow these steps:

1. Set the illumination and the objective that you will use for the image capture.

2. Show the camera a sample of white light. This step varies according to microscopy technique and sample type, as shown in the table below:

Image Type	White Balance Technique
Brightfield-transmitted light	Position the slide so that the specimen is not in the field of view, but light is going through the slide near the specimen.
Darkfield-transmitted light	Perform the white balance using the brightfield mode on your microscope. See Brightfield – transmitted light above.
Fluorescence	Use the Match Color feature. Refer to Ch. 8 – Viewing, Editing, and Annotating Images for more information.
Nomarski DIC/Hoffman	**For Nomarski DIC:** Adjust the Nomarski prism until the image is in either the "brightfield" or the "gray scale" view. **For Hoffman:** Perform the white balance directly on the Hoffman image.
Phase contrast	Position the slide so that the specimen is not in the field of view, but light is going through the slide near the specimen.
Polarized light	Pull all polarizers and compensators out of the light path and then position the slide so that the specimen is not in the field of view, but light is going through the slide near the specimen.
Brightfield-reflected light	Replace your sample with a mirror.
Darkfield-reflected light	Perform the white balance using the brightfield mode on your microscope. See Brightfield – reflected light above.
Stereo/Macro	Using an EPI-illuminator (shining a light on the top of your specimen from off to one side), replace your sample with a white sheet of paper.

3. From the Camera menu, select the Compute White Balance Values option, or use the toolbar and click on the Compute White Balance Values button. The Compute White Balance window appears.

4. Click on Begin to start the calculation. The message **Computing white balance values** displays, as the camera samples the light. When the camera finishes calculating the white balance values, SPOT displays the computed white balance values.

> 🖉 *Note: Due to the inherently poor blue light sensitivity of CCD chips and the energy distribution of halogen bulbs, the blue value is often much higher than the red and green values.*

5. To change the setup that the new white balance values are saved to, select a setup from the drop - down box.

6. Click on OK. The program returns to the main editing and viewing window.

7. To edit the white balance values that you saved, open the image setup (i.e., Brightfield from the previous example). The values should appear in the Red, Green, and Blue selection bars. Edit the values as needed.
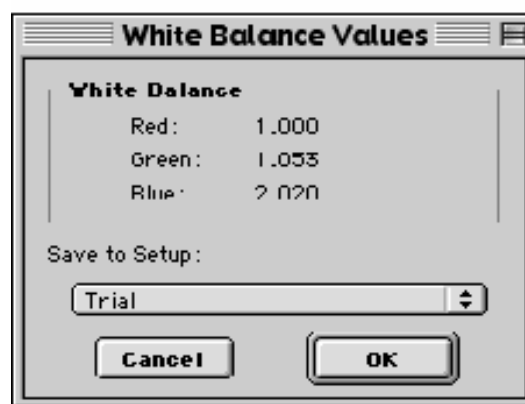
Spot Metering Area

Similar to the way photographers spot meter their cameras on a particular location within their subject image plane, the SPOT user can do the same within the microscope's imaging plane. The spot metering

feature allows the user to best select an area of the image to be used as the region of interest for the automatic exposure level determined by the software.

Users may select any rectangular image area less than or equal to the full image area. This can be accomplished in one of two ways. The user may drag an area of interest on the captured window screen. This automatically will insert the four side pixel values in the spot metering area of the image setup window. Alternatively, the user can input the four side values directly.

For a further discussion of the step by step details of these procedures, please refer to Chapter 7.

Manual Exposure Window

When you select Manual Exposure, the following Window options are available.

Exposure Time

There are three options for entering user-defined exposure times. With the SPOT camera, you can:

- Manually enter the value.
- Allow the camera to calculate the value.
- Recall the exposure time and the gain used for the previous image capture.

When you select the User-Defined option on the Image Setup, a default value appears for the Exposure Time.
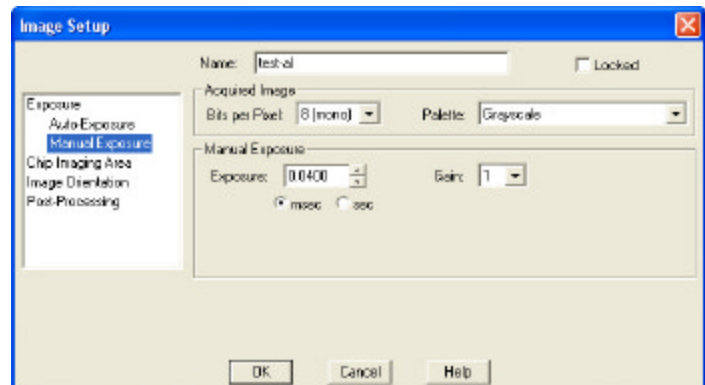
To manually enter new exposure value(s) follow these steps:

1. Ensure that the User-Defined Exposure box is selected.

2. Select either the **msec** (milliseconds) option or the **sec** (seconds) button.

3. Either use the selection arrows or type in a value (in seconds) for the exposure time.

    – The minimum exposure time is **1 millisecond (.001 seconds)**.
    – The maximum exposure time is **536,000 milliseconds (536 seconds)**.

4. Click on OK to save the settings.

To have the camera calculate the exposure time, follow these steps:

1. Close the Image Setup window.

2. Close the Image Setups dialog box.

3. From the Camera menu, select Compute Exposure.

4. The Exposure Settings for the current image setup appear. If you decide to change the settings, you can do so from the Compute Exposure window. Any changes that you make will be saved to the current setup.

5. Click on Begin. The Status message changes to **Computing exposure…** as the camera samples the light. When the camera finishes, the Exposure window displays the calculated exposure time and gain.

> ✎ *Note: For information on entering Image Type, Gain Limit, Adjustment Factor, and White Balance, refer to the appropriate section in this chapter.*

6. Select an image setup from the Save to Setup scroll bar. The current image setup is the default.

7. Click on OK. When you open the Image Setup window, the new exposure value(s) appear.

> *Note: When you save the calculated exposure value to an image setup, it automatically changes the setup from Auto – Exposure to User Defined.*

Gain

When you define your own Exposure, you can enter the precise gain that the camera will use for an image capture. You can enter a specific gain in one of three ways:

- You can manually enter the gain by selecting one of the five gain options from the selection list.

- You can let the camera compute the best gain (and exposure times) by using the Compute Exposure feature (see the previous section).

- You can use the Recall Previous Exposure feature to recall the gain from the previous image capture (see the previous section).

To manually enter a gain, follow these steps:

1. Ensure that the User-Defined Exposure option is checked.

2. Select one of the five gain options from the list. If the pixel bit depth is set to 12 bpp or 36 bpp, the gain value of 16 is unavailable.

To use the gain that the camera selects, follow the steps (in the previous section) listed for computing exposure times. The Exposure window, in addition to the optimal exposure times, displays an optimal gain. This gain is automatically added to the image setup that you specify in the Save to Setup box.

> *Note: The Compute Exposure window displays the Gain limit defined for the current image setup. This limits the gain options that the camera can choose from. If you do not want to use the Gain Limit shown, it can be changed from the Compute Exposure window.*

Chip Imaging Area

SPOT allows you to use any rectangular portion of the CCD chip for image capture. Using a portion of the chip is helpful in cases where you want to:

- Keep file sizes small.
- Shorten download times.
- Avoid areas of the image with optical problems (e.g., vignette areas).

There are three image area options:

- **Full Chip** – Capture the entire area of the CCD chip.

- **Center** – Capture a rectangular part of the CCD chip's area that has the same height - to - width ratio as the full chip, and is centered on the center of the full chip. The percentage that you enter is the percentage of full chip width that the new area's width will be.

  To capture a centered proportion of an image, follow these steps:
  a) On the Image Setup window, check the Center option.

  b) Enter the percentage of the image that you want to capture. The minimum percentage is 10 and the ma ximum is 100.

  c) Click on OK to save the image setup.

- **Region** – Capture a specific part of the CCD chip.

  If you have pre-determined the exact pixel values of the area that you want capture, follow these steps:
  a) On the Image Setup window, check Region.

  b) Enter the pixel values for the Left and Right columns and the Top and Bottom rows that form the boundaries of the region that you want to define.

  c) Click on OK. The camera captures the region that you selected.

  If you do not know the exact pixel values of the area that you want to capture, do the following:
  a) Click on the Full Chip option.
  b) From the Camera menu, select the Get Image (exposure from setup) option. The image capture appears on the screen.
  c) Position the cursor at the upper left edge of the image region that you want to define.
  d) Press and hold the left mouse button, and drag the cursor across the screen. SPOT highlights the area that you selected with a dotted border.
  e) From the Camera menu, select the Set Chip Imaging Region option. The Chip Imaging Region window displays the pixel values for the region that you selected.
  f) Either click OK to save the region values to the current image setup, or select a different image setup.

Image Orientation

Rotate

The Rotate option allows you to define an image capture's on-screen orientation. There are two options:

- **Right** – The image is rotated clockwise 90 degrees.
- **Left** – The image is rotated counterclockwise 90 degrees.
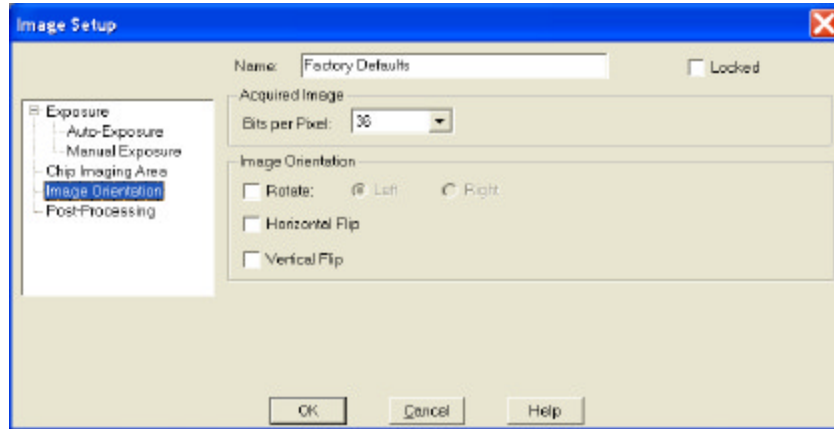
Horizontal Flip

To flip an image display from left to right, check the Horizontal Flip box..

> ✎ *Note: Horizontal Flip is also available as an Edit menu option – Flip Horizontal.*

Vertical Flip

To flip an image from top to bottom, check the Vertical Flip box.

> ✎ *Note: Vertical Flip is also available as an Edit menu option – Flip Vertical.*

Post-Processing

The final aspect of defining an image setup is to determine the post-processing adjustments (corrections) that you want the SPOT camera/software to make. Each option is described in the sections that follow. Some options are available only for the RT Color camera or only for the RT B/W (monochrome) camera - this is indicated parenthetically next to the heading.
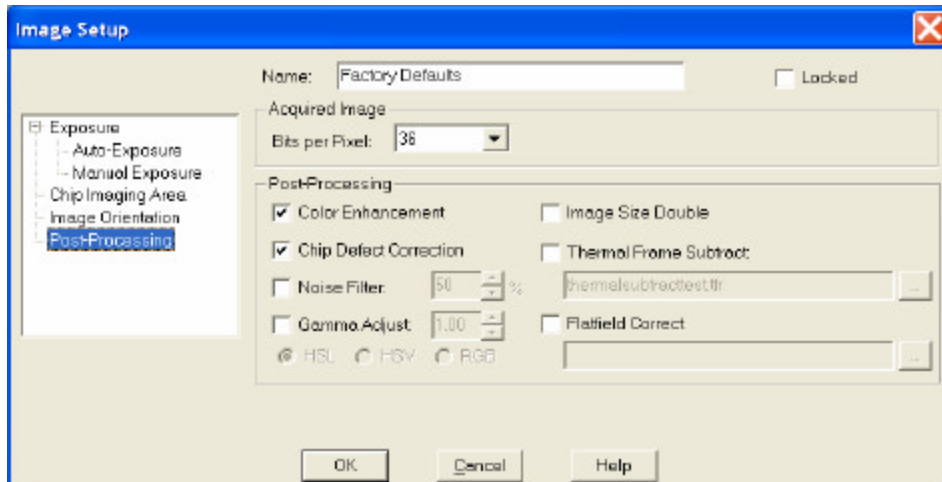
Black Level Subtract (12 BPP B/W)

The amplifier in the SPOT camera has been adjusted so that the average black (no light) pixel value is between 20 and 25, rather than zero for 12 bit (4,095 full scale) downloads. This offset is called the **black level** (sometimes referred to as the "pedestal"). Before each picture, the camera samples the black level by taking a picture with the shutter closed. The software then downloads the pixel values in the first row of the CCD chip, averages them, and stores this value as the black level. When capturing 8-bit monochrome, 24-bit RGB and 36-bit RGB images, the software automatically subtracts the black level from all pixel values so that black pixels are 0 (black) rather than 20-25 (dark gray), and colors are represented accurately.

When capturing 12-bit monochrome images, you can turn off the automatic Black Level Subtract. This is a useful option in cases where you need high accuracy brightness measurements. For example, you may want to adjust the black level of an image on a pixel-by-pixel basis by subtracting a second picture, taken with no light (a "dark frame"), from the original image. When high accuracy measurements are needed, the automatic Black Level Subtract is a problematic because the Black Level is an *average* of a sampling of black pixel values. Some values are higher, and some values are lower than the average. Subtracting this average value from a black pixel value that is lower than average would normally result in a negative value. But, since there are no negative values, the result is arbitrarily set to zero and the actual value is lost. This diminishes the accuracy of measurements.

There are two situations in the SPOT software where the automatic Black Level Subtract is *always* turned off:

- When you are acquiring background images (the Get Background Image menu option) and 12 bit is selected as the Pixel Bit Depth on the Image Setup window.

- When you are capturing 12 bit mono images and the Background Subtract option is selected on the Image Setup window.

When you define a new 12 bit monochrome image setup, the Black Level Subtract box is, as a default, checked. To turn off the program's automatic Black Level subtraction, deselect the Black Level Subtract box.

Color Enhancement (Color; 3-Shot)

The Color Enhancement option optimizes image captures by closely matching the final capture to the image as seen through the microscope. To optimize the final image, the SPOT program processes the raw image data to produce a final image that matches the view seen through the microscope.

> ✎ *Note: Color Enhancement adjustments are not reflected in the live image view –they appear only in captured images. Refer to Ch. 6 – Using Live Images for more details.*

Chip Defect Correction

When you capture an image, the SPOT program automatically compensates for CCD chip defects, so that they do not appear on your images. The Chip Defect Correction option allows you to turn off this automatic correction. This can be useful in cases when you are performing quantitative measurements of pixel brightness values and need to see precisely where chip defects are.

The Chip Defect Correction box is, as a default, checked when you define a new image setup. To turn off the program's automatic chip correction, deselect the Chip Defect Correction box.

Noise Filter

The Noise Filter corrects for the effects of electrical or thermal noise. The Noise Filter checks each pixel on the CCD chip as described below:

1. The program compares the value of each red pixel with the values of the eight neighboring red pixels.

2. If none of the eight neighboring red pixels has a value within ± X % (X being the selectable threshold value that the SPOT program uses) of the value for the red pixel being checked, the value for the red pixel being checked is replaced with the average value of the eight neighboring red pixels.

3. The program repeats this procedure for the values of the green and blue pixels being checked.

To use the Noise Filter, follow these steps:

To filter noise, follow these steps:

1. Check the **Noise Filter** box.
2. Enter a value between **10%** and **100%**. The Threshold value is set to a default of the last selected and saved value. Refer to the procedure described above for an explanation of the Threshold value.

3. When you capture an image, the program automatically adjusts the image using the Noise Filter value that you entered.

> ✎ *Note: You can also use the* Filter Noise *Edit menu option to adjust captured images, as described in Ch. 8 – Viewing, Editing, and Annotating Images.*

Gamma Adjust

Gamma Adjust smoothly lightens darker areas of an image without burning out bright areas or lightening black areas. This is especially useful for capturing/editing images with a wide range of brightness values (dynamic range) such as:

- Fluorescence specimens
- Macroscopic objects with glints, such as printed circuitry

When the bright areas of these types of images are correctly exposed, the darker areas can be so dark that they are in effect invisible. Gamma Adjust can remedy this problem. The figures below illustrate the effect of a gamma adjust on a fluorescent image. The image on the left is the original image prior to the gamma adjust. The image on the right has been gamma adjusted to a value of 1.75. In this instance, the gamma adjustment results in a better display of detail by lightening the darker areas without burning out bright areas or lightening black areas:
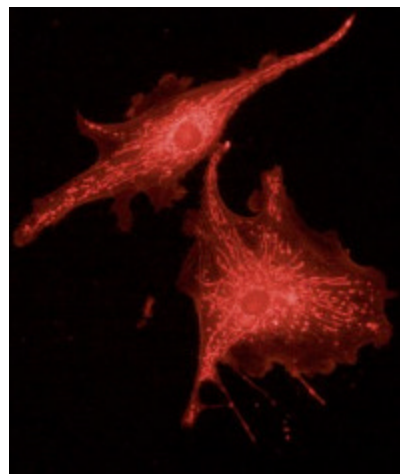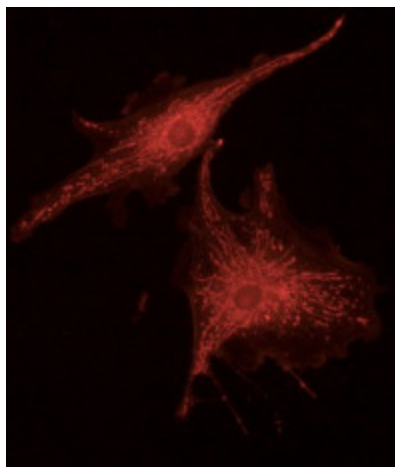
Fluorescence image, no gamma adjustment   *Fluorescence image, gamma adjusted to 1.75*

Figure 5- 4: Effects of Gamma Adjustment on a Fluorescence Image

Gamma adjustment corrects an image by creating a new version of the original. To create the new image, the Gamma Adjust function reassigns the RGB values of each pixe l in the image according to the curve in the graph at right. This graph demonstrates the basic principles of gamma adjustment:

- Black (pixel value = 0) remains black at all gamma values.

- White (pixel value = full scale) remains white at all gamma values.

- Ga mma values greater than one lift the darker areas of the original image into the brighter areas of the new image.

- A gamma curve is smooth - there are no unexpected jumps or cutoffs. This means that when viewing a gamma -adjusted image, you will be able to see the details (intensity differences) in both the black and white areas of the image.
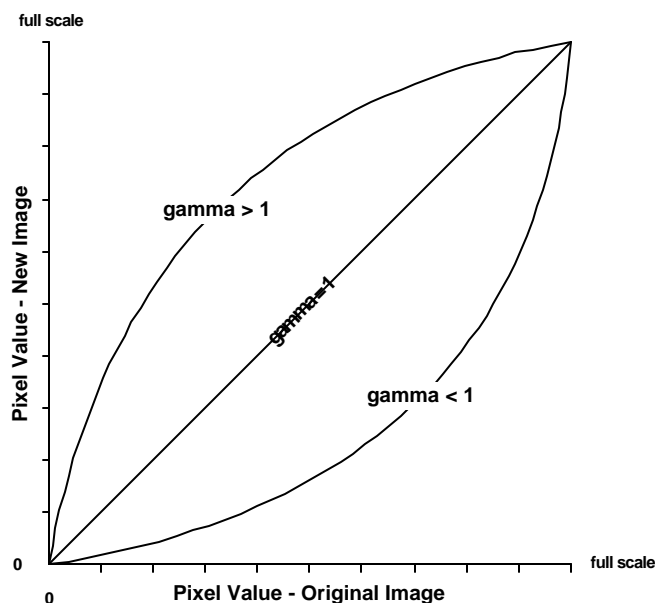
On the Image Setup window, you can specify whether or not to automatically apply gamma adjustment to an image capture. This process works well if you know in advance

Figure 5- 5: Gamma Curve

which gamma value works well for a specific class of images. If you are not certain of the best gamma value, you can acquire non-gamma adjusted images, and then use the HSL, HSV, or RGB gamma adjust options on the Edit menu and experiment with the gamma adjust options to find an optimal value.   Refer to Chapter 8 - Viewing, Editing, and Annotating Images for details on using the gamma adjust options.

> ✎ *Note: Gamma adjustments in live imaging are controlled exclusively via the Live Image Controls dialog. If you are using the* **Snap** *option to capture an image via the Live Image window, the Gamma Adjust setting that you enter on the Image Setup window has no effect on the final image capture.*

To automatically gamma adjust an image after it is captured, follow these steps:

1. Check the Gamma Adjust box on the Image Setup window.

2. Enter a gamma value between .1 and 4.0. Although there is no formula for determining a gamma value, gamma values are typically between 1.3 and 1.7

> ✎ *Note: Although the gamma adjust option is typically used to lighten the mid-tones of an image, entering a value between .1 and 1 will darken the mid-tones.*

3. Select one of the following options: **HSL**, **HSV**, or **RGB**.

> **RT B/W**   *If you are using an RT B/W camera, the color space options are not available. Gamma adjustment is automatically set to use RGB, the recommended color space for monochrome image editing.*

HSL (Hue Saturation Luminance), HSV (Hue Saturation Value), and RGB (Red Green Blue) are three different *color spaces*. A color space is any coordinate system that is used to mathematically represent color.

- The **HSL color space** is an intuitive model based on the three attributes that are common to all colors: hue, saturation, and luminance. The HSL color space assigns a value for each color attribute (e.g., hue, saturation, and luminance). In this color space the brightest saturated color is considered to be 50 % luminance. Above 50 % luminance, saturated colors transition through pastel shades before reaching pure white at 100 % luminance. This process mimics the natural perception of objects illuminated by broadband light sources.

- The **HSV color space** is similar to the HSL color space except that it uses value instead of luminance. In this color space, the brightest saturated color is considered to be 100 % value. This allows you to modify images that contain only saturated colors (e.g., fluorescence) without changing the bright, saturated areas to pastel or white.

- The **RGB color space** assigns a red, green, and blue value to each pixel in an image. This is the method that color computer monitors use to display images.

The process by which the program applies gamma to an image capture differs according to the color space that you select. The following table briefly describes how gamma is applied in each of the three color spaces and lists situations where the use of one color space would be preferable to another.

Color Space	Gamma Adjustment Process	Recommended Usage
HSL	1. RGB pixel values are translated to *HSL* (hue, saturation, and luminance) values. 2. Gamma is applied to the luminance. 3. The HSL values are translated back to the RGB pixels.	The HSL option is ideal for all color images except fluorescence images.
HSV	1. RGB pixel values are translated to *HSV* (hue, saturation, and value) values. 2. Gamma is applied to the value. 3. The HSV values are translated back to the RGB pixels.	The HSV option is ideal for fluorescence images.
RGB	Gamma is applied separately to the red, green, and blue pixel values.	The RGB option is ideal for monochrome images.

4. Click on OK to save the Gamma Adjust settings to the Image Setup. When you capture an image using the setup, the program automatically gamma adjusts the image according to the settings that you defined.

Image Size Double

When you select the Image Size Double feature, the camera *interpolates* between pixels to create an image that is twice as large. The program reads the red, green, and blue brightness values for the original image

and interpolates those values to create a new image that has double the number of columns and rows. The red, green, and blue values for the additional pixels are calculated based on the RGB values of the original pixels.

The Image Size Double feature helps to reduce the *pixellation* of fine detail that often results when you use lower magnification objectives. When used with low magnification images, the additional pixels created via the Image Size Double feature help to smooth out details; this results in a higher resolution appearance.

Dark Current, System Offset, and Scientific Imaging

Dark Current is a component of any image captured with a CCD.

What it is:	Dark current is the rate of accumulation of electrons in a pixel due to thermal action. Dark current is related to the temperature of the camera CCD. Dark current is higher at higher CCD temperatures. The level and type of impurities in the silicon of each pixel will affect the dark current for that pixel. Pixels with pure silicon will have lower dark current. Pixels with impurities will have higher dark current.
What it is not:	Dark current is not the result of photons (light) from the target specimen striking the CCD and therefore, does not represent true data related to the target image.

The accumulation of electrons in each pixel due to dark current is repeatable and predictable for any combination of time and CCD temperature.

In scientific imaging, the goal is to capture image data strictly related to the light gathered from the target specimen. Dark current is additional unwanted data (electrons) that can degrade the image data desired. Get Bias Frame and Get Thermal Frame are features that can eliminate the effect of this unwanted dark current, leaving the user with a better representation of the true data associated with the target specimen.

In scientific imaging, pixel values of zero are not desired. Therefore, the camera electronics add an "offset" to all pixel values so that a pixel value that would normally be zero (no light) is offset to a higher positive number. The amount of this offset is referred to as the "system offset".

Dark Frames, Bias Frames and Thermal Frames: Definitions

A dark frame is any image taken with no light hitting the CCD.

A bias frame is a short exposure dark frame. Each pixel in the bias frame has a value equal to the system offset plus any dark current electrons accumulated by the pixel in the time between the end of exposure and when that pixel's charge is measured by the camera's electronics. Since the pixels are read one at a time, the first pixel read would have the least amount of dark current electrons and the last pixel read would have the most.

Subtracting a bias frame from any image will subtract the system offset and the dark current electrons that accumulated in each pixel while it was waiting to be read out.

A thermal frame is a longer exposure dark frame with the bias frame subtracted. All that is left after subtraction of the bias frame is the accumulation of dark current electrons in each pixel that happened during the exposure time of the dark frame.

Subtracting a thermal frame from any image of the same exposure time will remove the dark current electrons that accumulated during the exposure time. Since the accumulation of dark current electrons is directly proportional to exposure time (twice the exposure time results in twice as many dark current electrons) the thermal frame is "scalable". This means that one thermal frame (at a known exposure time) can be scaled up or down to predict the amount of dark current electrons that will accumulate in each pixel of an image of ANY exposure time.

Subtracting both a bias frame and a (scaled) thermal frame should erase the system offset and eliminate all electrons due to dark current and leave an image that has pixel values due only to light striking the CCD. This is a highly useful way to "clean up" an image.

Get Bias Frame (Bias Frame Subtraction)

Get Bias Frame allows you to collect and store a bias frame. Bias frame subtraction is a process that uses the stored bias frame, subtracting it from a captured image to remove both the system offset and the dark current electrons that accumulated in each pixel while it was waiting to be read out. When this subtraction process has been applied to a <u>short exposure image</u>, the remaining data (and image) is mostly representative of the true image data being captured.

You may select a bias frame to use in the subtraction process within the image set-up post-processing window. Select the checkbox for Bias Frame and then browse to select a Bias Frame correction file from those that have been previously developed and stored.

For a step-by-step explanation for acquiring bias frames, refer to Ch. 7 – Taking a Picture.

> *Note: Bias frame subtraction is available only when a monochrome camera is set up in 12 bit capture and black level subtract is not selected.*

Get Thermal Frame (Thermal Frame Subtraction)

Get Thermal Frame is a function that allows you to collect data to eliminate the dark current accumulated during the exposure time of your captured image. By determining the rate of dark current accumulation in each pixel, we can scale the quantitative effect of the dark current for each pixel in the image plane (the Thermal Frame) based on the time of image exposure and subtract it from the final image. The end result is a processed image that more closely reflects the true data that is collected from the specimen during the image capture.

The SPOT program accomplishes this method of thermal frame characterization by collecting one, or an average of a series of images that reflect the electrons accumulated due to the temperature of the CCD. This information is then stored as the Thermal Frame data. For a given experimental condition (i.e. temperature in the room), normally only one set of Thermal Frame images must be collected, with its data later scaled to correspond to the time of the exposure.

> *Note: Collecting thermal frames should be done from time to time depending upon the changing nature of the environment that the camera is in.*

The Thermal Frame correction data is stored as a file. The user must select it in the Image Setups options prior to image captures. Select the checkbox for Thermal Frame and then browse to select a Thermal Frame correction file from those that have been previously developed and stored.

You may select a thermal frame to use in the subtraction process within the image set-up post-processing window. For a detailed description of the steps involved in acquiring a Thermal Frame, refer to Ch. 7 – Taking a Picture.

> **RT B/W**   *If 12 bit monochrome is selected as the Pixel Bit Depth on the Image Setup window, the automatic Black Level Subtract process is always turned off when acquiring background images.*

Get Flatfield Image (Flatfield Correction)

For all other SPOT camera lines, flatfield correction is used to correct for display problems associated with uneven intensity or coloration in illumination, or to correct for art ifacts (e.g., dust) in the optical system. Flatfield can still be used for this purpose with SPOT RT cameras.   But, because SPOT RT cameras use a color mosaic chip, Flatfield correction serves a new primary purpose. In the production process for color mosaic CCD chips, the RGB colors are applied directly to the pixels on the chip. This process often results in an uneven color density around the outer 30-40 rows of the CCD chip, causing a "halo" appearance in some image captures, particularly those with low contrast. The Flatfield option can be used to correct this problem.

> *Note: Flatfield correction with the SPOT camera refers only to the correction of uneven lighting. It should not be confused with the correction of optical field flatness.*

Get Flatfield Image is the first step in the Flatfield Correct process described in Ch. 5 - Preparing to Take a Picture. Flatfield correction works by taking a pic ture of the brightfield background without the specimen in the field of view. This image lets the SPOT software know, on a pixel-by pixel basis, which areas in the field of view are receiving less illumination than the brightest areas. When taking a picture that contains a specimen, the software uses this information to accurately increase the brightness of each pixel in the dimly illuminated areas. The resulting image gives the appearance of an even illumination.

For a detailed description of the steps involved in the flatfield correction process, refer to refer to Ch. 7 – Taking a Picture.

Ch. 6 – Using Live Images

QUICK REFERENCE:

➲ View a real-time preview of your specimen/ image capture… p. 103

➲ Transfer a real-time image preview directly to the editing window… p. 104

➲ Pause the Live Image display… p. 105

➲ Zoom in on a live image… p. 105

➲ Adjust the real-time viewing settings… p. 106

➲ View of real-time preview of a "piece" of an image… p. 108

➲ Adjust the real-time preview for a dim image… p. 109

Using Live Images

The SPOT software allows you to view real-time black and white (RT B&W) or color (RT Color) previews of your specimens – i.e., your potential image captures. Using the Live Image preview, you can scan, zoom, focus, and frame images so that they are optimally adjusted for the image capture. After focusing and positioning the image, you can then capture it using one of five options, generating one of three types of images:

- **Get Image (exposure from Setup)** - Capture the image using the settings from the current Image Setup and ignore the Live Image Controls values. This allows you to use one set of values for framing and focusing an image and a different set of values for capturing the image. Get Image can be accomplished in one of three ways: from the dropdown menu (under Camera), from the toolbar, or by creating and using a Taskbar button (Chapter 12) for Get Image.

- **Snap** - Capture the image using the **area** and **exposure** values from the Live Image Controls dialog (see below). The **Snap** feature is handy because it enables you to adjust the live image until you see what you want, and then use those settings to capture the image – "what you see is what you get." Also because the **Snap** option uses Live Image exposure values, it allows you to avoid the exposure computation that typically precedes image captures.

  Depending on how **Live Image** Preferences (see Ch. 4) are defined, the **Snap** option either uses or disregards the Post Processing options from the current Image Setup (see Ch. 5).

  – *If the **Snapped Images** box is selected*, the Snap option uses all of the Post Processing options from the current Image Setup, except for Background Subtraction and Flatfield Correction.

  – *If the **Snapped Images** box is unselected*, the Snap option ignores the Post Processing options for the current Image Setup, with one exception: if the **Chip Defect Correction** option is selected for

the current image setup, the corrections will appear in the image capture, regardless of the Snapped Images preference.

- **Transfer** - You can **Pause** the live image view and **Transfer** (see below) the preview directly to the SPOT editing window.   This option is helpful in situations with rapidly changing samples. If you see something that you like, you can pause the live image preview before it changes. Then, you can transfer the paused image to a SPOT editing window and save it to file. Images saved in this way have two restrictions. First, all transferred images are 8 bit per channel (color). Second, because live images are read out via two amplifiers, the right and left sides of the image might not be perfectly matched.

  Depending on how **Live Image** Preferences are defined, the **Transfer** option either uses or disregards the Post Processing options from the current Image Setup (see Ch. 5).

  - *If the **Transferred Images** box is selected*, the Transfer option applies the Noise Filter, Color Enhancement, and Image Size Double options as specified for the current Image Setup.

  - *If the **Transferred Images** box is unselected*, the **Transfer** option ignores the Post Processing options for the current Image Setup, with one exception: if the **Chip Defect Correction** option is selected for the current image setup (SPOT and RT cameras only), the corrections will appear in the image capture, regardless of the Transferred Images preference.

Note also the following:

- Image orientation adjustments (i.e., flipping and rotating) specified on the Image Setup are always reflected in the live image view and Snap capture.

- If you crop or zoom in on an image, Snaps and Transfers capture only the cropped or zoomed portion.

✐ *Note: With some video cards, you might experience system crashes or display problems in live image mode. To correct these problems, lower the Graphics Acceleration setting in Windows, as described in the* Video Card Related Problems *section of the* Installation Guidelines *chapter.*

✐ *Note: Occasionally, when viewing a dim live image, you might notice that the right half of the image is brighter or dimmer than the left half. To display images in real time, the RT camera (with the exception of the RT-SE6) uses two separate amplifiers to simultaneously read the right and left halves of the CCD chip. With some images, particularly dimmer samples, the brightness of the two halves may not match. This results in a "split" appearance. To avoid this problem with captured images, the RT camera reads the whole CCD through a single amplifier. A "split" Live Image view is* **not** *indicative of what your image capture will look like.   Refer to Appendix A: Electronic Imaging Theory and SPOT Cameras for details.*

The Live Image View

 To open the Live Image window, either click on the Live Image toolbar button, or select the Live Image option from the Camera menu.

- The **title bar** at the top of the Live Image window displays the status of the live image view - **Running, Computing Exposure**, or **Paused**. Double-click on the title bar to "roll up" the live image display. This pauses the live image display. Double-click again to display the whole window and resume the live image display.

- When the **Fit to Window** option is checked, the live image window **cursor** becomes a **zoom tool**. To zoom in on a portion of a live image, click and drag over the area that you want to zoom in on.   To return to the non-zoomed image, click on the **Reset Area** button on the bottom of the window.

- When the **Fit to Window** option is not checked, the cursor is a cropping tool. If you select a portion of an image, the program displays only the selected area and discards the rest of the image. To return to the non-cropped image, click on the **Reset Area** button on the bottom of the window.

> *Note: Due to a flaw in the Windows operating system, monitors that are set to higher resolutions cannot zoom to very high magnification levels. If zoomed in to a high magnification, the monitor displays white instead of the zoomed image.*

- The **Full Screen** option (accessed via the View menu or [F7]) can be used with live images. This option allows you to use the entire screen to view a live image, without the SPOT menus/toolbar and the Windows navigation bar.
  - To access live image viewing options in the full screen view, right click on the live image.
  - To exit the full screen view, either press [F 7] or move the cursor to the top of the screen and click on the **Exit Full Screen Mode** button that appears.

- There are nine option**s** at the bottom of the Live Image window.

Restart	Resample the brightness of the image, compute new exposure times, and restart the live image display. This is useful for updating exposure times when specimen brightness has changed.
Pause/Resume	Freeze the live image window. This is useful when you are changing specimens and want to preserve the last view. In addition, to use the Transfer option (see below), you must first Pause the live image view. Click on Resume to restart the live image view
Close	Close the Live Image window.
Controls	Open the Live Image Controls dialog box (see below). From this dialog box, you can define the viewing/capture area, and adjust gamma, brightness level, and dim image quality. You c an also elect to display either the full chip or a region of interest. Refer to the steps below for more

details on the Live Image Controls window.

Fit to Window	Size images to automatically fit the resizable display window.
Reset Area	Return to the non-zoomed view of the live image.
Transfer	Transfer the live image directly to the SPOT viewing window, where it can be edited and/or saved to file. The Transfer button is inactive until you **Pause** the real-time view. The Transfer option is limited in that all images are 8 bits per channel (i.e., because they are from live mode). The Transfer option should not be used for image captures that require highly accurate brightness measurements.
Snap	Capture the live image using the settings from the Live Image Controls dialog. When you click on the Snap button, the **Get Image** status dialogs appear, just as they do when you select **Get Image (exposure from setup)** from the Camera menu. When you use the Snap option to capture an image, you avoid the exposure computation sequence that typically precedes image captures.
Faster Readout	Faster Readout enables you to "speed up" the live image display (higher frame rates) in cases where you are willing to sacrifice some resolution for a higher frame rate.

Live Images are Different Than Captured Images

When you view a live image, keep in mind that the captured version of the image will not be identical to the live image view. This is due to two factors:

- Live images are raw pixel data. Captured images are digitally processed to give a more accurate color rendition. For individual image captures, there is enough time to process the data. For each image in the live image stream, there is not enough time to process the data.

- Live Image gamma adjustments are made in the RGB color space. Captured image gamma adjustments are made in the HSL color space (HSV for fluorescence). This is because gamma adjustments can be made quickly in the RGB color space, but are very slow in the HSV and HSL color spaces. Refer to Ch. 5 – Preparing to Take a Picture for more information on gamma adjustment and color spaces.

✎ *Note: HSL and HSV gamma adjustment for captured images applies only to images captured using the* Get Image (exposure from Setup) *option. Gamma adjustment for images captured using the* Snap *live image option is always made in the RGB color space.*

Live Image Controls

To adjust the live image view settings, click on the **Controls** button at the bottom of the Live Image window. The Live Image Controls dialog appears. Depending upon whether your image settings for exposure (Chapter 5) are set to Auto or Manual, one of two windows will appear, each slightly different. Each window, however, will have a common section including the following options.

Filter Color (RT 3-Shot)

Your selection should provide the highest level of contrast for the image. Because different colors provide different levels of contrast, the best way to determine a color is to preview it by clicking on the color and seeing how it affects the view of the specimen.

- **RGB** – View a color preview of your specimen

- **Red** – View a monochrome preview of your specimen using the red state of the LCD filter.
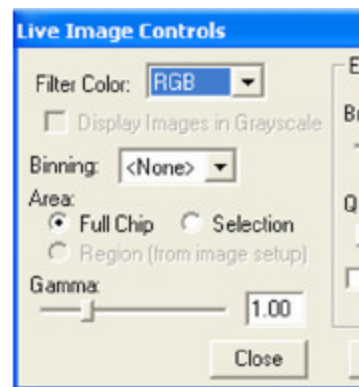
- **Green** – View a monochrome preview of your specimen using the green state of the LCD filter.
- **Blue** - View a monochrome preview of your specimen using the blue state of the LCD filter.
- **Clear** – View a monochrome preview of your specimen using the clear state of the LCD filter. Selecting the Clear option does not provide the entirely unfiltered image that is possible with the RT Slider camera. Although all three colors are transmitted, the polarizing filter, which is part of the liquid crystal filter, and the IR filter remain in the light path, blocking some of the light.   When using the Live Image window to focus or position dim images, RT Slider users can slide the filter out to provide higher frame rates and less noisy images.
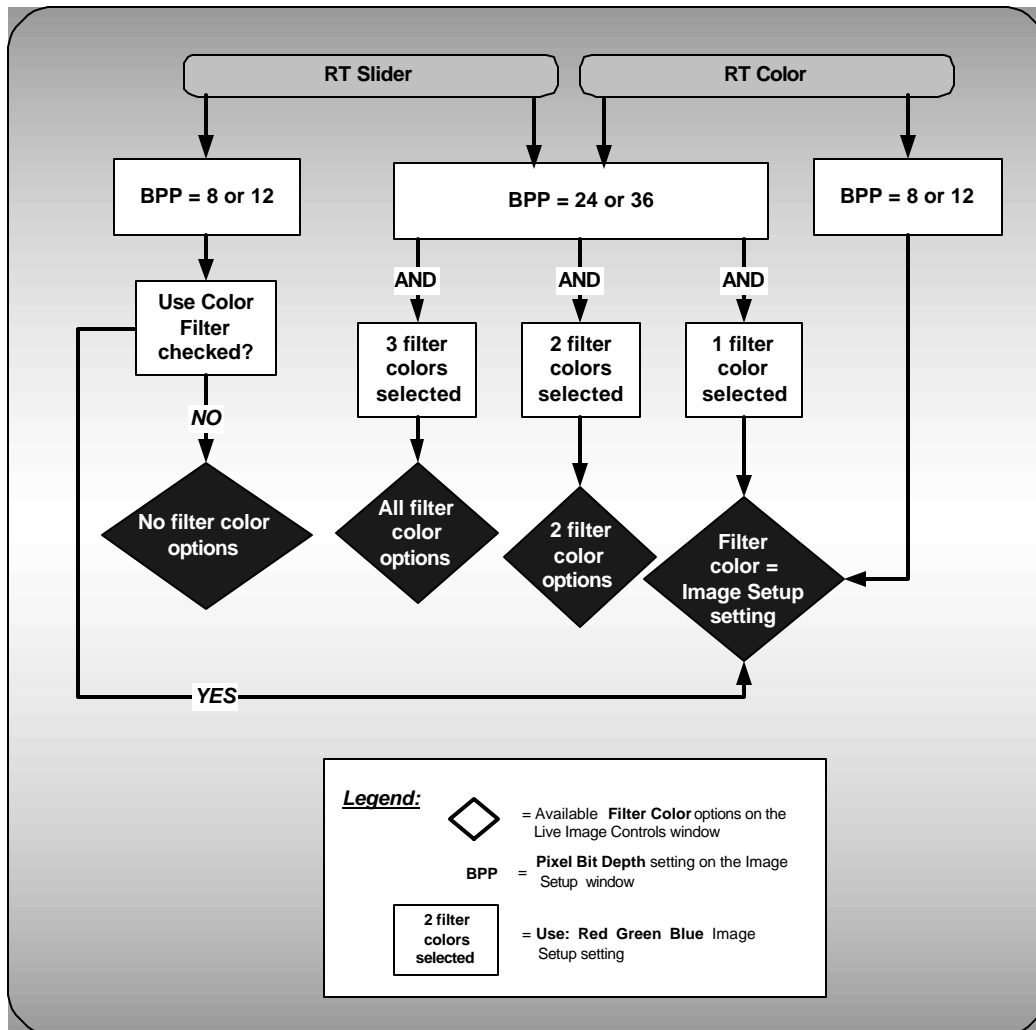
The available Filter Color options on the Live Image Controls dialog are dependent on the settings defined on the Image Setup. For example, if you select Red and Green for the **Use: ☑ Red ☑ Green ☐ Blue** option, the Live Image Controls dialog allows you to select, Red, Green, or both (i.e., by selecting RGB). The flow chart above illustrates the possibilities.

RT Slider	As noted in the above flow chart, Image setups with pixel bit depth settings of 24 bpp or 36 bpp allow you to select RGB and preview live images in full color. However, if you move the filter slider on the camera to the **B/W** position, you should also change the Filter Color setting to one of the monochrome options (e.g., Clear, Red, Green, or Blue). The **Monitor Camera Color Filter Position** preference monitors the color filter position only for image captures – it does not monitor color filter position when viewing live images.

Display Images in Grayscale (RT 3-Shot)

To counter the difficulty of viewing, focusing, and adjusting monochrome images in live mode, the **Display Images in Grayscale** option enables you to view a monochrome image in grayscale, as opposed to with the red, green, or blue filter. This option applies if the following are true:

- The **Bits per Pixel** option on the Image Setup is set to either 8 bpp or 12 bpp
- The **Filter Color** (see above) on the Live Image Controls dialog is set to Red, Green, or Blue.

Binning (RT B/W, RT 3-Shot)

Binning, as described in Ch. 5 - Preparing to Take a Picture, combines adjacent pixels on the CCD chip, thereby reducing the number of pixels used in the image capture. Binning is useful when you have dim images and want to increase sensitivity and reduce download time. This gives you higher frame rates and less noisy images. But, as binning level increases, image resolution decreases. All of these factors should be considered when you select a binning option.

Area

The Live Image Controls window allows you to view any rectangular portion of the CCD chip.

- **Full Chip -** View the entire chip.
- **Selection -** View a selection that you made with the cursor.
- **Region (from Setup) -** View the region (if any) that is defined for the current image setup.

Use the **Reset Area** button at the bottom of the Live Image window to return to the full chip view.

When you are viewing a live image **Selection**, how this selection is displayed, depends on whether the **Open Images in Fit to Window Mode** and **Always Show Full Chip in Live Image** preferences (see the **Preferences** section in Ch. 4) are checked. The following table illustrates the possibilities:

Fit to Window Checked?	Always Show Full Chip in Live Image Checked?	Selection Display	Live or Captured Image
YES	YES	When you select a portion of the chip, the selection is outlined with a **yellow rectangle**.	LIVE
YES	NO	When you select a portion of the chip the cursor is a **zoom tool**. To zoom in on a portion of a live or captured image, click and drag over the area that you want to zoom in on. To return to the non-zoomed image, click on the **Full Chip** option.	BOTH
NO	YES	When you select a portion of the chip, the selection is outlined with a yellow rectangle. However, because **Fit to Window** is not checked, you will not see the entire chip in the window.	LIVE

NO	**NO**	When you select a portion of the image, the cursor is a cropping tool. If you select a portion of an image, the program displays only the selected area and discards the rest of the image. To return to the non-cropped image, click on the **Full Chip** option.	**BOTH**

Gamma

Enter a **Gamma** value between **.1** and **4**.

Gamma adjustment smoothly lightens darker areas of an image without burning out bright areas or lightening black areas. This is especially useful for capturing/editing images with a wide range of brightness values (dynamic range) such as fluorescence specimens or macroscopic objects with glints, (e.g., printed circuitry).

- Values between **1** and **4** lighten the mid-tones of the image.

- Values between **.1** and **1** darken the mid-tones of the image.

Because images update very quickly in Live Image mode, gamma adjustments are only displayed in the RGB color space. Gamma adjustments made in the HSL or HSV color spaces would be too slow for live imaging display. Refer to Ch. 5 – Preparing to Take a Picture for more details on color spaces and gamma adjustment.

Live Image Controls – Auto Exposure Options

When you have selected auto-exposure in your image set-up, the Live Image Controls window will feature the following options.

Brightness

Enter a **Brightness** value between **.25** and **32**.

A brightness value of 1, the default value, gives you the correct image brightness for most images. The Brightness Adjustment control allows you to overexpose or underexpose (darken or lighten) the image area. In many cases, an image has varying levels of brightness; to see a specific area better you might want to lighten or darken the image. When using the Brightness control, the SPOT camera uses an algorithm that combines gain and exposure time to provide the fastest possible image update rate (shutter speed) in live preview mode:

- When overexposing images to make them brighter (i.e., entering values greater than one), the camera first selects the highest possible gain before lengthening the exposure time. Because gain also brightens an image, less exposure time is needed.

- When underexposing images to make them darker (i.e., entering values less then one) the camera uses the lowest possible exposure time before lowering the gain.
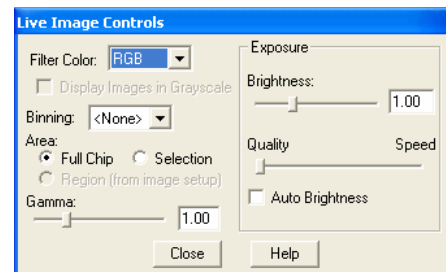
Quality/Speed

Use the slider to balance speed and quality when previewing dim images.
- Moving the slider toward the **Quality** side increases image quality and decreases frame rates.
- Moving the slider toward the **Speed** side increases frame rates and decreases image quality.

The Quality/Speed measurement corresponds to the Gain limit setting (see Ch. 5) on the Image Setup window, which allows you to set an upper limit on the gain options that will be available during the Auto-Exposure process.

- When the slider is at the left-most point, the Gain Limit is 1, which restricts the camera to a gain of 1, giving you higher quality pictures, but lengthier exposure times.

- When the slider is at the right-most point, the Gain Limit is 16, which allows the camera to select from the six gain options available in Live Image Mode. This shortens exposure times, but decreases image quality.

Note that the highest gain options may not be on the Image Setup window.

For more details on setting a Gain Limit, refer to Ch. 5 –Preparing to Take a Picture. For more information on the concept of gain, refer to Appendix A: Electronic Imaging Theory and SPOT Cameras.

Auto-Brightness

Available only when you are using **Auto** exposure, the **Auto-Brightness** option enables you to maintain a consistent brightness level for changing specimens.

Live Image Controls – Manual Exposure Options

When you have selected manual exposure in your image set-up, the Live Image Controls window will feature the following options.

Time

From the Live Image Controls window, you can enter a specific exposure time or times for the image. When you use a specific exposure time, the camera skips the auto-exposure sequence (Computing Exposure) that takes place when you open the Live Image window. This is very useful in cases where exposure times are lengthy, as with fluorescence samples.

To enter new exposure value(s) on the Live Image Controls window, follow these steps:

1. Ensure that the Manual Exposure box for the current image setup is selected.

2. Either use the selection arrows or type in a value in milliseconds for the exposure time.
   - The minimum exposure time is **.04 millisecond**.
   - The maximum exposure time is **536,000 milliseconds (536 seconds)**.
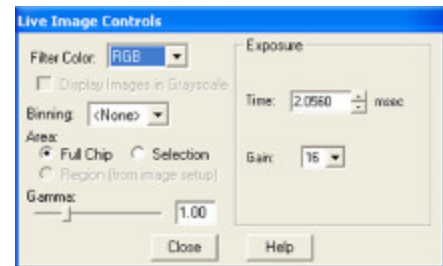
RT 3-Shot users can also use the **Adjust** option to modify the brightness of the image. Click on **Adjust** and enter an Adjustment Factor. The program then multiplies each exposure time by this factor. This option allows you to make an image brighter or darker without changing the color balance. When using the Adjust RGB option, verify that the adjusted Exposure Times fall between 1 and 536,000 milliseconds.

Gain

From the Live Image Controls window, you can enter the precise gain that the camera will use for an image capture. Use the drop-down list to select one of the five gain options: **1**, **2**, **4**, **8**, or **16**. Note that the highest gain options may not be an option on the Image Setup window.

Ch. 7 – Taking a Picture

Introduction: Three Ways to Take a Picture

There are three ways to take a picture with the SPOT camera:

- **Live Image Pause/Transfer**
  After pausing a live image, pressing the Transfer button saves the image to a window. This is useful for capturing rapidly changing samples. Images captured in this way are essentially "frozen" live images. As such, the right and left sides of the images are read out via separate amplifiers and are limited to 8 bit monochrome/24 bit color.

- **Snap**
  Pressing the Snap button when viewing a live image initiates an image capture where the entire image is read out via a single amplifier. Snap image captures avoid the time dedicated to computing exposure by using the exposure time and gain calculated for the live image.

- **Get Image (Toolbar button)**
  Pressing the Get Image toolbar button initiates an image capture where the entire image is read out via a single amplifier using only the parameters from the current image setup. This allows you to use the live image window purely as an aid to framing and focusing the image. You may also access Get Image from the dropdown menu (under Camera), from the toolbar, or by creating and using a Taskbar button (Chapter 12) for Get Image Adjustments made to the live image have no impact on the image capture.

These three types of image captures were designed to give you greater flexibility in working with different types of specimens and in how you use live images.

- For more details on the **Live Image Pause/Transfer** and **Snap** options, refer to **Ch. 6 – Using Live Images**.

- For more details on the **Get Image** option, refer to the **Get Image (exposure from Setup)** section below.

This chapter discusses the Get Image type of capture as used in a variety of different procedures. The chapter is divided into two sections:

- **Basic Image Captures** –Standard and sequential image captures.

- **Image Captures in Other Procedures –** Image captures where one capture is used to prepare for another capture.

Basic Image Captures

The SPOT software offers three primary image capture options:

- Get Image (exposure from setup)
- Get Image (last used exposure)
- Get Sequential Images

RT Slider	When the liquid crystal color filter has been moved out of the light path, RT Slider users may notice a diffuse bright area in the center of the image on non-fluorescence image captures. This is due to infrared light that is transmitted when the filter is out of the light path. Our infrared filter is bonded to the liquid crystal color filter. When the color filter is moved out of the light path, the infrared filter is also moved. To correct this problem, use an external infrared filter

Get Image (exposure from setup)

Get Image (exposure from setup) captures an image according to the specifications in the Current image setup. Press **[F12]** to view the current image setup or select it from the dropdown menu under Setup.

To capture an image using the current image setup, follow these steps:

1. Determine or define the image setup that you want to use, and set it as the Current setting (i.e., select the Current option from the Image Setups dialog box).

2. From the Camera menu, select the Get Image (exposure from setup option), or click on the Get Image (exposure from setup) button on the toolbar:

The Get Image window, which indicates capture status and gain, appears. The Status messages are as follows:

a) **Computing Exposure** – The camera is sampling brightness levels in the image in order to determine proper gain and exposure time.

b) **Getting Image** – The camera is downloading the image to the computer.

RT 3-Shot, Slider	RT 3-Shot and RT Slider Users (color mode) will see three messages – Getting Red Image, Getting Green Image, Getting Blue Image as the camera downs the separate color images to the computer.

c) **Exposing** – A countdown clock displays when the exposure will last longer than 2 seconds. This informs the user that the electronic shutter is open and counts down the minutes:seconds remaining until it closes.

d) **Processing** – The camera is preparing to display the image.

The newly captured image appears on screen. Following an image capture, several noticeable changes take place in the appearance of the main SPOT screen:

- The Edit and Windows menus become available.

- The Toolbar expands to offer more buttons and the zoom, pan window, save, and print related toolbar buttons become functional (available).

- The Get Image (last used exposure), Set Chip Image Area, Set Spot Metering Area, and Recall Previous Exposures options on the Camera menu are functional (available).

Get Image (last used exposure)

 The Get Image (last used exposure) option captures an image using the exposure times and gain from the previous exposure. In all other respects, it works in exactly the same way as the Get Image (exposure from setup) option. This option shortens the time required to capture an image because it skips the initial auto-exposure sequence. This is convenient when you are satisfied with the appearance of an image and want to use the same settings to capture a similar image in the same session. If you close the software, and re-open it, the Get Image (last used exposure) option is unavailable until you capture an image using the Get Image (exposure from setup) option.

To use the Get Image (last used exposure) option, follow these steps:

1. Prepare the microscope and specimen for the next image capture.

2. Either select Get Image (last used exposure) from the Camera menu,   or click on the
    toolbar button.

3. The Get Image status window appears, and the camera captures the image using the last used exposure
    times. It only displays the exposure time and the gain because the camera no longer needs to sample
    image brightness.

> *Note: This feature will retain the image settings from the previous capture even if you delete the*
> *previously captured image from the screen.*

Get Sequential Images

The Get Sequential Images option allows you to capture a sequence of images. SPOT's sequential image
capture process enables you to save an image sequence in two different ways:

*   You can save a sequence as a group of individual image files.
*   You can save a sequence as a single (TIF format), "sequence image file" that is composed of multiple
    images. Refer to Ch. 8 – Viewing, Editing, and Annotating Images for details on viewing and
    modifying image sequence files.

The available file name and save options on the Get Sequential Images window vary according to whether
you save the sequential images individually or as a sequence, as shown here:
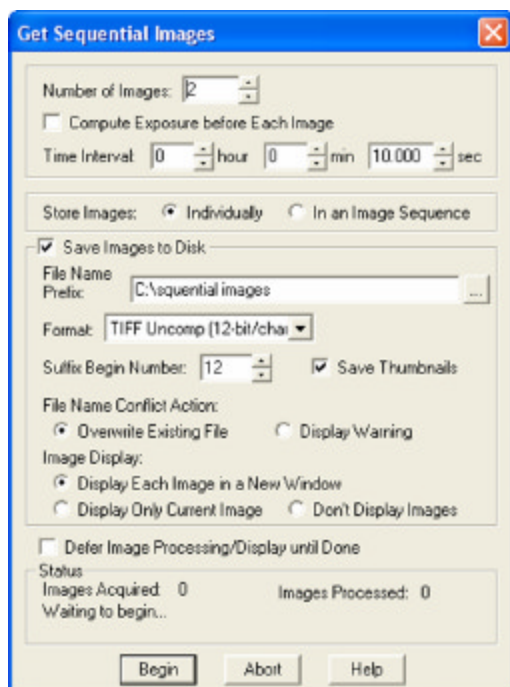
Save and name options – individual files   **Save and name options – image sequence**
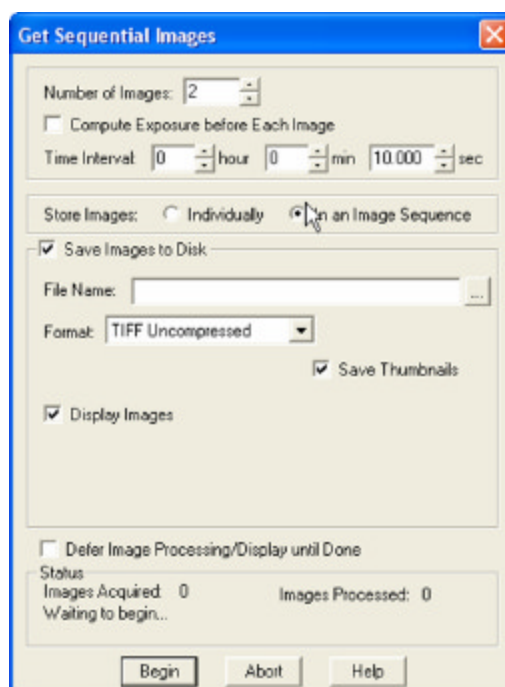
To use the Get Sequential Images option, follow these steps:

1. Select the image setup that you want to use for the sequential image captures. Either define a new image setup, or select an existing setup from the drop down list on the status bar (bottom right corner).

2. From the Camera menu, select the Get Sequential Images option. The Get Sequential Images window appears. The table on the following page explains each option.

Number/Timing Options	
Number of Images	Enter the number of images in the sequence of captures. This number can be set up to **2,000**.
Time Interval	Enter the time interval between the start of one image capture to the start of the next image capture in hours, minutes, and seconds.

Storage Options	
Store Images	Select either **Individually** or In **an Image Sequence**

- Individually – Store each image as a separate file.

- In an Image Sequence – Store all images in a single TIF format file.

The available save and name options depend on your selection here.

Save and Name Options	
Save Images to Disk	The **Save Images to Disk** box is checked as a default for the Get Sequential Images window.

- If you do not want to save the sequential images/image sequence to file, deselect the box. Deselecting the box makes the remaining save and display options unavailable. You can define the process/display option (**Don't Process or Display Images until Done**), and then click on **Begin** to start the sequence.

- If you want to save the sequential images/image sequence, leave the box selected, and enter the remaining file save and display options, as described below.

The file name of each image (individual images only) in the sequence is made up of two components:

- A **File Name Prefix** that you designate.

- An automatically indexed Suffix Number that starts with the **Suffix Begin Number** that you designate.

File Name Prefix (individual images) File Name (image sequences)	This prefix appears before the number of each image capture in the sequence. To enter the File Name Prefix/File Name, you can:

- Type only the name (e.g., **Growth Sequence**) in the box. The program automatically saves the image sequence to the last used directory. If you specify the prefix only, you cannot see exactly where the files are being saved. Thus, to avoid having to search for your files, you should verify the location of the last save.

- Type the full path and file name (e.g., **C:\Spotcam\Growth_Sequence**) in the box to specify the location of the image sequence. When entering the file path and name, you can also specify a new directory simply by entering it in the path (e.g., C:\Spotcam**Flowers**Growth_Sequence).

- Click on the browse button to select a new file location and file name (prefix). All of the image files are saved with the prefix that you enter. Thus, if your Suffix Begin Number (see below) is one, the file name for the first image in the sequence would be **Growth_Sequence0001**.

Save and Name Options (cont.)

Format   Select the file format for the sequence of images.

- For individual files, there are six options: **Windows Bitmap**, **JPEG**, **JPEG2000, Mac PICT**, **TIFF Uncompressed, or TIFF JPEG**.

- For image sequences there are two options: **TIFF Uncompressed** or **TIFF JPEG**.

- If the bits per pixel in your image setup is either 12 (monochrome) or 36 (color) your format save options will be reduced to the following:

  o For individual files, there are three options: **JPEG2000, TIFF Uncompressed 12 bit or 16 bit.**

  o For image sequences there is one option: **TIFF Uncompressed**.

Suffix Begin Number (individual images)   Select the beginning number for the sequence of images.
The Begin Number automatically defaults to the first number that follows the previous sequence of images. So, if you captured a sequence of 10 images, 11 would appear as the Begin Number when you next opened the Get Sequential Images window.

The program prefaces numbers with enough zeros to create a four-character suffix. For example, if you have 35 images, the first image suffix will be **0001** and the last image suffix will be **0035**. This enables Windows to correctly order images, so that Growth_Sequence0002 appears before Growth_Sequence1000, and not the other way around.

JPEG Quality (1-100)   JPEG quality is the level of compression for JPEG images: this option is available only when JPEG or TIFF JPEG (for image sequences) is selected as the image file format. Enter an integer between 1 and 100 to set the JPEG quality for images. A value of one is the most compressed (and lowest image quality), while a value of 100 is the least compressed (and highest image quality).

Compression (JPEG2000)   When the JPEG2000 file format is selected, the Get Sequential Images dialog displays compression options:

- **Lossless** – Information is not lost when the image is compressed.

- **Lossy –** Information is lost according to the ratio that you enter: the higher the ratio that you enter, the smaller the file size and the lower the quality of the image.

Save Thumbnails   Save thumbnails of each image in the sequence. This option is available only when JPEG, TIFF JPEG, or TIFF uncompressed is selected as the image file format.

File Name Conflict Action (individual images)   Determine how the program will respond if it finds an image file with the same name as an image in the current sequence. Select one of the following options:

- **Overwrite Existing File** – The program overwrites the existing file with the image from the sequence you are capturing.

- **Display Warning** – The program displays a warning message indicating that a file of the same name already exists, and stops the sequential image capture.

Image Display (individual images)   Determine how the sequence of images will be displayed. Select one of the following options:

- **Display Each Image in a New Window** – The program opens a window for each image in the sequence. This option works well if you have a short sequence; if you have a long sequence, however, displaying numerous images uses up memory and could interfere with your use of other programs.

- **Display Only Current Image –** The program sequentially displays each image in the same window. Each image displays only until the next image is captured. This is useful for lengthy sequences where you do not want to be concerned with running out of memory.

- **Don't Display Images –** The program automatically saves all of the sequential image files to the specified location without displaying them.

Display Images   Select this box to open an image sequence immediately after the capture. To save the

(image sequences)	image sequence to file without opening it, deselect the box.
Defer Image Processing/Display until Done	Wait until the entire sequence is completed before displaying or saving the images.

After you have entered all of the options, click on **Begin** to start the image capture sequence.   Refer to Ch. 8 – Viewing, Editing, and Annotating Images for details on viewing and modifying image sequence files

Image Captures in Other Procedures

In addition to the basic image captures, the Camera menu contains options in which one image capture is used to prepare the camera for the final image capture They include:

* Set Chip Imaging Area
* Set Spot Metering Area
* Compute Exposure
* Recall Previous Exposure
* Compute White Balance Values
* Get Bias Frame
* Get Thermal Frame
* Get Flatfield Image

RT B/W	RT B/W camera users will not see Compute White Balance Values option (or the corresponding toolbar button).

Because they are used prior to a final image capture, several of these options are discussed as a part of the image setup process. In such cases, the text of the manual refers back to the appropriate section of Chapter 5 - Preparing to Take a Picture.

Set Chip Imaging Area

The Set Chip Imaging Area selects a specific portion of the CCD chip. This option is available once an image has been captured. Using just a small portion of the available area on the CCD chip is useful because:

* The download time will be shorter.
* The file size will be smaller and easier to manage.

To avoid confusion when defining the region of the image that you want to capture, it is important to realize that there are two separate coordinate systems in use:

* The coordinate system of the CCD chip
* The coordinate system of the displayed image

The coordinates displayed on the Status Bar as the cursor is moved over a displayed image are always "displayed image" coordinates. The coordinates displayed in the Set Chip Imaging Area dialog box are always CCD chip coordinates. These coordinate systems match only if the orientation of an image has not changed (i.e., it has not been flipped or rotated).

Because the SPOT software tracks orientation changes, you can select a Chip Imaging Area on any displayed image, regardless of whether the orientation has changed: the Set Chip Imaging Area dialog box will correctly display the region of the CCD chip that you designated as the imaging region. However, because of the different coordinate systems, the coordinates that you see on the Status Bar (i.e., when moving the cursor over the displayed image) may not match the coordinates that appear in the Set Chip Imaging Area dialog box if the image was flipped or rotated.

To set an image region, follow these steps:

1. Position the cursor at the upper left edge of the image region that you want to capture.

2. Press and hold the left mouse button and drag the cursor across the screen. A dotted border appears around the region that you select.

3. From the Camera menu, select Set Chip Imaging Area. The Chip Imaging Area window displays the pixel locations for the borders of the region that you selected.

4. Click OK to save the region values to the current image setup, or select a different image setup. The region values are now saved to the designated image setup.

> ✎ *Note: The image setup uses the specified region values for all subsequent image captures. Refer to Ch. 5 – Preparing to Take a Picture for other region capture techniques.*
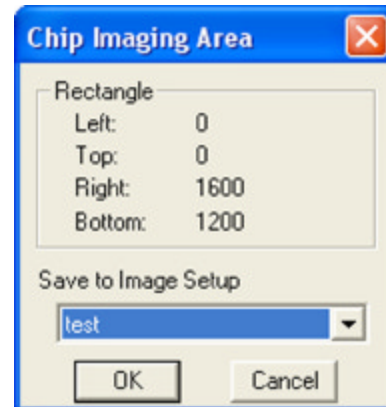
5. Recapture the image using either Get Image (exposure from setup) or the Get Image (last used exposure) option. The new image capture displays only the region that you selected.

Set Spot Metering Area

Spot metering is a method for selecting a region of interest on a desired image and then allowing the SPOT camera to automatically set the exposure time to allow for the best possible balance of image quality and contrast. This can be useful when the default autoexposure settings (full image sampling) do not provide a captured image that provides the detail you need for a particular region of your subject being viewed.

The Set Spot metering area feature in the SPOT software allows you to zero in on a rectangular region of the live image. There are two ways of setting the Spot Metering Area.

Method One – Drag the Area

1. Start by opening the live image window.
2. Select the area of the image by setting and then dragging the mouse across an area on the image.
3. Acquire that image using Snap.
4. Select Set Spot Metering Area.
5. Save it to an Image Set-up file for future image captures.

Method Two

1. Open the Image Setup Window from the Set-up dropdown menu.
2. Select the desired image setup file.
3. Select Auto-exposure from the menu selection window.
4. Select Region from the Set Metering Area box.
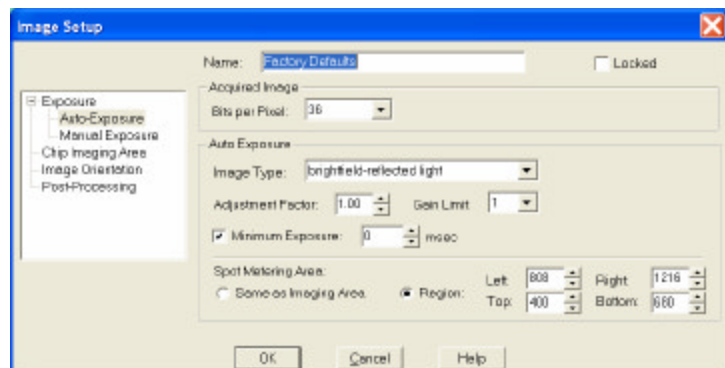
5. Type in or scale to the desired pixel points that will define the Spot Metering Area rectangle.

6. Save the Image Set-up modification.

You may also select the **Same as Imaging Area** option. That way, the camera will meter off of the entire image frame that you have selected (this is the default for autoexposure). You will also note that once you completed Method One, if you open the Auto-exposure box and choose region, the values defining the rectangle determined by the drop and drag method are resident in the Region values.

Compute Exposure

Compute Exposure determines the correct exposure time and gain for an image. By calculating exposure times and gain ahead of time, you can avoid the auto-exposure sequence that takes place each time that you use Get Image (e xposure from setup). Using Compute Exposure also changes your current image setup:

- The computed exposure values are added to your current image setup.
- The current image setup, if set to Auto-Exposure, is changed to User-Defined.

To compute exposure, follow these steps:

1. Select Compute Exposure from the Camera menu. The Compute Exposure window displays the image setup options (from the current setup) that directly affect the computation. The entries for these options can be modified from this window. Modifying the options also changes the current image setup.
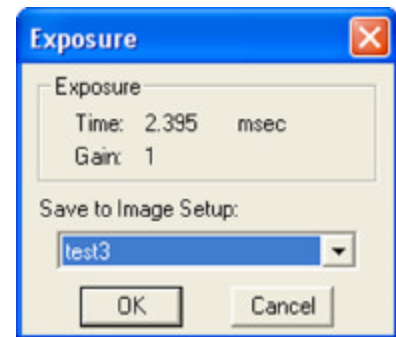
RT B/W	RT B/W camera users will not see white balance values on the Compute Exposure window.

📝 *Note: For information on entering Image Type, Auto-Gain Limit, Adjustment Factor, and White Balance, refer to Ch. 5 – Preparing to Take a Picture.*

Click on Begin. The Status message changes to **Computing exposure…** When the camera finishes sampling the light, the Exposure window displays the values.

2. Select an image setup from the Save to Setup drop-down box. The current image setup is the default.

📝 *Note: Saving an exposure value to an image setup changes the setup from Auto-Exposure to User-Defined Exposure.*

3. Click on OK. When you open the Image Setup window, the new exposure value displays.

4. Select Get Image (exposure from setup) to capture the image using the new exposure values.

Recall Previous Exposure

Recall Previous Exposure assigns the exposure time and gain fro m your last image capture to an image setup. The Recall Previous Exposure feature is available only after you have taken a picture.

To recall previous exposure values, follow these steps:

1. Capture an image.

2. From the Camera menu, select Recall Previous Exposure. The Exposure window displays the value from the last image capture (see above).

> ✎ *Note: If you change the current image setup, the Recall Previous Exposure option will be unavailable until you take another picture.*

3. Select an image setup from the drop-down box.
4. Click OK.
5. Use Get Image (exposure from setup) to capture an image with the new exposure and gain values.

Compute White Balance Values (RT Color Cameras)

White balance is the ratio of red, green, and blue exposure times necessary to achieve the proper color rendition for an image. Many factors impact the coloration of an image capture, including the lamp voltage, the coloration of the glass in the slide, and the coloration of the lenses in the microscope's objectives. To minimize the impact of these factors, use the Compute White Balance feature.   When you perform a white balance calculation, SPOT samples the color of the light from your light source after it has passed through the slide and the objective, and then calculates the exposure values needed to produce images where the white areas are white and the color areas are true.

You should perform a new white balance calculation when you:

- Start a new image capture session.
- Change the lamp voltage.
- Switch to a different objective on the microscope.

When you open an image setup, the Image Setup screen displays the default white balance values for that Setup, which are the values that were last saved to the Image Setup. If you capture an image without computing a new white balance, SPOT uses these default values. Refer to Ch. 5 – Preparing to Take a Picture for a step-by-step description of how to compute a white balance.

Get Bias Frame (Bias Frame Subtraction) (RT Monochrome and 3-shot in Monochrome mode)

Get Bias Frame allows you to collect data used to reduce the effect of noise in your captured image, specifically noise related to the bias (offset) used to adjust for the readout electronics.   For a more detailed discussion of Bias Frame Subtractions, refer to Chapter 5, preparing to…

To use Bias Frame Subtraction follow these steps:

1. From the Camera menu, select Get Bias Frame. The Get Bias Frame dialog box appears.

2. The program will ask for the number of image frames to capture to determine the Bias Frame reference noise level. You may select a number from 1-50 frames. The more frames you choose, the more accurate the result should be. However, the longer the process will take.

    The Get Bias Frame window will direct you to "Block all light to the camera".

3. Cover the optical train or find another method to block light to the camera's CCD.
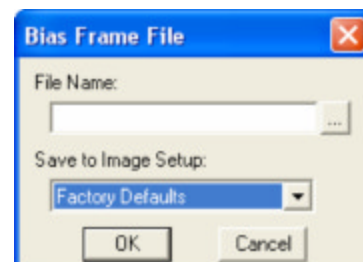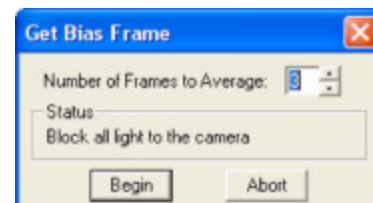
Click on OK. The program will acquire the number of short dark frame exposures required to calculate the Bias Frame.

4. A window will appear asking you to save the bias frame data to a file for use in a specific image setup. Input the information as required.

5. Move the specimen back into the field of view, and perform the image capture.

Get Thermal Frame (Dark Noise Subtraction)

Get Thermal Frame is a function that allows you to collect data to minimize the effect of thermal noise in your captured image. For a more detailed discussion of the theory and benefits of the Dark Noise Subtraction process, refer to Chapter 5, preparing to…

To use Thermal Frame Subtraction follow these steps:

1. From the Camera menu, select Get Thermal Frame. The Get Thermal Frame dialog box appears.

2. The program will ask for the number of image frames to capture to determine the Thermal Frame reference noise level. You may select a number from 1-50 frames. The more frames you choose, the more accurate the result should be. However, the longer the process will take.

3. The Get Thermal Frame window will direct you to "Move to Background". Do so and then click on Begin. The program will acquire the number of exposures required to calculate the Thermal Frame.

4. The Get Thermal Frame window will direct you to "Block all light from the camera"

5. Cover the optical train or find another method to block light to the camera's CCD.

6. Click on OK. The program will acquire the number of exposures required to calculate the Thermal Frame.

7. A window will appear asking you to save the Thermal frame data to a file for use in a specific image setup. Input the information as required.

8. Move the specimen back into the field of view, and perform the image capture.
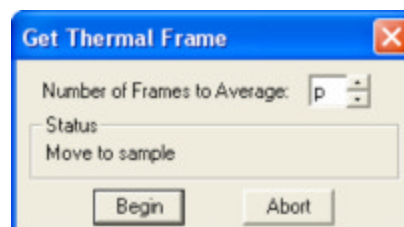
Get Flatfield Image

The Flatfield option can be used to correct for display problems associated with uneven intensity or coloration in illumination, or to correct for artifacts (e.g., dust) in the optical system. For a more detailed discussion of the theory and benefits of the Flatfield correction process, refer to Chapter 5, Preparing to Take a Picture.

Note: Flatfield correction with the SPOT camera refers only to the correction of uneven lighting. It should not be confused with the correction of optical field flatness.

To use Flatfield Correct, follow these steps:

1. Ensure that the specimen is under the desired objective and in focus.

2. From the Camera menu, select Get Flatfield Image. The Get Flatfield dialog box appears.

3. Remove the slide from the field of view.

4. Click on Begin. When the program finishes computing the exposure times and acquiring the flatfield file, the program prompts you to name and save the file:

    a) Enter a name in the File Name box. Because flatfield image files are different for each objective, you might want to name the file according to the objective's magnification (e.g., 10x).

    b) Specify the image setup that will be associated with this flatfield image file when using the Flatfield Correct option. The current setup is the default. When you open the setup, the Flatfield Correct option is selected.

5. Click on OK.

✎ *Note: The default file location varies, depending on where you installed the SPOT program.*

---

6. Move the specimen back into the field of view, and perform the image capture. Refer to Ch. 7 – Taking a Picture, for more details on the image capture process.

Ch. 8 - Viewing, Editing, and Annotating Images

Introduction

After capturing an image, you can choose from a variety of viewing, editing, and annotation options that allow you to customize the appearance of the final image. These features are described in the following sections:

- Viewing Captured Images
- Working with Image Sequences
- Editing Captured Images

✏️ *Note: These menus are available only when an image is on-screen.*

Viewing Captured Images

The View menu options include:

• Fit to Window	• Show Image Date/Time
• Set Zoom Mode	• Show Image Acquisition Settings
• Set Zoom Level	• Show Taskbar
• Show Grid Lines	• Full Screen
• Show Pan Window	
• Show Sequence Controls (available when an image sequence is open)	

```
✓ Fit to Window            Ctrl+F

  Set Zoom Mode            Ctrl+Z
  Zoom Level                       ►
  Show Grid Lines         Ctrl+G

  Show Pan Window         Ctrl+W

  Show Image Date/Time    Ctrl+T
  Show Image Acquisition Settings

  Show Taskbar
  Full Screen             F7
```

✏️ *Note: The Show Grid Lines option is available only after you have zoomed in (8x or 16x) on an image.*

Fit to Window

Fit to Window allows you to view the whole image, regardless of the window size. Typically, reducing the size of a window also cuts off part of the image. The Fit to Window option solves this problem by resizing the image so that it is always the same size as the window.

✏️ *Note: The View menu options supplement the standard Windows viewing options (e.g., Cascade, Tile Horizontal, etc.) that are found on the Window menu. Refer to the Windows on-line help for more information on these options.*

To use Fit to Window, select (i.e., click on) the image that you want to modify and, from the View window, select Fit to Window.

Set Zoom Mode

1. Either select Zoom Mode From the View menu, or click on the Zoom toolbar button:
2. The cursor changes to a magnifying glass, and the Zoom toolbar button becomes active. The precise reference point for the zoom location is indicated by the white dot in the center of the magnifying glass. The row and column numbers at the bottom of the screen indicate the exact position of the white dot as you move the magnifying glass over the image.

3. Use the mouse to zoom in or zoom out:

   a) Position the mouse over the center of the area that you want to zoom in on.

   b) Either left click to zoom in 2x, or right click to zoom out 2x.

   c) To return to the normal view mode, de-select the Zoom option.

The Zoom feature works in conjunction with the pan window (see **Show Pan Window** below), which allows you to see where you are in relation to the larger image while zoomed in.

> *Note: All of the standard save, view, and edit options, with three exceptions –* **Add Calibration Mark***,* **Add Measurement***, and* **Annotate** *– can be used while the program is in zoom mode. If you need to annotate a zoomed image, use the* **Zoom Level** *option (see below).*

> *Note: With some video cards, you might experience display problems when zooming in on captured images. To correct these problems, lower the Graphics Acceleration setting in Windows, as described in the* Video Card Related Problems *section of the* Installation Guidelines *chapter.*

Set Zoom Level

The **Zoom Level** option, like the **Zoom Mode** option enables you to view zoomed images. However, unlike the Zoom Mode option, you have the option to add annotations, including text, calibration marks, and measurements, to the zoomed image. To use the Zoom Level option, select **Zoom Level** from the View menu and select a percentage option from the list.

Like the Zoom Mode option, the Zoom Level option can be used in conjunction with the pan window (see **Show Pan Window** below), which allows you to see where you are in relation to the larger image while zoomed in.

> *Note: Zoom Level values are always referenced to the original image capture. The original image, however, does not always equal 100%. It will be 100% if you don't select the Fit to Window option in the Preferences window.*

> *Note: With some video cards, you might experience display problems when zooming in on captured images. To correct these problems, lower the Graphics Acceleration setting in Windows, as described in the* Video Card Related Problems *section of the* Installation Guidelines *chapter.*

Show Grid Lines

Show Grid Lines clearly defines pixel borders, which are often invisible in low contrast areas of an image. Clearly defined borders help in positioning the cursor at an exact row and column location (i.e., using the readouts at the bottom of the screen), which then allows you to view the brightness value for the individual pixel.

> *Note: When using the* **Zoom Mode** *option, all of the standard save, view, and edit options, with three exceptions –* **Add Calibration Mark***,* **Add Measurement***, and* **Annotate** *– can be used while the program is in zoom mode. If you want to annotate a zoomed image, use the* **Zoom Level** *option (see above).*

To show the grid lines on an image, follow these steps:

1. Position the magnifying glass in the center of the area that you want to zoom in on.
2. Zoom to either 8x or 16x. If you are using the **Zoom Level** option, select a percentage of 800 or greater.
3. From the View menu, select the Show Grid Lines option. Grid lines appear around each pixel.

Show Pan Window

Show Pan Window enables you to navigate around an image while zoomed in at high magnifications. When you select the Show Pan Window option, the program highlights your location in relation to the whole image. A "color negative" area represents the zoomed-in area of the image over the smaller version of the image in the Pan Window. By moving the highlighted area around the pan window, you can explore the image, piece by piece.

Without the Show Pan Window option, the process of moving to a new point while in high magnification would involve several steps:

1. Zooming in to the first area of interest.
2. Zooming out to determine where you are.
3. Determining a new area of interest.
4. Re-zooming in to the new area of interest.

To use the pan window, follow these steps:

1. From the View menu, select Show Pan Window:

> *Note: The pan window can be opened at any level of magnification; however, the obvious value of pan is only evident at higher levels of image magnification.*

The pan window appears, superimposed upon the image. The illustration to the right shows the pan window as it is used when zoomed in on an image.

2. Position the cursor on the image in the pan window: it changes from a magnifying glass to a four arrow pointer.

3. To move to a different area of the image, either left click on the rectangle and drag it to the new location, or left click on the new location.

Show Sequence Controls

The Show Sequence Controls option displays the play controls for image sequences. This option appears only when an image sequence is open. Using the Image Sequence dialog you can:

- Play all or part of an image sequence in forward or reverse order.
- Set the display interval between images.
- Set the action that takes when the image sequence finishes playing – i.e., stop, restart, or reverse.

Refer to the **Working With Image Sequences** section for details.

Show Image Date/Time

The Image Date/Time feature pulls up the date and time associated with the capture of an image. When you capture an image, the SPOT software remembers the date and time of the image capture. When you save the image, even if this is many hours later, the program sets the **Created** date and time of the image file to the date and time of the original image capture.

When image files are transferred over networks, the **Created** date and time is sometimes reset to the date and time of the transfer, making it an unreliable indicator of the image capture time. Because TIFF and JPEG format images have tags both for the Created date and the original capture date and time, they are not affected by network transfers. If reliable image capture date and time information is important to your work, you should save images in either TIFF or JPEG format.

When you select the Image Date/Time option from the View menu, a dialog box displays the date and time associated with the capture.

- For non-TIFF, non-JPEG format image files, this is the **Created** date/time, as explained above.

- For TIFF and JPEG format image files, this is the TIFF/JPEG tag date and time.

- For SPOT Database images this is the original date and time that was stored in the database file.

Show Image Acquisition Settings

The Show Image Acquisition Settings feature, when selected from the dropdown menu, will display the image capture settings that were associated with the open image file. The information displayed will include such settings as exposure time, gain setting, filter color (color cameras), binning setting, chip area, gamma setting, etc.

Show Taskbar

When you select this command from the dropdown menu, the taskbar will appear on the computer screen. If you have not yet developed a taskbar, the taskbar development window will appear along with a floating taskbar template without any commands. You may then decide to add functions to the taskbar. See Chapter 12 – Taskbars for a complete discussion regarding taskbar development and use.

Full Screen

View a full screen version of an image – i.e., without the SPOT menus or toolbars or the Windows navigation bar. This option allows you to utilize the entire window for previewing images. Press **[F 7]** to view a full screen image. Press **[F 7]** again to exit the full screen view. Alternatively, you can exit the full screen view by moving the cursor to the top of the window and clicking on the **Exit Full Screen Mode** button that appears. Mac users will exit the full screen mode from the top dropdown menu. While working in Full Screen mode, Windows users can access SPOT menu options using standard keystroke combinations, as illustrated in the table below:

[Alt] + **A**	=	Attributes (Report mode)	**[Alt]** + **H**	=	Help
[Alt] + **C**	=	Camera	**[Alt]** + **O**	=	Object (Report mode)
[Alt] + **D**	=	Database (Advanced mode)	**[Alt]** + **Q**	=	Sequence
[Alt] + **E**	=	Edit	**[Alt]** + **S**	=	Setup
[Alt] + **F**	=	File	**[Alt]** + **V**	=	View
			[Alt] + **W**	=	Window

Working with Image Sequences

There are three places from which you can edit and play image sequence files:

- The **Sequence** menu – Rearrange the order of a sequence, add and/or remove images to and from the sequence, extract images from the sequence, and play the sequence.

- The **Sequence Controls (Image Sequence)** dialog – Set view options and play the sequence.

- The **Edit** menu - Modify/enhance image sequences using the options on SPOT's Edit menu. Refer to **Editing Images in a Sequence** (below) for more information.

Refer to the sections below for details.

Sequence Menu Options

Image sequences are editable. The **Sequence** menu, which is available only when an image sequence is open, displays the available options. They are as follows:

Extract Images

Extract an image or images from a sequence file. Extracting creates new image(s), leaving the original sequence intact. Click on **Extract Images** and enter the number(s) of the image(s) that you want to extract. To extract multiple images from a sequence, separate numbers with commas and/or dashes - i.e., **5, 6, 7** or **5-7** or **1 – 5, 6, 9**.
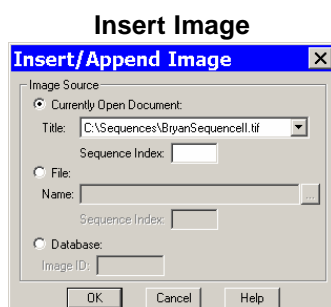
Insert Image

Add an image to the sequence. Using **Insert Image**, you can add an image to a sequence from one of three sources:

- **Currently Open Document** – Add an image from the currently open sequence file. Make sure to specify the **Sequence Index –** i.e., the number of the image in the sequence.

- **File** – Add an image from disk. If the image comes from a sequence file, make sure to specify the Sequence Index.

- **Database (Windows)** – Add an image from a SPOT database. To determine the Image ID, open the database and use the **Search** option. The search results table displays the Image ID for each record.

When you click on **OK**, a dialog prompts you to specify the point in the sequence where you want to insert the image.

Append Image
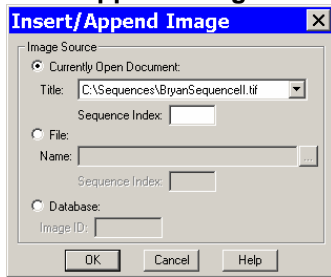
Add an image to the end of the sequence. Using **Append Image**, you can add an image to a sequence from one of three sources:

- **Currently Open Document** – Add an image from the currently open sequence file. Make sure to specify the **Sequence Index –** i.e., the number of the image in the sequence.

- **File** – Add an image from disk. If the image comes from a sequence file, make sure to specify the Sequence Index.

- **Database (Windows)** – Add an image from a SPOT database. To determine the Image ID, open the database and use the **Search** option. The search results table displays the Image ID for each record.

Remove Images
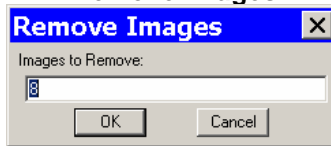
Remove an image or images from a sequence file. Removing an image deletes the image from the sequence, which is automatically reordered. Click on **Remove Images** and enter the number of the image(s) that you want to extract. To extract multiple images from a sequence, separate numbers with commas and/or dashes - i.e., **5, 6, 7** or **5-7** or **1 – 5, 6, 9**.

Move Images
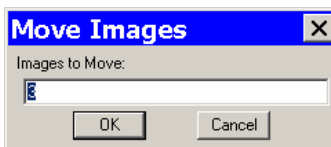
Move an image or image(s) to a different point in the sequence. Click on **Move Image** and enter the number(s) of the image(s) that you want to move. To move multiple images from a sequence, separate numbers with commas and/or dashes - i.e., **5, 6, 7** or **5-7** or **1 – 5, 6, 9**.

Play Forward	Continuously play the image sequence forward.
Play Reverse	Continuously play the image sequence backward.
Stop Play/Resume Play	Stop play if the sequence is running; resume play when it is stopped.
Step Forward	Move forward one image.
Step Reverse	Move backward one image.

Image Sequence Controls

The **Sequence Controls (Image Sequence)** dialog enables you to play image sequences and to set the play options.

Image Index

Use the **Image Index** options to specify the view parameters of the sequence.

- **First** - Enter the number of the image that should be first in the sequence.

- **Current** – This field displays the number of the image you are currently viewing. Enter a new number to view a different image.

- **Last** – Enter the number of the image that should be last in the sequence
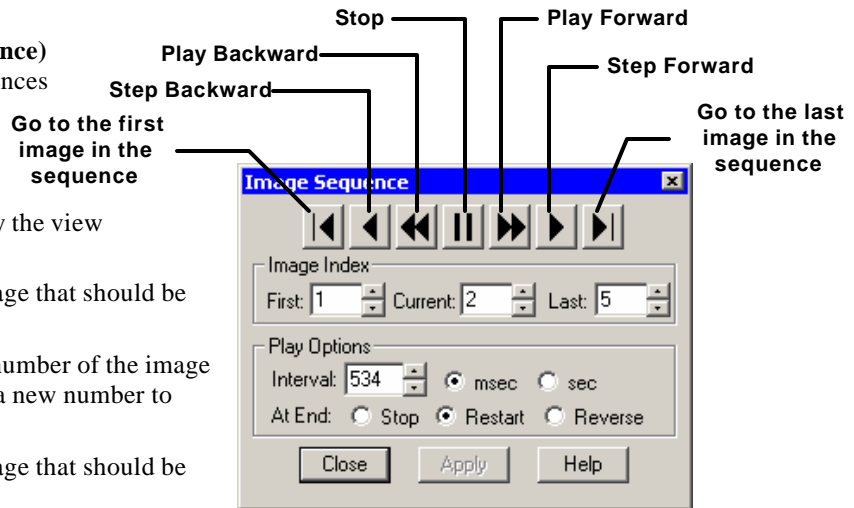
Play Options

Use the **Play Options** to control how the image sequence displays when playing.

- **Interval** – The time interval between each image when the sequence is playing. Enter a number in milliseconds or seconds.

- **At End** – The action that takes place when the sequence finishes playing.

    - **Stop:** The sequence stops at the last image in the sequence.
    - **Restart:** The sequence begins playing from the beginning.
    - **Reverse:** The sequence reverses play when it reaches the end.

Editing Images in a Sequence

Images from a sequential image file can be edited using the **Edit** menu options. This allows you to specify the individual image or images (including the entire sequence) to which the edits are applied.

- In some cases – e.g., **Combine Images** – the dialog prompts you to specify the **Sequence Index** – i.e., the number of the image within the sequence.

- In other cases, as with the **Adjust RGB** options, SPOT prompts you to specify whether the edit applies only to the current image in the sequence, all of the images in the sequence, or a specific subset of images in the sequence. For example, when editing an image sequence, if you select **Edit >Adjust RGB > Adjust Brightness (additive)**, the **Additive Brightness Adjust** dialog appears. After you make your edits and click on **OK**, the **Edit Images** window prompts you to specify the image or images that the edit will apply to, as shown at right.

    - **No Other Images** – The edit applies only to the current image – i.e., the on-screen image.

    - **All Other Images in Sequence** – The edit applies to all of the images in the sequence.
    Specified Image Sequence – The edit applies to the images that you specify. Enter the number(s) of the image(s) in the box at the bottom of the dialog. To specify multiple images, separate numbers with commas and/or dashes - e.g., **5, 6, 7**; or **5-7**; or **1 – 5, 6, 9**.

Edit

The Edit menu contains six categories of editing options, as illustrated in the following table:

Editing Category	Menu Option
Basic Editing Functions	Undo Copy to Clipboard
Image Sizing and Position	Resize Rotate Right Rotate Left Flip Horizontal Flip Vertical Crop
Image Quality	Adjust RGB Adjust HSL Adjust HSV Filter Make Negative Convert to Gray Scale Set Palette (monochrome) Match Color (RGB)
Customization	Change Pixel Bit Depth Subtract Image Merge Images Duplicate Images
Annotation Calibration and Measurement Marks Custom Image Annotation	Add Calibration Mark Add Measurement Annotate
Image Memos	Memo

Basic Editing Functions

The Edit menu contains several functions that are common to most operating systems.

Undo   Undo the last edit that you made. The Undo option only allows for one level of edits: in other words, you can only undo the immediately preceding edit. To Undo an action, select Undo from the Edit menu.

(cont.)

Copy to Clipboard (PC) Copy (Mac)	Copy all or part of an image onto the clipboard for insertion into another program. These options are identical to the Windows **[Ctrl C]** command and the Mac [⌘ **C**] command.

1.   Press and hold the left mouse button (PC)/mouse button (Mac).

2.   Drag the cursor over the image until the dotted rectangle covers the area of the image that you want to copy. If you copy without "cutting out" a specific portion of the image, SPOT selects the entire image.

3.   Either select the Copy to Clipboard/Copy option from the Edit menu, or press **[Ctrl C]** (PC)/[⌘ **C**] (Mac). The image is now ready to be pasted into another application.

4.   Open the application that you want to paste the image into, and select from the available pasting options.

Cut (Mac)	Cut text fields within the SPOT program.
Paste (Mac)	Paste text fields in the SPOT program. The Paste option is unavailable (grayed out) for image editing.
Clear (Mac)	Clear a text field in the SPOT program. The Clear option is unavailable (grayed out) for image editing.
Select All (Mac)	Select text for copying. The Select All option is unavailable (grayed out) for image editing.

Image Sizing and Position

From the Edit menu, you can:

- Re-size images

- Re-position images

- Match the on-screen orientation of a specimen to the mic roscopic view

- Crop an image to better reflect the area of interest

Resize...
Rotate Right
Rotate Left
Flip Horizontal
Flip Vertical
Crop...

Resize

Use the Resize option to reduce or enlarge an image. When you reduce or enlarge an image, the resolution and file size change accordingly. The SPOT program maintains aspect ratio so enlarged and reduced images will not be distorted. When you reduce or enlarge an image, keep the following in mind:

- Reduction - Resolution (number of pixels) and file size decrease.

- Enlargement - Resolution (number of pixels) and file size increase.

The trade off for higher resolution images is increased file size. The Resize option lets you specify the percentage by which you want to increase or decrease the linear dimensions of the image. The program reads the red, green, and blue brightness values for the pixels in the original image and uses those values to create a new image where the number of rows and columns is reduced or enlarged by the perc entage that you specify.

Two circumstances where you might want to enlarge or reduce an image are listed here:

- **You captured an image using a low magnification objective (e.g., 5x or below)** - In this case, enlarging the image adds pixels, which helps to smo oth out the pixellation of high contrast, fine details. This makes it easier to view and interpret the structure of these details. Enlarging also makes large format prints appear smoother and less pixellated.

- • **You want to save file space and resolution is not the primary concern** - In this case, reducing the image reduces the file size.

To resize an image, follow these steps:

1. From the Edit menu, select the resize option. The Resize Image window appears.

2. Do one of the following:

   − If you want to change the size of an image by a specific percentage, type in a value between 10% and 200%, or move the slider bar accordingly. The linear dimensions (width or height) of the image will change by this percentage factor.

   − If you want to specify the dimension in pixels, enter a number in either the Width or the Height box. The SPOT program automatically calculates the dimension not entered in order to maintain the proper aspect ratio.

3. Click on OK. The newly sized image replaces the original image on the screen.
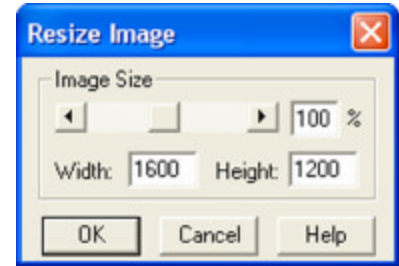
> ✎ *Note: If you want to create two differently sized images of an image for comparison purposes, use the Save As [Ctrl A] command to save the resized image with a different file name.*

Rotate Right

Rotate an image clockwise in 90 degree increments.

Rotate Left

Rotate an image counter-clockwise in 90 degree increments.

Flip Horizontal

Flip an image from right to left.

Flip Vertical

Flip an image from top to bottom.

Crop

Cut out a portion of an image. To crop an image, follow these steps:

1. Press and hold the left mouse button.

2. Drag the cursor over the image until the dotted rectangle covers the area of the image that you want to crop.

3. From the Edit menu, select Crop. The Crop Area window displays the pixel coordinates of the rectangle that you selected. From this window you can fine tune the crop area by modifying the pixel coordinates. If you select the Crop option directly from the menu (i.e., without specifying a crop area), the Crop Area window displays the values for the entire image.

4. Click on OK to crop the image. The cropped image appears on-screen.

> ✎ *Note: When you crop an unsaved image, the program automatically discards the area of the image outside the crop. To give yourself the opportunity to change the crop area, you should save the image before you crop it.*

Image Quality

The image quality editing options provide you with a wide array of powerful and easy to use tools for changing the appearance of an image after it has been captured. These tools enable you to enhance the appearance of an image in ways that are not possible with conventional photography.

Before using the SPOT editing features, review the following two sections:

- **Functional Features** – This section explains how thumbnail images work in the SPOT image editing windows.

- **Image Editing and the Concept of Color Space** – This section introduces the concept of color space and describes how it applies to the SPOT software.

Functional Features

With the exception of Make Negative and Convert to Gray Scale, all of the image quality editing options utilize *thumbnail* images to preview the edits being made to the main image. As illustrated at right, a thumbnail of the image capture appears in the adjustment window, and the **Modify Main Image** box is checked as the default.

- **If you leave the box selected…** As you make editing adjustments, the changes appear in both the main image and the thumbnail.

- **If you deselect the box…** As you make editing adjustments, the changes appear only in the thumbnail image. After you click on OK, the changes appear in the main image.

Properly utilized, thumbnail images can be very helpful as part of the image editing process. Consider the following:

- *You can use thumbnails to speed the editing process.* The SPOT software often cannot update all the pixels in the main image as quickly as you can adjust the edit controls. In cases where the thumbnail image is sufficient to judge the effect of the edits, you can save time by deselecting **Modify Main Image**, so that the main image is updated only after you close the editing window. Deselecting **Modify Main Image** also makes the editing feel more "real time."

- *The thumbnail can be used to display a smaller, magnified section of an image.* When you select a specific image area (i.e., by dragging the cursor) before selecting an edit function, only the selected area appears in the thumbnail. This is useful for Sharpen, an edit function that affects only the fine structure of an image. This is also useful when you are analyzing histograms, and want the histogram to represent only the section of the image that interests you.

Image Editing and the Concept of Color Space

A color space is a coordinate system that is used to mathematically represent color. In order to satisfy the imaging requirements of different microscopy techniques, the SPOT program allows you to adjust brightness, color, and contrast in three color spaces: RGB, HSL, and HSV.
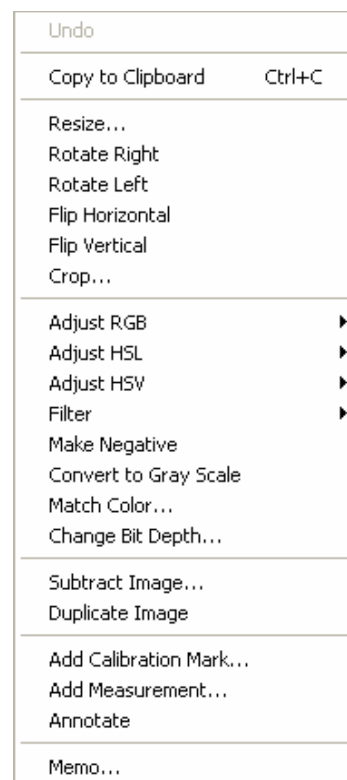
The color space options and their suggested uses are as follows:

- **Adjust RGB** (Red Green Blue) – Use for monochrome images
- **Adjust HSL** (Hue Saturation Luminance) – Use for all color images except fluorescence
- **Adjust HSV** (Hue Saturation Value) – Use for fluorescence images

Each color space is described below.

RGB The RGB color space operates by assigning a red, green, and blue value to each pixel in an image. Because red, green and blue are the primary colors for emissive light, this is the method that color computer monitors use to display images. However, the RGB color space is not intuitive to users.

HSL The HSL color space is a more intuitive model based on the three attributes that are common to all colors: hue, saturation, and luminance. Instead of assigning a red, blue, and green value to each pixel, the HSL color space assigns a value for each color attribute (e.g., hue, saturation, and luminance). These attributes are defined as follows:

- **Hue** – Hue is the designation for the actual color, as distinguished from others in the color spectrum. Hue is derived from a color wheel and is expressed in the color's angular location (in degrees) on the wheel. (e.g., $+180°$ to $-180°$ for the SPOT software).

- **Saturation** – Saturation is the proportion of perceived pure hue in the color. Saturation (in the SPOT software) is measured on a numerical scale of 0 to 100, where zero is pure gray and 100 is pure hue (e.g., day glow).

- **Luminance** – Luminance is the relative brightness or darkness of a hue. Luminance (in the SPOT software) is measured on a numerical scale of 0 to 100. A saturated color starts out as black at a luminance of 0, reaches full brightness as a saturated color at a luminance of 50, and then transitions through lighter pastel shades to white at a luminance of 100. This mimics the human perception of increasing brightness for images that are illuminated with light that contains a broad spectrum of wavelengths.

HSV The HSV color space was added to the SPOT software to optimize fluorescence image editing. Both the RGB and the HSL color spaces are problematic when used to edit color fluorescence images. Increasing gamma in RGB can cause dimmer sections of the image to lose saturation (look "washed out") and change hue. Increasing gamma or brightness in HSL causes the brighter areas of an image to look unnaturally pastel or even white. The HSV color space avoids both problems.

The HSV color space is similar to the HSL color space in that it also uses the intuitive concepts of hue and saturation. However, instead of luminance for brightness, it uses Value. Value is like Luminance in that a value of 0 is black. Value differs from Luminance in that a Value of 100 (in the HSV color space) is the brightest version of the original hue whereas a Luminance of 100 (in the HSL color space) is always white. For example, a dim cherry red changes to a bright cherry red when its Value is increased from 30 to 100. When its Luminance is increased from 30 to 100, a dim cherry red turns white.

HSV works well for fluorescence images because the dyes in fluorescence images emit single wavelength light. This light is, by definition, highly saturated. Editing in the HSV color space ensures that all areas of the image retain their saturated colors. Thus, using the HSV color space, images can be modified without causing the bright areas to look unnaturally pastel or white.

Adjust RGB/Adjust HSL/Adjust HSV

The process by which the program applies edits to an image capture differs according to the color space that you select. The following table briefly describes the edit process in each of the three color spaces and lists the recommended usage for each color space.

Color Space	Editing Process	Recommended Usage
RGB	Adjustments are applied separately to the red, green, and blue pixel values.	The RGB option is ideal for monochrome images.
HSL	1. RGB pixel values are translated to *HSL* (hue, saturation, and luminance) values. 2. Adjustments are applied to the hue, saturation, or luminance. 3. The HSL values are translated back to the RGB pixels.	The HSL option is ideal for all color images except fluorescence images.
HSV	1. RGB pixel values are translated to *HSV* (hue, saturation, and value) values. 2. Adjustments are applied to the hue, saturation, or value. 3. The HSV values are translated back to the RGB pixels.	The HSV option is ideal for fluorescence images.

The Adjust RGB, Adjust HSL, and Adjust HSV options are each made up of several image-editing functions. The table below lists the parallel RGB, HSL, and HSV functions:

| Adjust RGB	Adjust HSL	Adjust HSV
Adjust Brightness (additive)	Adjust Hue, Saturation, and Luminance	Adjust Hue, Saturation and Value
Adjust Brightness (multiplicative)	Adjust Luminance (multiplicative)	Adjust Value (multiplicative)
Adjust Contrast	Adjust Contrast	Adjust Contrast
Adjust Gamma	Adjust Gamma	Adjust Gamma
Stretch Bright and Dark Level	Stretch Bright and Dark Level	Stretch Bright and Dark Level
Adjust Histogram	Adjust Histogram	Adjust Histogram

Many of the Adjust edit functions are duplicated in all three color spaces. To avoid unnecessary repetition, we have grouped together discussions of edit functions that are the same in different color spaces. The color space associated with the option appears parenthetically in the heading.

Adjust Brightness (additive) (RGB)

Use Adjust Brightness (additive) to lighten or darken an entire image. This function adds or subtracts the specified number(s) from the red, green, and blue values for every pixel in the image. Because this is an additive function, it affects bright and dark areas equally. You can change an image's brightness either by adjusting all three values (e.g., red, green, and blue) together, or adjusting each value individually.

To Adjust Brightness (additive), follow these steps:

1. From the Edit menu, select Adjust RGB and Adjust Brightness (additive). The Additive Brightness Adjust window appears, with a thumbnail preview of the open image.
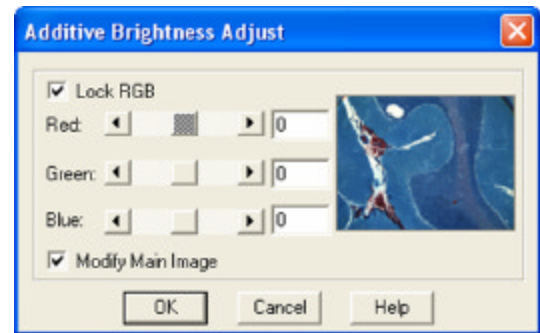
    The Lock RGB and Modify Main Image boxes are checked as defaults. To adjust the RGB values individually, deselect the Lock RGB box.

2. Change the brightness of the image.

    − To modify an image's brightness equally for red, green, and blue values, ensure that the Lock RGB box is checked, and either enter a number in any one of the three boxes, or use one of the slider bars to specify a number between -255 and 255 (8 bit) and –4095 and 4095 (12 bit).

    − To modify an image's brightness by specifying separate red, green, and blue values, deselect the Lock RGB box, and enter a value for each color.

3. Click on OK. The new image appears on-screen.

Adjust Hue, Saturation, and Luminance (HSL)
Adjust Hue, Saturation, and Value (HSV)

Use the Adjust Hue, Saturation, and Luminance and the Adjust Hue, Saturation, and Value options to make additive changes to the HSL or HSV values in an image. This process converts the RGB values for each pixel in the image to HSL or HSV values. After you make the adjustments, the program converts the HSL/HSV values back to RGB color space values.

To change the HSL or HSV values for an image, follow these steps:

1. From the Edit menu, select Adjust HSL, Adjust Hue, Saturation, and Luminance or Adjust HSV, Adjust Hue, Saturation, and Value:
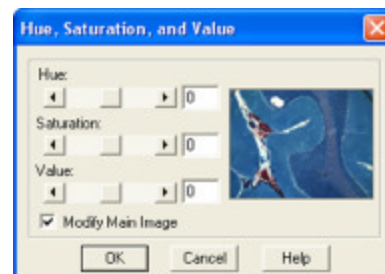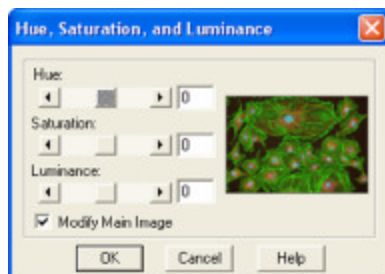
2. Either type in the HSL/HSV values, or use the slider bars to select the values. Each value uses its own scale, and must be entered separately.

    − **Hue** - Enter an angle value between -180° and 180°. This number represents the amount that the color wheel is rotated for each pixel in the image.
    − **Saturation** - Enter a number between -100 and 100. This number is added to the saturation value (e.g., a number between 0 - pure gray and 100 - day glow) of each pixel in the image.

- **Luminance or Value** - Enter a number between -100 and 100. This number is added to the Luminance/Value value (e.g., a number between 0 - pure black and 100 - pure white for Luminance/brightest version of hue if Value) of each pixel in the image.

> ✏️ *Note: The scales used to measure HSL/HSV values are not standardized. Thus, the scales used by the SPOT software might differ from those of other programs.*

3. Click on OK. The new image appears on-screen.

Adjust Brightness (multiplicative) (RGB)

Use Adjust Brightness (multiplicative) to make the bright areas of an image brighter or darker. This function multiplies the red, green, and blue values for every pixel in the image by the number(s) that you specify. Because this option is multiplicative, it affects bright areas more than dark areas: black areas tend to stay black while lighter areas get brighter. Like the Adjust Brightness (additive) option, the multiplicative option enables you to either adjust the RGB values together or as individual units.

To use the Adjust Brightness (multiplicative) function, follow these steps:

1. From the Edit menu, select **Adjust RGB > Adjust Brightness (multiplicative)**. The Multiplicative Brightness Adjust window appears, with a thumbnail preview of the open image.

   The Lock RGB and Modify Main Image boxes are checked as defaults. To adjust the RGB values individually, deselect the Lock RGB box.
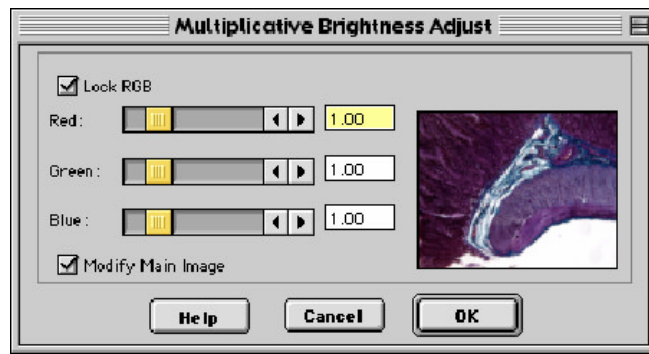

2. Change the brightness of the image.

   - To modify an image's brightness equally for red, green, and blue values, ensure that the Lock RGB box is checked, and either enter a number in any one of the three boxes, or use one of the slider bars to specify a number between 0 (darkest) and 5 (brightest).

   - To modify an image's brightness by specifying separate red, green, and blue values, deselect the Lock RGB box, and enter a value for each color.

3. Click on OK. The new image appears on-screen.

Adjust Luminance (multiplicative) (HSL)
Adjust Value (multiplicative) (HSV)

Adjust Luminance (multiplicative) and Adjust Value (multiplicative) make the bright areas of an image brighter or darker when editing in the HSL and HSV color spaces. These functions multiply the Luminance or Value values for every pixel in the image by the number that you specify. Because these options are multiplicative, they affect bright areas more than dark areas.

To Adjust Luminance (multiplicative) (HSL) or Adjust Value (multiplicative) (HSV), follow these steps:

1. From the Edit menu, select Adjust HSL, Adjust Luminance (multiplicative) or Adjust HSV, Adjust Value (multiplicative):

The Modify Main Image box is checked as a default.

2. Change the Luminance or Value of the image. Use the slider bar to specify a number between 0 (darkest) and 5 (brightest).

3. Click on OK. The new image appears on-screen.

Adjust Contrast (RGB)

Use Adjust Contrast to exaggerate or subdue the differences between the bright and dark areas in an image. The Adjust Contrast option, when accessed from the Adjust RGB menu item, enables you to make adjustments to the red, green, and blue values either together, or as individual units.

To adjust the contrast in an image, follow these steps:

1. From the Edit menu, select Adjust RGB and Adjust Contrast. The Contrast Adjust window appears.

   The Lock RGB and Modify Main Image boxes are checked as defaults. To adjust the RGB values individually, deselect the Lock RGB box.

2. Change the contrast of the image.

   – To change the contrast equally for red, green, and blue values, ensure that the Lock RGB box is checked, and either enter a number in any one of the three boxes, or use a slider bar to specify a number between 0 (pure gray - no contrast) and 5 (maximum contrast).

   – To modify an image's contrast by specifying separate red, green, and blue values, deselect the Lock RGB box, and enter a value for each color.
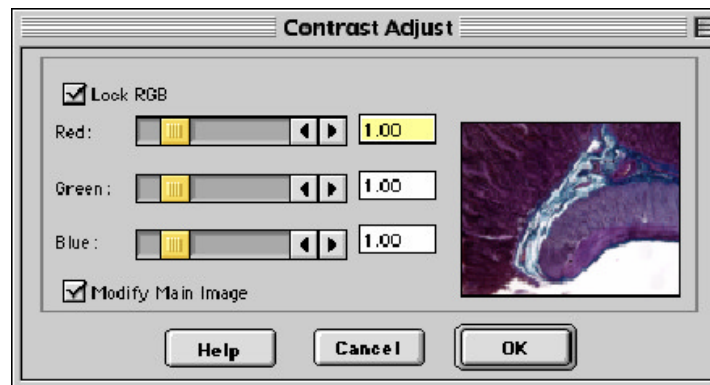
3. Click on OK. The new image appears on-screen.

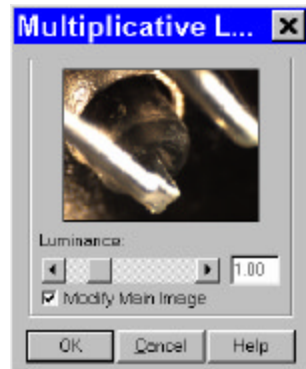## Adjust Contrast (HSL)
Adjust Contrast (HSV)

The Adjust Contrast (HSL) and Adjust Contrast (HSV) options, like the Adjust Contrast (RGB) option, exaggerate or subdue the differences between the bright and dark areas in an image. Unlike the Adjust

Contrast (RGB) process, the Adjust Contrast (HSL/HSV) process works only on the Luminance/Value values for an image as opposed to the specific red, green, and blue values.

To use Adjust Contrast (HSL/HSV), follow these steps:

1. From the Edit menu, select Adjust HSL and Adjust Contrast or Adjust HSV and Adjust Contrast.

The Modify Main Image box is checked as a default.

2. Enter a value between .1 and 4, or use the slider bar to select a value. The thumbnail changes as you adjust the value.

   – To exaggerate the contrast of an image, enter a value above one (the base value).
   – To subdue the contrast of an image, enter a value below one (the base value).

3. Click on OK. The new image appears on-screen.

Adjust Gamma (RGB)

Gamma Adjust smoothly lightens darker areas of an image without burning out bright areas or lightening black areas. This is especially useful for editing images with a wide range of brightness values (dynamic range). Gamma adjustment corrects an image by creating a new version of the original. Refer to Ch. 5 – Preparing to Take a Picture for a more detailed explanation of gamma adjustment.

To adjust gamma, follow these steps:

1. From the Edit menu, select Adjust RGB and Adjust Gamma. The Gamma Adjust window appears. The Lock RGB and Modify Main Image boxes are checked as defaults.

2. Gamma adjust the image.

   – To adjust the gamma equally for red, green, and blue values, ensure that the Lock RGB box is checked, and either enter a number in any one of the three boxes, or use a slider bar to specify a number between .1 and 4.

   – To gamma adjust an image by specifying separate red, green, and blue values, deselect the Lock RGB box, and enter a value for each color.
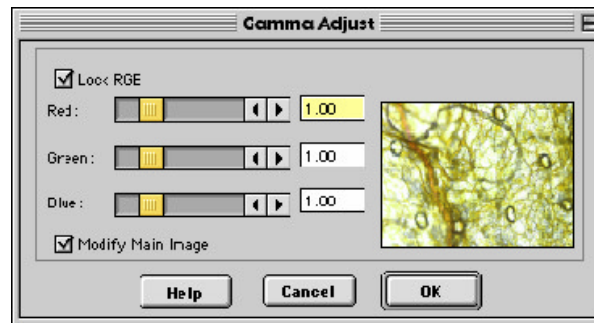
> ✎ *Note: The ability to deselect the Lock RGB box and enter specific RGB values distinguishes the use of the Adjust Gamma menu option from the Gamma Adjust option on the Image Setup window (see Ch. 5 – Preparing to Take a Picture).*

3. Click on OK. The new image appears on screen.

Adjust Gamma (HSL)
Adjust Gamma (HSV)

Like the Adjust Gamma (RGB) option, the Adjust Gamma (HSL) and Adjust Gamma (HSV) options allow you to smoothly lighten darker areas of an image without burning out bright areas or lightening black areas. This is useful when modifying images with a wide *dynamic range*, meaning the range of brightness values recorded by the CCD chip. Unlike the Adjust Gamma (RGB) option, Adjust Gamma (HSL) and Adjust Gamma (HSV) work only on the luminance or value values (respectively) for an image as opposed to the specific red, green, and blue values. Refer to Ch. 5 – Preparing to Take a Picture for a more detailed explanation of gamma adjustment.

To Adjust Gamma (HSL/HSV), follow these steps:

1. From the Edit menu, select Adjust HSL, Adjust Gamma or Adjust HSV, Adjust Gamma.
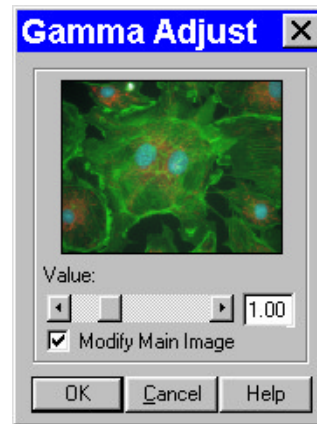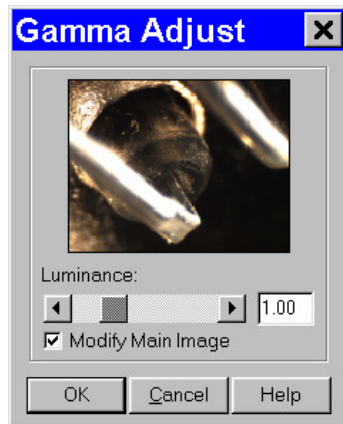
2. Enter a value between .1 and 4, or use the slider bar to select a value. The thumbnail preview of the image changes as you adjust the value.

   – To lighten the mid-tones of an image, enter a gamma value above one.
   – To darken the mid-tones of an image, enter a gamma value below one.

3. Click on OK. The new image appears on-screen.

Stretch Bright and Dark Level (RGB)

The Stretch Bright and Dark Level (RGB) option stretches the darkest level in an image to 0 (black) and the brightest level to full scale. Full scale is 255 for 8 bit monochrome and 24 bit RGB images, and 4,095 for 12 bit monochrome and 36 bit RGB images.

Stretching the bright and dark level (RGB) for a 24 bit RGB image with a range of brightness values between 50 and 150 has the following effects:

- The lowest value pixels (50) are stretched down to 0.
- The highest value pixels (150) are stretched up to 255.

This one-step process provides a quick and easy way to increase image contrast in low contrast specimens. To use Stretch Bright and Dark Level (RGB), select Adjust RGB, Stretch Bright and Dark Level from the Edit menu. The picture automatically adjusts itself.

Stretch Bright and Dark Level (HSL)
Stretch Bright and Dark Level (HSV)

Like Stretch Bright and Dark Level (RGB), the Stretch Bright and Dark Level options in the HSL and HSV color spaces stretch the darkest luminance or value level in an image to 0 (black) and the brightest luminance or value level to 100 (pure white if Luminance/brightest version of hue if Value). Using the

HSL or HSV (for fluorescence images) color space to stretch images, as opposed to the RGB color space, reduces the potential for washed out images and color shifts.

To stretch bright and dark level HSL or HSV, select Adjust HSL, Stretch Bright and Dark Level or Adjust HSV, Stretch Bright and Dark Level from the Edit menu. The picture automatically adjusts itself.

Adjust Histogram (RGB)

A *histogram* is a graphical representation of the number of pixels at each brightness level in an image. SPOT's histogram displays the red, green, and blue histograms on the same graph. It serves two main purposes:

- It is a point of reference for examining the distribution of brightness levels for the red, green, and blue components in an image.

- It can be used to reset the zero and full-scale points for an image. This is known as *stretching*. Like the aforementioned Stretch Bright and Dark Level function, the Adjust Histogram option enables you to stretch the darker areas of your image to pure black and at the same time lighten the brighter areas of the image.

> *Note: Full scale for 8 bit monochrome and 24 bit RGB images is 255. Full scale for 12 bit monochrome and 36 bit RGB images is 4,095.*

To adjust an RGB histogram, follow these steps:

1. From the Edit menu, select Adjust RGB, Adjust Histogram. The RGB Histogram window appears. Set the display parameters for the histogram:

   – Determine the color or colors that you want to display brightness values for. As a default, the histogram displays all three values. To display a different combination of colors, deselect the Show box for the unwanted color(s).

   – To display the histogram according to a logarithmic scale (as opposed to a linear scale), check the Log Scale box. This makes the vertical axis more sensitive to small numbers and compresses the range for larger numbers.
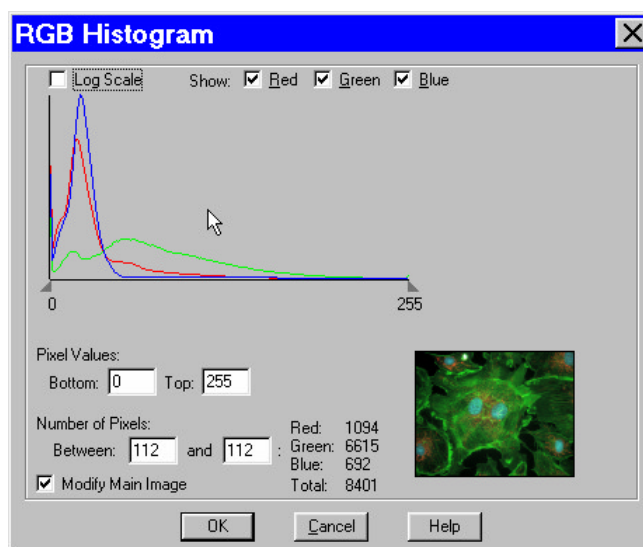
From the RGB Histogram screen you have three viewing/editing options.

- ***Examine the number of pixels at each brightness level.***
  Do one of the following:

  - Move the cursor along the graph to display the number of pixels with red, green, and blue values at each brightness level and the total number of pixels at that level.
  - Type the same brightness level into both boxes under Number of Pixels. The histogram readouts display the number of pixels with red, green, and blue values at that brightness level, as well as the total number of pixels at that level.

- ***Examine the number of pixels between two brightness levels.***
  Do one of the following:

  - Position the cursor over one brightness level on the graph, and left click and drag the cursor to the second brightness level. The histogram readouts display the number of pixels with red, green, and blue values between the two brightness levels as well as the total number of pixels.
  - Type the two brightness levels into the boxes under Number of Pixels. The histogram readouts display the number of pixels with red, green, and blue values between the two brightness levels, as well as the total number of pixels.

- ***Stretch the image.***
  Follow these steps

  a) Either drag the left triangle (on the X axis) to the brightness level that you want to define as zero, or enter the value in the **Bottom** box. All red, green, and blue values that are less than or equal to this level are now reset to zero.

  b) Either drag the right triangle (on the X axis) to the brightness level that you want to define as full scale, or type in the value in the **Top** box.   All red, green, and blue values that are greater than or equal to this level are now reset to full scale.

     The program automatically multiplies all red, green, and blue values between the new zero point and full scale values by the factor necessary to make the new full scale setting equal to actual full scale after the new zero point setting (if you have defined one) has been subtracted.

  c) Click on OK to save the image with the new zero point (Bottom) and full scale (Top) values.

Adjust Histogram (HSL)
Adjust Histogram (HSV)

HSL and HSV histograms are identical to RGB histograms, except that instead of displaying the number of pixels for the RGB components, they display the number of pixels at each luminance or value level. As with the RGB histogram, HSL and HSV histograms are typically used for two purposes:

- Examining the distribution of luminance or value levels in an image.

- Resetting the zero and 100 % luminance/value points in an image. This is known as *stretching*. The Adjust Histogram (HSL) and Adjust Histogram (HSV) options allow you to stretch the darker areas of your image to pure black and lighten the brighter areas to either pure white, if using the HSL color space, or the brightest version of the hue, if using the HSV color space.

To Adjust Histogram (HSL) or Adjust Histogram (HSV), follow these steps:

From the Edit menu, select Adjust HSL, Adjust Histogram or Adjust HSV, Adjust Histogram. The Luminance Histogram or Value Histogram window appears.

2. If you want to display the image's histogram according to a logarithmic scale, check the Log Scale box. Checking the Log Scale box makes the vertical axis more sensitive to small numbers and compresses the range for larger numbers.

From the Luminance/Value Histogram window you have three viewing/editing options.
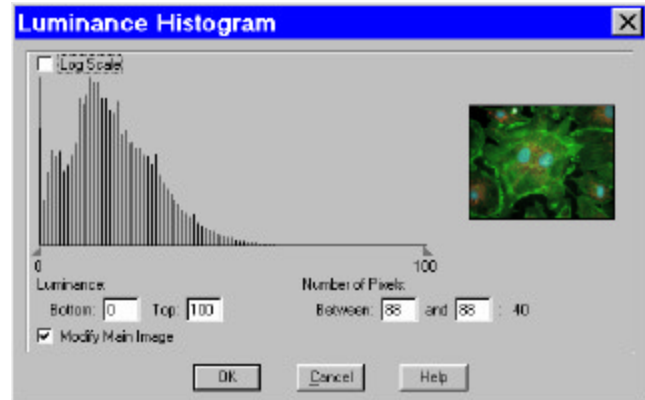
- *Examine the number of pixels at each Luminance/Value level.*
  Do one of the following:
  − Move the cursor along the graph to display the number of pixels at each Luminance/Value level.
  − Type the same Luminance/Value level into both boxes under Number of Pixels. The histogram readouts display the number of pixels at that Luminance/Value level.

- *Examine the number of pixels between two brightness levels.*
  Do one of the following:
  − Position the cursor over one luminance or value level on the graph, and left click and drag the cursor to the second Luminance/Value level. The histogram readouts display the number of pixels between the two levels.
  − Type the two Luminance/Value levels into the boxes under Number of Pixels. The histogram readouts display the number of pixels between the two Luminance/Value levels.

- *Stretch the image.*
  Follow these steps:

  a) Either drag the left triangle (on the X axis) to the Luminance/Value level that you want to define as black (0 % Luminance/Value), or enter the Luminance/Value number in the **Bottom** box. All Luminance or Value values that are less than or equal to this level are now reset to black (0 % Luminance/Value).

  b) Either drag the right triangle (on the X axis) to the Luminance/Value level that you want to define as full scale (100 % Luminance/Value), or enter the Luminance/Value number in the **Top** box. All Luminance/Value values that are greater than or equal to this level are now reset to full scale (100 % luminance/value).

  The program automatically multiplies all Luminance/Value values between the new black and full scale values by the factor necessary to make the new full scale value equal to 100 % Luminance/Value after the new black value (if you have defined one) has been subtracted. The thumbnail sample image updates automatically so you can gauge the effect of your adjustments.

  c) Click on OK to save the image with the new black and white Luminance/Value values.

Filter

The Filter menu option contains five options for modifying images:

- Smooth
- Sharpen
- Unsharp Mask
- Filter Noise
- Median Filter

Smooth

Use Smooth to smooth out grainy images. The smooth option works by lightening the darker side of contrast boundaries and darkening the lighter side. Because the Smooth option removes the high frequency details from an image, some fine image detail is also lost.

To smooth the appearance of an image, follow these steps:

1. From the Edit menu, select **Filter > Smooth**.

   To preview images on the thumbnail image only; deselect the Modify Main Image box.

2. Either use the slider bar to select a Strength value between one and 100, or enter the value in the box.

3. When you have determined a suitable value, click on OK. The smoothed image appears.

Sharpen

Use Sharpen to improve the appearance of soft or blurry looking pictures. The Sharpen option works by darkening the darker side of a contrast boundary and brightening the brighter side of a contrast boundary.

To sharpen the appearance of an image, follow these steps:

1. From the Edit menu, select **Filter > Shar pen**.

   To preview changes on the thumbnail image only, deselect the Modify Main Image box.

2. Either use the slider bar to select a Strength value between one and 100, or enter the value in the box.

3. When you have determined a suitable value, click on OK. The sharpened image appears.

Unsharp Mask

Use the Unsharp Mask filter to improve the appearance of an image's high frequency details. The Unsharp Mask option sharpens an image by subtracting a brightness-scaled, low-pass-filtered image from the original image.

To apply the Unsharp Mask filter, follow these steps:

From the Edit menu, select **Filter > Unsharp Mask.**

   To preview changes on the thumbnail image only, deselect the Modify Main Image box.

Either use the slider bar to select a Strength value between one and 100, or enter the value in the box.

When you have determined a suitable value, click on OK. The sharpened image appears.
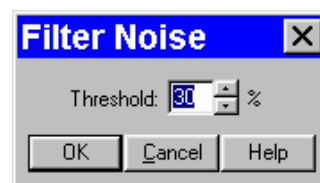
Filter Noise

The Noise Filter corrects for the effects of electrical or thermal noise. The Noise Filter checks each pixel on the CCD chip as described below:

1. The program compares the value of each red pixel with the values of the eight neighboring red pixels.

2. If none of the eight neighboring red pixels has a value within $\pm$ **X** % (X being the threshold value that the SPOT user specifies) of the value for the red pixel being checked, the value for the red pixel being checked is replaced with the average value of the eight neighboring red pixels.

3. The program repeats this procedure for the values of the green and blue pixels being checked.

To filter noise, follow these steps:

1. From the Edit menu, select **Filter > Filter Noise**.

2. Enter a value between **10%** and **100%**. The Threshold value will default to the last used setting. Refer to the procedure described at the start of the Filter Noise section for an explanation of the Threshold value.

3. Click on OK. The filtered image appears on-screen.

> 🖉 *Note: Image setups can be defined to automatically use the noise filter when capturing an image. Refer to Ch. 5 – Preparing to Take a Picture for details.*

Median Filter

The Median Filter corrects for the effects of shot noise. This filter functions by considering a sample pixel and its surrounding 8 pixels. The values of all nine pixels are compared and the median value of the group is substituted for the sample pixel. In this process, all pixels are always being modified, unless, of course, the sample pixel is the median value of the group.

Unlike the Filter Noise function, there is no need for any user inputs regarding the median filter process.

This approach negates any impact that a hot pixel might have on the filtered value of another neighboring (sample) hot pixel.

> 🖉 *Note: Filtering algorithms always utilize original data for each sample pixel being considered. In other words, filtered (substituted) pixel data is not used in the filtering process.*

Make Negative

Make Negative to make a color negative (with complimentary colors) of an image. From the Edit menu, select Make Negative. The Program automatically converts the image to a negative.

Convert to Gray Scale

Use Convert Gray Scale to convert images from color to gray scale. The Convert to Gray Scale option works only with color (24 bit and 36 bit) images.

RT B/W	The Convert to Gray Scale option is available only for RGB images that have been opened with the SPOT software/RT Color camera. For monochrome image captures, the Convert to Gray Scale option is unavailable.

From the Edit menu, select the Convert to Gray Scale option. The program converts the color image to a gray scale image.

Set Palette (monochrome images)

The Set Palette option enables you to apply a specific color palette to a captured monochrome image. There are four pre -defined color options: **Grayscale**, **Red**, **Green**, and **Blue**.
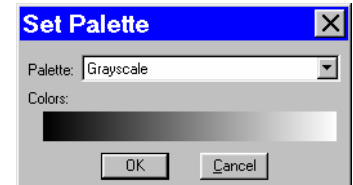
To define a custom palette, use the **Palettes** option (Setup>Palettes). Refer to Ch. 4 – The Advanced Program Mode for more details. **RT B/W** users can also apply palettes as part of the image capture process using the **Palette** option on the Image Setup window.

Match Color

The Match Color feature is typically used in two situations:

- You want to change the colors of an image on the screen to match the way that you think the specimen looks.

- You want to set the white balance for pictures taken with a microscopic technique that never has white in its images. A prime example of this situation is fluorescence microscopy.

> *Note: The Match Color white balance adjustment can be used as an alternative to the Compute White Balance Values option.*

To use the Match Color feature, follow these steps:

a)   From the Edit menu, select Match Color.

b)   Click on OK. The cursor becomes an eye-dropper.

c)   Use the mouse to move the eye-dropper to the pixel location of the color that you want to match. Refer to the status bar at the bottom of the window to ensure that each of the R, G, and B values are less than full scale (255 for 24 bit RGB and 4,095 for 36 bit RGB).

> *Note: If you select a pixel with a red, green and/or blue value that is saturated at full scale, the SPOT program prompts with a warning message indicating that you need to choose a pixel with RGB values less than 255 (24 bit RGB) or 4,095 (36 bit RGB). Saturated pixels do not provide a good reference point for correctly determining the RGB ratios needed for this function.*
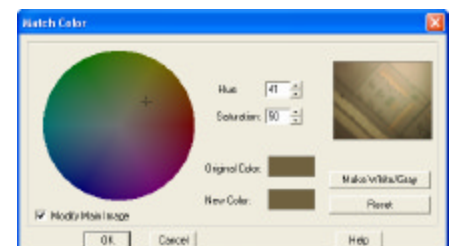
d)   Click on the pixel. The Match Color window appears.

> *Note: The Set White Balance button (not shown above) appears only for image captures that have not yet been saved and closed.*

RT B/W	RT B/W camera users will not see the Set White Balance button.

The window displays information about the pixel that you selected:

- **Hue** – This the actual color, as distinguished from others in the color spectrum. Hue is derived from a color wheel and is expressed in the color's angular location (e.g., 0 to 360 degrees).

- **Saturation** - The proportion of perceived pure hue in the color. Saturation (in the SPOT software) is measured on a numerical scale of 0 to 100, where zero equals pure gray and 100 equals pure hue (i.e., "day glow").

- **Original Color –** A sample "swatch" of the color that you selected. The cross hairs on the color wheel indicate the relative position of the color.

e)   The **Modify Main Image** box is checked as a default.

f)   Set the new color. This can be done in one of two ways, depending on whether the image should contain white:

- If you are trying to make the selected pixel in your image appear as white (or gray if the pixel is darker), click on the Make White/Gray button.
- If you are working with an image that does not have any white, modify the Hue and Saturation values so that the New Color "swatch" shows up as the color that you want. You can do this either by entering specific Hue and Saturation values, or dragging the cross-hairs on the color wheel to the desired location.

At this point there are three options:

- Click on **OK** to save the image with the new color rendition.

- Click on **Reset** to return the image to the original color rendition.

- For images that have not yet been saved and closed, click on **Set White Balance** to set the values so that colors are rendered correctly for future image captures. When you click on Set White Balance, a dialog box displays the white balance values. As a default, the values are saved to the current setup. If desired, you can select a different Setup and then click on OK.
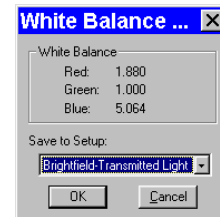
g)   If you reset the White Balance, you can now recapture the image with the new settings.

Customizing Images

In addition to the image editing features des cribed in this chapter, the SPOT software offers several options for customizing images.

- **Change Pixel Bit Depth** – Change from one bit depth to any of the other three options without having to recapture an image.

- **Subtract Image** – Subtract an image from a selected open image.

- **Merge Images** –Merge two or three images, assigning red, green, or blue to each image. The Merge Images feature is mainly used to combine two or three monochrome images into an artificially colored composite image.

Change Pixel Bit Depth

Use Change Pixel Bit Depth to change the bit depth of the currently open image. To change the pixel bit depth for an image, follow these steps:

1. From the Edit menu, select the Change Pixel Bit Depth option. The Change Pixel Bit Depth window displays the current Pixel Bit Depth for image.
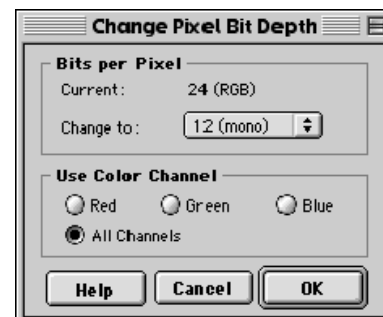
2.   Select the pixel bit depth that you want to change the image to.

   –   Changing from one monochrome bit depth to another (8 bit to 12 bit) or from one RGB bit depth to another (24 bit to 36 bit) does not require the addition of any color channeling information. In this case, select the new pixel bit depth and click on OK.

CAUTION!
If you are changing an image to either 12 bit monochrome or 36 bit RGB, you must save the image in TIFF or JPEG 2000 format before you can close it. Refer to step four for further instructions.

   –   Changing from an RGB (24 bit or 36 bit) color image to a monochrome image (8 bit or 12 bit) requires the entry of color channeling information. When you select the new pixel bit depth, the color channeling information appears.

   Select the color channel(s) that you want to use. Select either a single color or the All Channels option, which is the default.

   ♦   If you select a single color channel, the program uses only the values for that color (in the original image) when it creates the monochrome image.

   ♦   If you select All Channels, the program uses a mathematical algorithm to combine the red, green, and blue values from the original image when it creates the monochrome image.

CAUTION!
If you are changing an image to 12 bit monochrome or 36 bit RGB, you must save the image in TIFF or JPEG2000 format before you can close it. Refer to step four for further instructions.

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3.   Click on OK to save the image with the new pixel bit depth.

4.   If you changed the original pixel bit depth to either 12 bit or 36 bit, follow these steps before trying to save the image to disk:

   a)   From the File menu, select the Save As option. The Save As window appears. The Save as type dialog box displays **TIFF Uncomp (12-bit/channel) (TIFF)**.

   b)   The Save as type drop-down box has two options:

   ♦   **TIFF Uncomp (12-bit/channel) (TIFF) –** Select the 12 bit per channel option if you want to save the image as 36 bpp.

   ♦   **TIFF Uncomp (16-bit/channel) (TIFF) –** Select the 16 bit per channel option if you want to save the image as 48 bpp. This allows you to edit images with Adobe Photoshop, which cannot read 36 bpp images

   ♦   **JPEG2000 –** Select for the improved JPEG2000 compression algorithm that saves file space.

   c)   Enter the desired file name and location, and click on the Save button.
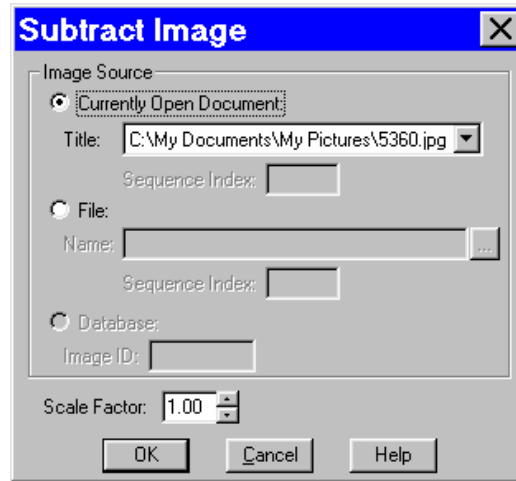
Subtract Image

Use Subtract Image to subtract an image from a selected, open image. Follow these steps:

1. From the Edit menu, select **Subtract Image**.

2. Select the Source of the image that you want to subtract from the currently open, selected image. The image can come from one of the following three sources:

   – A **Currently Open Document** – Subtract an image from a currently open image. If the path for the image you are editing does not appear in the Title box, select it from the Title drop-down list, which lists all open images. If the file is an image sequence, specify the number of the image in the sequence (the Sequence Index).

   > ✐ *Note: It is possible to subtract an image from itself.*

   – An **Image File** – Subtract a file from disk from the currently open, selected image. Either enter the file path in the box, or click on the browse button to the right of the box to search for and select the file. If the file is an image sequence, specify the number of the image in the sequence (the Sequence Index).

   – A **Database (Windows only)** – Subtract a file from a SPOT database from the currently open, selected image. To subtract an image from a SPOT database you must enter the Image ID for that image. You can determine the eight-digit Image ID number by searching the database for the image that you want to add. The search results display the image ID number.

   > ✐ *Note: To subtract a database file, the database must be open. For more information on using SPOT databases, refer to Chapter 9, Using SPOT Databases.*

3. Enter a Scale Factor between **.01** and **5**. The Scale factor adjusts the brightness of the image that you are subtracting:

   – Numbers between .01 and 1 darken the image.
   – Numbers between 1 and 5 lighten the image.

4. Click on OK to add or subtract the image. The combined image replaces the original.

   > ✐ *Note: Subtracting an image modifies the original file. This contrasts with the process of the* Merge Images *function (see below), which creates an entirely new file.*

Merge Images

Use Merge Images to combine two or three monochrome images into a single composite image.   The Merge Images option is used primarily in fluorescence imaging when specimens have been stained with more than one dye, and a triple filter cube is not available. In this circumstance, each dye can be excited individually, and a monochrome image can be captured for each excitation. The Merge Images feature then allows you to combine these images to create a composite image that shows all the dyes. The following call-outs illustrate the different aspects of the Merge Images dialogue:

Scale factor enables you to scale the brightness of a source image before it is merged.

Specify any position offset (in pixels) for the image. This offset is reflected in the final merged image.

Specify the source of the image that you are merging.

Display only the brightness scaling and positioning options for the source images.

Specify whether non-overlapping parts of the source images are included in the combined image.

Apply your settings to the merged image without closing this dialogue.

Automatically scale the brightness of the merged image so that it does not come out too bright.

> ✎ *Note: Merge Images is now found in the File dropdown menu. Past versions of SPOT software included Merge Images in the Edit dropdown menu.*

To merge images, follow these steps:

1.	**Define the source for each image.**	At least two **Source Images** must be selected. If the image that you select is part of an image sequence, the **Sequence Index** box is also active. The source can be:

- A **Currently Open Document** – Select Currently Open Document to merge a currently open image. Specify the number of the image in the sequence, if applicable.

- An image on **File** – Select File to merge a file from disk. Either enter the file path in the box, or click on the browse button (to the right of the box) to search for and select a file from disk. Specify the number of the image in the sequence if applicable.

- A **Database (Windows only)** – Select Database to merge a SPOT database image. To merge a database image you must enter the Image ID for that image. You can determine the eight-digit Image ID number by searching the database for the image that you want to add. The search results display the image ID number. In order to select a database image, the database must be open.

When you are merging monochrome images, the program uses whatever palette that you have set for that image. So, if you defined a specific palette for an image, that color appears in the merged image. If a palette has not been assigned to an image, the program uses the grayscale palette as a default.

Palettes cannot be applied to monochrome images through the Merge Images function. They must be applied prior to merging the image, either via the Image Setup window (pre-capture) or the Set Palette Edit menu option (post capture).

- For information on defining palettes, refer to Ch. 4 – Working in Advanced Mode.

- For information on applying a palettes to an image as part of the image capture process, refer to the Palette Image Setup option, as discussed in Ch. 5 – Preparing to Take a Picture (RT B/W users only).

- For information on applying a palette to a captured image, refer to the Set Palette Edit menu option, as discussed in this chapter.

2.	**Specify the brightness (Scale Factor) for individual images**	For each source image, specify a **Scale Factor** between **.01** and **5**.

- Numbers between .01 and 1 darken the image.

- Numbers between 1 and 5 lighten the image.

3.	**Specify the Position Offsets for individual images**	Changing filters for an image capture can cause its position to shift from that of the previous capture. This can be problematic when trying to merge two or three images captured with different filters. The **Position Offsets** for each individual image in the merged image allow you to compensate for such shifts by specifying **Horizontal** and **Vertical Position Offsets** in pixels.
4.	**If you have defined position offsets, specify whether the merged image will retain the non-overlapping areas.**	To retain the non-overlapping areas, ensure that the **Include Non-Overlapping Areas in Final Image** box is selected. If you do not select the box, the final image automatically discards any non-overlapping areas.
5.	**Specify the Auto-Scale brightness setting for the final merged image.**	Whereas the Scale Factors are applied to individual images before they are Merged, the **Auto-Scale Brightness** feature automatically adjusts the brightness of the merged image to prevent overlapping bright areas from burning out.
6.	**Preview your merged image.**	Click on **Apply** to apply your settings and view the merged image. When you click on **Apply**, the Merge Images dialogue remains open, allowing you to experiment with different Scale Factors and Position Offsets. When experimenting with different values, click on **Shrink** to view only the Scale Factors and Position Offsets – this minimizes the amount of space that the Merge Images dialogue takes up in the window.
7.	**Click on OK.**	When you are satisfied with the appearance of the merged image, click on **OK** to apply the settings and close the Merge Images dialogue.

Duplicate Image

Click on the Duplicate Image option to create a copy of the active (on-screen) image.

Annotation – Calibration and Measurement Marks

The Add Calibration Mark and Add Measurement options enable you to add calibration and measurement references to SPOT images.

Add Calibration Mark...
Add Measurement...
Annotate

Memo...

Add Calibration Mark

The Add Calibration Mark feature allows you to insert a calibration mark into an image capture. To add a calibration mark, follow these steps:

1. If you have not already calibrated the objectives on your microscope, do so. Refer to Ch. 4 – The Advanced Program Mode for details.

2. From the Edit menu, select Add Calibration Mark. The Calibration Mark window appears.

3. Select a **Calibration Setup** (e.g., 15x) for the objective that you are using.

4. Move the cursor to the point in the image where you want to insert the calibration mark and click at that point. The calibration mark appears with the default appearance settings.

📝 *Note: If you are not satisfied with the placement of the calibration mark, you can click on and drag the mark to a new position. Clicking on and dragging the bar moves both the bar and the number. Clicking on and dragging the number moves only the number.*
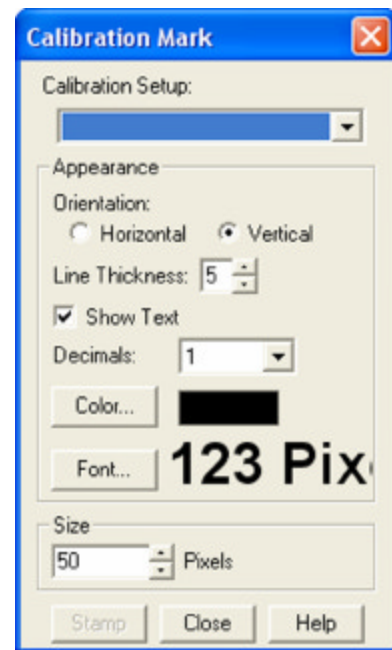
5. Using the on-screen mark as a reference, modify the Appearance options:

   a) **Orientation** – Check the appropriate box to select either a horizontal or vertical display for the calibration mark.

   b) **Line Thickness** – Select a line thickness between 1 (thinnest) and 5 (thickest).

   c) **Show Text** – Select this checkbox if you would like the calibration measurement text to be shown along with the calibration line.

   d) **Decimals** – Select the number of decimals that appear in the calibration mark number. Choose from a range of zero decimals (1) to five decimals (1.00000)

   e) **Color** – Click on the Color button to define the color of the calibration mark. You can either choose from a palette of basic colors or define a custom color to provide the best display.

   f) **Font** – Define the font, point size, and appearance.

6. **Size** - Enter the size of the calibration mark. You can modify the size either by entering the number in the Size box, or by using the cursor to stretch the on-screen calibration bar. To adjust the size of the calibration mark, move the cursor to the end of the calibration bar line. The standard cursor changes to a two-headed arrow. Drag the end of the bar to adjust the length. The calibration mark number changes to reflect the new length.

7. Click on **Stamp** to incorporate the calibration mark into the image.

Add Measurement

The Add Measurement option enables you to incorporate measurements into an image capture, including line, area, and angle measurements.

To add a measurement, follow these steps:

1. If you have not already calibrated the objectives on your microscope, do so. Refer to Ch. 4 – The Advanced Program Mode for details.

2. From the Edit menu, select Add Measurement. The Measurement window appears.

3. From the **Calibration Setup** drop-down list, select the setup (e.g., 15x) for the objective that you are using.

4. Check the type of measurement that you want.

    – **Straight Line** – Point to point distance measurement

    – **Curve Length –** Length of a freehand line

    – **Rectangle Perimeter** – Length around the outside edge of a rectangle

    – **Ellipse Perimeter** – Length around the outside of a perimeter

    – **Region Area** – Area of a freehand outline

    – **Rectangle Area** – Area of a rectangular shape

    – **Ellipse Area** – Area of an elliptical shape

    – **Angle** – Angle measurement

5. Move the cross-hair cursor to the point in the image capture where you want to begin the measurement.

6. Single click to activate the drawing function. ***Do not hold the mouse button down between the measurement points***.

    – For a **Straight Line**, move the cross-hair cursor to the end point of the measurement. Single click to deactivate the drawing function and display the length of the line.

    – For a **Curve**, use the cross-hair cursor to trace a freehand line on an image. Single click to deactivate the drawing function and display the length of the freehand line.

    – For an **Angle**, use the cross-hair cursor to specify the three points that define the angle. Single click to mark the end point of the first side. Move the cross hairs to the vertex, and single click again. Move the cross hairs to the end point of the second side, and single click. This last click deactivates the drawing function and displays the angle measurement.

    – For a **Region Area**, use the cross-hair cursor to trace a freehand outline around the area that you want to measure. Single click to deactivate the drawing function and display the measurement. If
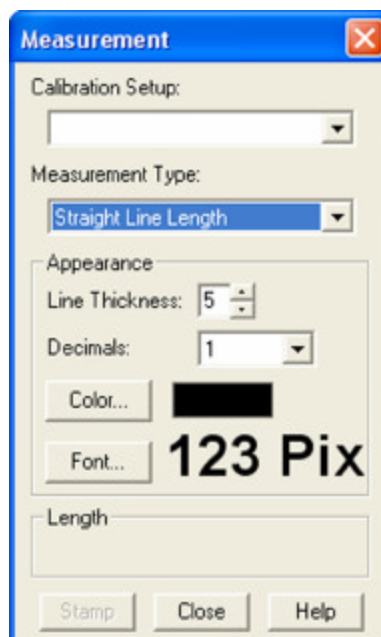
the area is not completely enclosed by the outline, the SPOT program automatically completes the path by connecting the end point to the starting point with a straight line.

When you complete a measurement, the drawing function is deactivated, and the cursor changes back to a standard arrow. At this point you can:

− Re-position the measurement number. When you position the cursor over the number, it changes to a four-point arrow. Click and drag on the number to move it to a new location.

− Erase the last measurement. Double-click anywhere on the image to erase the previous measurement. The cross-hair cursor re-appears for you to draw another measurement

> *Note: Although you may reposition the measurement number, you cannot reposition the measurement lines or curves.*

After you have completed steps one through six, you can modify the measurement as needed, using the Appearance options:

− **Line Thickness** – Select a line thickness between 1 (thinnest) and 5 (thickest).

− **Decimals** – Select the number of decimals that appear in the measurement. Choose from a range of zero decimals (1) to five decimals (1.00000)

− **Color** – Click on the Color button to define the color of the measurement. You can either choose from a palette of basic colors or define a custom color to provide the best display. If you are editing a monochrome image, you can select either black or white.

− **Font** – Define the font, point size, and appearance.

7. Click on **Stamp** to incorporate the measurement into the image. The cross-hair cursor reappears for you to add additional measurements to the image.

Annotation - Custom Image Annotation

Image annotation is useful when you want to prepare captures for formal presentation, or inclusion in an article or reference work. Using SPOT's image annotation features, you can:

- Add text to an image.
- Use an arrow, line, rectangle, or ellipse to emphasize a particular area in an image.
- Add a graphic stamp such as a company logo to an image.
- Add freehand lines to an image.

When you select the Annotate option on the Edit menu, several changes take place in the SPOT image editing environment:

- The **Edit** menu options change to reflect annotation editing, as opposed to the image editing discussed earlier in this chapter.

- The **Object** menu appears with options for inserting drawing objects, text, images, and image related information onto an image. Cursor functionality in SPOT reports varies according to which **Object** menu option is selected.
    - If **None** is selected, the cursor is an editing tool for selecting, sizing, and positioning objects. When creating new reports, **None** is the default option.
    - If a report object is selected, the cursor changes to a cross-hair icon for placing new report objects on the grid.

- The **View** menu displays two options:
    - **Show Pan Window** – Refer to the beginning of this chapter for a description of this function.
    - **Show Tools –** This option displays the Tools dialog box, which contains shortcuts for inserting annotation objects. Refer to the Adding Annotation Objects to SPOT Images section (below) for more details on using the Tools dialog box,

- The **Attributes** menu appears with options for specifying annotation qualities.

- The **Done!** menu (Windows)/ **Mode** menu (Mac) allow you to switch back from annotation mode to image capture/edit mode.
  - **Windows** – The **Done!** menu has only one option, **End Annotation Mode**. When you select this option, the SPOT program prompts you to merge the changes into the image.
  - **Mac** – The **Mode** menu has only one option, **Exit Annotation Mode**. When you select this option, the SPOT program prompts you to merge the changes into the image.

CAUTION!

If you select Yes after clicking on Done! The annotations become a permanent part of the image. If you want to preserve the original image, save the original image to a file. After annotating it, use Save As to save the annotated version to a different file.

## Adding Annotation Objects to SPOT Images

Annotation objects can be added to images in one of two ways:

- Through the **Object** menu.

- Through the Tools dialog box. To access the Tools dialog box, select the **Show Tools** option on the View menu. The Tools dialog box, as illustrated below, displays shortcut buttons to the eleven drawing objects on the Object menu:

Before discussing the individual objects, review the following general procedure for inserting an annotation object into an image:

Note: In some cases, the procedure varies to fit the object's characteristics. The specifics are noted in the descriptions of the individual objects.
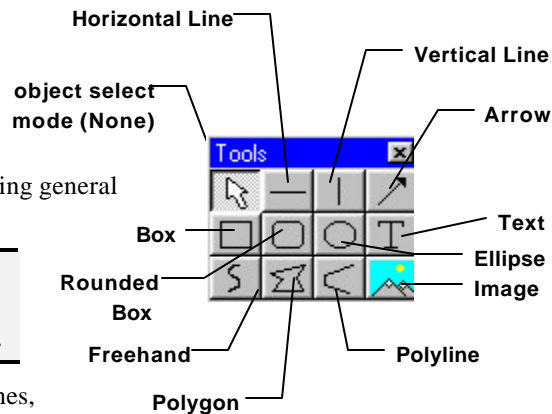
1. From the Object menu, select the type of item (i.e., Lines, Connected Lines, etc…) that you want to add to the image. The cursor's functionality varies according to whether an object type (i.e., line, text) is selected:.
   - If an object type is selected, the cursor changers to a cross-hair icon that is used to add new report objects to the grid.
   - If **None** is selected, the cursor is an editing tool that is used to select and position objects.

2. Use the mouse to move the cross hair to the location where you want to insert the object.

3. Left-click at the insertion point.
   - If you selected a line, box, or other shape, hold down the mouse button and drag the object to the desired size.
   - If you selected one of the other objects, the object will either appear (e.g., Image Time) or the program will prompt you to enter the associated information (e.g., Text, Image Memo, etc…).

4. After you have added the object, there are three options:
   - Add another object of the same type, as described in step 3.
   - Add a different type of object - select from the Object menu options, the toolbox.
   - Resize, reposition, or edit an object. To do so, select **None** from the Object menu.
     - **Resize** - Select (click on) the object and drag the handles in the direction of the resize.
     - **Reposition** – Left click and drag the object the new area.
     - **Edit** – Select the object and choose an option from either the Edit menu or the Attributes menu. You can also edit an object by **right clicking** on it.

The following sections describe each of the annotation objects.

None   Select **None** when you want to use the cursor to edit and position the objects.

Lines   **Unconstrained Line** - Add a straight line annotation to the image

Horizontal Line - Add a horizontal line annotation to the image.

Vertical Line - Add a vertical line annotation to the image.

Arrow – Add an arrow to the image. Either select Lines>Arrow from the Object menu, or click on the Tools box button. Left click on the image and drag toward the area that the arrowhead will point to.

Connected Lines   **Polygon** – Add a closed polygon to an image. Either select Polygon from the Object menu, or click on the Tools box button. Left click and release, and then drag the mouse to create a side. Left click again to start a new side. Double click to finish the polygon. The program automatically adds any additional lines needed to close the figure.

Polyline – Add an open polygon to an image. Either select Polyline from the Object menu, or click on the Tools box button. Left click and release, and then drag the mouse to create a side. Left click again to start a new side. Double click to finish the polyline.

Freehand - Add a freehand line to the image

Shapes   **Rectangular Box** – Add a square cornered box to the image.

Rounded Box - Add a round cornered box to the image.

Ellipse – Add an elliptical object to the image. Ellipses are drawn inside of the rectangular area that you define. Ellipses touch the top, bottom, right and left sides of this rectangle.

Either select Ellipse from the Object menu, or click on the Tools box button. To draw an ellipse, left click at the point where you want to position one vertex of the rectangle and drag the cursor to the point of the opposite vertex.

Images   **Image** – Add an image.

1.   From the Object menu, select Image.
2.   Left click at the point on the display grid where you want to insert the Image box, and holding down the mouse button, drag the cursor to create the desired shape and size for the image (it can also be resized/re-positioned at a later point). When you release the mouse button, the Select Image dialog box appears.

3.   Specify the file source for the image:

–   **Currently Open Image** – Add a currently open image. If you have one or more open images, SPOT lists the file name(s) and path(s) in the drop-down box. Select from the list.

–   **Image File –** Add an image from disk. Specify the file name and location either by entering the path in the Name box, or clicking on the selection box to the right.

–   **Database (Windows only)** – Add an image from a SPOT database. In order to insert a database image, the database must be open. Enter the Image ID for the file. If you do not know the database image ID, search the database – the display table indicates the Image ID for each file.

Click on OK. The image appears on your report grid.

Stamp – Insert a selected bitmap image onto the image capture. From the Object menu, select Stamp. The last selected bitmap appears on your image. To select a new bitmap image to insert, choose the Stamp Bitmap option from the Attributes menu.

Because the Mac edition does not yet have database functionality, Mac users will not be able to use the Database option. Diagnostic Instruments provides free software updates via the Web to all SPOT camera owners. Check the Diagnostic Instruments web site (www.diaginc.com) for the most recent update. For details on finding and downloading the latest version of the software, refer to the **Free Software Updates** section in "About This Manual."

Text

Text - Add text to the image.

1. Either select **Text>Text** from the Object menu, or click on the Tools box button.
2. Left click at the point where you want to insert the text. The Text window appears.
3. Enter the text.
4. Click on OK. The text box appears.
5. Resize the box as needed to display the text.

Current Date - Add the current date to the image. This date defaults to the date set on the computer you are using.

Current Time - Add the current time to the image. This time defaults to the time set on the computer you are using.

Image Date - Add a date to the image. This date defaults to the date that the image was captured. To change the date, double click on the box, and enter the desired date.

Image Time - Add the image capture time to the image. This time defaults to the time that the image was captured. To change the time, double click on the box, and enter a new time. The time that appears here varies according to whether the file was transferred across a network, and whether the file is a SPOT database image. Refer to the Image Date/Time section of this chapter for more details.

Image Title - Add a title to the image. The title automatically defaults to the file name and location of the open image, e.g., **C:\images\cells\fluor1**. To change the title, double click on the box and enter a new title.

Image Memo – Add a previously defined TIFF image memo. Click at the point where you want to insert the text box. To change the memo, double click on the box and modify the text as needed. Changing the text in the memo annotation does not affect a memo that is attached to the image file.

Image Sequence Index (sequence images) - Add the Sequence Index (number) to the image. This is the number of the image within the sequence – i.e., the Sequence Index for the first image in the sequence is "1," the second image "2," etc…

Image Sequence Size (sequence images) – Add the total number of images in the sequence to the image.

Image Sequence Elapsed Time (sequence images) – Add the elapsed time between the image being annotated and an image that you specify. After selecting **Object>Text>Image Sequence Elapsed Time**, the program prompts you to enter the **Reference Image Index Number**. If you specify a number that comes after the image being annotated, the elapsed time will be negative. For instance, if you are annotating image number five in a sequence of 10, and you specify a Reference Image… of eight, the elapsed time will be a negative value.

User Name – Add the user's name to the image. This defaults to the user who is logged on the computer.

AutoNumber – Adds numbering to the image starting at 1 and incrementing by 1 for each click of the mouse. Good for counting and tracking objects in an image. You may elect to change the starting number to a value greater than one by defining Autonumber's attributes (see section below).

Defining Annotation Attributes

All of the general style and appearance options can be accessed from the Attributes menu. Annotation attributes can be defined for an entire report or on an object-by-object basis (see the Adding Annotation Objects to SPOT Images section above).

- When an annotation object is selected, the attribute is defined for the selected object(s) only. In addition, when you right click on an object, the menu displays the attribute options for that object.

- When nothing is selected, the attribute is defined for all future, relevant objects.

The following table details each of the annotation attributes and the report objects that they affect.
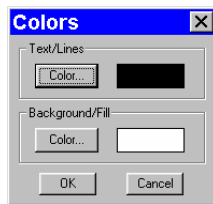
Colors

Define the foreground and background colors for all objects. Follow these steps:

1. From the Attributes menu, select Colors.

2. Define the **Text/Lines** color. This is the color of the text, the line, or the border around a text/image box. The default foreground color is black. To select a color click on Color.

3. The next window varies, depending on whether you are using a Windows or a Mac system.
    – Windows allows you to select from a palette of colors or enter specific HSL or RGB color values.
    – Mac OS allows you to select from a palette of colors, enter specific RGB, CMYK, HLS, or HSV values, and enter Internet specific colors.

4. Define the **Background/Fill** color. This is the color that appears behind text and in boxes. Lines and images do not display a background color.

Line Thickness

Determine line thickness for the following objects: **Line** , **Horizontal Line** , **Vertical Line** , **Arrow, Box**, **Rounded Box**, **Ellipse**, **Freehand**, **Polygon**, and **Polyline** .

1. From the Attributes menu, select Line Thickness.
2. Enter a Thickness value between **1** and **5** pixels.
3. Click on OK.

Note: In order to define a background/fill color for a Box or a Rounded Box, a fill must be selected. Similarly, in order to define a background/fill color for a text box, the background (see Text Border, Justification, and Background below) must be set to Opaque.

Box/Ellipse Fill

Define the appearance of the **Box**, **Rounded Box**, and **Ellipse** objects.

1. Specify whether you want a fill for the object.
    –   If you do not want the box to be filled (i.e., you want a transparent box), click on OK.
    –   If you want to specify a fill pattern for the box, check the Fill Box/Ellipse. The **Pattern** options appear.

2. Specify a pattern by clicking on one of the seven options.

3. Click on OK.

Image Border

Determine the line thickness for image borders. This option applies only to the **Stamp** and **Image** objects.

1. Select the object.

2. To create a border around the image, click on the Border box, and enter a **Thickness** value between **1** and **5** pixels..

3. Click on OK.

Arrow Size and Direction

Specify the size of the arrowhead and define the direction that the arrow points.

1. To specify the size of the arrowhead, enter a value between **1** and **10**.

2. Specify the direction of the arrow:
    –   To draw an arrow starting tail first and finishing with the point (head), select **Tail to Head**.
    –   To draw an arrow starting point (head) first and finishing with the tail, select **Head to Tail**.

3. Click on OK.

The Arrow Size and Direction attribute is not available for Mac users. For Mac users, arrows are always drawn **Tail to Head**.

Text Font
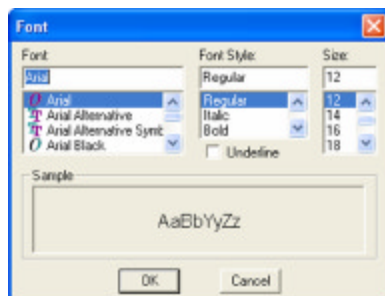
Define the appearance of the text for the **Text**, **Image Date**, **Image Time**, **Image Title**, **Image Memo** objects.

1. Select a **Font**, a **Font Style**, and a point **Size**. The Sample box displays a preview of the font.

2. Define any Underline effects.

3. Click on OK.

Text Border, Justification, and Background

Define the appearance of the text boxes for the **Text**, **Image Date**, **Image Time**, **Image Title**, **Image Memo** objects.

1. Specify whether the text box should have a border. To add a border, click on the **Border** box, and enter a Thickness value between **.05** and **10**.

2. Define the **Justification** of the text. This justification applies to the position of the text in the box, not the position of the text on the page.

3. Define the Background. Select either **Transparent** or **Opaque**.

4. Click on OK.

Title Options
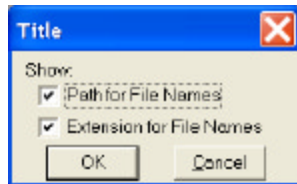
Define the appearance of the title associated with the captured image(s).

1. Specify whether the title should include the computer file storage path as part of the image title.

2. Specify whether the title should include the computer file name extension as part of the image title.

Click on OK.

Time Options

Define the appearance of the Time stamp associated with the image(s).

1. Specify whether the time stamp should include seconds.

2. Specify whether the time stamp should include milliseconds.

Click on OK.

Stamp Image
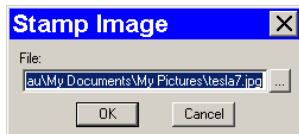
Use the Stamp Image option to select the image that you want to use for the Stamp option. To select the image, choose Stamp Image. The Stamp Image dialog box prompts you to enter a file name and path:

- If you know the name and location of the image file, enter it in the File box.
- If you do not know the name and location, of the image, click on the browse button to search for the file.

After you have selected the image file, click on OK.

AutoNumber Value
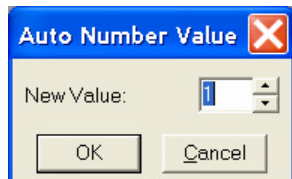
Define the starting number used in the AutoNumber feature.

1. Select a whole number that sets the beginning value once the AutoNumber feature is activated. (Numbers subsequent to the initial number will increment by 1).

Click on OK.

Editing Annotation Objects

Once you have annotated an image, you can edit the annotation objects as needed. There are three ways to edit objects:

- **Use the Attributes menu** - The Attributes menu, as noted in the Defining Annotation Attributes section of this chapter, allows you to either set the attributes for adding specific types of objects, or to select existing objects and modify them as needed. Refer to the Defining Annotation Attributes section for more details.

- **Use the Edit dropdown menu** - The Edit menu contains general editing features such as Undo, Copy, Cut, Send to Back, etc.

- **Right-click on the report object -** Right clicking on a report object brings up a customized editing menu that contains both general editing commands and the attributes that can be edited for that object.

You can also move and size annotation objects:

- To move an object, left click on it and drag the item to a new location. When you drag the item, the cursor becomes a four-pointed arrow.

- To size an object, left click on it and drag the handles to resize the object as needed.

The following sections describe the available editing options from the editing dropdown menu.

Undo	Undo the last action. Unlike the image capture/editing Undo feature, the annotation Undo undoes up to ten changes.
Bring to Front	Bring the selected annotation in front of all other annotations.
Move Forward	Bring the selected annotation one layer forward.
Move Backward	Send the selected annotation one layer back.
Send to Back	Send the selected annotation behind all other annotations.
Select All	Select all annotation objects.
Unselect All	Unselect all annotation objects.
Delete (Windows)	Delete the selected annotation object(s).
Clear (Mac)	Delete the selected annotation object(s).
Delete All	Delete all of the annotations on the image.
Copy to Clipboard	Copy the selected annotation clipboard.
Cut	Remove the selected annotation and place it on the clipboard.
Paste	Place the cut or copied annotation from the clipboard into the image.

Duplicate   Create additional copies of the annotation object. The program prompts you to enter the number of copies. When you click on OK, the duplicates appear at the top of the window.

Set Position, Alignment, and Size   The **Set Position**, **Alignment**, and **Size** option allows you to specify precise sizing and position values for single or multiple selected report and annotation objects.

The **Position** options enable you to:

- Align top/bottom edges of multiple objects.

- Align left/right edges of multiple objects.

- Align the centers of multiple objects.

- Center an object(s) on the image.

- Enter exact pixel values position the object.

The **Size** options enable you to enter:

- Exact width/height in pixels for one or more objects.

- Set the width/height dimension for multiple objects to the smallest or largest objects in pixels.
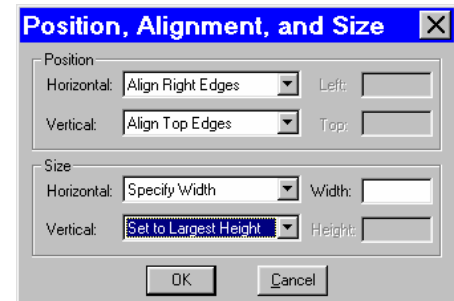
Memo

In the SPOT software, you can attach a memo to TIFF or JPEG type format image files.   To use the Memo option, follow these steps. From the Edit menu, select Memo.   The Image Memo window appears for you to enter a new memo or edit an existing memo.

1.   Enter the message.

2.   Click on OK to attach the memo to the image.
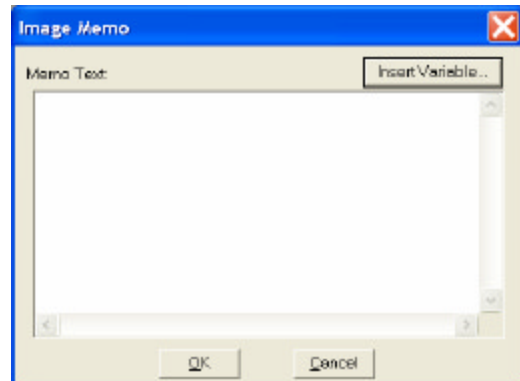
✎   *Note: If you attach a memo to an image that is not in either TIFF or JPEG format and then attempt to save the memo (i.e., when you close the image file), the SPOT program displays a warning message that asks you if you want to save the image without the memo. If you want to retain the memo, click on Cancel and use the* **Save As** *option to save the image in either TIFF or JPEG format.*

Insert Variable

You may insert a variable in your memo from a variety of preprogrammed options. Once the variable has been selected, the next time the memo box is opened. The variables value will be displayed as part of the memo message.

1. Select Insert Variable on the Memo Text box.

2. Choose a variable from the dropdown list. The variable box may change to reflect characteristics of or more information required to define that specific variable.

3. Input any additional information or directions as required.

4. Click on OK to insert the variable
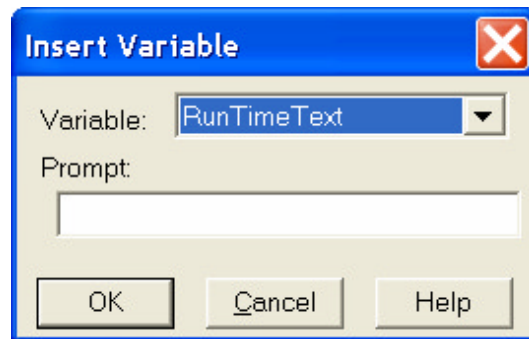
As an example, if we wanted to insert the current time in the memo, the development of the memo may be as follows:

1. Select Memo from the dropdown menu and begin typing your text message.
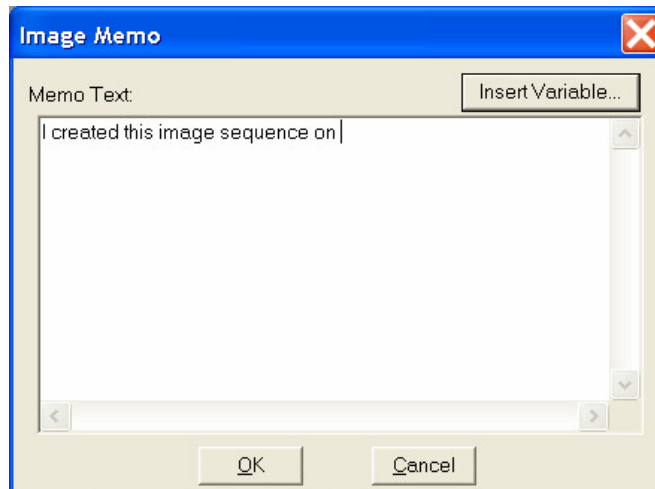
When you get to the point where the variable is to be inserted, select Insert Variable.

From the dropdown menu next to variable, select the appropriate variable. In this case, we select ImgDate (Image Date).
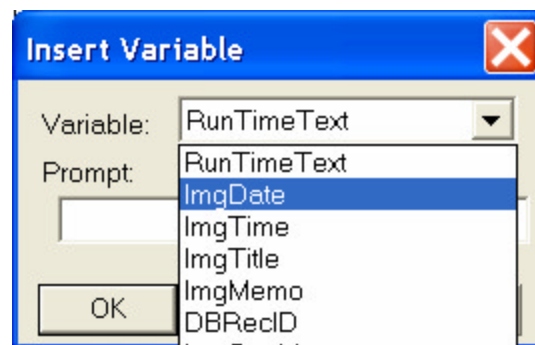
The Variable window changes to match the variable selected. In this case, no other information is required for this selection.
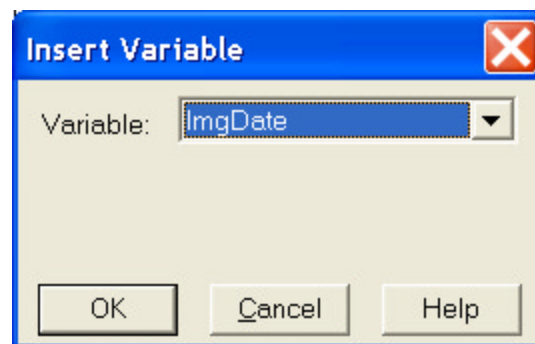
Select OK and the variable is inserted into the text memo in its shorthand form.
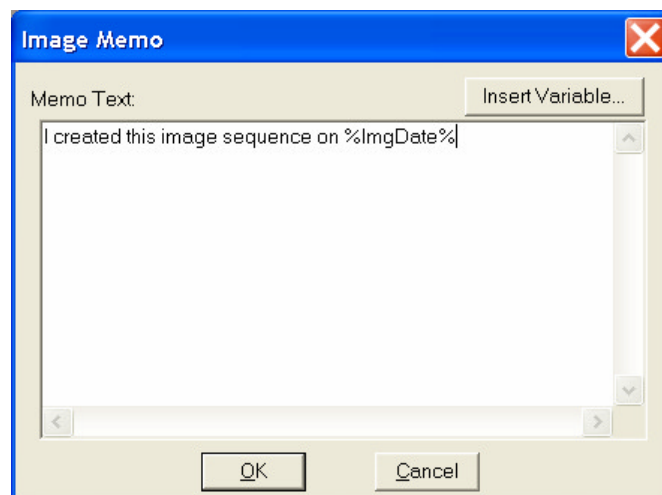
Click OK to accept the memo

The next time you access this memo, the variable value is displayed in lieu of the variable shorthand form.

Image Memo

Memo Text: Insert Variable...

I created this image sequence on 7/9/2003

[OK]   [Cancel]

Ch. 9 – Using SPOT Databases

QUICK REFERENCE:

Introduction

> ⓘ SPOT's image archiving database features are not yet available for the Mac edition of the program. As soon as this functionality is available, you will be able to download the updated version of the software from Diagnostic Instruments' web site, **www.diaginc.com.** Check the site periodically to determine the most recent update. For detailed steps on downloading the software, refer to the **Free Software Updates** section in "About this Manual".

In addition to the numerous image capture and editing features, the SPOT software provides you with an easy to use, fully featured image archiving database.

> ✎ *Note: If you are using SPOT databases from an earlier version of the SPOT software, you may need to convert the old databases to work with the latest version (4.0) of the software. Refer to Appendix E: Converting Previous Version SPOT Databases for detailed steps.*

This chapter is divided into the following sections:
- The Database Menu
- The SPOT Sample Database
- Creating and Modifying Databases
- Searching Databases

The Database Menu

The Database menu contains all of the options for accessing, creating, modifying, and searching SPOT databases. When you first open the SPOT program, only the first two options, **New** and **Open** are active. The other options remain inactive (grayed) until you either create a new database or open an existing database. In addition, **Edit Database Record** does not appear on the menu unless a database image is open. Each option is described below.

- **New** – Create a new SPOT database. Refer to the "Creating and Modifying Databases" section for details.

- **Open** – Open or delete an existing SPOT database.

> ✎ *Note: Only one database can be opened per session. If you try to open a second database, the program prompts you to close the open database.*

- **Close** – Close a SPOT database.

- **Password** – Define a password to protect a SPOT database.

- **Fields** – Define the text and numeric fields, look-up lists, and default options for your database.

- **Preferences** – Define the location and format of the image files in your database.

- **Search** – Define parameters for database searches.

- **Edit Database Record** - Edit information associated with a database image, including field data, time and date information, file save location, and memo text.

The SPOT Sample Database

For your reference, a fully functional sample database is included with your software. We recommend that you browse through this database to familiarize yourself with the features and functionality. The following section is a "visual tour" of the SPOT Sample Database.

Open the SPOT Sample Database

1. From the Database menu, select **Open.** The SPOT program prompts you to select the database.
2. Select **SPOT Sample Database**, and click on **Open**. SPOT prompts you for a password.
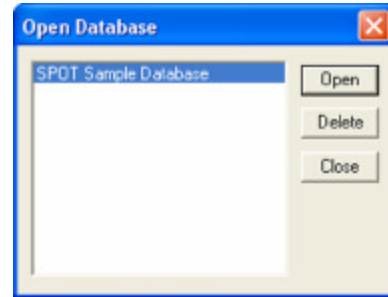
Note that the SPOT database icon appears at the bottom of your Windows screen, indicating that the database is open.

View the Database Structure

From the Database menu, select **Fields**. The **Database Fields** window illustrates the different types of fields that you can define for a SPOT database:
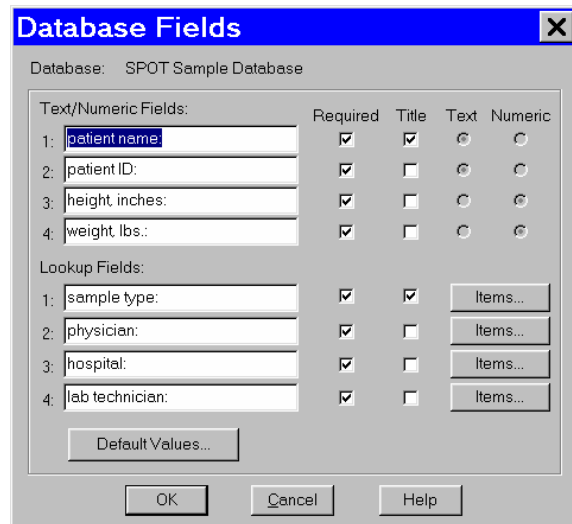
Click on the **Items** button for the **Sample Type** lookup field to view a list of the sample type options for the SPOT Sample Database.

From the **Lookup Field Items** window, you can Add, Modify, or Delete items in the lookup list.
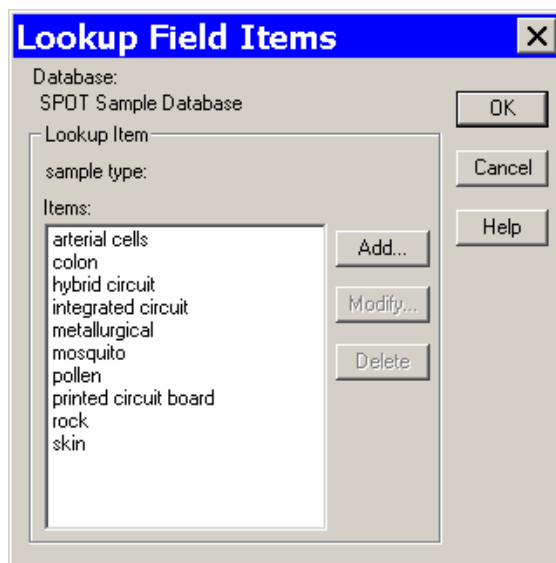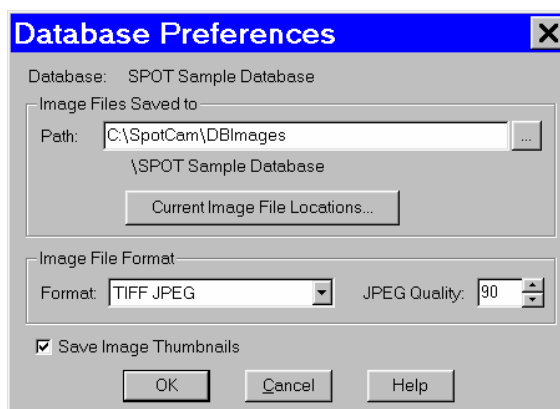
View Database Preferences

From the Database menu, select **Preferences**
The Preferences window is the starting point for defining:

- Image file/database location
- Image file format
- Thumbnail save options

Refer to the **Defining File and Save Preferences** section of this chapter for details.
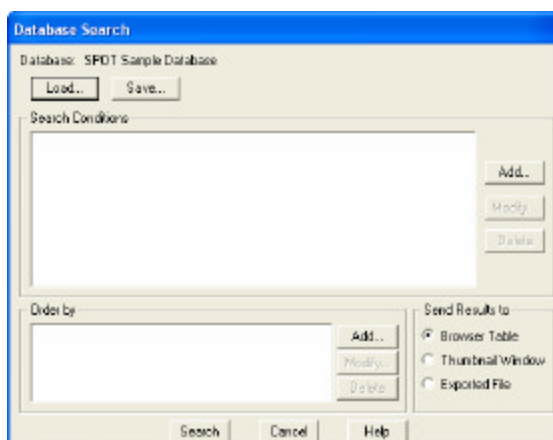
Database Preferences

Database: SPOT Sample Database

Image Files Saved to

Path: C:\SpotCam\DBImages

\SPOT Sample Database

Current Image File Locations...

Image File Format

Format: TIFF JPEG   JPEG Quality: 90

☑ Save Image Thumbnails
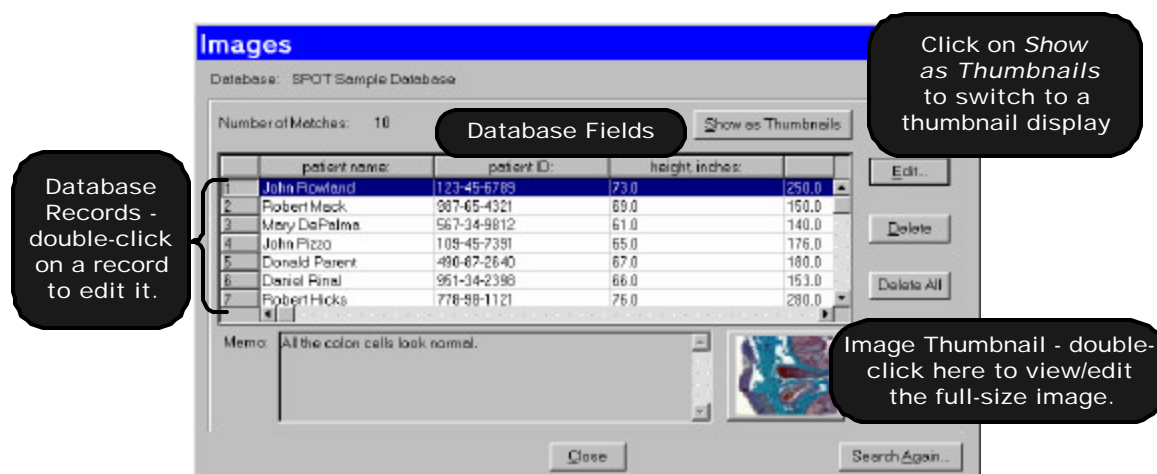
OK   Cancel   Help

Search the Database

From the Database menu, select **Search.** The Database Search window allows you to:

- Define search and sort parameters

- Save a specific set of search parameters

- Choose display options for search results (i.e., tabular or thumbnail display).

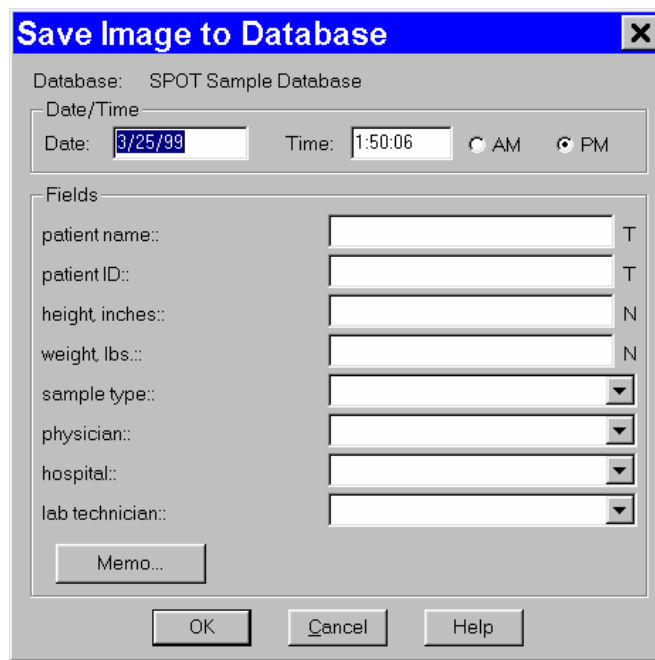Refer to the **Searching Databases** section of this chapter for details.

Database Search

Database: SPOT Sample Database

Load...   Save...

Search Conditions

Add...
Modify
Delete

Order by

Add...
Modify
Delete

Send Results to
⦿ Browser Table
○ Thumbnail Window
○ Exported File

Search   Cancel   Help

Click on Search. Clicking on Search without entering any conditions results in a display of all the records in the database:

Images

Database: SPOT Sample Database

Number of Matches: 10   Show as Thumbnails

Database Fields

Click on *Show as Thumbnails* to switch to a thumbnail display

	patient name:	patient ID:	height, inches:	
1	John Rowland	123-45-6789	73.0	250.0
2	Robert Mack	987-65-4321	69.0	150.0
3	Mary DePalma	567-34-9812	61.0	140.0
4	John Pizzo	109-45-7391	65.0	176.0
5	Donald Parent	490-87-2640	67.0	180.0
6	Daniel Rinal	951-34-2398	66.0	153.0
7	Robert Hicks	778-98-1121	76.0	280.0

Database Records - double-click on a record to edit it.

Edit...
Delete
Delete All

Memo: All the colon cells look normal.

Image Thumbnail - double-click here to view/edit the full-size image.

Close   Search Again...

Save an Image to the Database

Open an image file, and from the File menu, select Save to Database:

All of the required fields must be entered before the image can be saved.

Creating and Modifying Databases

The following sections outline the details involved in creating and/or modifying SPOT databases.

- Setting Up the Database
- Defining Database Fields
- Defining File and Save Preferences
- Saving Images
- Editing Database Image Records
- Moving Existing Database Images
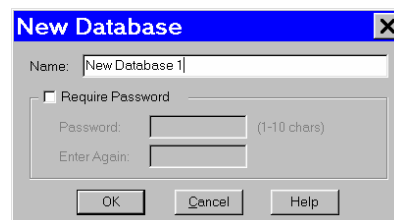
Setting Up a New Database

The initial set up for a new database consists of two steps:

- Naming the database (required)
- Defining a password for the database (optional)

> ✎   *Note: If you do not define a password during the initial definition, you can use the Password option on the Database menu to add a password at a later point..*

To set up your database, follow these steps:

1. From the Database menu, select **New**.
2. Enter a **Name** for the database.

3. Define a password for the database, if needed. Follow these steps:

   a) Check the Require Password box. The Password and Enter Again boxes are activated.

   b) Enter a 1-10 character alphanumeric password.

   c) Re-enter the password.

4. Click on **OK**. The **Database Preferences** window appears. At this point, you can:

   – Define the database preferences. Refer to the next section, **Defining File and Save Preferences**, for details.

   – Click on **OK to** skip defining **Database Preferences** and proceed directly to defining the database fields and lookup lists (i.e., the **Database Fields** window).   Refer to the **Defining Database Fields** section of this chapter for details.

   > ✎ *Note:* **Database Preferences** *can be defined/modified at any future time by selecting the* **Preferences** *option on the Database menu.*
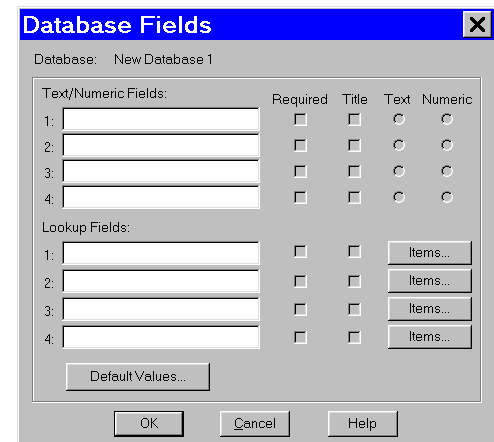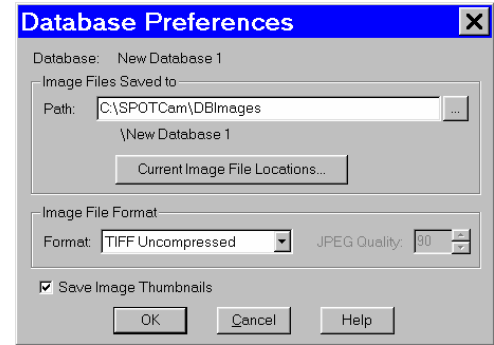
Defining File and Save Preferences

Before saving images to a database, you should specify your file and save preferences for the database. This includes:

- Location and path for database images
- Image file format
- Thumbnail save preference

All of the file and save preferences are defined from a single point, the **Database Preferences** window. The Database Preferences window automatically appears when defining a new database. If you want to define the file and save preferences at a later point, select the **Preferences** options from the Database menu.

Storing Archived Images – An Important Consideration

Before proceeding to define the file and save parameters for your database, you should carefully plan how and where you will store archived images, as well as how you will accommodate future images. Because each uncompressed image file ranges from between 1.3 Mb to 8 Mb, saving and storing large quantities of images requires planning. There are a number of options for storing/archiving images including your computer's hard drive and external storage devices including Zip, Jaz, DVD RW, external hard drives, mini-drives, and your system network. For more details on storage planning, contact your MIS department or a qualified computer or network specialist.
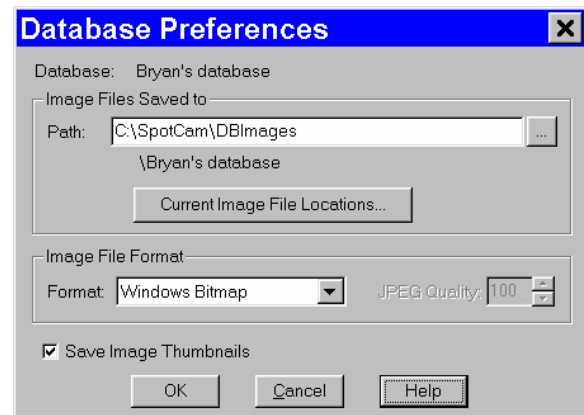
Location and Path of Database Images

SPOT databases are structured so that the database and the images stored in that database do not have to reside in the same location. This structure allows you to keep the relatively small database program files on your hard drive, while storing the larger image files in a different location. The name and location of the database - **SPOT Sample Database** in the illustration above - appears under the path for the database images; it cannot be changed from this window. The database always resides in the default location, **C:\SpotCam\DBImages**. Although the default location for image files is also **C:\SpotCam\DBImages**, we recommend that you define an alternative location such as a Jaz drive or a network drive. If you plan to save images to removable media such as ZIP or Jaz drives, you should, at this point, define the volume label for the disk.

Because a single database of images can span more than one disk, defining unique volume labels for each disk provides a reference so that you can conveniently access images in the future.

To define a volume label for a disk, follow these steps:

1. Ensure that the ZIP or Jaz disk is in the drive.

2. From the Windows 98SE, ME, and XP Explorer, single click on the ZIP or Jaz drive icon.

3. From the File menu, select the **Properties** option; or, right click on the drive and select the Properties option. The Properties window appears. If you do not see a view similar to that in the illustration at right, click on the **General** tab.

4. Enter a name in the **Label** box. For ease of reference, you should name the volume so that it tells you both the name of the database and the disk number.

5. Click on **OK**.

6. Write the volume label on the ZIP or Jaz disk. This way, when you search a multi-disk image database, and the program prompts you to enter the disk number, you will be able to find the information.

7. Repeat steps 1 – 6 for each disk of images associated with a database.

If you are saving images to an existing database, you can click on the **Current Image File Locations** button to display the location of the saved image files and change the location and/or volume label for the disk. The Database Image File Locations window appears.

To change the File Path and/or the Volume label, either double click on the File Path/Volume information, or select the information and click on Modify. The **Image File Location** dialog box appears.

From this window you can:

- Enter a new location for the database images in the Path box; or, click on the button next to the Path box to browse for a new location.

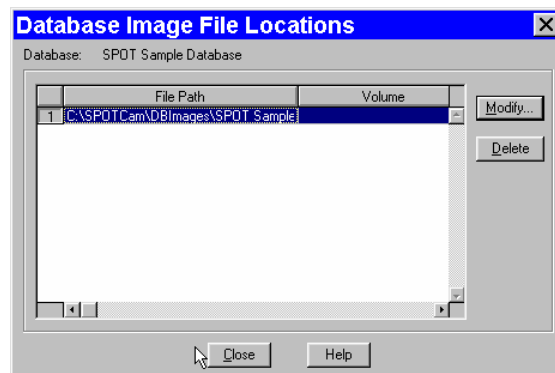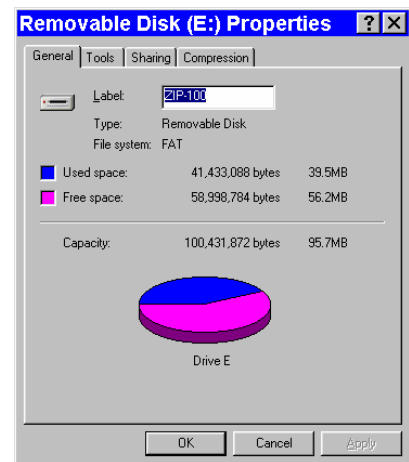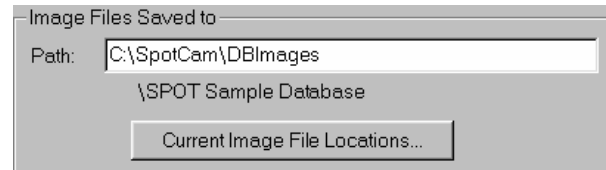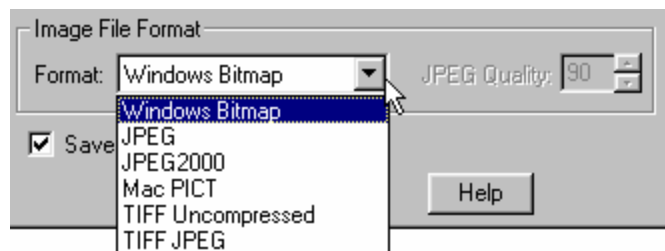- Enter a new Volume label for the disk.

Image File Format

From the Preferences window, you can define the graphic format of the images saved to a database. There are six options:

- Windows Bitmap
- JPEG *(allows for image compression)*
- JPEG2000 *(allows for image compression)*
- Mac PICT
- TIFF Uncompressed
- TIFF JPEG *(allows for image compression)*

Windows Bitmap is the default option. To define image file format, select one of the formats from the drop-down list. Selecting either the JPEG or the TIFF JPEG option activates the JPEG Quality box. This option allows you to compress images (i.e., lower their resolution) so that you can save disk space in cases where high-resolution images are not needed.

To define JPEG Quality, select a value between 1 and 100:

- A value of 1 represents the lowest resolution and the smallest file size.
- A value of 100 represents the highest resolution and the largest file size.

For JPEG2000 Quality, select between lossless (minimal comp ression) and lossy. Select a ratio for lossy compression. A higher number equates to greater compression with greater chance of data loss.

Thumbnail Save Preference

Determine whether you will **Save Image Thumbnails** for the database. The Save Image Thumbnails option saves a thumbnail of each image in the database. This allows you to visually browse through the images in a database. You can also open a database image from a thumbnail.


CAUTION!

When you select the Save Image Thumbnails option, the thumbnail images are saved to the SPOT database file, which resides on your hard drive at C:\Spotcam\Database\[name of your database].db. Each thumbnail increases the size of the database file by approximately 6 Kb.

Defining Database Fields

The Database Fields window is a centralized point for defining the details associated with a database. There are three processes associated with the definition of database fields:

- Naming and defining **Text/Numeric Fields** – The textual/numeric fields that you define represent the data you want to be associated with an image. Typical text fields include patient name and ID number. Typical numeric fields include height and weight.

- Naming and defining **Lookup Fields** – Lookup fields also represent data that you want to be associated with an image. Lookup fields are used in cases where the data item has a limited number of discrete options (e.g., blood type). Each lookup field is associated with a user-defined list of items, enabling you to select an option from a list, as opposed to manually entering it each time an image is saved to the database.

- Setting **Default Values** for the fields – To speed the process of saving images to a database, you can define default values to automatically appear for a field. This helps you to avoid multiple entries of the same item when numerous images have the same data for a particular field.
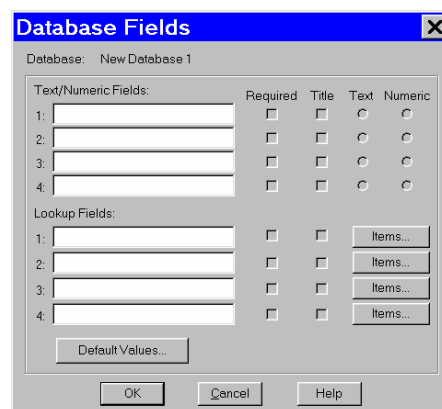
Text/Numeric Fields

To define a textual/numeric field, follow these steps.

1. On the Database Fields window, enter the name of the field in the Text/Numeric Fields box. You can use up to 30 alphanumeric characters. Entering information in the box activates (un-grays) the four variables to the right of the box.

2. Define the four variables associated with each field.

    −   **Required** – Check (click on) the Required box to make the field a required entry when saving images to the database. When this box is checked, images can not be saved until the field has an entry.

    −   **Title** – Check the Title box to ensure that the data from the text field appears in the title of the image window and under any thumbnail of the image.

    −   **Text** – Click on the Text button to define the field as a text field. This is the default setting for the field type.

    −   **Numeric** – Click on the Numeric button to define the field as a numeric field.

    The field type (i.e., text or numeric) is important in terms of how the program searches the database. Text field searches look for textual strings of information while numeric field searches look for a range of values. So, a database field that contains numbers should not automatically be defined as a numeric field. For instance, if your field is a number such as a patient ID, it should be defined as a Text field because it is not a measurement and it is not within a specific range of values.

CAUTION!

After you have defined the Text/Numeric fields, you cannot change the Text or Numeric setting. Although you can make changes to an existing field name (i.e., to correct a spelling error or make a more intuitive name), you should not change the meaning of the field. This will render the field useless for searches performed on all previously stored images.

Lookup Fields and Lists

Defining a Lookup Field consists of three actions:

*   Naming the Lookup Field

*   Selecting the Required and Title variables

*   Creating the items in the Lookup Field list

The SPOT database allows you to perform these actions in different orders, depending on your situation. Consider the following scenarios:

*   You are the database administrator, and you want to define a new database with multiple Lookup Fields for others to use when saving images to the database. You know ahead of time which Lookup Fields you want to define, and you also know all of the items that will be associated with each Lookup Field.

    In this scenario, you can define the Lookup Fields, the Required and Title options, and some or all of the items in Lookup Field list.

*   You are an end user and know which Lookup Fields you want to define, but prefer to add items for each field, as needed, when saving images to the database.
    You can define one or more Lookup Fields without adding any of the Lookup Field list items. The Lookup Field items can be added either at a later time, or during the process of saving images to the database.

To define a Lookup Field and the associated list, follow these steps:

1. Enter the name of the first Lookup Field.

2. Select the **Required** and **Title** options.

   – **Required** – Check (click on) the Required box to make the field a required entry when saving images to the database. When this box is checked, images cannot be saved until the field has an entry.

   – **Title** – Check the Title box to ensure that the data from the text field appears in the title of the image window and under any thumbnail of the image.

   You can also leave one or both options unchecked. If you do not want to enter the Lookup Field list items at this time, you can enter any remaining Lookup Fields and click on **OK** to save the database.

3. Click on the **Items** button. The **Lookup Field Items** window appears with the name, as shown at right.

4. Click on **Add**. The **Lookup Item** dialog window appears for you to enter the list item.

5. Enter the name of the list item in the box.

6. Click on OK. The program returns you to the Lookup Field Items window. In the illustration to the right, the user has added the **A +** item to the Blood Type Lookup Field.

   From the Lookup Field Items window, you can:
   – Click on **Add** to add additional items to the Blood Type lookup list.

   – Select a lookup list item and click on **Modify** to edit the item or **Delete** to remove the item from the list.

   – Click on **OK** to save the lookup list and the associated items. The program returns you to the Database Fields window.

7. Follow steps 1-7 to enter additional Lookup Fields. You can enter up to four per database.
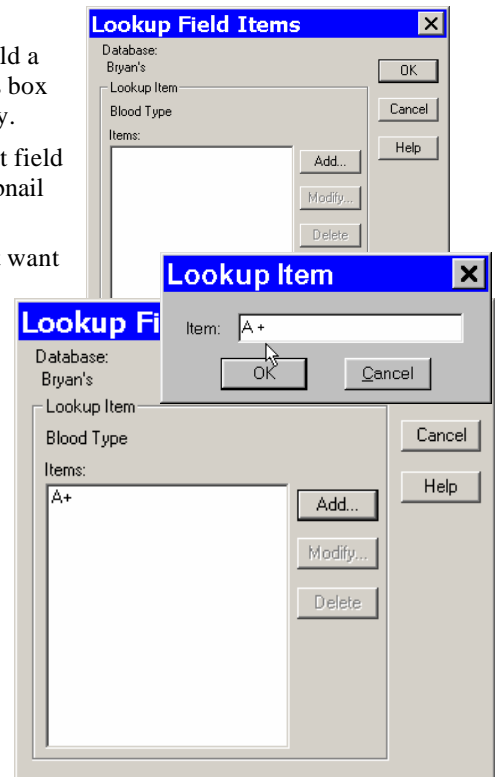
Setting Default Values

As part of the process of defining database fields, you have the option to define default values for each field. The values that you define will appear as default field entries each time you save an image to the database. If desired, you can edit the default entries before saving the image. To enter default values for a field, follow these steps:

1. After you have defined your text/numeric and lookup fields, click on **Default Values** (at the bottom of the Database Fields window). **The Default Field Entries** window appears, as shown at right. Unless specified, the SPOT database uses the Last Value for each field. To change this default for a Field, check the box next to the field name. Checking the box activates the Use: options.

2. Select the option that you want:

   – **Last Value**
     When saving an image to a database, the program uses the data entered for the last image as the entry for the same field in the current image. The last entered data always appears in the entry box for the image that you are saving. Thus, if you select Use: Last for the
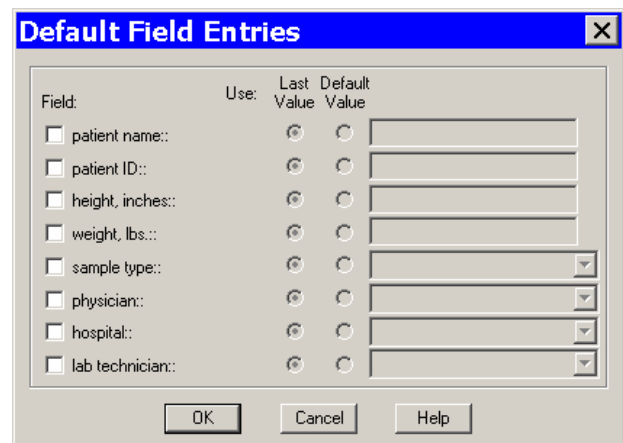
physician field, and you select Dr. Jarvis for the first image, Dr. Jarvis appears in all subsequent images until you make a different selection.

To select the Use: Last option, click on the Last button for that field and click on OK to save the settings.

−   **Default**
    When saving an image to a database the program uses the entry that you make (in the box next to the Default button). If desired, you can edit the default entries before saving the image.

    To select the Use: Default option, click on the Default button for the field and enter/select the default entry. Click on OK to save the settings.

If you do not specify a default value option for a field, all of the fields must be entered/selected each time an image is saved to the database.

Saving Images

Once you have acquired the images that you want to store in your database, the SPOT program makes it easy to save the information. To save an image to a database, follow these steps:

1. From the File menu, select the Save to Database option. The Save Image to Database window appears. The program automatically displays the date and time that you first saved the image. This information can be modified as needed.

2. Enter the information associated with the image in the database fields.
    −   **T** next to a field indicates that the field is Textual.
    −   **N** next to a field indicates that the field is Numeric.

To enter data for a lookup field that is not contained in the drop-down list options, simply type the information into the field.

> ✎   *Note: When you save an image to a SPOT database, the program automatically creates an image ID that is stored in the image path folder. This is a reference number that the program uses to access images and associated data.*
>
> ✎   *Note: If you defined a data field as* **Required** *on the Database Fields window, the program will not let you save the image without the information.*

3. Click on the Memo button to associate a memo with an image.   The Image Memo window appears.
4. Enter the text for the memo, and click on **OK** to save the memo.
5. Click on **OK** at the Save Image to Database window. If you entered a lookup list item that was not in the drop-down list of options, as described in step 2, the program prompts you to add the item to the list.
6. Click on Yes to add the new item to the lookup list.

Editing Database Image Records

Once you have created a database and saved images to that database, you can edit records as needed. To edit a database image record, follow these steps:

1. Ensure that the database is open.

2. From the File menu, select **Open Database Image**. The dialog box prompts you to enter the Image ID. If you do not know the Image ID, you can search the database, as described in the Searching the Database section that follows.

3. Click on OK to open the database image.

4. From the Database menu, select the Edit Database Record option. **The Image Database Record** window appears.

5. Change the Date/Time settings as needed. Use the following format:
   mm/dd/yy.

6. Edit the Fields data as needed.

   – For the text and numeric fields, simply enter the new data.

   – For the lookup fields, either select an item from the drop-down list or enter a new item. When you close the Image Database window, the program prompts you to add the new lookup list item.

7. Edit the Image Memo, if necessary. Click on **Memo**, enter the information, and clic k on OK.
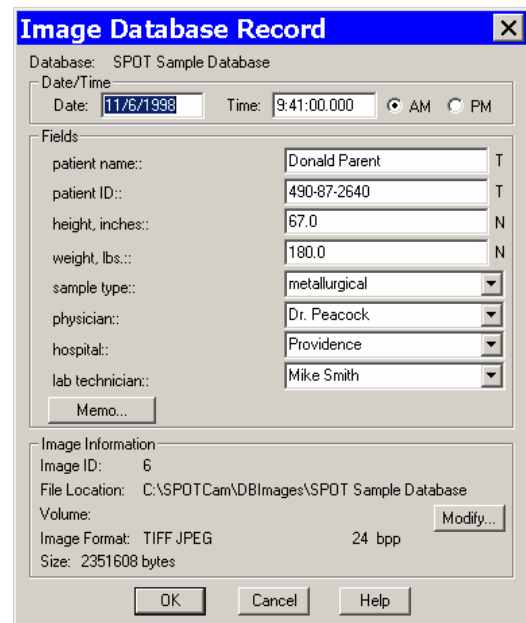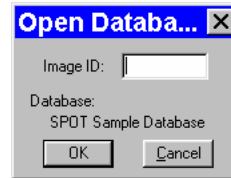
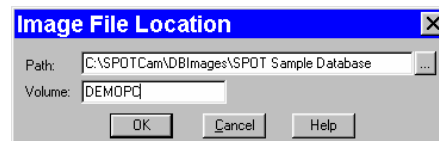In addition to the data associated with a database image, the Image Database Record window also allows you to modify the image file location/path information. For example, if you have moved a database image to a new file location, you would need to modify the record to reflect this.

8. To modify the location/path information for a database image, click on Modify. The Image File Location window appears.

9. Either enter the new file path in the Path box, or click on the button to browse for the information.

10. Enter the new Volume label.

11. Click on OK.

12. Click on OK at the Image Database Record window to save the changes.

> 🖉 *Note: The image itself cannot be edited while the Image Database Record window is open.*

Moving Database Images

When saving images to a database, the default save location is your computer's hard drive – **C:\SpotCam\DBImages** (or wherever you installed the SPOT software). To move images in a database from your hard drive to a removable storage medium such as a zip disk or a CD, follow these steps.

1. **Determine the file space needed for the database images**.

   a) Open the Windows Explorer.

   b) Navigate to the database image files. In the example at right, you can see that the image files associated with the **Histology** SPOT database are located in the **C:\SPOTCam\DBImages\Histology** directory. Note that the status bar at the bottom of the screen displays the number of objects in the **Histology** folder (45) and the disk space used by these objects (58.2 MB).
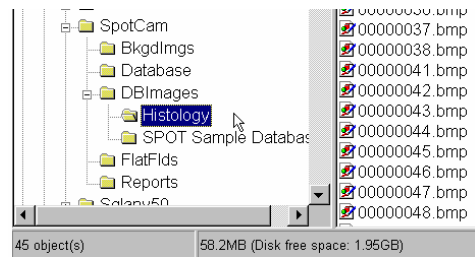
2. **Define the volume label for your zip disk**.
   In some cases, you will be able to store all of a database's images on one disk. At a future point, you might want to add additional images to the database that will not fit on the same disk. Defining a volume label enables you to access SPOT databases that span more than one Zip disk (or any other removable media). To define a volume label, follow these steps:

   a) Insert the Zip disk (or other removable media) in the drive.

   b) In Windows Explorer, right click on the drive letter for the Zip disk.

   c) Select the Properties option. You should see a view similar to that shown at right. If you do not see the pie shape, click on the **General** tab.

   d) Enter a name for the Volume Label in the **Label** box. You should use a name that indicates both the name of the database and the disk number (e.g., HISTO1).

   e) Click **OK**.

   f) For future reference, label the disk or CD.
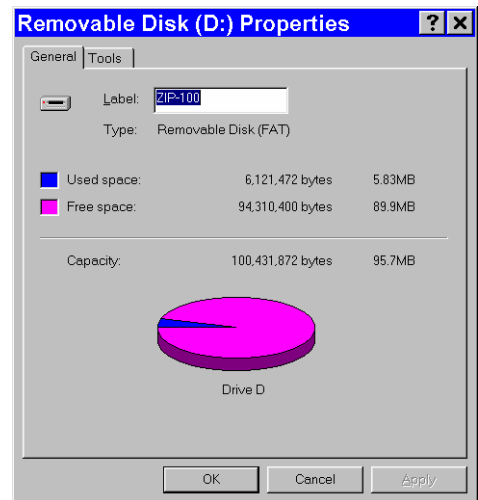
3. **Move the database images to the Zip disk**.

   a) If it is not already open, open the SPOT program.

   b) Open the database that contains the images that you want to move. From the Database menu, select **Preferences**.

   c) At the Database Preferences window, click on **Current Image File Locations**. The Database Image File Locations window displays the current file path and volume, as shown at right.

   d) Click on the **Modify** button. The Image File Location window appears, with the current path and volume information for the database images, **c:\SpotCam\DBImages\Histology**.

   e) Either click on the square box next to the file path to navigate to the folder that you want to move the images to, or enter the file path in the box. When you click on the square box next to the Path box, the SPOT program prompts you with an Explorer view. Navigate to the **D:** drive (or whichever letter is used by your PC).
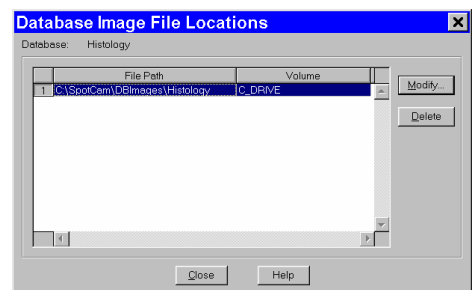
f) Click on the Create New Folder tab, as shown at right.

> 📝 *Note: If you simply enter the Path* **D:\Histology** *on the Image File Location dialog (above), the SPOT program will not create the folder. You must create the folder manually, either from the Windows Explorer, or from the Select Folder dialog.*
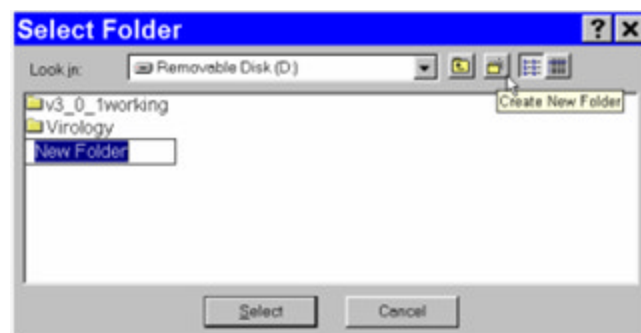
g) Enter the name of the new folder, e.g., **Histology**.

h) Click on **Select**. The Image File Location dialog reappears.

i) Enter the **Volume** label that you defined for the Zip disk in step 2, e.g., HISTO1

j) Click on **OK**. The Database Image File Locations window reappears.

k) **Close** the Database Image File Locations window.   The Database Preference window reappears. Note that the **Image Files Saved To** path has not changed – it is still C:\SpotCam\DBImages\Histology. Unless you change this setting, new images added to the database will still be saved to this location.

l) Click on **OK**.

4. From the Windows Explorer, cut and paste the image(s) from the original location (e.g., C:\SpotCam\DBImages\Histology) to the new location (e.g., D:\Histology).

   a) Navigate to the C:\SpotCam\DBImages\Histology folder, and click on Histology.

   b) From the **Edit** menu, choose **Select All**.

   c) Cut the images **[Ctrl X]**.

   d) Click on the D:\Histology folder.

   e) Paste the images **[Ctrl V]**.
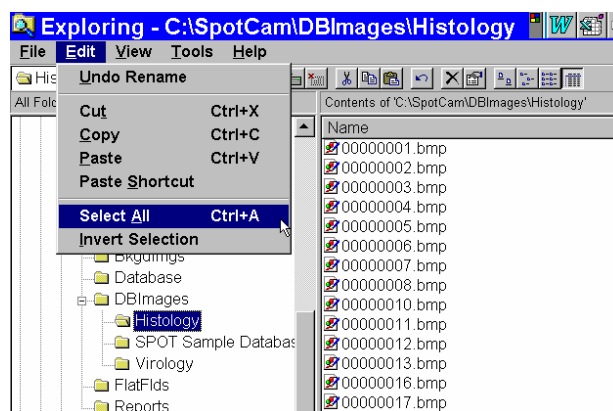
Steps one through four describe how to move an entire database of images. However, it is possible to move individual images or groups of images. When moving individual or groups of images from a SPOT database, you must change the path of *each* image file that you want to move.

To do so, follow these steps:

1. Search the database to find the image(s) that you want to move. For details on entering search conditions, refer to the Searching Databases section below.

2. From the search results display table (the Images window), select an image that you want to move, and click on Edit. You can select only one image at a time for editing. The Image Database Record window appears.

> ✎ *Note: You can also access the Image Database Record window by opening a database image (i.e., File>Open Database Image) and then selecting the* **Edit Database Record** *option from the Database menu.*

3. Note down the Image ID number – the first item in the Image Information section.

4. Click on the Modify button. The Image File Location dialog appears.

5. Enter the Path and Volume information as described in steps 2d – 2i above.

6. Repeat steps two through five for each image that you want to move.

7. From the Windows Explorer, cut and paste the images from their original location to the new location defined in step five.
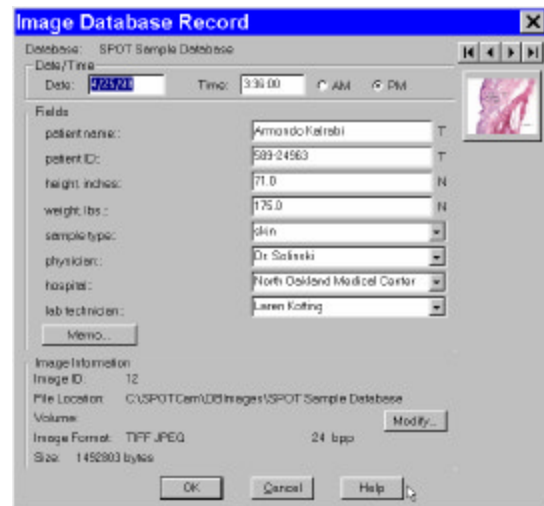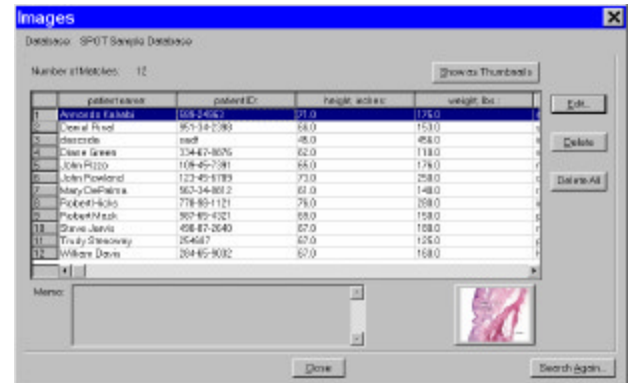
Searching Databases

The SPOT program offers a robust, yet easy to use database search function. The **Database Search** window is the central point from which search and display parameters are entered. From this window, you:

- Define search conditions
- Order search results
- Display and edit search results
- Save search criteria
- Load previously saved search criteria

To access the Database Search window, select Search from the Database menu.
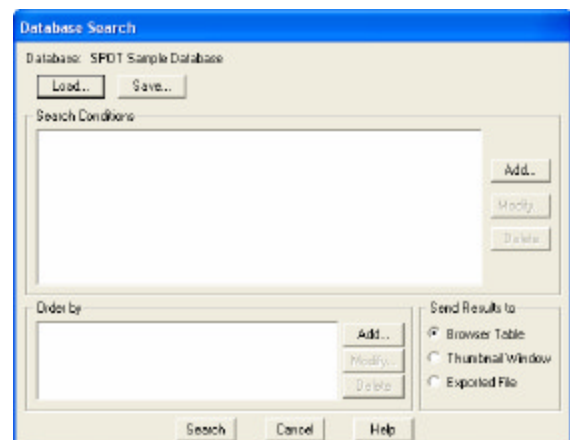
Defining Search Conditions

To define database search conditions, follow these steps.

1. Click on the Add button (for Search Conditions) on the Database Search window. The Search Condition window appears.

2. Select the database field that you want to base the search on. The left drop-down box contains a list of all the textual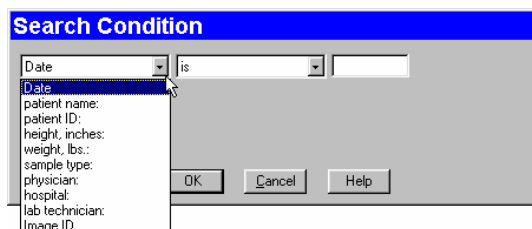/numeric and lookup fields that you defined for the database. In addition to the fields that you defined, the Date associated with the image and the Image ID appear in the list.

3. Select the criteria for the search from the center drop-down box. The list options vary according to the type of field that you selected, as shown below:

Field Type	Options	Field Type	Options
Date	is is is not is before is on or before is after is on or after is between is not between is one of is not one of	**Numeric**	is equal to is equal to is not equal to is less than is less than or equal to is greater than is greater than or equal to is between is not between is one of is not one of
Text	is is begins with doesn't begin with contains doesn't contain is like (use * and ?) is not like (use * and ?) is one of is not one of	**Lookup**	is is is not is one of is not one of

4. Enter the final search parameter in the right hand box. This entry will vary according to the search criteria that you selected. For **Text** and **Numeric** field searches, you are required to enter the information, while for **Lookup** field searches, you can select from a list of the lookup items
   For Text fields, asterisks (*) and question marks (?) can be used as "wild card" characters with the **is like** and **is not like** options.

   – ***** - The asterisk wild card tells the program that any number of characters can be used with this text field.

   For example, if you want to search all patients with "smith" in their name, you can enter ***smith***. This search will bring up names such as John Smith, Jane Smithfield, and Joe Hammersmith.

   – **?** - The question mark wild card tells the program that any single character can be used where the question mark appears.

   For example, if you want to search for patients with either the name Smith or Smyth, you can enter *** sm?th**. The asterisk and space in the front of sm?th tells the program to find all first names associated with sm?th. The question mark tells the program to search for one-letter difference variations on the name Smith. This search will bring up names such as John Smith, Anne Smyth, and Fred Smoth, but it will not bring up Jane Smithfield, Harry Hammersmith, or Abigail Smythe.

5. Click on OK to save the search parameters. The Database Search window reappears with the search conditions in the box.

   After you have entered a search condition, you can:
   – Click on **Add** to add another search condition.
   – Select a condition and click on **Modify** to change it.
   – Select a condition and click on **Delete** to remove it from the search.

6. Define how the search results will be ordered. Refer to the next section for details.

Ordering Search Results

After you have defined the search conditions, the **Order by** options allow you to set the display order of the search results.

To order search results, follow these steps:

1. Click on the Add button to the right of the Order by box. The Record Ordering window appears.
2. Select from the list of drop-down list options to determine how the database search results will be ordered. In addition to the database fields that you defined, the options include Date/Time and Image ID.
3. Check either the Ascending or Descending option:
   – Ascending lists display results beginning with the lowest value and progressing to the highest value.

   – Descending lists display results beginning with the highest value and descending to the lowest value.
4. Click on OK. The options that you selected appear in the Order by box:

   After you have entered an ordering condition, you can:

   – Click on **Add** to add another ordering condition.

   – Select a condition and click on **Modify** to change it.

   – Select a condition and click on **Delete** to remove it from the search.
5. Choose the display options. Refer to the next section for details.
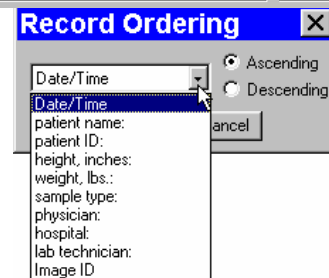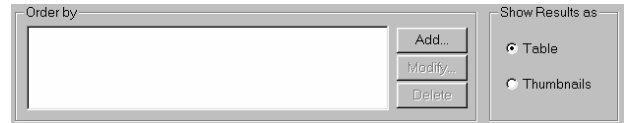
Displaying Search Results

The SPOT software allows you to display database searches either in tabular form or as thumbnails. From the **Send Results to** box, select either Table or Thumbnails. When all the search conditions have been entered, click on Search. The following sections detail the display and editing functionality.

Send Results to: Table

Display the search results in a table. From left to right, the column headings for the table are:

- The user-defined database fields
- Date/Time
- Image ID
- Image Format
- Bits per Pixel
- Size (in bytes)

The illustration at right shows a search that resulted in one record.

From the table window, you can:

- **Open a database image** – Select the database record item, and click on the thumbnail at the bottom of the window. The image opens for viewing and modification.

- **Edit a database record** – Either select the database record item and click on Edit, or double-click on the database record item. The Image Database Record window appears. Refer to the Editing Database Image Records section below for details.

- **Delete a single database record** – Select the database record item and click on Delete.

- **Delete all of the database records -** Select a database record item and click on Delete All.

- **Switch to the thumbnail view** – Click on the Show as Thumbnails button.

- **Perform another search** – Click on the Search Again button to return to the Database Search window, where you can add or modify search conditions.
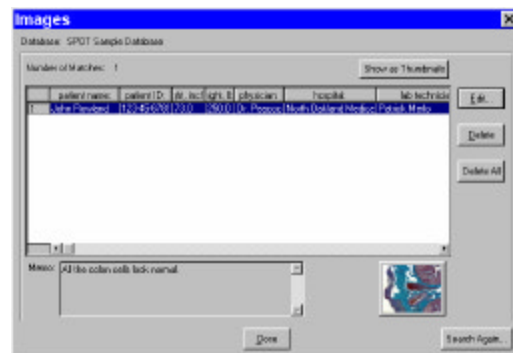
Send Results to: Thumbnails

Display the search results in the form of image thumbnails. The illustration to the right shows the thumbnail view of a database search that produced eleven records.

- *Double-click* on a thumbnail to open an image for viewing or modification.

- *Right-click* **(Windows)/[Ctrl] Click (Mac)** on a thumbnail to display a list of options, as shown at right. The right-click menu enables you to perform a variety of actions directly from the thumbnail window. You can:

  - **Select** an image.
  - **Open** a database image.
  - **Delete** an image from the database.
  - **Print** an image.
  - **Edit Database Record -**Edit the database image record.
  - **Add Image to Image Sequence –** Add a database image to an image sequence or create a new image sequence.
  - **Add Image to Report Template** – Add a database image to a selected report template. When you select this option, SPOT prompts you to select from a list of reports. If you have not defined a report, SPOT automatically creates a new report for you and inserts the image as a **Static Image** object. Refer to the **Creating Reports** section of Ch. 10 - Reporting for more details.
  - **Select all images** in the thumbnail view. When you use this option, the right-click menu expands to include the above options for multiple images.
- *Click and drag with the mouse* to select two or more images.

Send Results to: Exported File

Display the search results as fields exportable to a database.

- Include field information from the selected database to a list of information that is to be exported to another database application.

- Select a filename and one of the file type options from the dropdown menu.

- When you click on Begin, the export will take occur, placing the file in the location defined by your path. Use the browse option to quickly define a path. You may also choose to either overwrite or append an existing document with the same path and filename.
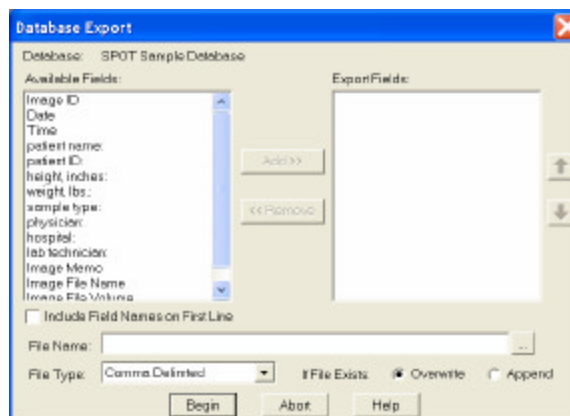
🖉 *Note: The file you export will be created as a delimited file as selected by you in the dropdown menu. Once the file has been created with the exported data, it may be opened with a variety of applications, depending upon the preferences defined in your operating system.*

Saving and Reusing Search Criteria

After you have defined the search and display parameters, you can save that set of criteria to be used for future searches on that database. Once saved, you can then reuse the search criteria clicking on the Load button.

To save a search, follow these steps:

1. Enter the search and ordering parameters.

2. Click on **Save**. The program prompts you to name the database query.

3. Name the query.

4. Click on **OK**. At this point, you can:

   − Run the search by clicking on **Search**.
   − Close the **Database Search** window and reopen the search at a future time.

   To reopen the search at a later time, click on the **Load** button, and select a search (**.qry**) file. Click on Open. The Database Search window displays the parameters for that search query.
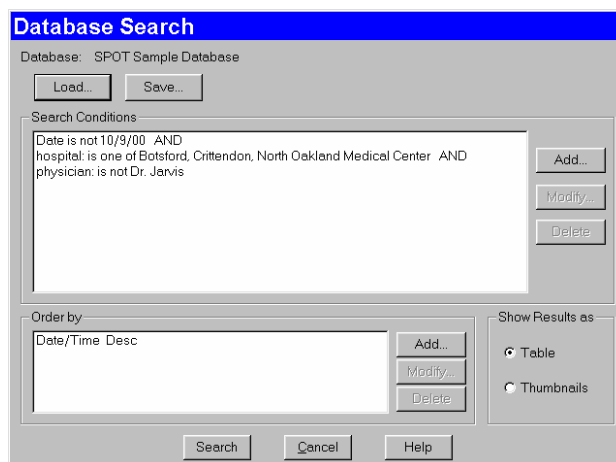
Editing Database Image Records (from a search)

The SPOT program provides two ways to access and edit database image records:
- Via the Edit Database Record option on the Database menu
- Via the display windows of database search results

In both cases, the Image Database Record window allows you to change the information associated with the database image record.

However, the functionality differs slightly, depending on how the window is accessed:

Accessed via the Database Menu	Accessed via a Search Results Display
Modify date/time, database field entries, image memo, and file/path information.	
Modify the database record for the open image only.	Modify the database record for any image in the database.
Edit the database record only.	Edit the database record and/or the image itself.

To edit a database image record from a search results display window, follow these steps:

1. Open the Image Database Record window.

   − If your results are displayed in a table, either double-click on the record in the table, or select the record and click on Edit.

   − If your results are displayed as thumbnails, right-click on the thumbnail.

   The Image Database Record window appears. Notice that navigational arrows appear above the thumbnail.

2. Change the Date/Time settings as needed. Enter the information in **mm/dd/yy** format

3. Edit the Fields data as needed.

   − For the text and numeric fields, simply enter the new data.

   − For the lookup fields, either select an item from the drop-down list or enter a new item. When you close the Image Database window, the program prompts you to add the new lookup list item.

   − Edit the Image Memo, if necessary. Click on the Memo button, enter the information, and click on OK.
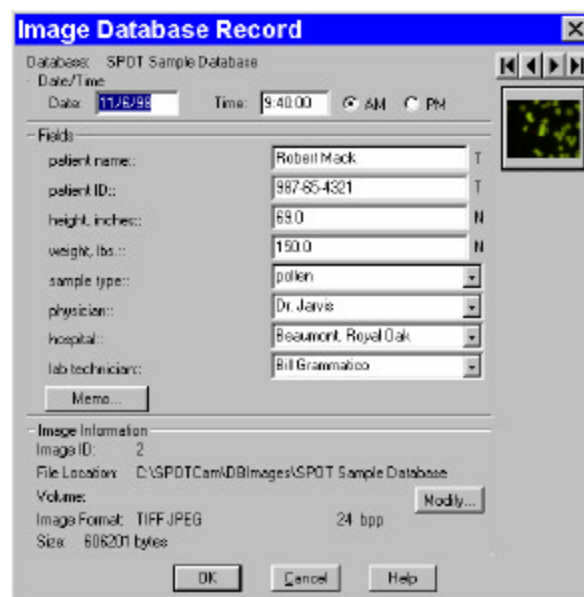
In addition to the data associated with a database image, the Image Database Record window also allows you to modify the image file location/path information. For example, if you have moved a database image to a new file location, you would need to modify the record to reflect this.

   − To modify the location/path information for a database image, click on Modify. The Image File Location window appears.

   − Either enter the new file path in the Path box, or click on the button to browse for the information.

   − Enter the new Volume label.

   − Click on OK.

At this point, you can either save the changes to the image record or proceed to make changes to another database record.

   − To save the changes and return to the search results display, click on OK.

   − To edit another database record without closing the Image Database Record window, use the navigational arrows at the top of the window to browse for the record.

      • Use the two middle arrows to scroll one image at a time through the database.

      • Use the left arrow to go back to the first image in the database.

      • Use the right arrow to go forward to the last image in the database.

4. Repeat steps one through seven to modify additional image database records.

Ch. 10 - Reporting

QUICK REFERENCE:

- ➲ "Tour" the SPOT sample report... p. 188

- ➲ Edit report objects... p. 203

- ➲ Run a report... p. 205

- ➲ Save a report to file without previewing or printing it... p. 207

- ➲ Create an "instant" report without having to save or preview... p. 208

- ➲ Distribute a report electronically to a user who does not have the SPOT program... p. 208

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Introduction

The SPOT software includes a built-in reporting functionality that can be used either by itself or in conjunction with a SPOT database (Windows) to collect, organize, and present information related to your image captures. This chapter discusses the process of creating and running reports. It is divided into the following sections:

- Overview: The SPOT Sample Report (Windows)
- Creating a Report
- Running a Report
- Distributing Reports

Overview: The SPOT Sample Report

When you create a report in SPOT, you are building a template to be applied to specific situations. Running the report applies the template to an image or images. For example, you might have one template called Histology Report to document histology samples and another template called Cell Pathology Report to document different cell cultures. Similarly, you might want to produce reports for production line analysis. In this case, you could define different templates for different parts, such as Part A Analysis Report, Part B Analysis Report, etc…

For your reference, a fully functional sample report is included with the SPOT software. This report can be used:

- As an introduction to SPOT's reporting features
- As a basis from which to build additional reports

The following section is a "visual tour" of the SPOT Sample Report.

Open the SPOT Sample Report

The SPOT Sample Report, **Pathology Report**, is a database report: it is based on the information associated with the images in the SPOT Sample Database. To open the SPOT Sample Report, follow these steps:

1. Because the SPOT Sample Report is based on the SPOT Sample Database, you must first open this database:

   a. From the Database menu, select Open.

   b. Select SPOT Sample Database.

   c. Click on Open.

2. Open an image from the SPOT database.

   a. From the Database menu, select Search. The Database Search window appears.   Do not enter any search conditions at this point.

   b. In the Send Results to box, select **Thumbnail Window**.

   c. Click on Search. The search results display.

   d. Click on the **John Rowland colon** thumbnail to open a database image to report on.

3. Open the SPOT Sample Report. From the File menu, select Open Report. The Open window appears for you to select the file. The file path and name for the SPOT sample report is **C:\Spotcam\reports\sampleDBreport.rpt**.
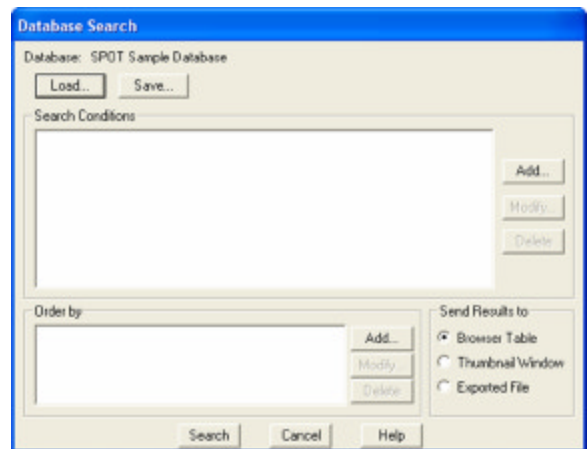
The report appears in a new window and the menus and menu options in the main window change to reflect SPOT's report editing functions.

View the SPOT Sample Report Objects

When you open the SPOT Sample Report (or any SPOT **rpt** file), you are actually opening a template of user-defined report objects that the program uses to create the report output. The call outs on the following illustration of the SPOT Sample Report template identify the different objects used in the design of this report. Refer to the **Defining Report Objects** section for descriptions of each object.
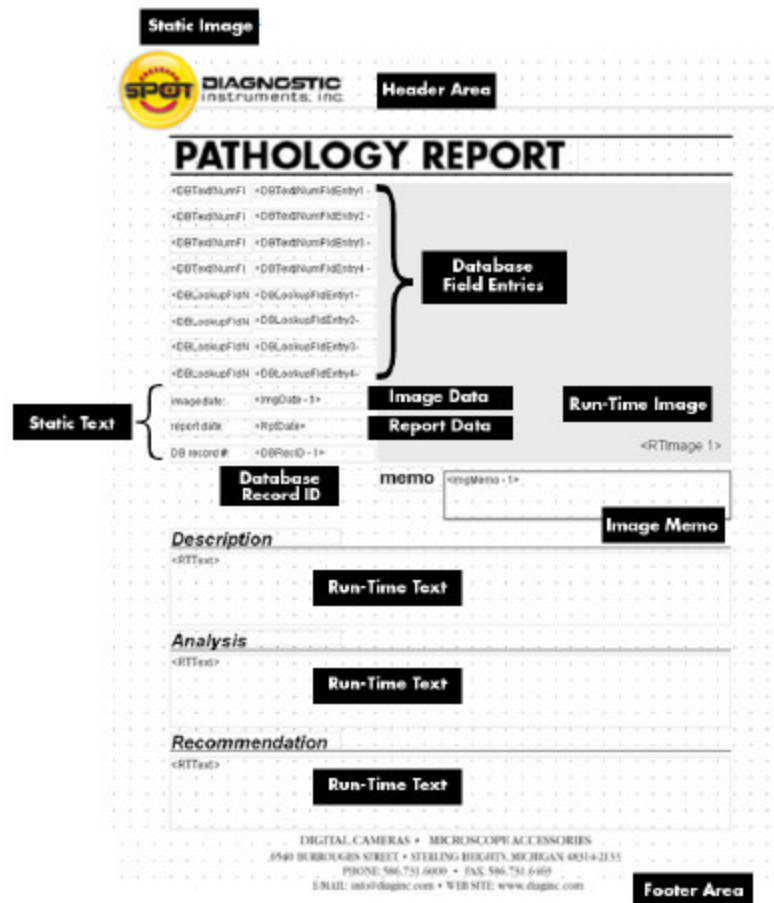
Run the SPOT Sample Report

To run the Pathology Report for the **John Rowland – colon** image from the SPOT Sample Database, select **Run Report** from the File menu. The report sequence is as follows:

1. The Run Report window prompts you to define the Output destination for the report. Select **Preview Window**.
2. Click on **Run**.

3. The report then prompts you with the run-time text prompts that have been defined for this report. Because there are three run-time text prompts for the SPOT Sample Report (i.e., to enter the information for the Description, Analysis, and Recommendation fields), you will be prompted to enter run-time text for each field. In the illustration at right, the run-time text has already been entered, but if you were reporting on the image for the first time, you would see only the prompt. Click on OK at each window. When you click on OK at the third Run-Time text window, the report appears, as shown below.

4. Click on Close to close the report without saving or printing it. Refer to the Running Reports section for more details.

Creating a Report

To create a new SPOT report, select **New Report** from the File menu. The following changes take place in the main window:

- The report definition window appears as a separate window. If an image or image(s) were open, they remain open.
- The **Object** menu appears. Use these options to specify the fields, text, and images that appear in the report.
- The **Attributes** menu appears. Use these options to define the appearance and style of the report.
- The **View** menu options change to reflect report definition function.
- The **Edit** menu options change to reflect report-editing functions. These options are available only after one or more report objects has been added to the New Report window.

✎   *Note: When you move between an image window and the report definition window, the menu options change accordingly.*

This section is divided as follows:

- **Working in Report Mode** – general reporting features and functionality
- **Defining View Options** – the view of the report definition screen
- **Defining Report Attributes** – the style and appearance of the report
- **Defining Report Objects** – the text, field, and image information that appears in the report
- **Editing Report Objects** – edit options for the report objects

Working in Report Mode

The report mode contains functionality that differs from the image capture and editing functionality. The following is a brief overview of these features.

Cursor Functionality

Cursor functionality in SPOT reports varies according to which **Object** menu option is selected.

- If **None** is selected, the cursor is a an editing tool for selecting, sizing, and positioning objects. When creating new reports, **None** is the default option.
- If a report object is selected, the cursor changes to a cross-hair icon for placing new report objects to the grid.

Sizing and Positioning Report Objects

When sizing and positioning a report object in the report window, you must first select the object by single clicking on it. When an object has been selected, handles appear at the corners; for line objects, the handles appear at each end. You can select multiple report objects by holding the mouse button down and dragging the cursor around the objects. Handles appear around both objects. When selected, report objects can be sized or positioned as needed.

- To size objects, move the cursor either to a point on the object's border or to one of the handles. The cursor changes to a two pointed arrow

  - To resize one dimension of a box, position the cursor on a vertical or horizontal line; click and drag the side to the new size.
  - To proportionally resize a box, position the cursor on a handle; click and drag the box to the new size.

- To position objects, move the cursor over the selected object. The cursor changes to a four-point arrow. Click and drag the object to a new location. If an object is selected, you can use the arrow keys to fine-tune its position.

> ✎ *Note: When multiple objects are selected, they can be sized and positioned as a single object.*

Using Right-click and Double-click Functionality

When you are in report mode, you can use the right-click and double-click mouse functions as editing shortcuts.

- Double clicking on a report object enables you to edit the object's attributes – e.g., the thickness of a line.

- Right clicking (Windows)/ [Ctrl] clicking (Mac) on a report object calls an object specific edit menu that contains both general editing options and attribute editing options specific to that object. Right clicking on an Image object, for example, gives you a list of editing options, as shown at right.

- Right clicking (Windows)/ [Ctrl] clicking (Mac) on the report grid gives you three options: **Paste** an object that you copied or cut; **Select All** of the objects on the grid, or **Delete All** of the objects on the grid.

Refer to the Defining Report Attributes and Editing Report Objects sections for more details.
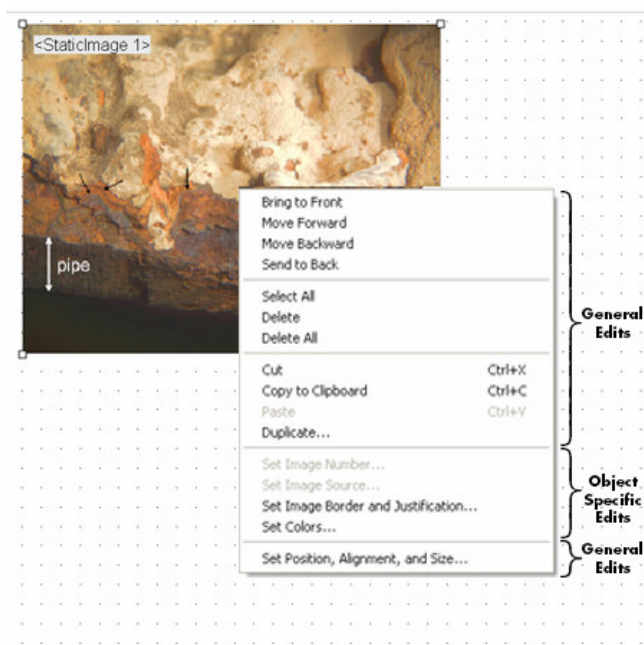
Defining View Options

The **View** menu provides you with eight options, each of which is described on the following pages.

Zoom Level
Zoom in or out to six different sizes. From the View menu, select Zoom and enter the desired percentage.

First Page
Go to the first page in the report. (only available when multiple report pages have been created)

Previous Page
Go back one page in the report. (only available when multiple report pages have been created)

Next Page
Go forward one page in the report. (only available when multiple report pages have been created)

Last Page
Go to the last page in the report. (only available when multiple report pages have been created)

Set Display Grid Size
The report definition window includes an adjustable grid (i.e., the dots on the screen) to assist you in placing and aligning report objects on the report page. By default, the grid is turned on. To turn off or modify the grid, follow these steps:
1. From the View menu, select Set Display Grid Size.

– To turn off the display grid, deselect the Show Grid box.
– To modify the spacing follow steps two through four.
2. Select either inches or millimeters.
3. Enter the spacing for the display grid. The number that you enter represents the space in inches or millimeters between the dots on the grid.
    – If the measurement is **inches**, enter a number between **.04** and **10**.
    – If the measurement is in **millimeters**, enter a number between **1** and **25**.
4. Click on OK. The display grid appears.

Set Object Grid Size
In addition to the display grid, you can define a second grid specific to report objects. The object grid controls the movement of objects as you work with them on the display grid. When you select a report object and move it, the object automatically "snaps" to the neares t line in the object grid, allowing you to more precisely align and arrange the objects on your report page.
1. From the View menu, select Set Object Grid Size.

2. If desired, check the Snap to Grid option.
3. Define the spacing for the object grid. In many cases, the object grid and the display grid will have the same spacing. In other cases, you might not want the display grid to be as finely spaced as the object grid (i.e., so the dots do not interfere with the view of the report definition window).
    – If the measurement is **inches**, enter a number between **.04** and **10**.
    – If the measurement is in **millimeters**, enter a number between **1** and **25**.

    (The measurement values will convert automatically as you toggle between English and metric units)
    The default spacing value for the Object Grid is the last entered value.
4. Click on OK.

Show Tools

Click on the Show Tools option to display a floating palette of navigation buttons and report objects, as shown here. Refer to the Defining Report Objects section of this chapter for more details.
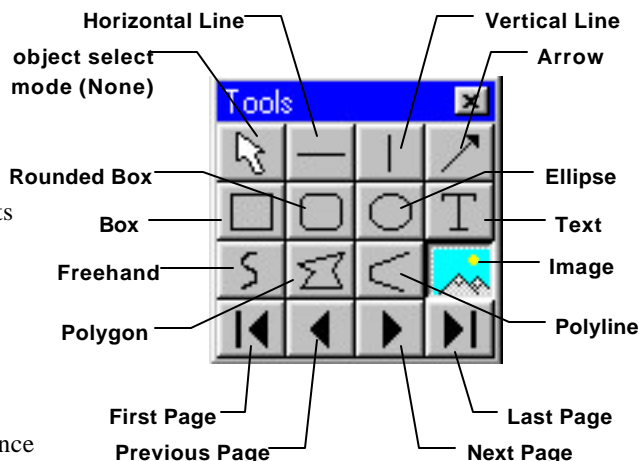
Horizontal Line — **Vertical Line**

object select mode (None) — **Arrow**

Rounded Box — **Ellipse**

Box — **Text**

Freehand — **Image**

Polygon — **Polyline**

First Page — **Last Page**

Previous Page — **Next Page**

Defining Report Attributes

All of the general style and appearance options (report attributes) can be accessed from the Attributes menu. Report attributes can be defined for an entire report or on an object-by-object basis (see the Defining Report Objects section below).

- When a report object is selected, the attribute is defined for the selected object(s) only.

- When nothing is selected, the attribute is defined for all future, relevant objects.

The following sections detail each of the report attributes and the report objects that they affect.

Line Thickness
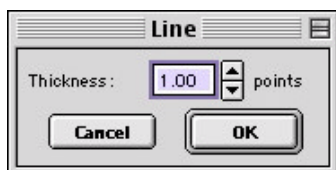
Determine line thickness for the following report objects: **Line**, **Horizontal Line**, **Vertical Line**, **Arrow, Box**, **Rounded Box**, **Ellipse**, **Freehand**, **Polygon**, and **Polyline**.

1. From the Attributes menu, select Line Thickness.
2. Enter a Thickness value between **.05** and **10** points.
3. Click on OK.

Colors

Define the foreground and background colors for all objects. Follow these steps:

1. From the Attributes menu, select Colors.
2. Define the **Text/Lines** color. This is the color of the text, the line, or the border around a text/image box. The default foreground color is black. To select a color click on Color.
3. The next window varies, depending on whether you are using a Windows or a Mac system.
   – Windows allows you to select from a palette of colors or enter specific HSL or RGB color values.
   – Mac OS allows you to select from a palette of colors, enter specific RGB, CMYK, HLS, or HSV values, and enter Internet specific colors.
   – Define the **Background/Fill** color. This is the color that appears behind text and in boxes. Lines and images do not display a background color.

✎ *Note: In order to define a background/fill color for a Box or a Rounded Box, a fill must be selected. Similarly, in order to define a background/fill color for a text box, the background (see* Text Border, Justification, and Background *below) must be set to Opaque.*

Box/Ellipse Fill

Define the appearance of the Box, Rounded Box, and Ellipse objects.

1. Specify whether you want a fill for the object.
   - If you do not want the box to be filled (i.e., you want a transparent box), click on OK.
   - If you want to specify a fill pattern for the box, check the Fill Box/Ellipse. The **Pattern** options appear.
2. Specify a pattern by clicking on one of the seven options.
3. Click on OK.

Image Border and Justification

Determine the line thickness for image borders and define the justification of the image within the display box. This option applies only to the **Image** report object.

1. To create a border around the image, click on the Border box, and enter a **Thickness** value between **.05** and **10**.
2. Select the **Justification** for the image. This value is the justification of the image within its display box, not the justification on the report page.
3. Click on OK.

Arrow Size and Direction

Specify the size of the arrow head and define the direction that the arrow points.

1. To specify the size of the arrow head, enter a value between **1** and **10**.
2. Specify the direction of the arrow:
   - To draw an arrow starting tail first and finishing with the point (head), select **Tail to Head**.
   - To draw an arrow starting point (head) first and finishing with the tail, select **Head to Tail**.
3. Click on OK.

Text Font

Define the appearance of the text for the **Text**, **Date**, and **Time** objects.

1. Select a **Font**, a **Font Style**, and a point **Size**. The Sample box displays a preview of the font.
2. Define any Underline effects.
3. Click on OK.

Text Border, Justification, and Background

Define the appearance of the text boxes for the **Text**, **Date**, and **Time** objects

1. Specify whether the text box should have a border. To add a border, click on the **Border** box, and enter a Thickness value between **.05** and **10**.

2. Define the **Justification** of the text. This justification applies to the position of the text in the box, not the position of the text on the page.

3. Define the Background. Select either **Transparent** or **Opaque**.

4. Click on OK.

Title Options

Specify how the title of an image will appear on a report. This attribute applies only to the **Image Title** report object. If you have not added an image title to your report, the Title Options do not matter.

-   To display only the file name – e.g., Healthy1 – leave both options unselected.

-   To display the entire path for an image – e.g., C:\My Images\Cells\Healthy1 – check the **Show Path for File Names** box.

-   To display the file name with the file type extension – e.g., Healthy1.jpg – check the **Show Extension for File Names** box.

-   To display the file path and the file name with the extension – e.g., C:\My Images\Cells\Healthy1.jpg – check both boxes.

Specify whether the **Image Time** report object will include seconds and milliseconds in addition to the hour and minute.

-   To display only seconds, check the **Show Seconds** box.

-   To display seconds and milliseconds, check both the **Seconds** and **Milliseconds** boxes.

Time Options

Defining Report Objects

All of the components of your report, including the formatting, text, images, and fields are known as objects. Using the objects, you can specify the information that your report will contain, as well as how the report will work.

-   You can, using run-time images, produce reports for images as they are captured. This is a good option for situations where you are capturing many images in one sitting.

-   You can produce reports for images that are saved to a SPOT database. Refer to Ch. 9 – Using SPOT Databases for details on creating and modifying SPOT databases.

-   You can produce ad-hoc reports for single or multiple saved images.

Before discussing the individual report objects, review the following general procedure for inserting an object into a report:

1. From the Object menu or by clicking on the appropriate Toolbox button, select the type of item (i.e., Lines, Connected Lines, etc…) that you want to add to the image. The cursor's functionality varies according to whether a report object type (i.e., line, text) is selected.

   – If a report object type is selected, the cursor changers to a cross-hair icon that is used to add new report objects to the grid.
   – If **None** is selected, the cursor is a an editing tool that is used to select and position objects.

2. Use the mouse to move the cross hair to the location where you want to insert the object.

3. Left-click at the insertion point.

   – If you selected a **Line** (unconnected line) or a **Shape**, hold down the mouse button and drag the line/box to the desired size.
   – If you selected any of the other report objects, the object will appear and you will be prompted to enter the associated information (e.g., static text, the location of an image file, the database field, etc…).

4. Once it appears on screen, you can resize, reposition, and edit an object as needed.

   – **Resize** - Select (click on) the object and drag the handles in the direction of the resize.
   – **Reposition** – Left click and drag the object the new area.
   – **Edit** – Select the object and choose an option from either the Edit menu or the Attributes menu. You can also edit an object by **right clicking** (Windows)/**[Ctrl] clicking** (Mac) on it.

For Connected lines use the following procedure. Select the appropriate **Connected Lines** object from the Object menu, or on the Tools box button. Move the cursor to your first location. Left click and release, and then move the mouse to create a side. Left click again to start a new side. Double click to finish the polyline, polygon, or freehand shape.

Refer to the table below for descriptions of the SPOT report objects.

None	Select **None** when you want to use the cursor to edit and position the objects in your report.
Lines	**Unconstrained Line** – Add a straight-line annotation to the report.
	Horizontal Line - Add a horizontal line annotation to the report.
	Vertical Line – Add a vertical line annotation to the report.
	Arrow - Add an arrow to the report. Either select Arrow from the Object menu, or click on the Tools box button. Left click on the report and drag toward the area that the arrowhead will point to.
Connected Lines	**Polygon** – Add a closed polygon to the report.
	Polyline – Add an open polygon to the report.
	Freehand - Add a freehand line to the report.
Shapes	**Rectangular Box** - Add a square cornered box to the report.
	Rounded Box – Add a round cornered box to the report.
	Ellipse - Add an elliptical object to the report. Ellipses are drawn inside of the rectangular area that you define. Ellipses touch the top, bottom, right and left sides of this rectangle.

Images

<u>Static Image</u>

A static image is an image that you want to appear on all productions of the report. A logo is a good example of a static image. To define a static image, follow these steps:

1. From the Object menu, select Images>Static Image.

2. Left click at the point on the display grid where you want to insert the Static Image box. As you insert the box , you can also size it by dragging a corner or side.

3. The Static Image box then prompts you to enter the source for the image file. Select from one of the three options:

    –   **Currently Open Image**
        If you have more than one image open, select the image from the drop-down list.

    –   **Image File**
        A file from disk. Either enter the file name and path, or click on the browse button (to the right of the Image File box) to search for the file. If you select a **sequence** file, specify the image number (i.e., the order in the sequence) in the **Sequence Index** box. Note that, if the **Store Image in Report Template** is selected, you can view the image from within the report template as well as in the report itself. This feature allows you to quickly generate printed reports, without having to first save the report to file and/or run a preview report. Refer to Instant Reports in the Running a Report section of this chapter for details.

    –   **Database (Windows)**
        An image file from a SPOT database. In order to use an image from a database, that must be open. If you are not sure of the Image ID, search the database and view the results table – the image ID appears there.

4. Click on **OK**. The static image object (or the actual image if you checked the Store Image in Report Template box) appears on the display grid, where you can move or resize the box.

When you run the report, the image is automatically proportioned to fit the dimensions of the box.

<u>Run-Time Image</u>

A run-time image is an image that is open at the time that you run the report. Run-time image objects allow you to report on images as you capture them, without having to save the images to file or to a database.

To define a run-time image, follow these steps:

1. From the Object menu, select Images>Run-Time Image.

2. Left click at the point the on the display grid where you want to insert the Run-Time Image box. As you insert the box, you can also size it by dragging a corner or side. The Run-Time Image window prompts you to specify the image.

3. Specify which image (if you have multiple open images) will be the run-time image. The entry in the Offset from Top Image Window box defaults to 0. If you have only one image open, click on OK.
    If you have more than one open image, you will need to specify the number for that image. For example, if you have five open images, there are five values, from 0 – 4 that correspond to the images, as illustrated in this cascaded view of multiple image windows: If you have multiple open images, selecting a cascade view of the images can help you to determine the proper number. To do this, select the Cascade option from the Window menu

4. Click on OK. The Run-Time image box appears on the display grid, where you can move or resize the box. When you run the report, the image is automatically proportioned to fit the dimensions of the box.
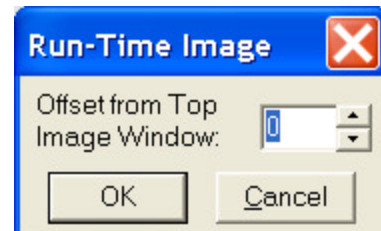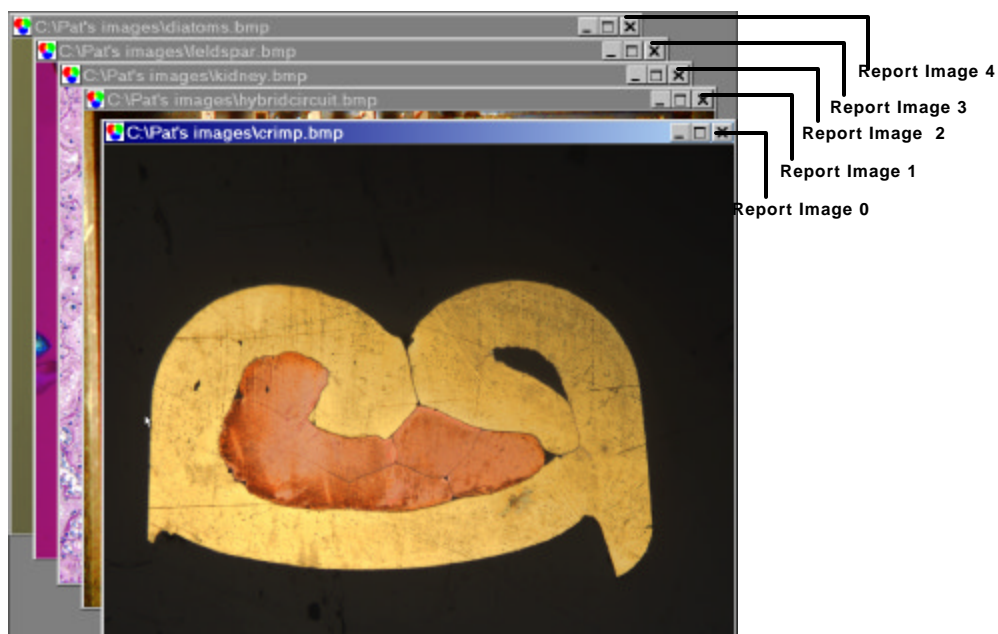
✏️ *Note: Offset is related to the current order of any cascaded images. In other words, if you select an offset value, and then change the order of the image cascade (i.e. which image lies on top of another) the offset value will not reset. Instead, a different image may be selected by virtue of the offset.*

Images (cont.)

<u>Selected Image</u>

The Selected Image options allows you to select one or multiple images to be included on a report at the time that you run the report. Selected images can be:

- Single or multiple open images
- Single or multiple images from file
- Single or multiple database images

To define a selected image or images, follow these steps:

1. From the Object menu, select Images>Selected Image.

2. Left click on the point in the display grid where you want to insert the selected image.

3. Size and position the image box as desired.

4. Add any additional report objects needed to complete the report.

5. From the File menu, select Run Report.

6. Select the report template either from the **Currently Open Template**, or from **File**.

7. Select the **Output** format. The default choice for running a report is to use the Preview Window, which allows you to view the report before saving or printing it. For more details on the Run Reports options, refer to the **Running Reports** section of this chapter.

8. Click on Run. The Selected Images window appears.

9. Define the Selected Images.

   – **Top Open Image** – The report displays only the top- most image, if multiple images are open.

   – **All Open Images** – The report displays all open images.

   – **Image File(s)** – The report displays the image file or files selected from disk. When you check the Image File(s) option, and click on OK, the Report Images window appears for you to select one or more images to be included in the report. To individually select multiple image files, hold the **[Ctrl]** key and click on each file.

   – **Database (Windows)** – The report displays one or more images from a database. When you check the Database option and click on OK, the Database Search window appears. Add search conditions and ordering criteria, as needed, and click on Search. The report displays the Search Results. Refer to Ch. 9 – Using SPOT Databases for detailed steps on how to define search and order conditions.

10. Click on **OK**.
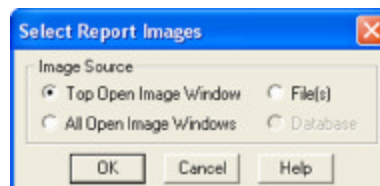
Text   <u>Static Text</u>
Use the Static Text option to add text that will be the same for all of the images in a report. Typical static text fields include:
- The title of the report
- Report field headings

To define Static Text, follow these steps:
1. From the Object menu, select **Text>Static Text**.
2. Left click at the point where you want to insert the text. The program prompts you to enter the text. You may also include variables in the Static Text box that will be recalled when the report is run.
3. After you have entered the text, click on **OK**. The Static Text appears in a box.
4. To edit the text, double-click on the box. The Static Text box reappears for you to modify the text.

<u>Run-Time Text</u>
Use the Run-Time Text option to define the data entry prompts that will appear when you run an image report. For instance, if a static field on your Blood Samples report is Blood Type, you can define a Blood Type? Run-time field that prompts you to enter the blood type for each sample, as the report is run.

To define Run-time Text, follow these steps:

1. From the Object menu, select Text>Run-Time Text.

2. Left click at the point where you want to insert the text. The program prompts you to enter a prompt and default run-time text.

3. Enter the Prompt. When you run a report on an image, you will be prompted to enter information for the prompt.

5. Enter the Default Text, if desired. The Default Text field is useful in cases where the same item is entered for multiple image reports. For example, one lab technician might have drawn the sample for numerous blood samples. In this case, you could enter this technician's name as the Default Text. You may also include variables in the Run-TimeText box that will be recalled when the report is run.

4. Click on OK. The object displays on the report grid.

5. To edit the text, double-click on the box. The Run-Time Text dialog box reappears for you to modify the text.
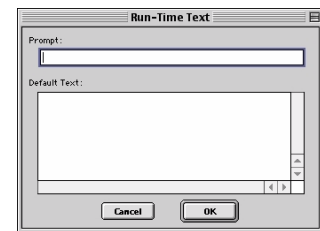
<u>Page Number</u>
Add a page number to a report page.
<u>Page Count</u>
Add a display of the total number of pages in your report.
<u>Record Number</u>
Add the number of the record (selected image/s) to the report. When you run the report, the number for each record is displayed on the page.
<u>Record Count</u>
Add the total number of records (selected image/s) in the report. When you run the report, the Record Count appears on each page.
<u>Report Date</u>
Insert the current date in your report.
<u>Report Time</u>
Insert the time that you ran the report.

Text (cont.)

<u>Image Date</u>
Include the date that an image was captured.

1. After you have inserted an image in the Report, select **Text>Image Date** from the Object menu.
2. Left click at the point where you want the Image Date to appear. The ImgDate box appears on the display grid.

   The number following ImgDate is associated with the image or images in your report. Thus, if the report has more than one image, the program prompts you to define the image that the date should be associated with.
3. Enter the Report Image Number. When you insert images, the program automatically assigns it a number. The first image you insert is 1, the second image 2, etc…
4. Click on OK. When you run the report, the image date displays.

<u>Image Time</u>
Include the time of day that an image was captured.

1. After you have inserted an image in the Report, select **Text>Image Time** from the Object menu.
2. Left click at the point where you want the Image Time to appear.

   The number following ImgTime is associated with the image or images in your report. If the report has more than one image, the program prompts you to define the image that the time should be associated with.
3. Enter the Report Image Number.
4. Click on OK. When you run the report, the Image Time displays.

Note: For the Image Date and Image Time objects, the date/time that appears here varies according to whether the file was transferred across a network, and whether the file is a SPOT database image. Refer to the Image Date/Time section of Ch. 8 for more details.

<u>Image Title</u>
Insert the file path and name that is associated with an image.

To insert the Image Title, follow these steps:

1. After you have inserted an image in the Report, select **Text>Image Title** from the Object menu.
2. Left click at the point where you want the Image Title to appear.

   The number following ImgTitle is associated with the image or images in your report. If the report has more than one image, the program prompts you to define the image that the title should be associated with.
3. Enter the Report Image Number.
4. Click on OK. When you run the report, the file and path for the image displays.

You might have to resize the ImgTitle box to display the entire file path.

<u>Image Memo</u>
Insert the memo that you entered for a TIFF, JPEG, or SPOT database image. Follow these steps:

1. After you have inserted an image in the Report, select **Text>Image Memo** from the Object menu.
2. Left click at the point where you want the Image Memo to appear. The number following ImgMemo is associated with the image or images in your report. If the report has more than one image, the program prompts you to define the image that the memo should be associated with.
3. Enter the Report Image Number.
4. Click on OK. When you run the report, the memo displays.

<u>Image Sequence Index</u>
Insert the number of a specific image when reporting on an image sequence file.

<u>Image Sequence Size</u>
Add the total number of images in the sequence to the image.

<u>Image Sequence Elapsed Time</u>
Insert the elapsed time between two images. When you run a report that contains the Image Sequence Elapsed Time, the program prompts you to specify both the **Reference Image Index Number** and the sequence number of the image you are reporting on. The Reference Image Index number is any number within the image sequence, typically one that precedes the image being reported on. If you specify a number that comes after the image that you are reporting on, the elapsed time will be a negative value.

<u>Database Record ID (Windows)</u>
Include the record number of a database image in your report. The Database Record ID can only be used with run-time or selected images.

To insert the database record ID for a database image, follow these steps:

1. After you have inserted a database image in the Report (either as a run-time image or a selected image), select **Text>Database Record ID** from the Object menu.

2. Left click at the point where you want the record ID to appear. The **DBRecID** box appears on the display grid. The number following DBRecID is associated with the image or images in your report. If the report has more than one image, the program prompts you to define the image that the database record should be associated with.

3. Enter the Report Image Number.

4. Click on OK. When you run the report, the record ID of the database image displays.

<u>Database Field Name</u> **(Windows)**

The Database Field Name and Database Field Entry objects allow you to run reports that parallel some or all of the information from a SPOT database. Use the Database Field Name object to insert a text/numeric or lookup field from an open database.

1. After you have inserted a database image in the Report (either as a run-time image or a selected image), select **Text>Database Field Name** from the Object menu.

2. Left click at the point where you want the field name to appear. The program prompts you to select the field that you want in the report.

3. Select an entry from the **Field** drop-down list. The drop-down list options are generic i.e., Text/Numeric – 1, Text/Numeric – 2, etc.…

4. Click on OK.

5. If the report has more than one image, the Image Number dialog box prompts you to define the image that the database field name should be associated with.

6. Enter the **Report Image Number**.

7. Repeat steps one through seven to add additional database fields to the report.

<u>Database Field Entry</u> **(Windows)**

The Database Field Name and Database Field Entry objects allow you to run reports that parallel some or all of the information from a SPOT database. Use the Database Field Entry object to insert the data from a text/numeric or lookup field.

To insert a Database Field Entry in your report, follow these steps:

1. After you have inserted a database image in the Report (either as a run-time image or a selected image), select **Database Field Entry** from the Object menu.

2. Left click at the point where you want the field name to appear. The program prompts you to select the field that contains the entry (data) that you want to appear in the report:

3. Select an entry from the **Field** drop-down list.

4. Click on **OK**. The box at right appears.

5. If the report has more than one image, the program prompts you to define the image that the database field entry should be associated with.

6. Enter the **Report Image Number**.

7. Click on **OK**. When you run the report, the database field entry displays.

8. Repeat steps one through seven to add additional database field entries to the report.

User Name   **User Name** – Add the user's name to the image. This defaults to the user who is logged on the computer.

Editing Report Objects

Once you have created a report, you can edit the report objects as needed. There are three basic ways to edit a report object:

- **Use the Attributes menu** - The Attributes menu, as noted in the Defining Report Attributes section of this chapter, allows you to either set the attributes for adding specific types of objects, or to select existing objects and modify them as needed. Refer to the Defining Report Attributes section for more details.

- **Use the Edit menu** - The Edit menu contains general editing features such as Undo, Copy, Cut, Send to Back, etc…

- **Right-click (Windows)/[Ctrl] click (Mac) on the report object -** Right clicking/[Ctrl] clicking on a report object brings up a customized editing menu that contains both general editing commands and the attributes that can be edited for that object. Refer to the Working in the Report Mode section for more information on the right click function.

The Edit Menu

The Edit menu provides general editing features for arranging, copying, and sizing report objects. Edit menu options are active only when a report object is selected. The following sections describe each of the Edit menu options. As is explained in the sections below, not all of the options are available at all times.

Undo Undo the last action. The Undo feature allows you to undo up to ten actions.

Bring to Front Bring a selected object in front of all other objects.

Move Forward Bring a selected object one layer forward.

Move Backward Send the selected object one layer back.

Send to Back Send a selected object behind all other objects.

Select All Select all report objects.

Unselect All Unselect all report objects.

Delete Delete the selected object(s).

Delete All Delete all of the objects in the report.

Copy to Clipboard Copy the selected object(s) to the Windows clipboard. You can then paste the object(s) into:

- A new SPOT report
- A different location in the same report

This feature is particularly useful when you are creating a new report and want to use objects from an existing report.

Cut Cut the selected object(s) for pasting into a new report or to a new location in the existing report.

Paste Paste the object that you cut or copied to its new location.

Duplicate Create additional copies of a report object. To duplicate a report object, follow these steps:

1. Select the object(s) that you want to duplicate.
2. Select Duplicate from the Edit menu. The program prompts you to enter the number of duplicate copies.
3. Enter the number of copies, and click on OK.
4. The duplicate object(s) appear at the top of report window.

Set Position, Alignment, and Size The **Set Position**, **Alignment**, and **Size** option allows you to specify precise sizing and position values for single or multiple selected report and annotation objects.

The **Position** options enable you to:

- Align top/bottom edges of multiple objects.
- Align left/right edges of multiple objects.
- Align the centers of multiple objects.
- Center an object(s) on the page.
- Enter exact values in inches or millimeters to position the object.

The **Size** options enable you to enter:

- Exact width/height in inches or millimeters for one or more objects.
- Set the width/height dimension for multiple objects the to smallest or largest object's value in inches or millimeters.

Add Page Add a page to your report. When you add a page, the program prompts you with the Add Page dialog box:

1. Specify whether the page that you want to add will be inserted Before or After a specified page.

2. Specify the Page Number that the additional page will precede or follow.

3. Click on OK.

Delete Page Delete a page from your report. When you select the Delete Page option, the program prompts you to specify the page number.

Add Static Images Add a static image object to the report. Refer to the Static Image object description in the Defining Report Objects section above for details on this process.

Order Run-Time Text Prompts When you run a report with multiple run-time text prompts (see the Defining Report Objects section), the prompts, by default, appear in the order that you defined them. Use the Order Run-Time Text Prompts option to rearrange the order as needed.

To re-order the run-time text prompts for a report, follow these steps:

1. From the Edit menu, select Order Run-time Text Prompts. The current order of the run-time text prompts displays.

2. Click and drag the prompts to rearrange them in the desired order.

3. Click on OK. When you run the report, you will be prompted to enter the run-time text in the newly defined order.
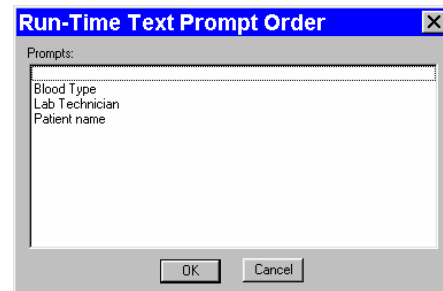
Running a Report

To run a SPOT report, follow these steps:

1. From the File menu, select Run Report. You can either run an open report or a report from file:

   – **Currently Open Template**
     If more than one report template is open, select from the drop-down list.

   – **File**
     Either enter the file name and path for the report that you want to run, or click on the Browse button to the right of the Name box to search for the report file.

2. Select the output format for the report.

   – **Printer** –The report is sent directly to the printer. The program first prompts you to select a printer/define print settings. Refer to Ch. 11 – Printing Images for details on printing from the SPOT program.

   – **Preview Window** –The report is displayed on screen, where it can be viewed, printed, and/or saved. If you have not run any previous reports, this is the default option. Otherwise, the program defaults to the output format for the last run report.

   – **File** – The report is saved directly to a file directory as a **Run Report (.rpd)** file. The program prompts you for the file location. The report can be opened for viewing or printing at a later time.

   The output options are discussed in more detail in the sections below.
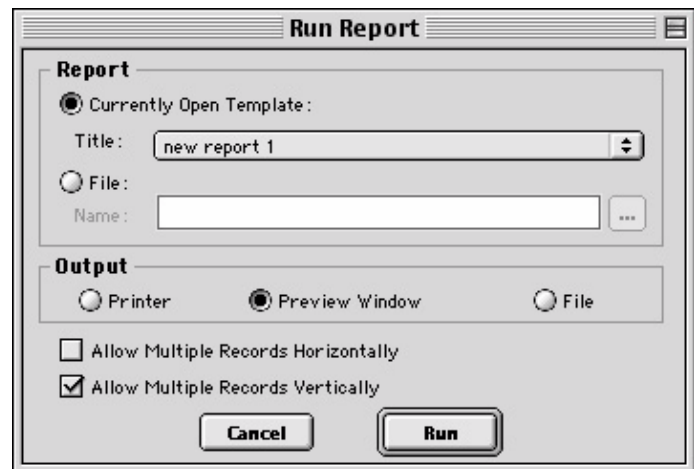
3.   Specify the **Allow Multiple Records Horizontally** and **Allow Multiple Records Vertically** options.
     When a report contains multiple Selected Image objects, SPOT separates each image with a page
     break: the report displays only one image per page. The **Allow Multiple Records Horizontally** and
     Allow Multiple Records Vertically options allow you to display multiple images on a page for cases
     such as a report on a database or a sequence image file.

     –   To display multiple
         images in a row,
         check **Allow
         Multiple Records
         Horizontally**.

     –   To display the
         multiple images in a
         column, check
         **Allow Multiple
         Records Vertically**.

     –   To display the
         images in rows and
   columns, check both
         options.

     In addition to selecting these options from the **Run Report** dialog, you can select them from the
     Preview window (see **Previewing a Report** below)

4.   Click on Run. The prompts that you see depend on the type of report objects that you defined. Refer
     to the Defining Report Objects section in this chapter for more information.

Previewing a Report

When you run a report in the Preview Window, the report appears with a navigation toolbar at the bottom
of the preview window, as shown here:
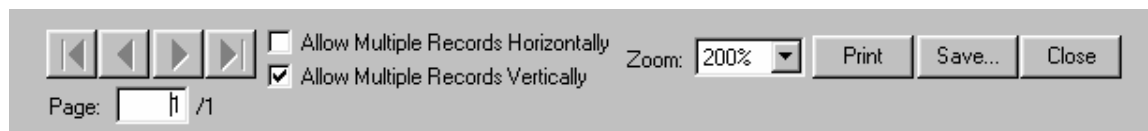
From left to right, the toolbar buttons are as follows:

- **Navigation arrows** - Move forward or backward in a multi-page report, or to the first page or last
  page in the report.

- **Page display** – See which page of the report you are viewing.

- **Allow Multiple Records Horizontally** – Display multiple selected images in a row.

- **Allow Multiple records Vertically** – Display multiple selected images in a column.

- **Zoom** – Zoom in or out on the report. There are six options.

- **Print** – Print the report without saving it.

- **Save** – Save the report as a Run Report (.rpd) file.

- **Close** – Close the report preview without printing or saving it.

Printing a Report

When you run a report with an output to Printer, the program prompts you to enter print information:

Follow these steps:

1. Select the printer.
2. Click on Properties to define the printer's properties, if needed.
3. Specify the page range if you are not printing an entire report.
4. Specify the number of copies.
5. Click on OK.

Saving a Report to File

When you run a report with an output to File, the program prompts you to save the file. Follow these steps:

1. When saving a new report (**.rpd** file type), SPOT prompts you with the default file location – i.e., **C:SpotCam\Reports** folder (Windows) or **Users\Shared\SPOT Files\Reports** (Mac) . Either save to the default location or specify a new location. If you plan to save many reports without compressing the image files, you should save them to a Jaz drive or to a network drive.

> ✏ *Note: Mac users will see the* **.rpd** *file type extension only if the* **Add Extensions to File Names on Save** *option is selected on the Preferences window*.

2. Enter a file name for the report.
3. Define the JPEG Compression for the image(s) in the report. The **Use JPEG2000 Compression for Images** box is checked as a default. To select any other compression format, check its corresponding box.

For JPEG2000 format,

- Lossless will give you the truest reproduction of data, but will also create the largest file size for any JPEG2000 compression scheme. Typical compression ratios range from 2:1 to 3:1.
- Lossy will compress the file to a level specified by the user through a compression ratio. The larger this ratio, the smaller the file, but also the greater chance that some digital information will be compromised. Lossy compression can achieve file compressions of up to 100 times smaller file size than lossless, yet still provide good resolution. (see examples at end of chapter)

For JPEG format,

- A value of 1 is the most compressed. File size and image resolution are decreased to the maximum extent.
- A value of 100 is non-compressed. File size remains large and the image resolution remains high.

4. Click on Save. You can now use the SPOT program to open and view the report at a later time.

Instant Reports

To quickly preview and print a simple report, without first running a preview report or saving the report to file, use the new **Store Image in Report Template** feature. The Store Image in Report Template feature allows you to view a Static Image report object in the report template: you do not need to run a preview report to see what the final printed product will look like.
Follow these steps:

1. Open a new report template. From the File menu, select **New Report**.
2.   Add a report image that to the template. From the **Object** menu, select **Images>Static Image**. Left-click and drag the cross-hairs cursor to size and position the Static Image box. When you release the button, the **Static Image** window appears.
3. Select **File** for the Image Source. Click on the button to the right of the **Name** box to select an image.
4. If you have selected a sequence file, specify the **Sequence Index** – i.e., the number of the image in the sequence.
5. Ensure that the **Store Image in Report Template** box is checked.
6. Click on **OK**. The Static Image appears in your report, where it can be sized, positioned, or annotated as needed.
7. Add additional static images as needed. Follow steps one through seven.
8. From the File menu, select **Run Report**.
9. For the Output Destination, select **Printer**.
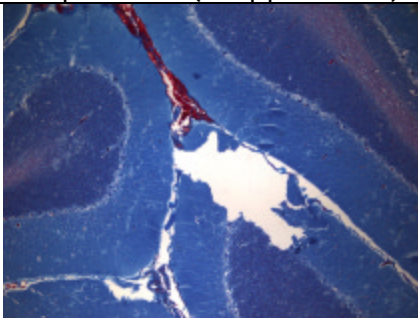10. Click on **Run**.
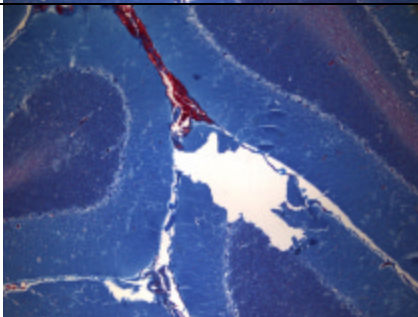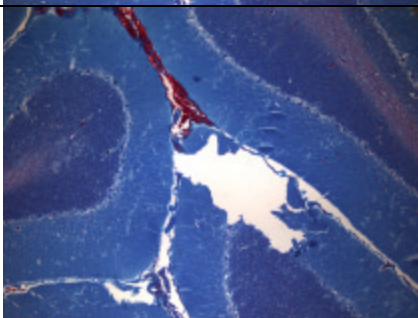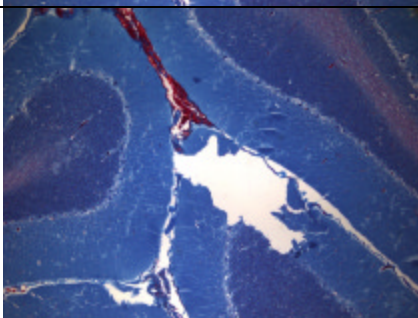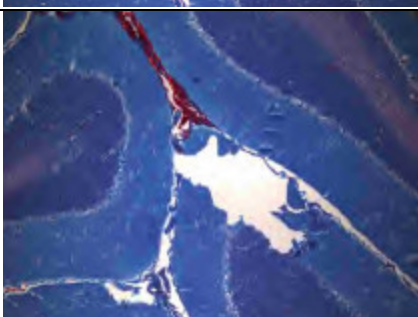
Distributing Reports

If you intend to distribute SPOT image reports to people who do not have the SPOT program, we recommend that you purchase Adobe Acrobat. Using Acrobat, you can convert the SPOT report (.rpd) files to PDF files, which can be distributed to users who do not have the SPOT software. They can view the reports with the free Acrobat Reader software, which is included on the SPOT software CD. The latest version of the Acrobat Reader can also be downloaded from the Adobe's web site www.Adobe.com.
Mac users may be able to use the Acrobat writer feature inherent in the Mac OSX version of Apple's operating system to generate PDF files without having to purchase Adobe Acrobat.

File Compression Comparison

Using JPEG2000 and various compression values

The following table indicates differences in image quality for image files saved in different compression levels using the JPEG 2000 format. Saved image file sizes are listed in the rightmost column.

File Compression Format	Captured Image after compression (Cropped area)	Resulting Full Image File Size
JPEG2000 Lossless		2,500,00 bytes
JPEG2000 Lossy 1:1 ratio		1,600,000 bytes
JPEG2000 Lossy 10:1 ratio		568,000 bytes
JPEG2000 Lossy 100:1 ratio		64,000 bytes
JPEG2000 Lossy 1000:1 ratio		1,200 bytes

Ch. 11 - Printing Images

QUICK REFERENCE:

- ➲ Print a single image… p. 212

- ➲ Print multiple images on one page… p. 212

- ➲ Add images from file and/or SPOT database images for printing… p. 212

- ➲ Arrange how multiple images appear on the page… p. 214

- ➲ Print an image sequence… p. 214

Introduction

The printing features in the SPOT program fall into two categories:

- **Printer Setup** - The standard Windows dialog boxes that are used to specify:
    - Printer and setup
    - Page setup
    - Orientation
    - Paper size and source.
- **Print** - The SPOT printing options allow you to print one or multiple diagrams on a page.

Printer Setup

After you have installed and setup your printer, the procedure for setting up and configuring a printer in SPOT is the same as in any other Windows program.

   ✎ *Note: Because the information contained in this section is standard Windows functionality, the options are not discussed in detail. For more information on Windows printing features, refer to the Windows on-line help.*

To choose and configure a printer, follow these steps:

1. **Choose a printer.** From the File menu, select Printer Setup. The Page Setup window appears. To select the printer you want to use, click on the Printer button at the bottom of the window. The program prompts you to select a printer.

2. **Modify the default settings of the printer, if necessary.**
Click on Properties. From this point you can modify printer specific options such as color settings, fonts, graphic settings, etc… The printer properties window and the available options will vary according to the printer that you are using. Refer to the on-line help or your printer's manual for more information. When you finish entering the property settings, click on **OK**.

3. Click on **OK**. The program returns you to the original Page Setup window.

4. **Define the page setup that you want to use for the image(s).**
This includes the page orientation (portrait or landscape), paper size, paper source, and margins.

5. **Save the page layout specifications that you entered.**
Click on OK.

   ✎ *Note: You can also access the Page Setup screen from the Print screen (i.e., From the File menu, select Print, and click on the Setup button.*
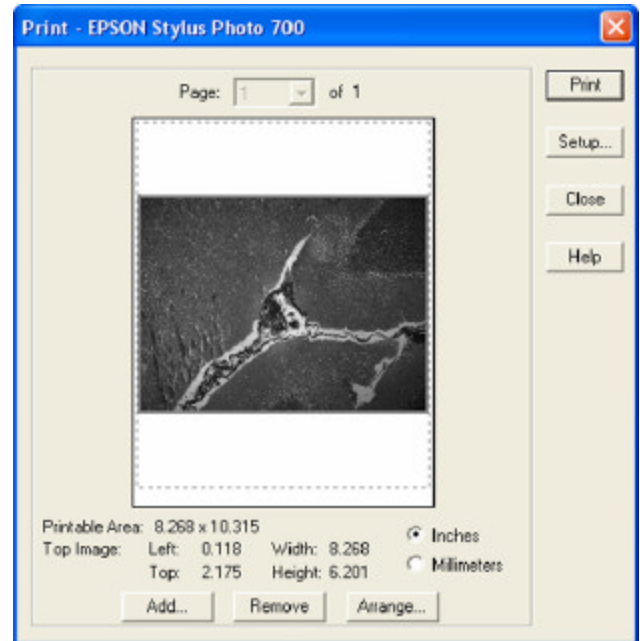
Print

After you have defined the printer and page layout, you are ready to print. With the SPOT software, you can print a single image to a page or you can print multiple images on a page for comparison or display.

Printing a Single Image

To print a single image from the SPOT software, follow these steps:

1. Either select Print from the File menu, or click on the Print toolbar button. The Print window appears with a preview of the active image. The Print window displays the following information:

    − **Printable Area:** The printable dimensions of the page.

    − **Top Image:** The position and size of the top-most image (if more than one image will be printed on the page). They are:

        • **Left** - The distance between the left side of the printable area and the left side of the image.

        • **Top -** The distance between the top of the printable area and the top of the image.

        • **Width** - The width of the image.

        • **Height** - The height of the image.

2. Check either **Inches** or **Millimeters** to specify the units of measurement.

3. Adjust the size of the image:

    a) Position your cursor on a side or a corner of the image. The cursor turns into a two-ended arrow.

    b) Using the two-ended arrow cursor, drag a side or corner to enlarge or reduce the image. The resized image maintains the height to width ratio of the original image.

4. Adjust the position of the image.

    a) Position the cursor on the image. When on the image, it appears as a four pointed arrow.

    b) Left click and drag the image to the desired location.

5. Click on the Print button. The Printing dialog box displays the job. To stop the print job, click on the Abort button.

Printing Multiple Images on a Page

The SPOT program also enables you to print multiple images on a single page. The additional images do not need to be open; you can add additional images from a file directory or a SPOT database.
To add and arrange multiple images for printing, follow these steps:

1. From the File menu, select the Print option. The currently open, selected image appears in the Print window.

2. Add an image to the page for printing. Follow these steps:

    a) Click on the Add button. The Add Image window appears.

    b) Select the image that you want to add. Images can be added from one of three places:

- From a **Currently Open Image** – Add one or all of the currently open images. Select either the desired image or **<All Images>** from the Title drop-down list. Ensure that you enter the **Sequence Index** (i.e., the number of the image in the sequence) if you are adding an image from a sequence file.

- From an **Image File** – Add a file from disk. Check the **Image File** option, and click on the square next to the box. Browse for the desired image, and click on Select. Ensure that you enter the **Sequence Index** (i.e., the number of the image in the sequence) if you are adding an image from a sequence file.

- From a SPOT Database – Add an image file from an open SPOT database. To add an image file from a SPOT database, you must know its Image ID number. After you have determined the number, check the Database option, and enter the ID number.
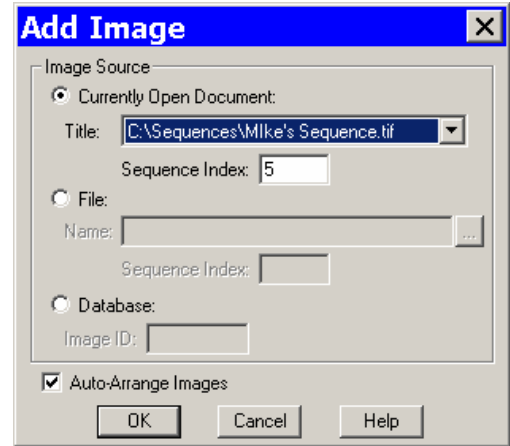
c) Set the auto-arrange option. If you check the Auto-Arrange Images box, the SPOT program automatically sizes and positions the images to fit on the print page.

d) Click on OK. The program adds the images to the print page and returns to the Print window. The images appear in the preview box.

- **If you selected the Auto-Arrange Images option**, the images will be arranged in a print-ready format. Click on Print to print the page.

- **If you did not use the Auto-Arrange Images option**, the images will be stacked on top of each other for you to arrange. Go to step 3 for details on manually arranging the images.

   To add additional images, repeat step 2.

To arrange how multiple images will appear on the page. Follow these steps:

a) Click on the Arrange button. The Arrange Images window appears.

b) Select the number of row(s) and column(s).

c) Enter the Spacing between the images on the printout in inches or millimeters.

d) Select **Apply to all pages** to make the arrangement applicable to every page of a multi-page report (This checkbox will not appear for a single page report).

e) Click on OK. The Print screen reappears with a preview of your print arrangement. The screen capture at right illustrates four images arranged in a two row, two-column format.
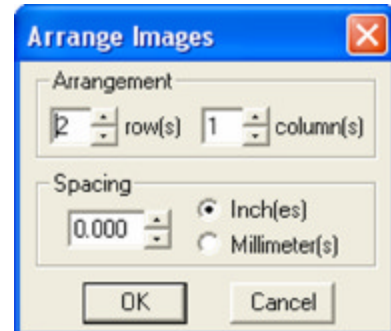
The SPOT program automatically resizes images to fit on the page, so you can add multiple images without having to resize them. For example, if you add five images, the preview will show six images (the original and five added) of equal size, distributed proportionally across the page.

Note: You can reposition and resize multiple images on the preview page, just as you can with a single image.

From the print screen, you can:

- Click on **Print** to print the page.
- **Add** an image as described in step two.
- Delete an image by selecting it and clicking on **Remove**.
- Reposition and/or resize the images as described in the previous section.
- Use the **Page** drop-down list to navigate to a different page view (when printing multiple pages.
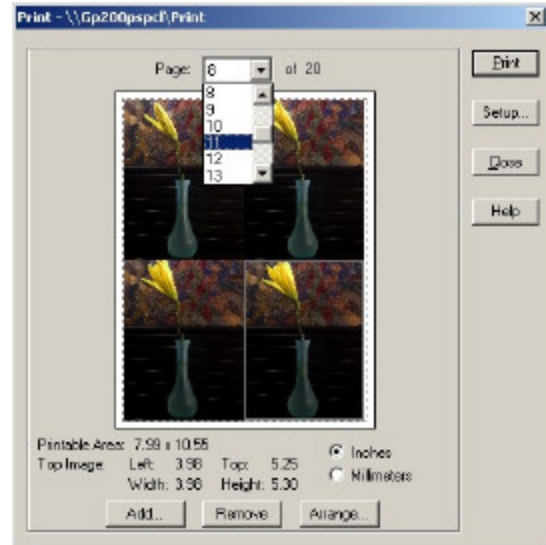
Printing Sequential Images

The procedures for printing sequential images in the SPOT software are identical to those described in the **Printing Multiple Images on a Page** section (see above), with one exception. When you select the **Print** option for a sequential image file, the program prompts you to specify the image(s) in the sequence that you want to print, as shown at right.

- If you select **All Images in Sequence** and click on OK, the program prompts you to specify the number of images per page.

- If you select **Specified Images**, you can select either a single image or multiple images. Separate multiple images with commas and/or dashes – e.g., **1, 2, 3**; **1-3**; or **1, 2, 3-6**.

Ch. 12 – Taskbars

QUICK REFERENCE:

⊃ Three ways to access the Taskbar Setup Window…p. 216

⊃ Adding buttons to the taskbar…p. 217

⊃ Modifying the look and function or taskbar buttons… p. 218

⊃ Deleting taskbar buttons… p. 219

⊃ Modifying the taskbar to view it more clearly or to create more space on the computer screen… p. 219

⊃ Why some of the taskbar buttons don't seem to work… p. 219

⊃ Autosave feature of Taskbar… p. 220

Introduction

SPOT software allows you to customize your program by developing a Floating Taskbar containing timesaving onscreen buttons that can initiate functions or start a macro. The Taskbar serves as an onscreen repository of the functions or Macros that you commonly use in your imaging/post processing work, where, with a single click, those functions can be easily initiated without searching through dropdown menus.

As an example, let's assume that you commonly need to Open image files, Save image files, add image borders, cut objects from images, use Annotation Tools, and/or run your favorite Macro program.

You can develop a taskbar with buttons that give you quick access to all of these functions and Macro programs. It might look like the taskbar below.
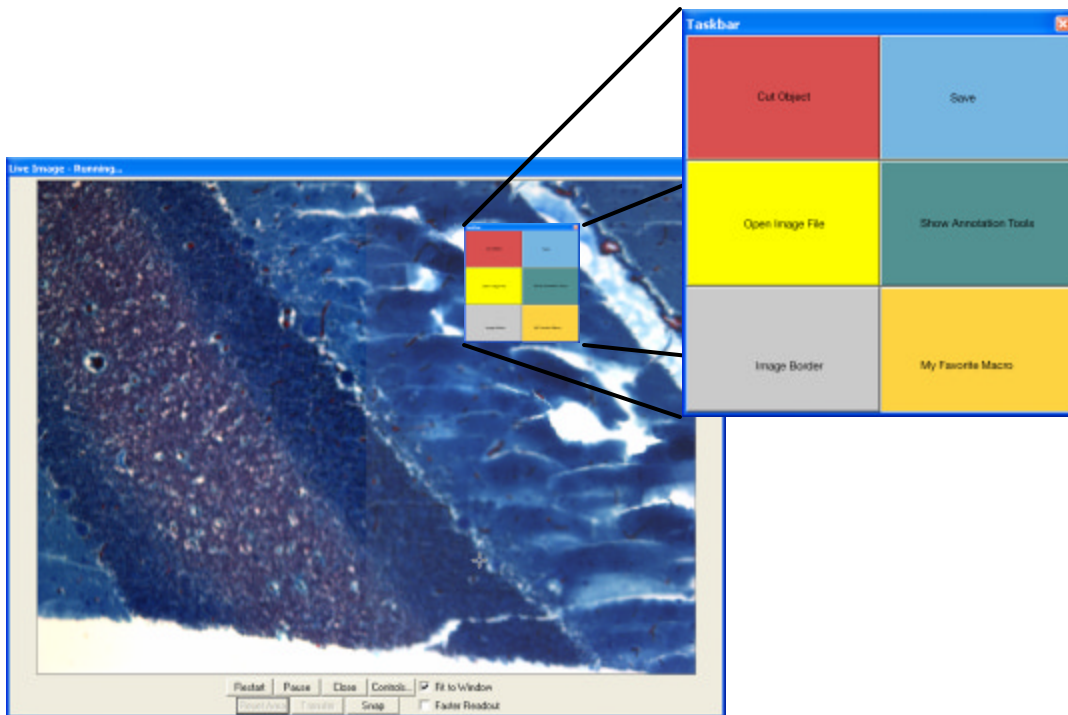
Developing a Taskbar

Accessing the Setup window

Developing a taskbar involves adding, deleting or modifying function buttons or macro buttons on the taskbar. All of these options are available in the Taskbar Setup window. You may access the taskbar setup window in one of three ways. The first method is only available the first time you develop the taskbar (or if you have previously completely deleted the buttons on an existing taskbar). The second is available either the first time you develop the taskbar or to modify an existing taskbar. The third is available only after you have developed and have opened a taskbar.

1. You may access the taskbar through the View dropdown menu. The first time you select *Show Taskbar* through the View menu, two events will occur in rapid succession. First, an empty taskbar window will open on the screen.
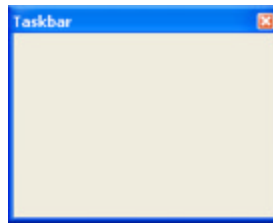
Almost simultaneously, the Taskbar Setup window will open. Again, this only happens when the taskbar is "empty", without any functions or Macros assigned to it.
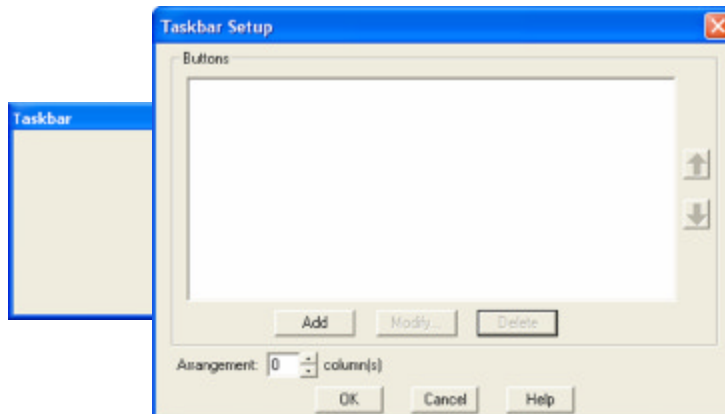
2. An alternate approach for developing your first taskbar involves selecting Taskbar from the dropdown menu under Setup. With this approach, the Taskbar setup window is immediately opened and is ready for the user to develop the taskbar. The floating taskbar will not automatically appear using this approach.

3. The third method for accessing the taskbar setup window requires the taskbar to be open onscreen. Place the cursor on the taskbar title bar and right click. A popup menu will appear. Select setup from the menu and the taskbar setup window will appear.

Developing the Taskbar for the first time

From the setup window, begin by pushing the Add button on the Taskbar Setup window. Another window will open named the Taskbar Button screen. This screen offers all of the functions that can be applied to the Taskbar. Additionally, the screen allows for the selection of a previously developed Macro as a Button on the Taskbar.

For example, the first function added to the taskbar might be the image rotation function. In order to add this function to the taskbar, select "Rotate Image Left" from the Edit Image menu in the Taskbar button window.
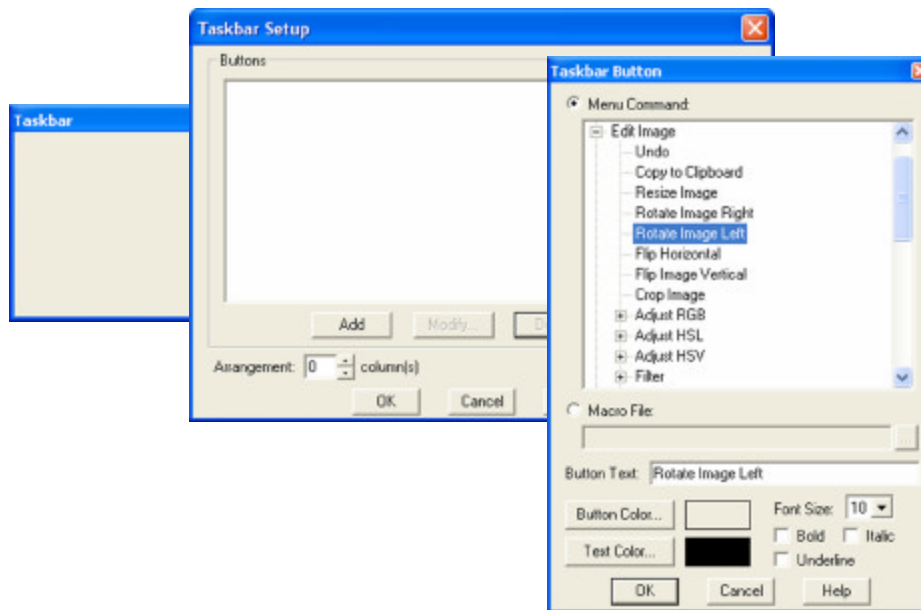
Note that the button text box defaults to Rotate Image Left. You may customize this button name to whatever you choose without changing the function of the button itself.

Next, select the color of the button background and the text along with the font size and style from the options available at the bottom of the taskbar button screen.

Click the OK button and the first function will be added to the Taskbar Setup window.
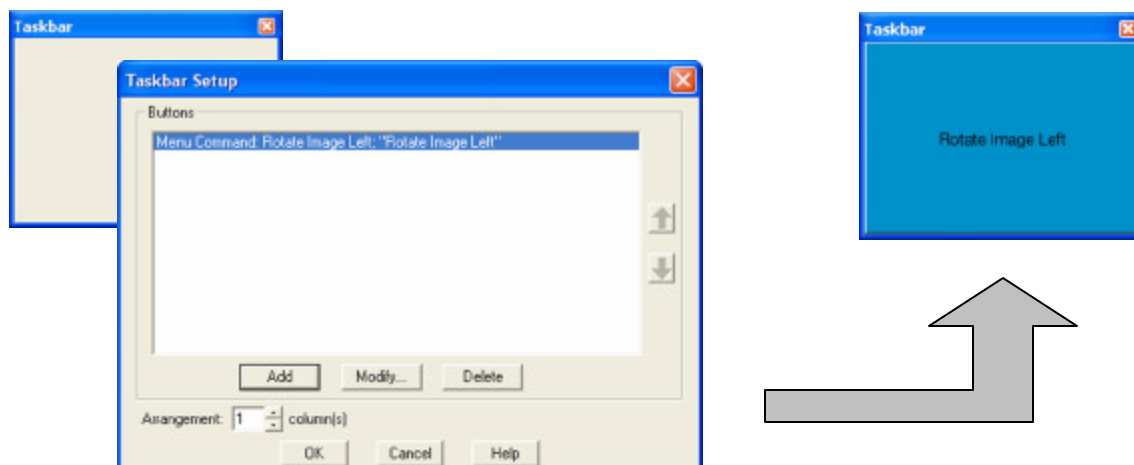
Then, if you click OK on the Taskbar Setup window, your first Taskbar button will be added to the taskbar.

Continue to add buttons to the taskbar in the same fashion until you are satisfied that you have added all of your favorite functions.

Note: The Macro taskbar pushbutton uses a defined computer path to search for the stored Macro file. If you decide to relocate the Macro file, you must update the taskbar button by redefining the path.

Note: You are limited to a taskbar with 50 buttons.

Modifying the Taskbar Appearance

After developing the taskbar, you may choose to modify its appearance by changing button colors, button names, text fonts, the position of the buttons on the taskbar, or the appearance of the taskbar.

Any of these changes can be easily accomplished through the Taskbar Setup window. Simply select the function or macro that you wish to change by highlighting it from the list. Then select the modify button, make the changes using the same procedures you used to develop the buttons originally. Click the OK button when you are finished and the Taskbar will be immediately modified.

You can change the relative position of the buttons on the taskbar by simply highlighting the function and moving its position up or down on the Taskbar Setup screen. Additionally, you may change the number of columns that the taskbar window is divided into using the column feature in the taskbar setup window.

Note: If you choose a number of columns that does not divide equally into the number of functions and macro buttons you have developed, the grid of the floating taskbar window will automatically fill the "extra boxes" with non-functional buttons in the default beige color.

You will notice, at times, that the font on certain buttons will appear faded. This indicates that the particular function is not available due to other circumstances present at the time. For example, if an image has not been captured yet, certain edit features will not be available. However, as soon as an image is acquired, the font on those buttons will become more contrasted to the button color, indicating that the function is now available.

Note: Until you select annotate, any buttons that relate to annotation will not be functional, even after you have captured an image. Once you select annotate from the drop down menu or as a taskbar button of its own, the annotation function buttons will become functional.

## Taskbar Size and Location

As an onscreen tool, the taskbar window can "float" on the screen in front of any open image windows (captured or Live images). The position of the Taskbar is not arbitrary, but instead can be set by the user. If the taskbar hinders your viewing of any images, you can do one of four things: modify its size, change its location, "roll it up", or make it disappear from the screen.

Modify the Taskbar size

Like most windows, simply highlight the taskbar and resize it by dragging one of its handles to make it larger or smaller. A side handle will change the width of the taskbar, a top or bottom handle will change the height of the taskbar, and a corner handle will change both height and width proportionately.

Change the Taskbar location

Simply grab the taskbar's title bar by placing the cursor over it and holding down the left button (PC). Then move the taskbar around the screen until you are satisfied with its location. Release the mouse button.
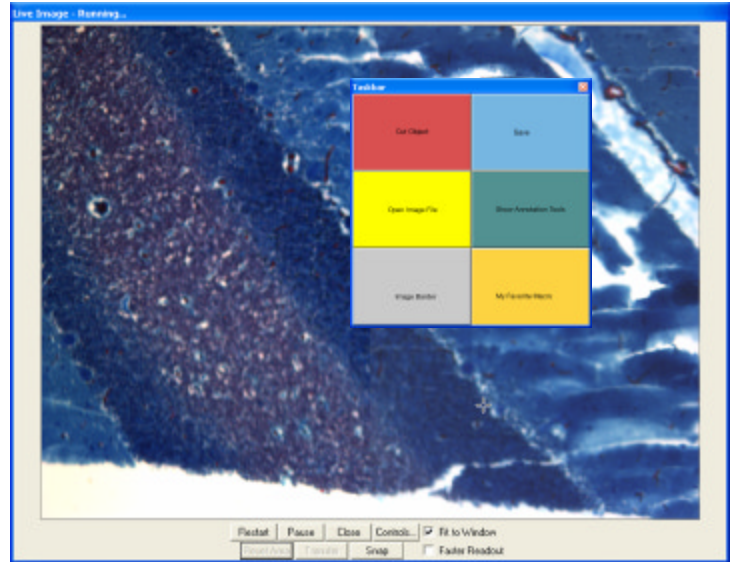
Roll up the Taskbar

Double click on the taskbar's title bar and the buttons will roll up under the title bar. You may also right click on the title bar and select roll up from the pop up menu.

Repeating these procedures will roll out the buttons from under the title bar.

Make it disappear

Click on the close box in the right upper corner of the taskbar. Alternately, right click on the title bar and select close from the pop up menu. In either case, you will need to access the View drop down menu and select show taskbar to make it reappear on the screen.

Saving the Taskbar

Once you have developed a taskbar, it is automatically stored as the default taskbar in the SPOT program. There is no need to perform any save function for the taskbar feature.

✎   *Note: When you close the SPOT program without closing the taskbar, the taskbar will be present onscreen the next time you open the SPOT program.*

Chapter 13 - Macro Scripting

QUICK REFERENCE:

➲ Why would I want to use Macro Scripting?… p.222

➲ How do I start to develop a Macro?…p.222

➲ What commands can I place in my Macro?… p.224

➲ I know the procedure to collect my images, but I don't like line-by-line programming to develop my macro. What can I do? …p.232

➲ I want to modify my existing Macro… p.233

➲ What is the purpose of the new commands that are specifically available in the Macro Scripting program?… p. 235

➲ Can I place any other programs within the Macro?… p. 253

Introduction

A new feature of the SPOT software is the addition of Macros Scripting (hereafter referred to as Macros). Macros offer the user the ability to automate many of the repetitive tasks associated with image capture and processing. As a result, the user can spend more time evaluating and analyzing results, and less time manipulating test set-ups, variables and other settings.

> **Macro** – *a program to automate a task or set of tasks that you perform repeatedly or on a regular basis. It is a series of commands and actions that can be stored and run whenever you need to perform that task.*

Benefits

A robot benefits an industrial user by accurately repeating redundant procedures. Macros can provide the same benefits to researchers and scientists for capturing images. Like a robot, a Macro requires the preparation of a program of commands that perform an operation. Once saved, that program can be modified and supplemented to create multiple procedures that do not require the researcher to replicate all of the program steps again and again.

> **Benefits of using Macros**
>
> - *Time savings*
>
> - *Accurate reproduction of image capture techniques*
>
> - *Macros are modifiable, allowing the user to maintain an original macro while modifying it for a slightly different result, with minimal effort*

We will begin by discussing directions for accessing, navigating, and using the Macro Window. New commands introduced in the Macro menu will be discussed.

Creating a Macro

There are two methods for creating a macro. The first involves utilizing a Macro Window to develop a macro program by adding commands line-by-line. The second involves using a record feature within Macros that develops a macro by monitoring and recording, on-the-fly, the individual commands that the user selects while in the process of carrying out an imaging procedure.

After your first macro is completed and saved, further macros can be developed as new, recorded, or can be developed by modifying an existing macro and saving it under a new filename (see Edit).

Both methods of macro development can be initiated through the Macro drop down menu. We will begin by discussing the first method, line-by-line development, in order to gain a greater understanding of the Macro Window.

Let's get started.

Accessing and Navigating the Macro Window

From the drop down menu on the Spot Advanced Software Screen, select Macro. The drop down menu will offer four choices. They include the following:

- New
- Edit
- Record
- Play

You can access the Macro Window directly by selecting the New option. You may also access it by selecting Edit and then selecting a macro file to edit from your computer (Once you have created and saved a macro).

New

Select New from the Macro menu. The Macro Window will open.
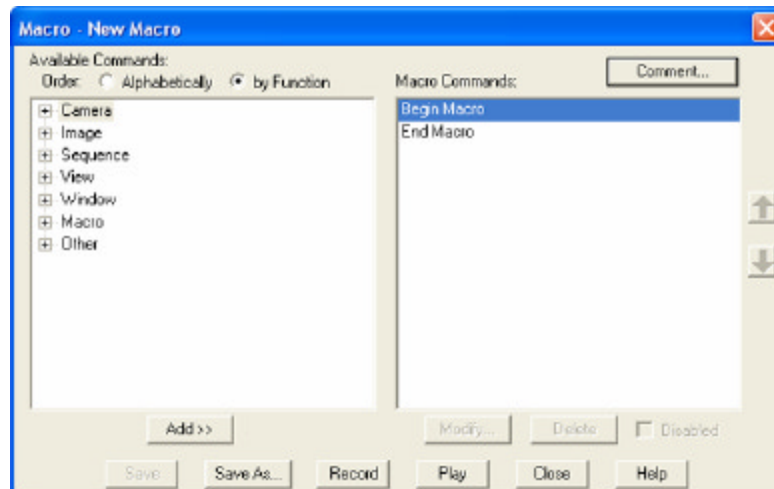
Macro Window

You will note that the Macro Window is a split into two active panes. The pane to the left includes a selection menu and provides all of the available commands that can be incorporated into any macro. The pane on the right represents the active macro development pane or a reflection of the Macro program, command line by command line. When "New Macro" is selected, the screen will open with the default commands of Begin Macro and End Macro on the right window. All commands are placed in between these commands when developing your personal macro.

The menu screen on the left can be formatted in two different ways. The first is logically grouped into seven main functions. They include:

1. Camera
2. Image
3. Sequence
4. View
5. Window
6. Macro
7. Other

Each of the main topics can be expanded by clicking on the [+] next to the topic name, similar to an expandable Windows menu. For example, the Camera topic expands as indicated below.

Command selection Pane

Active **Macro Development Pane**
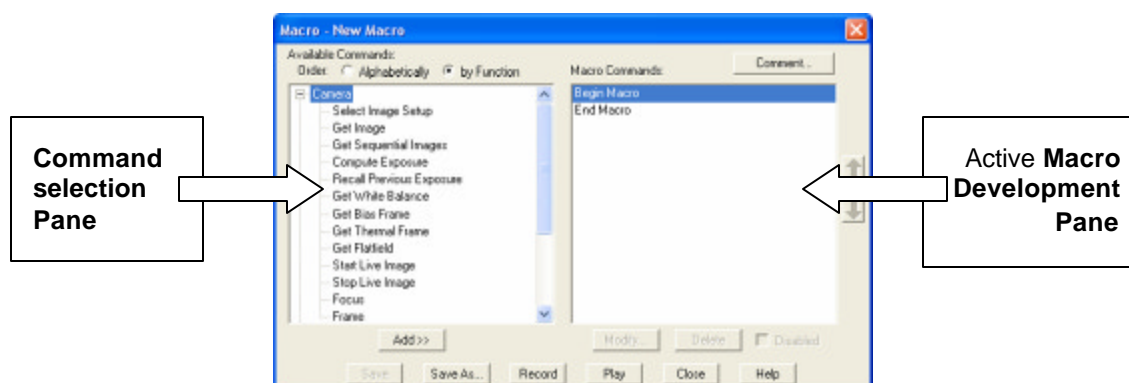
Macro Commands Available

The menu expands to show all of the available macro commands related to the main topic. Table A. indicates all of the commands available under each main topic.

Table A. Expanded Menu with Command Selections

Topic	Command	Interactive Mode
1. Camera	Select Image Setup	Yes
	Get Image	
	Get Sequential Images	Yes
	Compute Exposure	Yes
	Recall Previous Exposure	Yes
	Get White Balance	Yes
	Get Bias Frame	Yes
	Get Thermal Frame	Yes
	Get Flatfield	Yes
	Start Live Image	
	Stop Live Image	
	Focus	
	Frame	
2. Image	Open Image File	Yes
	Save Image File	
	Save Image File As	Yes
	Save Image to Database	Yes
	Print Images	Yes
	Resize	Yes

Topic	Command	Interactive Mode
	Rotate Right	Yes
	Rotate Left	Yes
	Flip Horizontally	Yes
	Flip Vertically	Yes
	Crop	Yes
	Adjust RGB Additive Brightness	Yes
	Adjust RGB Multiplicative Brightness	Yes
	Adjust RGB Contrast	Yes
	Adjust RGB Gamma	Yes
	Adjust RGB Bright and Dark Level	Yes
	Adjust RGB Histogram	Yes
	Adjust Hue Saturation and Luminance	Yes
	Adjust HSL Multiplicative Luminance	Yes
	Adjust HSL Contrast	Yes
	Adjust HSL Gamma	Yes
	Adjust HSL Bright and Dark Level	Yes
	Adjust HSL Histogram	Yes
	Adjust Hue Saturation and Value	Yes
	Adjust HSV Multiplicative Value	Yes
	Adjust HSV Contrast	Yes
	Adjust HSV Gamma	Yes
	Adjust HSV Bright and Dark Level	Yes
	Adjust HSL Histogram	Yes
	Smooth	Yes
	Sharpen	Yes
	Unsharp Mask	Yes
	Filter Noise	Yes
	Median Filter	Yes
	Make Image Negative	Yes
	Make Grayscale	Yes
	Set Palette	Yes
	Change Bit Depth	Yes
	Subtract Image	Yes
	Merge Images	Yes
	Duplicate Image	
	Add Calibration Mark	Yes

Topic	Command	Interactive Mode
	Add Measurement	
	Add Annotation	Yes
	Set Image Memo	Yes
3. Sequence	Create New Image Sequence	Yes
	Extract Sequence Images	Yes
	Append Sequence Images	Yes
	Play Sequence Forward	
	Play Sequence Reverse	
	Step Sequence Forward	
	Step Sequence Reverse	
4. View	Set Fit To Window	
	Set Zoom Level	
	Show Pan Window	
	Show Image Date/Time	
	Set Full Screen	
5. Window	Cascade Windows	
	Arrange Windows Horizontally	
	Arrange Windows Vertically	
	Close Image Window	
	Close all Image Windows	
	Close all Image Sequence Windows	
	Close all Document Windows	
6. Macro	Block	
	Loop	
	Escape Block/Loop	
	Delay	
	WaitUntil	
	If	
	Else	
	Set Variable	
	Increment Variable	
	End Macro	
7. Other	Play Sound	
	Prompt User	
	Run Report	Yes
	Execute OS Command	

The menu screen can also be formatted in alphabetical order as shown in the figure below.
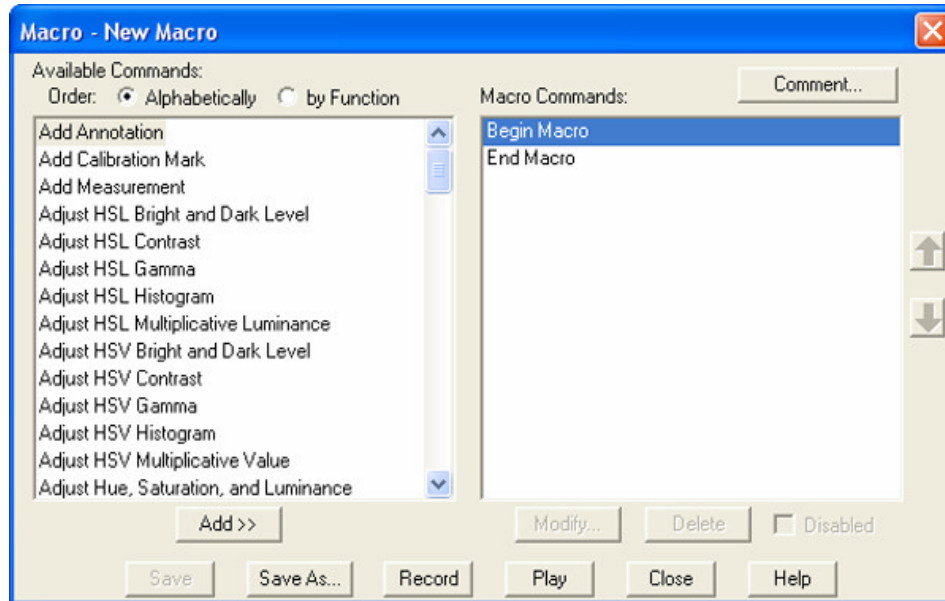
Interactive Mode

As listed in the previous table, many of the accessible commands in the Macro Command Selection Pane include an option that is used to prompt the user to provide input during the playback of the Macro. This is referred to as an Interactive Mode.

When a command with an interactive mode option is selected, the command window will appear with an unchecked box in the upper left corner. The user must select the Interactive feature of the command in order to be asked for data (in the case below, the filename) during the playback of the Macro program. When the interactive mode is not selected, the Macro Window may require immediate input before it can accept the command as part of the macro being constructed.

In the example below, if the interactive mode is not selected, a filename must be input before this window can be closed and added to the Macro program.
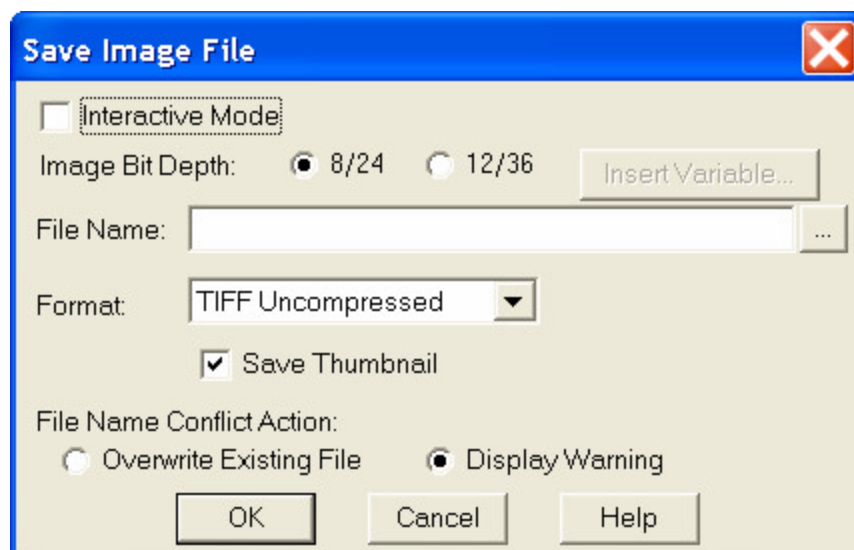
Developing a macro in the Macro Window

To begin developing your macro, you will want to copy commands from the left command selection pane into your active macro development screen in the right pane. You may do this by highlighting the command you would like to add from the left command selection pane and then clicking the **Add** button just below the pane. The first added command will be copied between Begin Macro command and the End Macro command.

As a second option, Windows users may double click on the command in the command selection pane. The command will be placed into the Macro in the development pane on the right.
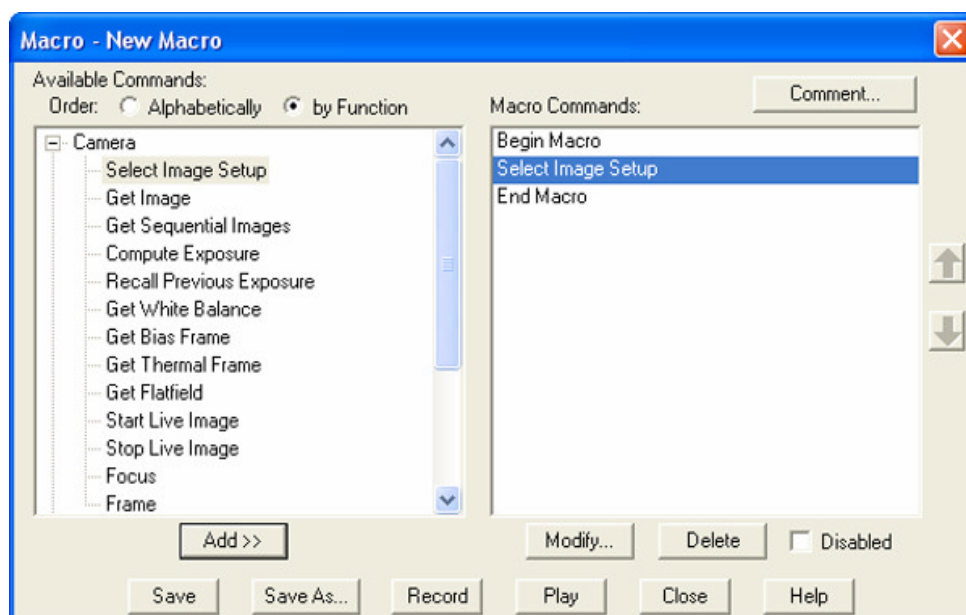
After the first command has been added, you will note that the Modify and Delete buttons become active on the right side of the Macro Window. The default Begin Macro and End Macro commands cannot be modified or deleted, however, all commands added in between them can be.
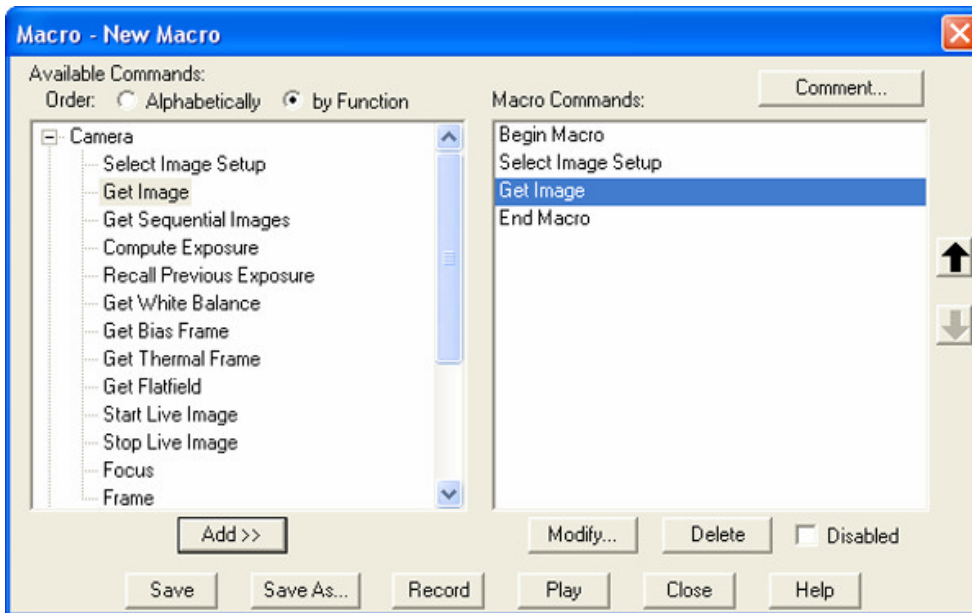
You will also note that any subsequent commands you add will be placed just below the highlighted command in the development pane. When a command is in a position and you would like to change its position, simply highlight that command and use the up and down arrow buttons to the right of the development pane to reposition it. You will note in the case above, that the Get Image command can only be repositioned upward. A downward reposition would place it outside of the End Macro command, which is not allowed.

Other Options on the Macro Window

Comment – The user may also add comments to the Macro through the use of the comment button located just above the development pane on the right.
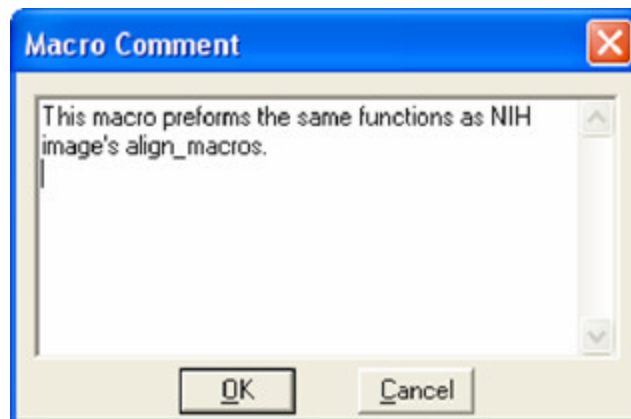
Once a comment is placed with the Macro, it remains hidden during editing and playback and can be accessed only when the comment button is pushed. It is associated with the Macro, and not any particular command lines in the Macro. It is simply a virtual notepad for the user to add a reminder that becomes part of the specific Macro program. It can be edited at any time by accessing the Macro comment window, modifying the comment, and saving the updated Macro program.

Modify – This command allows the user to modify any of the settings associated with any command in the development pane. The modify command can only be accessed with commands that have underlying user defined settings.

Delete – The delete button will delete any and all highlighted commands within the Macro development pane. The only exception to this is deletion of the Begin Macro and last End Macro command in the Macro. These cannot be deleted.

Disable – The disable command box located just below the development pane allows the user to selectively turn off (disable) any commands located between the Begin Macro and End Macro commands. This allows a user to temporarily remove certain procedures from a Macro without having to Delete them.

If you would like to select a group of commands, highlight the first in the group in the development pane, hold the control key (Windows) and continue to highlight all other commands you would like to disable. Once all commands are selected, activate the Disabled box. All selected commands will be placed in

parentheses, thus indicating that they are no longer commands that will be executed as part of the Macro.

For example, in the following Macro, the Rotate Left and Sharpen commands have been selected and disabled.

This macro can still be played, however, the Rotate Left and Sharpen commands will be ignored.

Save – The user may save the macro at any time by selecting this button. Like any program or application being developed, this saves all modifications being made to limit the loss of data in the event of a program interruption or crash. The user will be prompted for a filename only once when a new macro is being developed. After that, Save automatically saves to the active filename.

Save As – Like the save command, this allows the user to actively save the modified program at any time. However this command is best utilized for saving the modified file under a new file name, thus maintaining the original file structure with the original filename.

In this case, the user will be prompted to select a filename for the Macro to be saved. The default filename will be the current filename of the open Macro.

Record – This allows the user to quickly exit the Macro Window and begin developing the macro through use of the record feature. This feature is explained in greater detail in the following pages.

Play – This command allows the user to immediately begin playing the active macro or any other macro without having to manually close the macro Window.

Close – This command allows the user to close the Macro Window. If commands have been added to a new macro or if an existing macro has been modified, a window will appear and ask if the user would like to save the macro.

Help – This command allows the user to access the online help area of the SPOT software without closing the macro window.
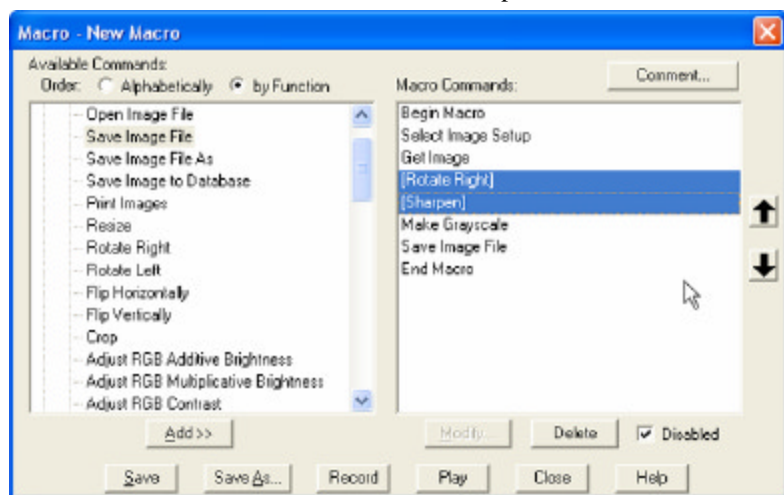
Macro Window - Quick Reference

Comment –   allows for the addition of a stored comment to the macro program

Add –   adds a command to the macro from the command selection pane

Modify –   allows modification of the settings associated with the selected command in the macro development pane

Delete –   deletes the selected command in the macro development pane

Disable –   makes the selected command(s) inactive when the macro plays

Save –   saves the macro program immediately under the current filename

Save As –   saves the macro program under a modified filename

Record –   activates the macro recording mode of macro development

Play –   plays the active macro immediately

Close –   closes the active macro

Help –   activates the help window in the SPOT program

Record

This handy feature allows the user to develop a macro without using the macro window to add commands line-by-line. Instead, the user selects record from the macro dropdown menu, a taskbar button, or from within the Macro Window. A window will appear informing the user that a new macro is recording.
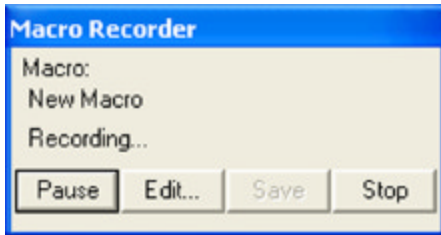
Once this has been indicated, all commands selected from the Spot Software menus, toolbar, or taskbar (except other macro programs) are sequentially added to the developing macro.

Developing a Macro Using the Record Feature

For example, if the first command is to be Get Image, the Camera dropdown would be opened, and the user would select the Get Image command.
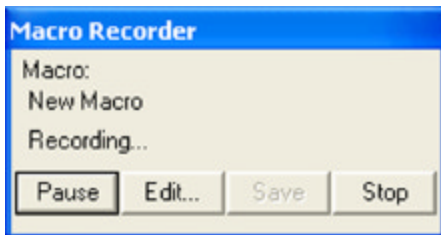
This command would automatically be added as the first command following the Begin Macro command in the macro development.

Thereafter, all selected commands would be added after the Get Image command, in sequence, until you choose to end the macro. To end the macro, simply choose the stop button on the Macro Recorder window.

When you choose to end the macro recording, another window will prompt you by asking whether you would like to save the recorded macro. If you select yes, a subsequent window will appear asking you to name and save the macro.

Recording a macro is an excellent way for developing a "rough draft" of a macro program. Once the macro is recorded, you can use the edit macro command to review all of the commands you recorded and modify the macro program in order to refine it.

Spot software users who are use to developing their imaging procedures using the dropdown menus may find this to be a comfortable and expedient method for initially developing a macro.

Edit

This command is used to review and edit an existing macro that has been saved. When the user selects the edit option from the macro dropdown menu, an Open Macro Window will appear, prompting the user to select a stored macro or browse for a stored macro.

Once the macro is found, open it by double clicking on the name (Windows users), single clicking on the name (which places the name in the File Name box) followed by clicking the open button, or by typing the name into the File Name box followed by clicking the open button. SPOT macros can be identified by their "**.spm**" filename extension.
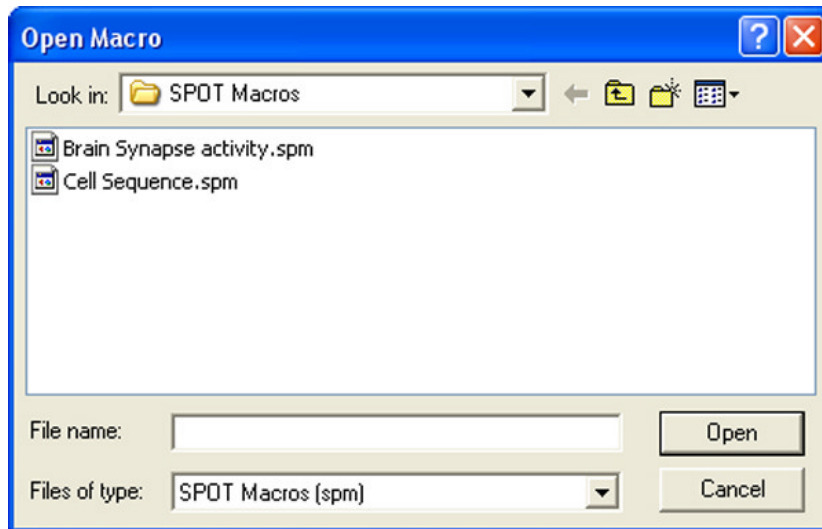
Once selected, the Macro Window will open and the Macro program will show in the development pane on the right.

At this point, the user can choose to simply review the program or choose to edit any or all of the available commands. The range of editing options includes adding new commands or deleting existing commands, disabling (but not deleting) commands, modifying commands (available only when subcommand windows are attached to the command), resequencing commands, defining or redefining variables, and adding or revising comments.

You can also choose to simply play the macro from this window. This allows the user to review the macro to verify that it is the proper procedure prior to setting up an experiment, playing the macro, and obtaining resulting images.

Play

The play option automatically initiates the macro program. For the user who knows which macro is the exact procedure to use to get the desired results, this is the fastest way to gain them (short of setting up a taskbar button to play the specific macro – See Taskbars). Once Play is selected from the macro dropdown menu, a window will appear prompting the user to select the desired macro to be played. Once the macro is selected, it begins to play immediately, without further prompting or warning.
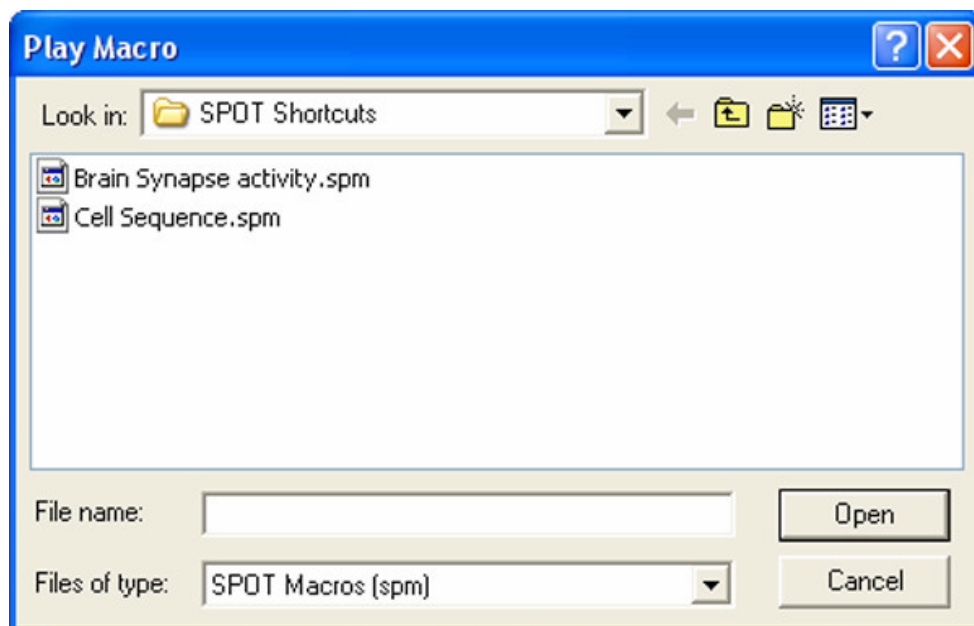
A Macro Playing window will appear anytime the macro is processing commands. You may interrupt the macro program at anytime by selecting the Stop button on the Macro Playing Screen.
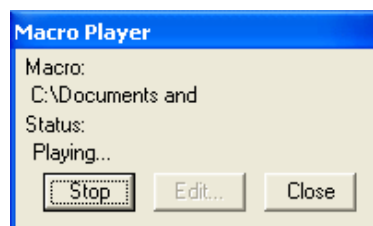
When the macro has completed playing on its own, the Macro Player window Status changes to Done, and the stop button changes to a Play button.
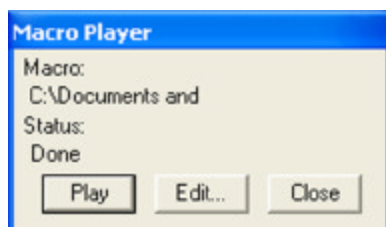
New Macro-specific Commands

The Macro feature in the Spot program introduces a number of new commands specific to the development of the macro program. In the following section we will review and further explain the commands. The following approach will be used with each command:

[**COMMAND NAME**]

- *Purpose and Use*

- *The Macro Window and [Command Name]*

- *Windows specific to [Command Name]*

- *Generic Example using [Command Name]*

Purpose and Use – Explains the basic function of the command and why and when it is most useful in developing a macro program.

The Macro Window and [Command Name] – Describes what happens on-screen in the macro development pane when the command is selected in the command selection pane.

Windows specific to [Command Name] - Describes the appearance, function, and how to input data for any window that results from selecting one of the new macro commands. Note that some, but not all, new commands have a data input window associated with the command.

Generic Example using [Command Name] - Provides one or more examples of the use of the specified command in conjunction with other generic commands.

The new commands associated with the Macro Scripting feature can be classified into four groups. They include the following:

- Conditional Commands.....................................IF, ELSE, LOOP, BLOCK, ESCAPE BLOCK/LOOP

- Variable Commands..SET VARIABLE, INCREMENT VARIABLE

- Timing Commands..DELAY, WAITUNTIL, END MACRO

- Communication Commands.............................PLAY SOUND, PROMPT USER, RUN REPORT, EXECUTE OS COMMAND

Conditional Commands

Conditional Commands arise from the IF Command and relate to what the Macro program will do next, once it has evaluated whether an IF Command is true or not true.

IF

Purpose and Use

The IF Command is used to write a conditional statement, otherwise known as a test statement. The result of the IF Command test is always yes (true) or no (false). When the result of the IF test proves to be true, the macro program will continue by executing the next program line after the IF Command. When the result of the IF Command proves to be false, the macro program will skip the subsequent command (or command set – see BLOCK and LOOP) and continue with the next command following the subsequent command.

The IF Command is a powerful tool when your macro program contains a number of different options as part of your imaging routine, but you only want to complete certain options for a given experiment condition.

The Macro Window and IF

When the IF Command is selected from the command screen, it is added to the development pane below the highlighted command in the pane.

Window specific to IF

When the IF Command is chosen from the command selection pane (left pane of the Macro Window), another window will open on top of the Macro Window. It will appear as shown below.
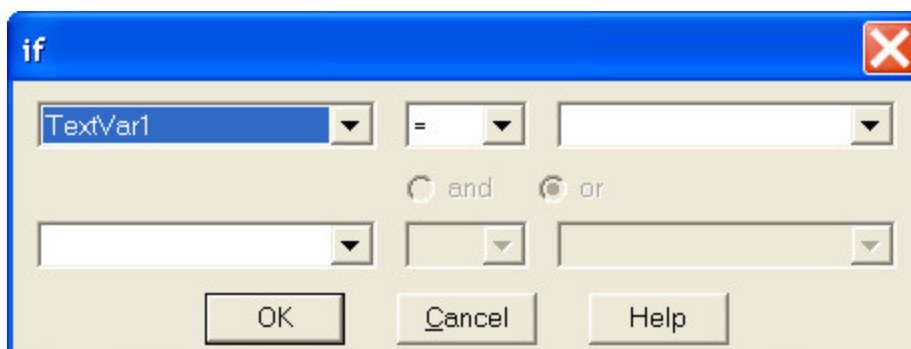
The purpose of this window is to develop the IF Command that will be proven true or false when evaluated. You will note the following features of the IF window.

Name of the Variable (drop down menu available)

Operator of Conditional Command (drop down menu available)

Value of Variable (drop down menu available)
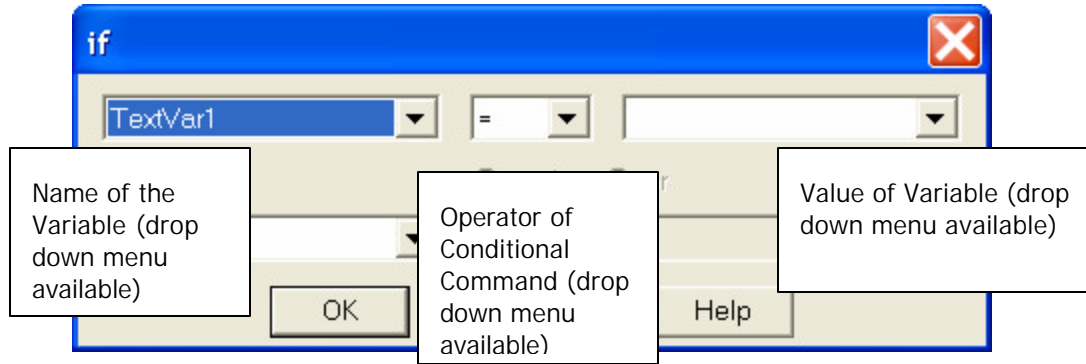
When a name is entered in the second variable name box, you will note that the corresponding operator and variable value boxes become active. Additionally, the and/or option becomes available. This allows for an expansion of the IF Command to include two conditions that must both be met (if "and" is used) or two conditions, either of which can be met (if "or" is used) for the IF Command to be proved true.

Name of 2 nd variable (drop down menu available)

Operator of 2 nd Conditional Command (drop down menu available)

Value of 2nd Variable (drop down menu available)
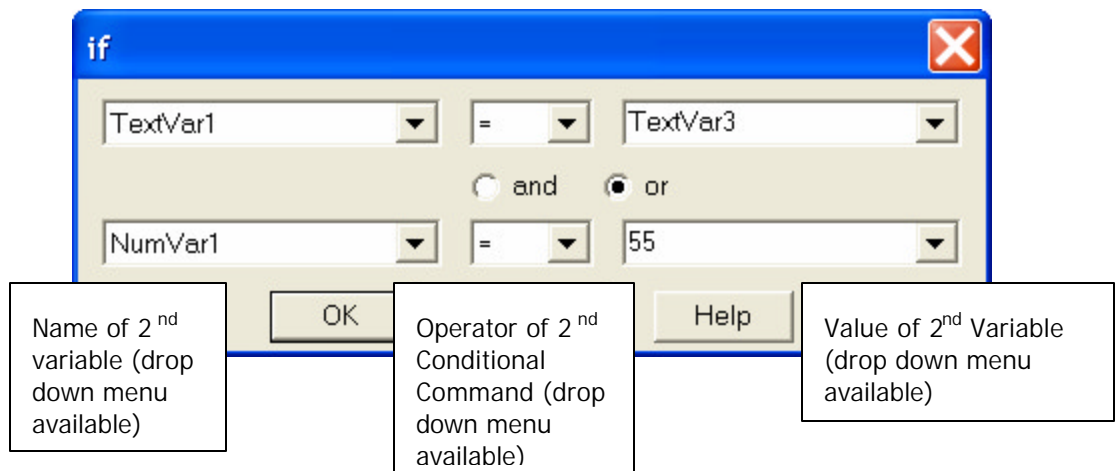
Generic Example using IF

Example X

IF Command
Command A1
Command B1
Command C1
Command…

When the IF Command is true, the program will execute Command A1, followed by Command B1, Command C1, etc.

Should the IF Command prove to be false, the macro will skip the <u>next command line or command set </u>following the IF Command, and will continue with the first command following that command or command set.

In the preceding Example X, when the IF Command is false, the program will skip Command A1 and will execute Command B1, followed by Command C1, etc.

If a group of commands are to be executed after the IF Command is evaluated, the user should use the BLOCK Command to execute the group (see BLOCK Command).

Example Z

```
IF Command
 Begin Block
  Command A1
  Command A2
  Command A3
 End Block
 Begin Block
  Command B1
  Command B2
  Command B3
 End Block
 Command C1
 Command…
```

In Example Z, when the IF Command is true, the program will execute the block of commands including A1, A2 and A3. It will follow by executing the block of commands including B1, B2, and B3. Once that block is completed, the program will execute Command C1, etc.

When the IF Command is false, the program will skip the block of commands including A1, A2 and A3 and will execute the block of commands including B1, B2, and B3. Once that block is completed, the program will execute Command C1, etc.

ELSE

Purpose and Use

The Else Command is used in conjunction with an IF Command, allowing a choice between two subsequent commands depending upon the outcome of the IF Command evaluation.

The Macro Window and ELSE

When the ELSE Command is selected from the command screen, an Else command is placed in the macro program below the existing highlighted command.

Window specific to ELSE

No specific window appears to further define the ELSE Command.

Generic Example using Else

When the ELSE Command follows an IF Command, it is the default command should the IF Command turn out to be false. In this "false" case, the command following the ELSE Command is executed. Interestingly, Example X, cited earlier (and below), includes an implicit ELSE Command. When the IF Command was proven false, Command A1 was omitted, and Command B1 was carried out. Could the program just as well been written as the following Example X.1?

Example X

IF Command
Command A1
Command B1
Command C1
Command…

?

Example X.1

IF Command
Command A1
Else
Command B1
Command C1
Command

The answer is no! There is a difference. In Example X, Command B1 is carried out after Command A1 when the IF Command is true or when it is false. In the Example X.1, Command B1 will not get carried out if the IF Command proves to be true. Instead, Command A1 will be carried out, followed by Command C1, etc. In Example X.1, with the Else Command, the subsequent command (Command B1) is only carried out if the IF Command is false. Therefore, in Example X.1, a choice between Commands A1 and B1 is made. In Example X, the only choice is whether to carry out Command A1 or not. Command B1 will always be implemented.

The same use of the Else Command can be applied with blocks.

Example Z

IF Command
Begin Block
Command A1
Command A2
Command A3
End Block
Begin Block
Command B1
Command B2
Command B3
End Block
Command C1
Command…

?

Example Z.1

IF Command
Begin Block
Command A1
Command A2
Command A3
End Block
Else
Begin Block
Command B1
Command B2
Command B3
End Block
Command C1
Command…

We already know from an earlier discussion of Example Z that the only choice is whether to carry out Command Block A1-A3. Block B1-B3 will be carried out regardless of the result of the IF Command.

However, in Example Z.1, when the IF Command is true, the program will execute the block of commands including A1, A2 and A3. It will skip the following block of commands including B1, B2, and B3 and will continue the program by executing Command C1, etc.

When the IF Command is false, the program will skip the block of commands including A1, A2 and A3 and will execute the block of commands including B1, B2, and B3. Once that block is completed, the program will execute Command C1, etc.

LOOP

Purpose and Use

The LOOP Command instructs the Macro program to complete a series of commands (within the loop) and then repeat those commands a defined number of times (iterations) before continuing the Macro program.

This can be quite helpful when a repetitive procedure defined by a series of commands must be completed.

The Macro Window and LOOP

When the LOOP Command is selected from the command selection pane in the Macro Window a Begin Loop and an End Loop command will be inserted into the Macro program in the development pane. The Begin Loop command will be highlighted in the development pane, indicating that the next selected command will be placed just below the Begin Loop command. Other commands to be carried out as part of the loop must be placed between the Begin Loop and End Loop commands.

After all commands are added between the Begin Loop and End Loop commands, the loop procedure is defined. Upon playing the Macro, the program will proceed through the series of Loop commands until it reaches the End Loop command. It will then return to the Begin Loop command and repeat the Series of commands for as many times as defined.
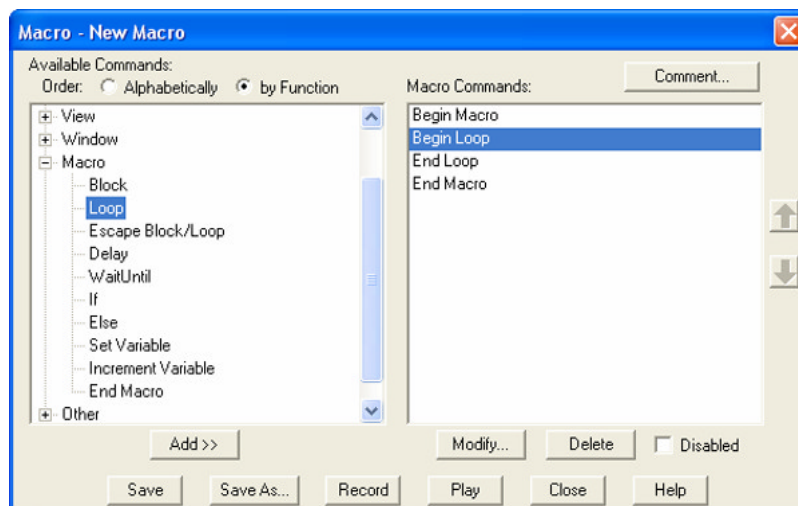
Window specific to LOOP

When LOOP is selected a Loop window will appear over the Macro Window. It will ask the user to define the number of loop iterations that will be carried out prior to the continuation of the remainder of the Macro program. The Loop window will look like this:
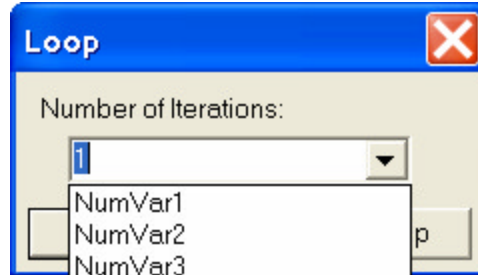
The Loop window includes a drop down menu that allows for 5 user-defined variables (NUMVAR1 through NUMVAR5). Additionally, any integer number may be placed in the Number of Iterations box in the Loop window. The value in the box defaults to 1 when the Loop window appears. When selecting the user-defined variables, the SET VARIABLE Command would be placed before The LOOP Command.

Generic Example using LOOP

Once you have selected the LOOP Command in the Macro Development pane, the command sequence entered might be:

```
Begin Macro (automatically added when the window is opened)
Begin Loop
 Command A1
 Command B1
 Command C1
 Command D1
End Loop
Command E1
End Macro (automatically added when the window is opened)
```

This macro would process Commands A1, B1, C1 and D1 in sequence, repeating the sequence for as many iterations as were specified in the LOOP Command. Once that number of iterations is completed, the program will move on and process Command E1.

BLOCK

Purpose and Use

The BLOCK Command is used to group a series of commands together, to be performed sequentially prior to any further commands being performed.

The BLOCK Command is very useful in conjunction with IF Commands (see IF Command) as it allows for a group of commands to be skipped should the IF Command prove to be false.

Essentially, the BLOCK Command is similar to the LOOP Command with an implied iteration value of one (see LOOP Command).

The Macro Window and BLOCK

When the BLOCK Command is selected from the command screen, both a Begin Block and an End Block command are simultaneously added, and the Begin Block command will be shaded in the development pane, indicating that the next command selected will be placed below the Begin Block command.
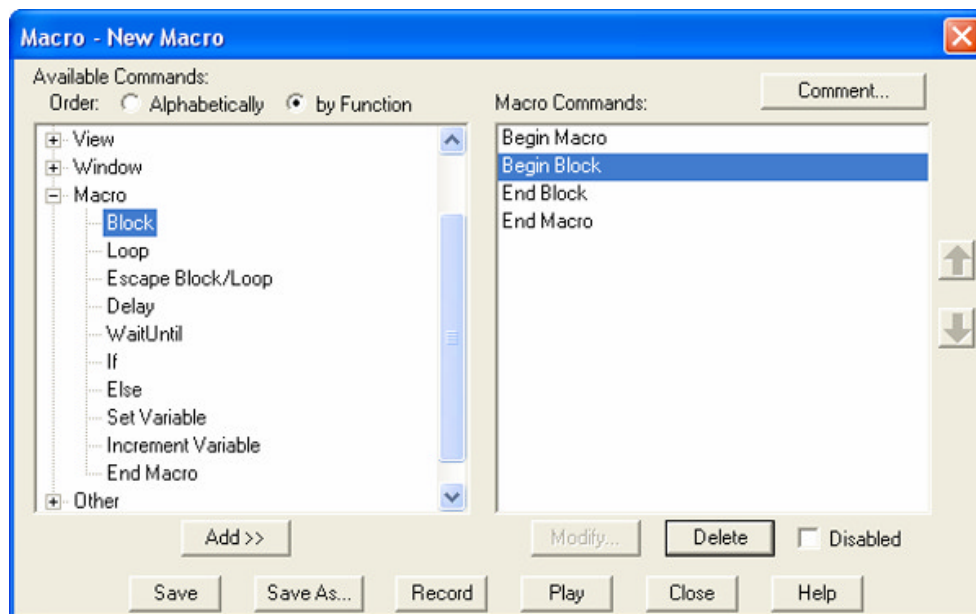
Window specific to BLOCK

No specific window appears to further define the BLOCK Command.

Generic Example using BLOCK

A simple command sequence using BLOCK might be:

```
Begin Macro (automatically added when the window is opened)
Begin Block
 Command A1
 Command B1
 Command C1
 Command D1
End Block
 Command E1
End Macro (automatically added when the window is opened)
```

In this example, the macro would process Commands A1, B1, C1 and D1 in sequence prior to any other subsequent commands being processed. Once the block A1 through D1 is completed, the program will move on and process Command E1.

This is not a terribly effective use of the BLOCK Command. Why? Because even without the Begin Block and End Block commands, the macro would process Commands A1 through E1 in the same sequence.

The real power of the BLOCK Command can be seen when used in conjunction with an IF Command.

Example Z

```
Begin Macro (automatically added when the window is opened)
IF Command
 Begin Block
  Command A1
  Command A2
  Command A3
 End Block
 Begin Block
  Command B1
  Command B2
  Command B3
 End Block
Command C1
Command…
```

In this example, the program evaluates the IF Command to determine whether it is true or not true. Should the IF Command prove true, the block of commands A1 through A3 will be carried out, followed by B1 through B3. Should the IF Command prove to be false, the program will skip block A1-A3, and the command block B1 through B3 will be carried out. In either event, the next command processed will be C1.

Using the Else Command between the command blocks provides a different option (See ELSE).

ESCAPE BLOCK/LOOP

Purpose and Use

The ESCAPE BLOCK/LOOP command is used to leave a programmed block or loop prior to completion of the block or loop.

The ESCAPE BLOCK/LOOP Command can be useful when placed in a block following an IF Command, allowing the program to exit the loop should a predetermined condition be met.

The Macro Window and ESCAPE BLOCK/LOOP

When the ESCAPE BLOCK/LOOP Command is selected from the command screen, it is added to the development pane below the highlighted command in the development pane.
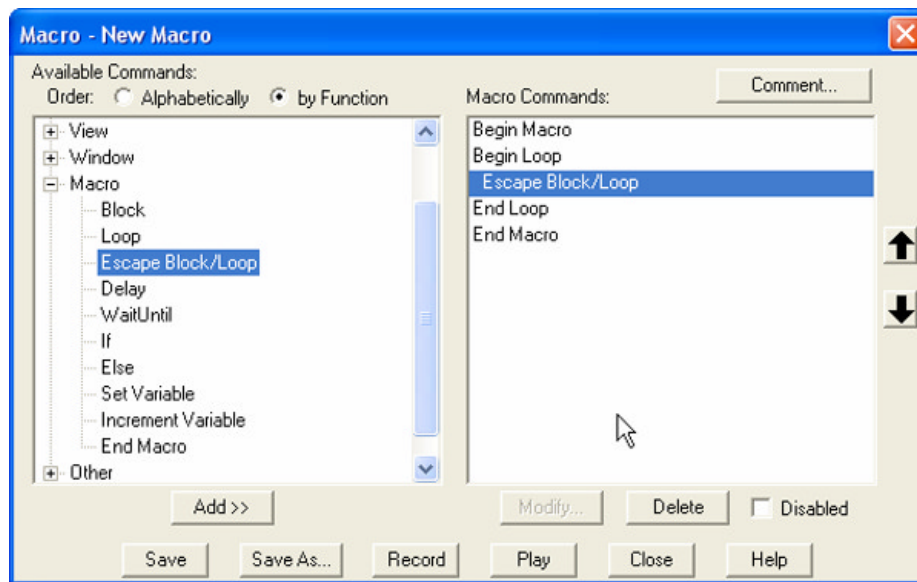
Window specific to ESCAPE BLOCK/LOOP

No specific window appears to further define the ESCAPE BLOCK/LOOP Command.

Generic Example using ESCAPE BLOCK/LOOP

```
Begin Macro (automatically added when the window is opened)
Begin Loop
 Command A1
 Command B1
 IF Command
 Escape Block/Loop
 Command C1
 Command D1
End Loop
Command E1
End Macro (automatically added when the window is opened)
```

In the generic example, the macro will begin the loop series by completing Command A1 and B1. Once the IF Command proves to be true, the ESCAPE BLOCK/LOOP Command will be carried out. At that point, it will leave the loop series and will continue by processing Command E1. Should the IF Command be false, Command C1 and D1 are carried out before the loop ends.

Variable Commands

SET VARIABLE

Purpose and Use

The SET VARIABLE Command allows the user to associate a variable label with a text or numeric value.

This can be useful when the value of a variable changes from one imaging procedure to another and the user wants to be able to modify that value without modifying the remainder of the macro program.

The Macro Window and SET VARIABLE

When the SET VARIABLE Command is selected from the command selection pane in the Macro Window a SET VARIABLE Command will be inserted into the Macro program in the development pane below the highlighted command.

Window specific to SET VARIABLE

When SET VARIABLE is selected a Set Variable window will appear over the Macro Window. It will ask the user to select the variable to be defined and also to place an initial value on that variable.

The Variable label box has a drop down menu allowing you to select from a list of preset variable labels: TextVar1…TextVar5 or NumVar1…NumVar5. Select from the list or insert your own Variable label.

The right hand box allows the user to define the initial value of the Variable label selected. If a text variable is selected, the user can assign a text or numeric value in the right side box. The numeric value will be treated as text. If the user selects a numeric label on the left hand box, the user may only assign a numeric value in the right hand box.

Text Label Example

Text or Number can be entered here. Numbers are treated as text.

Numeric Label Example
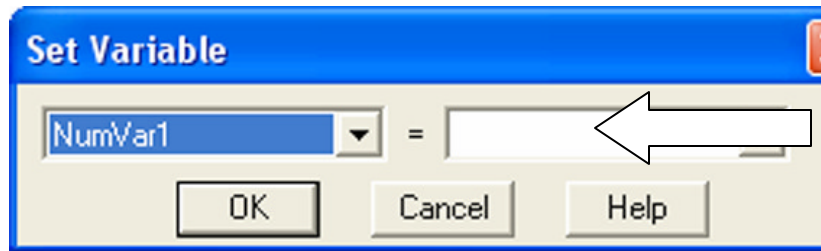
Only Numbers can be entered here. Numbers are treated as integers. **If you use a non-integer number, the SPOT program will round to the nearest integer during playback**

The value in the box defaults to TextVar1 when the Set Variable window appears.

Generic Example using SET VARIABLE

Please refer to the Generic Example that follows in the discussion of the INCREMENT VARIABLE Command

INCREMENT VARIABLE

Purpose and Use

The INCREMENT VARIABLE Command instructs the program to reset the value of a specified variable by an incremental amount equal to 1. The only variable that can be incremented using this command is a NumVar.

This can be quite helpful when the user wants the program to automatically keep track of (and/or record) the number of times a procedure has been carried out by the macro (such as a loop iteration), or to automatically rename images as they are collected. This latter use is more directly available in the Save As window using the Increment option.

The INCREMENT VARIABLE window is only of value after the SET VARIABLE Command has been utilized.

The Macro Window and INCREMENT VARIABLE

When the INCREMENT VARIABLE Command is selected from the command selection pane in the Macro Window an INCREMENT VARIABLE Command will be inserted into the Macro program in the development pane.

Window specific to INCREMENT VARIABLE

When INCREMENT VARIABLE is selected an Increment Variable window will appear over the Macro Window. It will ask the user to define which variable label is to be incremented. The drop down menu will only offer numeric value variables (NumVar1 through NumVar5). NumVar1 is placed in the box as the default value. The Increment Variable window will look like this:

User Guide to the SPOT RT Camera
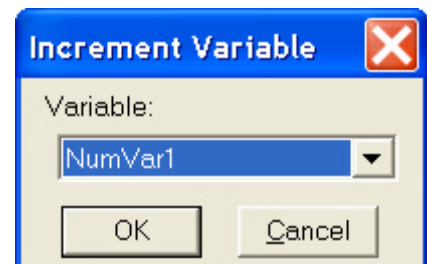
Generic Example using Increment Variable:

An example of the use of the SET VARIABLE and INCREMENT VARIABLE Commands is presented in the following generic macro.

```
Begin Macro (automatically added when the window is opened)
SetVariable (set NumVar1=1)
Begin Loop
 Command A1
 Command B1
 Command C1
 Command D1
 SaveImage (save as Bob'sImageNumVar1)
 IncrementVariable (increments NumVar1 by 1)
End Loop
Command E1
Command…
```

In this example, NumVar1 becomes part of the name of the images saved by the macro. The macro initially defines NumVar1 to be equal to 1. Commands A1 through D1 are carried out and the resulting image is saved as "Bob'sImage1". The variable NumVar1 is then incremented by 1 and now becomes 2. The loop is then repeated and the second image is saved as "Bob'sImage2". This process is repeated for as many iterations as were set when the loop was defined. Once the iterations are completed, Command E1 is carried out.

Timing Commands

DELAY

Purpose and Use

The DELAY Command is used to pause the playing of the macro program for a set period of time.

The DELAY Command can be useful when the user needs a prescribed delay period between commands being carried out and may be especially useful when carrying out a repeating series of commands within a loop where there is a desire for a time delay between loop iterations (for example, in order to manually move a stage).

The Macro Window and DELAY

When the DELAY Command is selected from the command screen, it is added to the development pane below the highlighted command in the development pane.

Window specific to DELAY

When the DELAY command is selected, in
addition to the Macro Window command
insertion noted above, a Delay window will
appear over the Macro Window. It will require
the user to define the amount of time that the
macro program should wait before carrying out
the next command in the sequence.
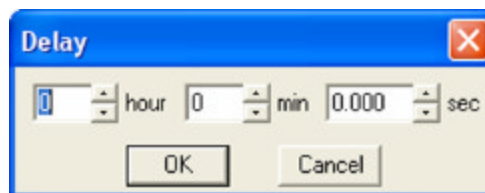
Generic Example using DELAY

```
Begin Macro (automatically added when the window is opened)
Begin Loop
 Command A1
 Command B1
 Command C1
 Command D1
 Delay
End Loop
Command E1
End Macro (automatically added when the window is opened)
```

In the generic example, the macro will begin the loop series by completing Command A1 through
D1 on the first iteration of the loop. At that point, the program will pause for a prescribed period
of time before initiating loop iteration #2. The delay will also occur prior to the program leaving
the loop and processing Command E1, once all of the loop iterations have been completed.

WAITUNTIL

Purpose and Use

The WAITUNTIL Command is used to pause the playing of the macro program until a specific
restart date and time.

The WAITUNTIL Command can be useful when the user needs to prescribe a defined start time
for a series of commands that follow the WAITUNTIL command. This command can allow a
user to automatically start an imaging sequence even while he/she is away from the imaging set-
up.

The Macro Window and WAITUNTIL

When the WAITUNTIL Command is selected from the command screen, it is added to the
development pane below the highlighted command in the development pane.

Window specific to WAITUNTIL

When the WAITUNTIL Command is selected, a window specific to this command opens over the
Macro Window. The user is required to define a date and time

Wait Until

Date:

Time: 12:00:00.000

○ AM ● PM

OK Cancel
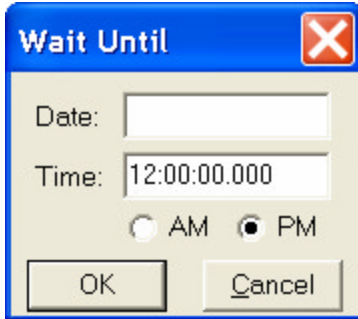
The date format can be entered in various numerical formats as long as the month, the date, and the year are separated by spaces, dashes, forward slashes, commas, or periods <u>and</u> they are in <u>that</u> order. Other formats will generate an Invalid Format message.

🖉 *Note: The WAITUNTIL Command is referenced to the time and date set in your computer's operating system. Please make sure that the operating system time and date are accurate before using this function.*

Generic Example using WAITUNTIL

Begin Macro (automatically added when the window is opened)
WaitUntil
Begin Loop
Command A1
Command B1
Command C1
Command D1
End Loop
End Macro (automatically added when the window is opened)

In the generic example, the macro will begin running, but will pause until the date and time specified by the WAITUNTIL command. Once that time is achieved, the loop series will begin for the prescribed number of iterations before completing the macro.

END MACRO

Purpose and Use

The END MACRO Command defines an endpoint to the Macro subroutine. This command is automatically placed as the macro endpoint when a new macro is created. This END MACRO command is supplemental and can be added anywhere throughout the macro program.

This command can be effective when the user would like to end a macro before it has completed all of its program steps. This is useful as a temporary interrupt to a rather large and complex macro that can yield effective results by just running a portion of the macro. The intermediate END MACRO Command can be switched on and off using the disable feature. This allows the flexibility during playback without significant modification to the base complex macro.

More commonly, however, the intermediate END MACRO Command can be used following an IF statement. This allows the user to exit a macro once a specific condition has been met.

The Macro Window and END MACRO

When the END MACRO Command is selected from the command selection pane in the Macro Window the command will be inserted into the Macro program in the development pane, just below the previously highlighted command.

Window specific to END MACRO

There is no window specific to this command.

Generic Example using END MACRO

```
Begin Macro (automatically added when the window is opened)
Begin Loop
 Command A1
 Command B1
 Command C1
 Command D1
End Loop
IF Command
End Macro
Command E1
Command F1
Command …
End Macro (automatically added when the window is opened)
```

In the generic example, the macro will begin the loop series by completing Command A1 and D1 for as many iterations as were defined. If a condition is proved true for the IF Command, the macro will end prior to carrying out Commands E1, F1, etc.

Communication Commands

PLAY SOUND

Purpose and Use

The PLAY SOUND Command instructs the computer to play an audible sound.

This can be quite useful in notifying the user when a point in the macro program has been reached. This point might indicate a need for user input (interactive mode) or may simply indicate that a portion of the imaging process has been completed.

The Macro Window and PLAY SOUND

When the PLAY SOUND Command is selected from the command selection pane in the Macro Window the command will be inserted into the Macro program in the development pane just after the previously highlighted command.

Window specific to PLAY SOUND

When PLAY SOUND is selected a Play Sound window will appear over the Macro Window. It will ask the user to define the sound file (.wav) to be used during playback. The Play Sound window will look like this:
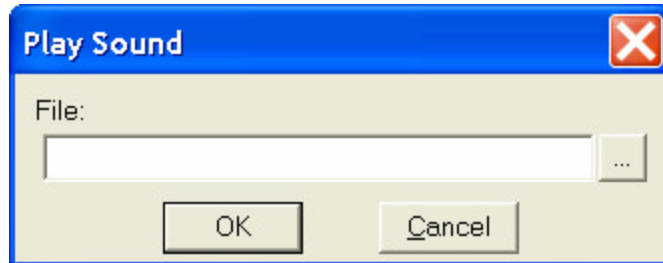
The user may define the path used to access the sound file, or may use the browse button to search for a particular sound file located on the computer or the network, selecting it from the browsing window.

Generic Example using PLAY SOUND

Begin Macro (automatically added when the window is opened)
Begin Loop
Command A1
Command B1
Command C1
Command D1
End Loop
Command E1
Play Sound
End Macro (automatically added when the window is opened)

In the generic example, the PLAY SOUND command will access and play an audio file to indicate to the user that the macro program has been completed.

PROMPT USER

Purpose and Use

The Prompt User Command prompts the user to take an action and/or to input data at a certain point in the macro program playback. Entered data is then stored in one of the pre-defined data labels (NumVar or TextVar) for use later in the program.

Prompt User can be used by itself, or more effectively, when preceded by the Play Sound function. This combination cues the user both audibly and visually of the need for an action or data input.

The Macro Window and PROMPT USER

When the PROMPT USER Command is selected from the command selection pane in the Macro Window the command will be placed just below the previously highlighted command in the Development pane of the Macro Window.

Window specific to PROMPT USER

When PROMPT USER is selected, a Prompt User window will appear over the Macro Window. It will ask the user to define a number of inputs needed to make the PROMPT USER functional during playback. The Prompt User window has many different looks and may appear like the following, depending upon the Input Type selected from the dropdown menu.

The Prompt User window includes a drop down menu that allows for 6 predefined options. These options include OK, Yes/No Buttons, User Text, User Numeric, Push Buttons, or Choice List (see Table B. Prompt User Options for more details about this feature).

Generic Example using PROMPT USER

Begin Macro (automatically added)
Begin Loop
Command A1
Command B1
Command C1
Command D1
End Loop
Command E1
Prompt User
End Macro (automatically added)

In the generic example, the PROMPT USER command will generate an on-screen message associated with an OK button. Once pushed, the OK button allows the macro to move on to the next command, in this case, to end the macro.

Table B. Prompt User Options

Prompt Type	Records your response? *(to what data location)*	General Use	General Response on Macro Playback	Selectable Default Response Available with this command?
OK Button	No	To confirm that an action or point in time has occurred in the macro. Allows for a customized message (Prompt Text) to indicate the action or point in time.	Click OK	None
Yes/No button	Yes *(to a text or numeric variable)*	Asks a question (Prompt Text) that requires a yes or no answer.	Click Yes or No	Yes - Selectable between yes and no as the default.
User Text	Yes *(to a text variable)*	Allows for a statement or question (Prompt Text) and a place to add a comment or an answer.	Add a comment or answer and click OK	Yes - Allows for a default answer to be place in the response area that can be overwritten.
User Numeric	Yes *(to a numeric variable)*	Allows for a statement or question (Prompt Text) and a place to add a numeric response.	Add a numeric value	Yes - Allows for a default value to be place in the response area that can be overwritten.
Push buttons	Yes *(to a text or numeric variable)*	Allows for a statement or question (Prompt Text) and a user-defined response via pushbuttons.	Click on one of the user-defined pushbuttons	Yes - Selectable between any of the user-defined buttons as the default.
Choice List	Yes *(to a text or numeric variable)*	Allows for a statement or question (Prompt Text) and a user-defined response from a dropdown list.	Click on one of the user-defined choices in the dropdown list	Yes - Selectable between any of the user-defined choices in the dropdown list as the default.

RUN REPORT

Purpose and Use

The RUN REPORT Command instructs the program to automatically execute a report file.

The Macro Window and RUN REPORT

When the RUN REPORT Command is selected from the command selection pane in the Macro Window the command will be inserted just below the previously highlighted command in the development pane of the Macro Window.

Window specific to RUN REPORT

When RUN REPORT is selected, a Run Report window will appear over the Macro Window. It will ask the user to define a number of inputs needed to define the report to be accessed.

Generic Example using RUN REPORT

```
Begin Macro (automatically added when the window is opened)
Begin Loop
 Command A1
 Command B1
 Command C1
 Command D1
End Loop
Command E1
Run Report
End Macro (automatically added when the window is opened)
```

In the generic example, the RUN REPORT Command will initiate the opening and display (or printing) of the specified report.

EXECUTE OS COMMAND

Purpose and Use

The EXECUTE OS COMMAND instructs the program to run an executable file from outside of the SPOT program, and allows the user to automatically open that file as part of the macro program. Users who are familiar with DOS will recognize this as a DOS command line.
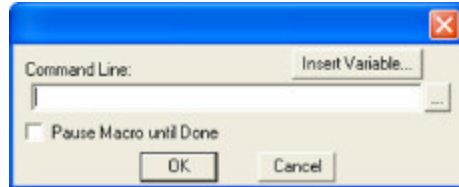
The Macro Window and EXECUTE OS COMMAND

When the EXECUTE OS COMMAND is selected in the command selection pane, the command will be placed just below the previously highlighted command in the development pane of the Macro Window.

Window specific to EXECUTE OS COMMAND

When this command is selected, a window will appear over the Macro Window. It will ask the user to define a path to access the executable file. There is a browsing feature that allows the user to search the computer for the appropriate executable filename. Selecting from the browse window automatically determines the path for the macro program.

If the name of the file includes a variable, insert it into the path by using the Insert Variable button and selecting the appropriate variable. Select the Pause Macro until Done checkbox to pause the macro program until the executable file has been completed.

Generic Example using EXECUTE OS COMMAND

| Begin Macro (automatically added when the window is opened) |
| Begin Loop |
| Command A1 |
| Command B1 |
| Command C1 |
| Command D1 |
| End Loop |
| Execute OS command |
| End Macro (automatically added when the window is opened) |

In the generic example, the EXECUTE OS COMMAND opens a program after the loop command series has been completed.

Ch. 14 – Using Other Windows Imaging Applications in Advanced Mode

QUICK REFERENCE:

- ➲ Set up the camera to work with another imaging application (define the TWAIN interface)… p. 256

- ➲ Define an image setup from the SPOT TWAIN interface… p. 256

- ➲ Preview an image in the SPOT TWAIN interface…. p. 259

- ➲ Transfer a captured image to a TWAIN compliant application… p. 260

- ➲ Capture an image and have it open in the TWAIN application… p. 260

Introduction

The SPOT software is TWAIN compliant. The TWAIN interface, as an alternative to the stand-alone SPOT software, allows Windows users to use other imaging programs (e.g., Photoshop or Image Pro) to view, and edit images that were captured using the SPOT camera and software. For example, using the TWAIN interface and the SPOT camera, you can capture an image from within Photoshop and then transfer the image into PhotoShop to be edited and saved. The following chapter details the use of the SPOT TWAIN interface via Adobe Photoshop 7.0.

   ✎ *Note: Adobe Photoshop was selected as the example to illustrate SPOT's TWAIN features because it is one of the most commonly used "TWAIN capable" image editing programs.*

This chapter is divided into two sections.

- Preparing to Take a Picture
- Taking a Picture

   ✎ *Note: Because the image setup and image capture functionality is identical in the stand-alone and TWAIN interface versions of the software, these sections reference other chapters in the manual.*

Preparing to Take a Picture

The TWAIN interface provides you with the same image setup options as the stand-alone version of the SPOT software. There are two main tasks in preparing to capture an image.

- Establishing the TWAIN Interface
- Defining an Image Setup

Establishing the TWAIN Interface

To use the SPOT camera and software via Photoshop, establish the TWAIN interface by defining the SPOT software as the default TWAIN source. Follow these steps:

1. Open Photoshop.

2. Define the source of the program that you want to interface with (in this case the SPOT software).
    From the **File** menu, select **Import.**

3. Select **SPOT RT Camera (Advanced)** Camera, and click on it.

> 🖉 *Note: To work in the Basic mode TWAIN interface, select* **SPOT RT Camera (Basic)**. *Refer to Ch. 13 – Using Other Windows Imaging Applications in Basic Mode.*

4. The SPOT Camera window appears within Photoshop, as shown at the right. You are now ready to capture images using the TWAIN interface in Photoshop.

Refer to the next sections, Defining Live Image Preferences and Defining Image Setups, for details on how to define imaging settings using the TWAIN interface.

Defining Live Image Preferences

The TWAIN window includes a **Preferences** button, which allows you to define live image viewing, exposure and other preferences. The window includes the following options:

- Live Images Window
    - Always Show Full Chip
    - Maximum Exposure time
    - Do Post-processing selection
    - Close window on snap option
- Bus Window
    - Bandwidth size
- Miscellaneous Window
    - Keep TWAIN Window on top
    - Make Shutter Sound
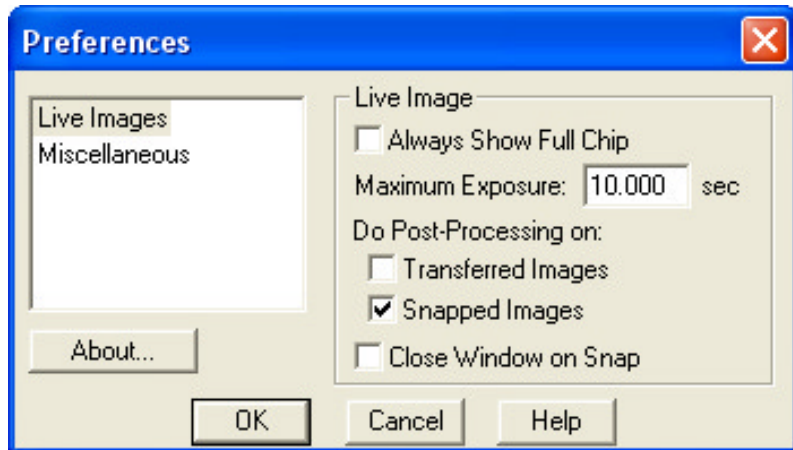
Live Images Window

Always Show Full Chip

Always display the full chip in live image mode. If you select a region, it will be displayed in a rectangular outline within the full-chip view.

Maximum Exposure

The Maximum Exposure option helps you to avoid unnecessarily long exposures when viewing a live image. Consider the following situation: When you change from a lower resolution objective to a higher resolution objective, the camera detects a brief interval of blackness and compensates by modifying the exposure time to maintain a constant brightness level. This results in you having to wait for the camera to compute a lengthy and incorrect exposure for the black interval. Using the **Maximum Exposure** option, you can limit this exposure time. For most cases, Diagnostic Instruments recommends a value of 0.5 seconds. Similarly, the Live Image Maximum option can also be used in cases where the microscope's light path selector is incorrectly set.

Do Post-Processing on

Specify whether to apply the Post Processing options for the current Image Setup to images captured from the live image view.

Transferred Images

- *If the Transferred Images box is selected*, the Transfer option applies the Noise Filter, Color Enhancement, and Image Size Double options as specified for the current Image Setup.

- *If the Transferred Images box is unselected*, the Transfer option ignores the Post Processing options for the current Image Setup, with one exception: if the Chip Defect Correction option is selected for the current image setup (RT and Enhanced cameras only), the corrections will appear in the image capture, regardless of the Transferred Images preference.

Snapped Images

- *If the Snapped Images box is selected*, the Snap option uses all of the Post Processing options from the current Image Setup, except for Thermal Frame Subtraction and Flatfield Correction.

- *If the Snapped Images box is unselected*, the Snap option ignores the Post Processing options for the current Image Setup, with one exception: if the Chip Defect Correction option is selected for the current image setup (RT and Enhanced cameras only), the corrections will appear in the image capture, regardless of the Snapped Images preference.
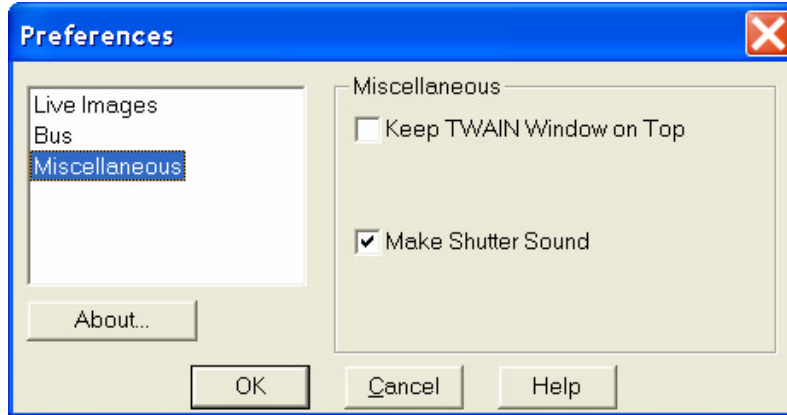
Close Window on Snap

Selecting this option will close the Live Image Window each time you capture an image using the Snap button in that window. If this option is not selected, captured images from snap will "stack up" behind the live image window.

Miscellaneous Window

Keep TWAIN window on top

The **Keep TWAIN Window on Top** option allows you to preview and transfer or acquire multiple images without having to re-open the SPOT window.
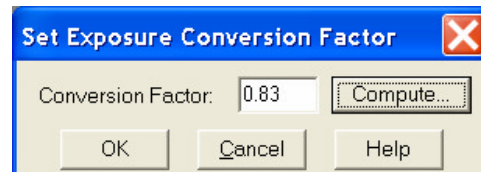
- If the Keep This Window on Top box is checked, the SPOT window remains open.

- If the box is not checked, the SPOT window automatically closes after each image is transferred or acquired.
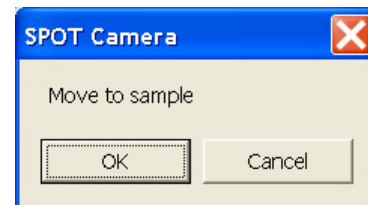
Set Exposure Conversion Factor

RT camera users may have noticed in the past, that image brightness differences existed between Live Images and Captured Images when using the Snap method of capturing an image in the Advanced program mode. The SPOT program now allows for users to correct for this difference by using the **Set Exposure Conversion Factor** function.

This function is used to adjust the relative image brightness between the Live Image and the captured image. You may elect to have the SPOT program compute the conversion factor or you may select the conversion factor yourself.

If you choose to have the SPOT program compute the conversion factor, the program will prompt you with a window asking you to "move to sample". You should set the imaging area to a moderately bright sample. Once you have done so and select OK, the SPOT program does the rest and places the computed exposure factor in the Conversion Factor window.

If you choose to set the factor yourself, you may want to open a Live Image window, set the conversion factor, and then use Snap to capture an image. Compare the two images. If you are satisfied that the two are at a similar brightness level, leave the conversion factor. If you are not satisfied, reset the conversion factor and Snap another image. Repeat this process until you are satisfied.

Setting a conversion factor less than 1 will decreases the brightness of the captured image when compared to the live image. Setting a conversion factor greater than one will increase the brightness of the captured image relative to the live image.

This value is retained for future use, even if you shut down the SPOT program. You may reset it at any time.

Make Shutter Sound

The user may select whether to utilize the audible shutter sound when capturing an image.

Monitor Camera Color Filter Position (RT Slider)

Determine whether to monitor the position of the color filter. The Monitor Camera Color Filter Position box is checked as a default. If this option is left checked, the SPOT program displays error messages when the sensors in the camera head detect that the color filter is not in the correct position. This option **does not** monitor the position of the filter slider in Live Image mode. In Basic mode, the program will give you an error message if you are capturing an image and the sliding color filter is in the **B/W** position.

Device

The Device option allows you to select from a variety of image acquisition devices for previewing and capturing images through the TWAIN interface. These can be selected and changed "on the fly". Once you have selected a different device, the TWAIN window will update automatically to include options consistent with the selected device.

The Palettes option allows Palettes (RT B/W)

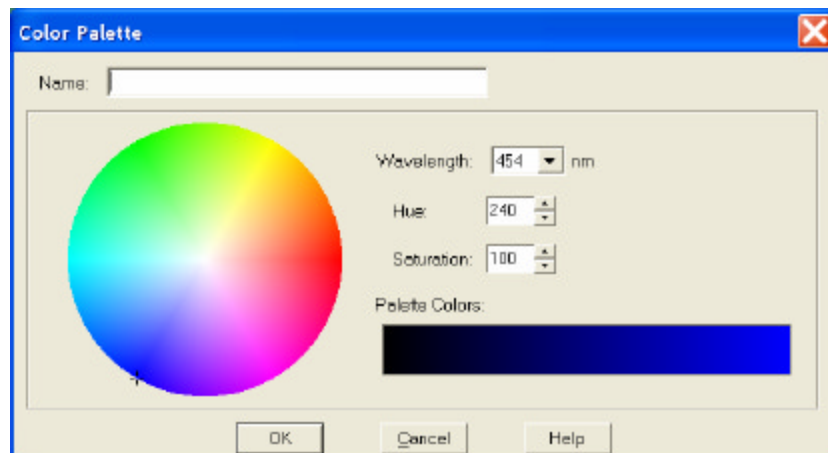The Palettes option allows you to define new palettes and edit existing palettes.

SPOT color palettes allow you to apply specific hue and saturation values to monochrome (8 bit or 12 bit) images. Palettes are useful in fluorescence applications where a sample is fluorescing at a single wavelength, and you want the acquired image to have the correct color. For such a case, you could create a palette to represent the color of the fluorescent dye and then apply that palette to the captured image. There are two ways to apply a palette to an image:

- You can associate a palette with an Image Setup, as discussed in Ch. 5 - Preparing to Take a Picture.

- You can apply a palette to a captured image by selecting the **Set Palette** option from the **Edit** menu. Refer to Ch. 8 – Viewing, Editing, and Annotating Images for more details.

To define a new palette, follow these steps:

1. From the **Setup** menu, select **Palettes**.

2. Click on **Add**. The Color Palette dialog appears as shown here:

3. Use the **Hue** and **Saturation** selection lists, select a **Wavelength**, or move the cross hairs

on the color wheel to enter the values.

- **Wavelength** allows the user to specify the exact wavelength ranging in value from 400nm to 700 nm. Wavelengths will automatically correspond to various Hues at full (100%) saturation.
- **Hue** is the designation for the actual color, as distinguished from others in the color spectrum. Hue is derived from the color wheel and is expressed in the color's angular location (in degrees) on the wheel. (e.g., 0° to 360°).
- **Saturation** is the proportion of perceived pure hue in the color. Saturation (in the SPOT software) is measured on a numerical scale of 0 to 100, where zero is pure gray and 100 is pure hue (e.g., day glow).

4. Click on **OK**.

Defining Image Setups

An image setup serves two crucial purposes:

- It defines the way a picture will be taken.
- It defines the way the program automatically modifies a picture after it is taken.

Image setups are defined to optimize the picture taking process for different types of images or microscopy techniques. They can be created, saved, and recalled as needed. To define a new image setup, you may select one of 11 pre-defined image types as a template for defining the new setup (auto-exposure) or define the settings yourself (manual exposure). The 11 pre-defined image types (in addition to the Factory Defaults setting) correspond to commonly used microscopy techniques.
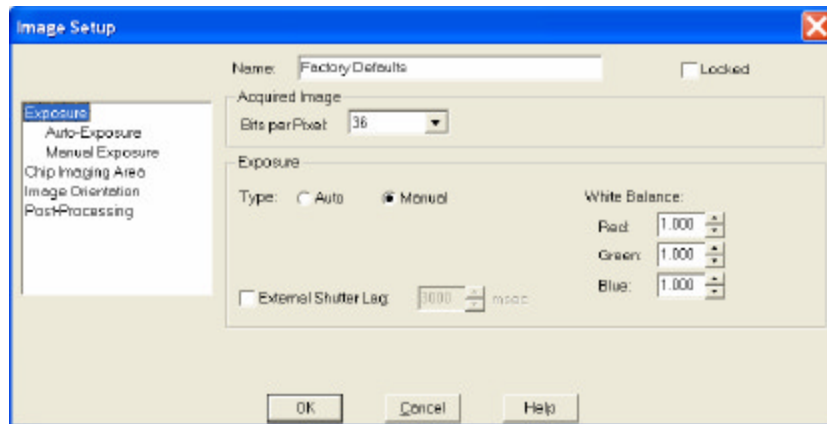
The Image Setup window is now comprised of 6 separate windows. Refer to Ch. 5 - Preparing to Take a Picture for a table of the different image types.

- To modify the current image setup, click on the **Modify** button.
- To delete an image setup, select the image setup from the list, and click on the **Delete** button.
- To define a new image setup, click on the **Add** button. The New Image Setup window appears with a list of pre-defined and user-defined templates (image types). Brackets indicate pre-defined image types < >. Select a template, and click on OK.

When you add a new image setup or modify an existing setup, the Image Setup window appears, as shown at right. Refer to Ch. 5 – Preparing to Take a Picture for a detailed explanation of the Image Setup options.

RT B/W	RT B/W and Color Mosaic camera users will not see the fluorescence – red, fluorescence – green, and fluorescence – blue Image Type options.

Taking a Picture

From the SPOT TWAIN interface, you can perform both initial image captures (i.e., for computing exposure, white balance, etc…) and final image captures.

Capturing Initial Images

The toolbar at the left of the SPOT Camera window enables you to quickly access the functions that are used prior to the capture of the final image.

RT B/W	RT B/W camera users will not see the Compute White Balance Values toolbar button.

Capturing Final Images

From the SPOT TWAIN interface, you can capture an image in two different ways. You can preview the image and transfer it to Photoshop, or you can acquire the image, which sends the captured image directly to Photoshop.

- **Preview**– Capture an image and preview it within the SPOT TWAIN window.
- **Transfer** – Transfer a captured image to Photoshop.
- **Acquire -** Capture an image and have it automatically transfer to (open in) Photoshop.

Ch. 15 – Using Other Windows Imaging Applications in Basic Mode

Introduction

The SPOT software is TWAIN compliant. The Basic TWAIN interface, as an alternative to the stand-alone SPOT software, allows Windows users to use other imaging programs (e.g., Photoshop or Image Pro) to view, and edit images that were captured using the SPOT camera and software. For example, using the Basic TWAIN interface and the SPOT camera, you can capture an image from within Photoshop and then transfer the image into Photoshop to be edited and saved. As with the stand-alone version of the SPOT software, the TWAIN interface can be used in both Basic and Advanced modes. The following chapter describes only the **Basic TWAIN** Interface.

Establishing the TWAIN Interface

To use the SPOT camera and software via a TWAIN compliant imaging application (e.g., Photoshop, Image-Pro, etc…), you must first set the SPOT software as the default TWAIN source.
Follow these steps:

1. Open the TWAIN compliant application e.g., Photoshop, Image-Pro, etc…

2. Specify the source of the program that you want to interface with (i.e., the SPOT software). The procedure for this step will vary according to the software you are using, but you should see a list of TWAIN devices. In many cases, the application asks you to specify a scanning device.

3. Choose **SPOT RT camera (Basic Mode)**. This establishes the SPOT software as the default TWAIN source.

4. Open the TWAIN interface. Again, this step varies according to the software you are using. The SPOT Camera window appears within the application, as shown at right.

Refer to the next sections for descriptions of the available options on the TWAIN interface.

Working in the Basic TWAIN Interface

The Basic TWAIN interface is a one-stop point for previewing and capturing images. For example, using the Basic TWAIN interface, a typical image capture session might involve the following steps, as illustrated below:

Preview your specimen

Use the live image as a point of reference to focus and position the image and to scan for areas of interest.

A Live Image preview of your specimen displays here when you first open the Basic TWAIN interface
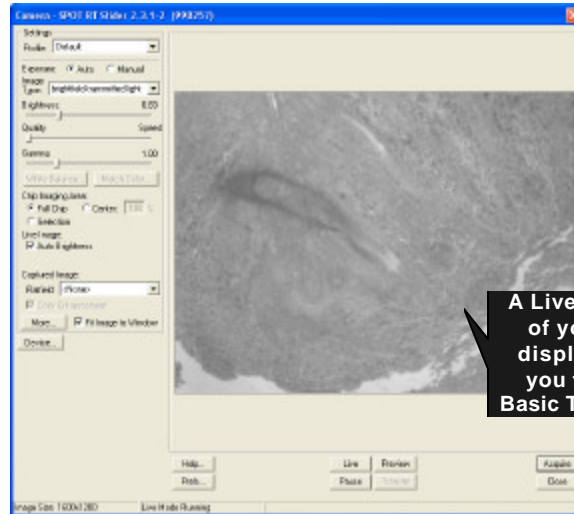
Create a Profile

Profiles allow you to create, modify, and reuse specific exposures settings that are tailored to your image capture needs. To create a new profile, right click on the Profile box.
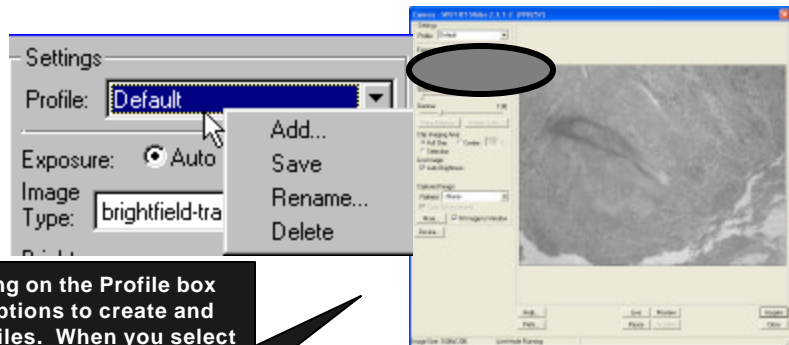
Right-clicking on the Profile box gives you options to create and modify Profiles. When you select "Add," you are prompted to name the new Profile. And, as you modify view and capture settings, they are saved to that Profile.

Define Settings

Define exposure settings for live and captured images. Each of these settings is discussed later in this chapter.

Capture an Image

All of the image capture options are located on the bottom portion of the window, as illustrated here

Defining Preferences

In the Basic TWAIN Interface, you can define three capture/view preferences. Click on the **Prefs** button to open the **Preferences** window (see right). Each option is described below:

Live Images Window

Always Show Full Chip

Always display the full chip in live image mode. If you select a region, it will be displayed in a rectangular outline within the full-chip view.
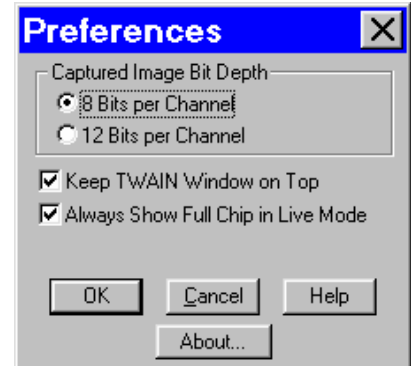
Miscellaneous Window

Keep TWAIN window on top

The **Keep TWAIN Window on Top** option allows you to preview and transfer or acquire multiple images without having to re-open the SPOT window.

- If the Keep This Window on Top box is checked, the SPOT window remains open.

- If the box is not checked, the SPOT window automatically closes after each image is transferred or acquired.
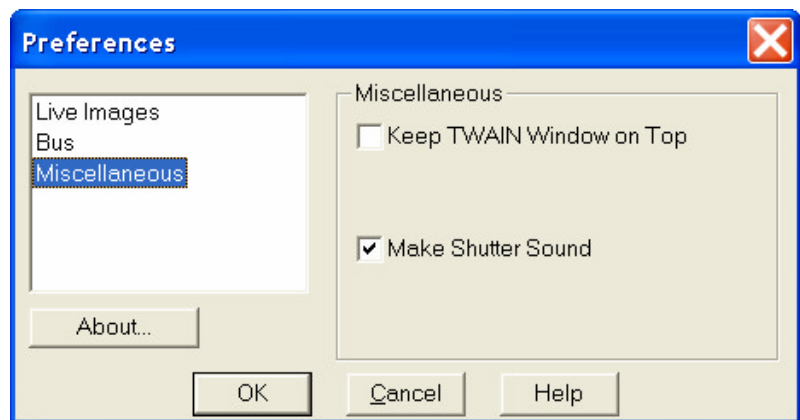
Captured Image Bit Depth

This option enables you to specify a different image bit depth when saving an image captured in the SPOT program.

- **8 Bits per Channel** – The 8 bits per channel option provides 256 gray levels per color:
    a) With RT B/W cameras, captured images are 8 bits per pixel monochrome.
    b) With RT Color cameras, captured images are 24 bits per pixel RGB color.

    This option results in smaller, more manageable image sizes, and is best for images that do not require extensive modification.

- **12 Bits per Channel** – The 12 Bits per channel option provides 4,096 gray levels per color.
    c) With RT B/W cameras, captured images are 12 bits per pixel monochrome.
    d) With RT Color cameras, captured images are 36 bits per pixel RGB color.

    This option reduces banding problems that can result with modified images, but also results in larger file sizes.

When saving 12 bit per channel images, SPOT prompts you to select either 12 bits per channel or 16 bits per channel. If you use Adobe's Photoshop for image editing, you should select the 16 bit per channel option because Photoshop cannot read 12 bit per channel images.

Set Exposure Conversion Factor

RT camera users may have noticed in the past, that image brightness differences existed between Live Images and Captured Images when using the Snap method of capturing an image in the Advanced program mode. The SPOT program now allows for users to correct for this difference by using the **Set Exposure Conversion Factor** function.

This function is used to adjust the relative image brightness between the Live Image and the captured image. You may elect to have the SPOT program compute the conversion factor or you may select the conversion factor yourself.

If you choose to have the SPOT program compute the conversion factor, the program will prompt you with a window asking you to "move to sample". You should set the imaging area to a moderately bright sample. Once you have done so and select OK, the SPOT program does the rest and places the computed exposure factor in the Conversion Factor window.

If you choose to set the factor yourself, you may want to open a Live Image window, set the conversion factor, and then use Snap to capture an image. Compare the two images. If you are satisfied that the two are at a similar brightness level, leave the conversion factor. If you are not satisfied, reset the conversion factor and Snap another image. Repeat this process until you are satisfied.

Setting a conversion factor less than 1 will decreases the brightness of the captured image when compared to the live image. Setting a conversion factor greater than one will increase the brightness of the captured image relative to the live image.

This value is retained for future use, even if you shut down the SPOT program. You may reset it at any time.

Make Shutter Sound

The user may select whether to utilize the audible shutter sound when capturing an image.

Monitor Camera Color Filter Position (RT Slider)

Determine whether to monitor the position of the color filter. The Monitor Camera Color Filter Position box is checked as a default. If this option is left checked, the SPOT program displays error messages when the sensors in the camera head detect that the color filter is not in the correct position. This option **does not** monitor the position of the filter slider in Live Image mode. In Basic mode, the program will give you an error message if you are capturing an image and the sliding color filter is in the **B/W** position.

F-Keys

The following function keys are available as shortcuts in the Basic TWAIN interface:

Function Key	Action	TWAIN Interface Option
[F 4]	Restart the live image view.	Live
[Alt] + [F 4]	Close the SPOT Camera window.	Close
[F 9]	Capture an image and automatically transfer it to the imaging application.	Acquire
[Ctrl] + [F 10]	Compute white balance values.	White Balance
[Alt] + [F 10]	Compute white balance values without displaying the informational dialog.	*Not available from the window*

Using the Live Image to Define Image Capture Settings

When you open the Basic mode TWAIN Interface, the Live Image window is, by default, running, as indicated by the **Live Mode Running** message in the status bar. The settings options in the TWAIN interface window enable you to optimize the live image view, and then capture an image using these settings. In the TWAIN interface, you can:

- Create profiles to use as pre-defined image capture settings.

- Modify exposure settings

- Define a specific portion of the chip to be used in the image capture.

- Modify the screen orientation of the specimen.

The following sections describe all of the Live Image/Image Capture Settings.

Profile

The **Profile** option allows you to create and re-use sets of live image/image capture settings. When you open the TWAIN interface, the **Default** Profile is selected. When you make changes to any of the live image/image capture settings, these changes can be saved to a Profile – either the Default or one that you have defined. For example, if you gamma adjust a live image and then decide that the result is ideal for the type of specimen you are working with, you could create a new Profile with the gamma and exposure values and name it for that type of specimen – i.e., "Diseased Cells."

When you close the **SPOT Camera** window, the program prompts you to save changes. Carefully consider the effect of your choice:

- Clicking **Yes** modifies the Profile according to the adjustments that you made. Future image captures taken with that Profile will reflect these changes. For example, if you rotated a live image, and then clicked Yes after closing the window, all future captures taken with that Profile would be rotated in the same way.

- Clicking **No** retains the original values of the Profile. This allows you to maintain a unique set of image view and capture settings that can be re-applied to future captures of similar specimens.

To add, edit, or delete Profiles, **right-click** on the Profile box. Right clicking gives the following options:
- **Add –** Add a new Profile. When you add a new Profile, the program prompts you to name it.
- **Save –** Save a Profile that you have modified.
- **Rename –** Rename an existing Profile.
- **Delete -** Delete a Profile. This option appears only after you have defined a second Profile in addition to the Default Profile.

Exposure: Auto or Manual

The **Exposure** option gives you the choice of having the camera calculate exposure time (**Auto**), or setting the exposure on your own (**Manual**).   The exposure options will vary according to whether you select **Auto** or **Manual**, as illustrated below.

Auto

Manual

When you enter one of the above exposure settings, note that it applies **both** to the live image that you are viewing and the captured image.

Auto Exposure options

Image Type

An Image Type tells the SPOT program how to expose and process image captures. Each of the 11 available Image Types (see below) corresponds to a microscopy technique: select the Image Type that matches the technique you are using. The camera will capture the image accordingly. The table below describes typical situations for each image type.

Image Type	Description
Brightfield-reflected light	Polished metallurgical specimens and semi-conductors using a microscope with a vertical illuminator.
Brightfield-transmitted light	Stained specimens on glass slides using transmitted light microscopes.
Darkfield-reflected light	Bright areas of specimens being viewed with darkf ield, reflected light techniques.
Darkfield-transmitted light	Bright specimens on dark backgrounds using transmitted light darkfield technique.
Fluorescence	Fluorescence specimens not fluorescing in a single red, green, or blue primary color
Nomarski DIC/Hoffman	Specimens being viewed with either Nomarski DIC or Hoffman techniques.
Phase contrast	Specimens being viewed using the phase contrast technique.
Polarized light	Specimens being viewed with polarized light techniques.

(cont.)

Image Type	Description
Stereo/macro-metallic glints	Metallic specimens with intense glints.
Stereo/macro–no glints	Matte specimens without bright glints.
Stereo/macro–shiny glints	Shiny specimens with bright glints.

Brightness

Enter a **Brightness** value between **.24** and **34**.

A brightness value of 1, the default value, gives you the correct image brightness for most images. The Brightness control allows you to overexpose or underexpose (darken or lighten) the image area. In many cases, an image has varying levels of brightness; to see a specific area better you might want to lighten or darken the image. When using the Brightness control, the SPOT camera uses an algorithm that combines gain and exposure time to provide the fastest possible image update rate (shutter speed) in live mode:

- When overexposing images to make them brighter (i.e., entering values greater than one), the camera first selects the highest possible gain before lengthening the exposure time. Because gain also brightens an image, less exposure time is needed.

- When underexposing images to make them darker (i.e., entering values less then one) the camera uses the lowest possible exposure time before lowering the gain.

Quality/Speed (Auto)

Use the slider to balance speed and quality when previewing dim images.

- Moving the slider toward the **Quality** side increases image quality and decreases frame rates.

- Moving the slider toward the **Speed** side increases frame rates and decreases image quality.

The Quality/Speed measurement corresponds to the Auto-Gain limit setting, which allows you to set an upper limit on the gain options that will be available during the Auto-Exposure process.

- When the slider is at the left-most point, the Auto-Gain Limit gives you higher quality pictures, but lengthier exposure times.

- When the slider is at the right-most point, the Auto-Gain Limit allows the camera to select from all available gain options. This shortens exposure times, but decreases image quality.

Refer to the "Auto-Gain Limit" section in Ch. 5 – Preparing to Take a Picture for a detailed explanation of gain and exposure.

Gamma

Enter a **Gamma** value between **.1** and **4**.

Gamma adjustment smoothly lightens darker areas of an image without burning out bright areas or lightening black areas. This is especially useful for capturing/editing images with a wide range of brightness values (dynamic range) such as fluorescence specimens or macroscopic objects with glints, (e.g., printed circuitry).

- Values between **1** and **4** lighten the darker areas of the image.
- Values between **.1** and **1** darken the mid-tones of the image.

Gamma adjustments on live images are made in the RGB color space, the only color space where adjustments can be made quickly enough to keep up with live imaging. Refer to Ch. 5 – Preparing to Take a Picture for more details on gamma adjustment and color spaces.

Manual Exposure Options

Exposure Time(s)

Use the slider-bar to specify an exposure time (or times) between **.04 ms** and **526.746 sec**.

When you specify an exposure time, the camera skips the automated exposure sequence that takes place

when you open the live image view – i.e., the TWAIN interface. This is very useful in cases where exposure times are lengthy, as with fluorescence samples.

Gain

Enter the precise gain that the camera will use for an image capture. Use the slider-bar to select one of the gain options: **1**, **2**, **4**, **8**, or **16**. The gain value displays to the right of the slider-bar. Refer to the "Auto-Gain Limit " and "Gain" sections of Ch. 5 – Preparing to Take a Picture for details on gain and exposure.

Gamma

The manual Gamma adjustment is identical to the auto Gamma adjustment (see above.)

White Balance

White balance is the ratio of red, green, and blue gains necessary to achieve the proper color rendition for an image. Many factors impact the coloration of an image capture, including lamp voltage, the coloration of the glass used in the slide, and the coloration of the glass used in the lenses of the microscope's objectives. To minimize the impact of these factors, use the White Balance feature.   When you perform a white balance calculation, SPOT samples the color of the light from your light source after it has passed through the slide and the objective, and then calculates the red, green, and blue gains needed to produce images where the white areas are white and the color areas are true.
You should perform a new white balance calculation when:
- Starting a new image capture session.
- Changing the lamp voltage.
- Switching to a different objective on the microscope.
To perform a white balance, follow these steps:

1. Set the illumination and the objective that you will use for the image capture.

2. If you have not done so, select your **Image Type** (see above). Each Image Type has its own set of white balance values. Any white balance values that you compute for an image type remain linked to that image type until you compute new values. When selecting a new image type, remember that you will be using the white balance values linked to that image type.

3. Click on the **White Balance** button.

4. Show the camera a sample of white light. This step varies according to microscopy technique and Image Type (sample), as shown in the table below:

Image Type	White Balance Technique
Brightfield-transmitted light	Position the slide so that the specimen is not in the field of view, but light is going through the slide near the specimen.
Darkfield-transmitted light	Perform the white balance using the brightfield mode on your microscope. See Brightfield – transmitted light above.
Fluorescence	Use the Match Color feature (see above)
Nomarski DIC/Hoffman	**For Nomarski DIC:** Adjust the Nomarski prism until the image is in either the "brightfield" or the "gray scale" view. **For Hoffman:** Perform the white balance directly on the Hoffman image.
Phase contrast	Position the slide so that the specimen is not in the field of view, but light is going through the slide near the specimen.
Polarized light	Pull all polarizers and compensators out of the light path and then position the slide so that the specimen is not in the field of view, but light is going through the slide near the specimen.
Brightfield-reflected light	Replace your sample with a mirror.
Darkfield-reflected light	Perform the white balance using the brightfield mode on your microscope. See Brightfield – reflected light above.
Stereo/Macro (all three options)	Using an EPI-illuminator (shining a light on the top of your specimen from off to one side), replace your sample with a white sheet of paper.

5. Click on **Begin** to start the calculation. The camera computes the white balance values, and they are automatically saved to the image settings for the image type that you selected.

Match Color

The Match Color feature is typically used in two situations:

- You want to change the colors of an image on the screen to match the way that you think the specimen looks.

- You want to set the white balance for pictures taken with a microscopic technique that never has white in its images. A prime example of this situation is fluorescence microscopy.

To use the Match Color feature, follow these steps:

1. Click on the **Preview** button to capture an image. The Match Color option remains inactive until a preview image is captured.

2. Select **Match Color.** The cursor becomes an eyedropper.

3. Using the mouse to move the eyedropper, position it on the pixel location of the color that you want to match. The R, G, and B values of the pixel that you select must all be less than 255.

4. Click on the pixel. The **Match Color** dialog appears with information about the pixel that you selected:

e) **Hue** – The actual color, as distinguished from others in the color spectrum. Hue is derived from a color wheel and is expressed in the color's angular location (e.g., 0 to 360 degrees).

f) **Saturation** - The proportion of perceived pure hue in the color. Saturation (in the SPOT software) is measured on a numerical scale of 0 to 100, where zero equals pure gray and 100 equals pure hue (e.g., "day glow").

5. Move the cross hairs to the desired color either by clicking on the new location or clicking and dragging. As you move the cross hairs, the color in the image changes accordingly, allowing you to preview the color before you save the image. Alternatively, you can click on the **Make White/Grey** button to automatically white balance the image.

6. Click on **Ok**
.

Chip Imaging Area

SPOT allows you to use any rectangular portion of the CCD chip for image capture. Using a portion of the chip is helpful in cases where you want to:

- Keep file sizes small.
- Shorten download times.
- Avoid areas of the image with optical problems (e.g., vignette areas).

There are three image area options:

- **Full Chip** – Capture the entire area of the CCD chip.

- **Center** – Capture a rectangular part of the CCD chip's area that has the same height - to - width ratio as the full chip, and is centered on the center of the full chip. The percentage that you enter is the percentage of full chip width that the new area's width will be.

    To capture a centered proportion of an image, follow these steps:
    g) Check the Center option.
    h) Enter the percentage of the image that you want to capture. The minimum percentage is 10 and the maximum is 100.
    i) Capture the image.

- **Selection** –Capture a specific part of the CCD chip.

    a) Check the **Selection** option.
    b) Use the mouse to select a region for capture. Click and drag to define the selection. Note that the new pixel dimensions display in the status bar.
    c) Capture the image.

When you are viewing a live image **Selection**, how this selection is displayed, depends on two other settings: the **Fit to Window** option (see below) and the **Always Show Full Chip in Live Mode** preference (see above). The following table illustrates the possibilities:

Fit to Window Checked?	Always Show Full Chip in Live Mode Checked?	Selection Display	Live or Captured Image
YES	YES	When you select a portion of the chip, the selection is outlined with a **yellow rectangle** .	LIVE
YES	NO	When you select a portion of the chip the cursor is a **zoom tool**. To zoom in on a portion of a live or captured image, click and drag over the area that you want to zoom in on. To return to the non-zoomed image, click on the **Full Chip** option.	BOTH
NO	YES	When you select a portion of the chip, the selection is outlined with a yellow rectangle. However, because **Fit to Window** is not checked, you will not see the entire chip in the window.	LIVE
NO	NO	When you select a portion of the image, the cursor is a cropping tool. If you select a portion of an image, the program displays only the selected area and discards the rest of the image. To return to the non-cropped image, click on the **Full Chip** option.	BOTH

Auto-Brightness (Auto, Live Images)

Available only when you are using **Auto** exposure and viewing a live image, the **Auto-Brightness** option enables you to maintain a consistent brightness level for changing specimens.

Faster Readout (Live Images)

Faster Readout enables you to "speed up" the live image display (higher frame rates) in cases where you are willing to sacrifice some resolution for a higher frame rate.

Flatfield (Captured Images)

For all other SPOT camera lines, the Flatfield Correct option is used to correct for display problems associated with uneven intensity or coloration in illumination, or to correct for artifacts (e.g., dust) in the optical system.

> ✎ *Note: Flatfield correction with the SPOT camera refers only to the correction of uneven lighting. It should not be confused with the correction of optical field flatness.*

Flatfield correction works by taking a picture of the brightfield background without the specimen in the field of view. This image lets the SPOT software know, on a pixel-by pixel basis, which areas in the field of view are receiving less illumination than the brightest areas. When taking a picture that contains a specimen, the software uses this information to accurately increase the brightness of each pixel in the dimly illuminated areas. The resulting image gives the appearance of an even illumination.

To use the **Flatfield** option, follow these steps:

1. Ensure that the specimen is under the desired objective and in focus.
2. Right click on the **Flatfield** drop-down list and click on the **Add** option. The **Get Flatfield** dialog appears as shown at right.
3. Remove the slide from the field of view.
4. Click on Begin. When the program finishes computing the exposure times and acquiring the flatfield file, the **Flatfield** dialog prompts you to name the file:
   a) Enter a name. Because flatfield image files are different for each objective, you might want to name the file according to the objective's magnification (e.g., 10x).
   b) Click on OK. If you do not specify a location, the program automatically saves the flatfield file to the **c:\SpotCam\Fltflds** directory.

> ✎ *Note: The default file location varies, depending on where you installed the SPOT program.*

5. Move the specimen back into the field of view, and perform the image capture, using either the **Preview** or the **Acquire** option.

Color Enhancement (Captured Images)

The Color Enhancement option optimizes image captures by closely matching the final capture to the image as seen through the microscope. To optimize the final image, the SPOT program processes the raw image data to produce a final image that matches the view seen through the microscope.
If you want an image capture to match the live image view, you will want to leave Color Enhancement off. Color Enhancement applies only to captured images.

Fit to Window

In addition to automatically resizing images to fit within the live image window, the Fit to Window option also determines cursor behavior in the live image view.

- When the **Fit to Window** option is checked, the live image window **cursor** becomes a **zoom tool**. To zoom in on a portion of a live image, click and drag over the area that you want to zoom in on. To return to the non-zoomed image, click on the **Full Chip** option.

- When the **Fit to Window** option is not checked, the cursor is a cropping tool. If you select a portion of an image, the program displays only the selected area and discards the rest of the image. To return to the non-cropped image, click on the **Full Chip** option.

These points apply *only* when the **Always Show Full Chip in Live Mode** (see above) is *not selected*. For more details, refer back to the **Chip Imaging Area** section (see above).
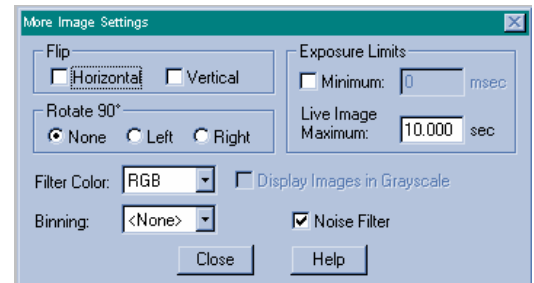
> ✎ *Note: Due to a flaw in the Windows operating system, monitors that are set to higher resolutions cannot zoom to very high magnification levels. If zoomed in to a high magnification, the monitor displays white instead of the zoomed image.*

More (Image Settings)

When you click on **More**, the program displays a dialog of additional orientation and exposure settings, as shown at right:

> ✎ *Note: The screen capture at right is from an RT 3-Shot/Slider – the Filter Color, Binning, and Display Images in Grayscale options are not available with other RT models.*

Flip

Specify whether images will be flipped in the live image view and during the image capture process.

- Select **Horizontal** to flip the image from left to right.
- Select **Vertical** to flip the image from top to bottom.
- Select both options to flip the image from left to right and from top to bottom.
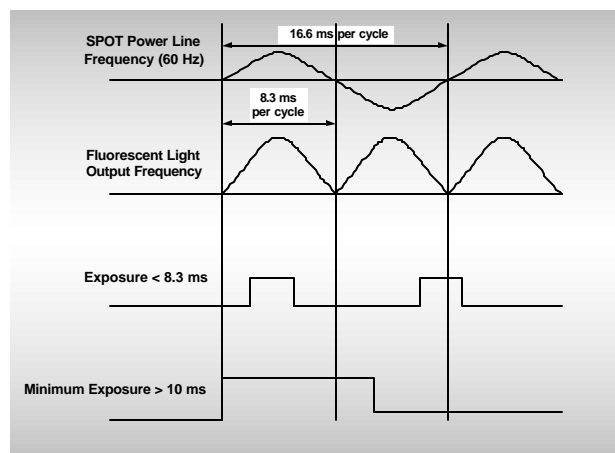
Rotate 90°

Specify whether images will be rotated in the live image view and during the image capture process.

- Select **None** if you do not want the image to be rotated.
- Select **Right** to rotate the image clockwise 90 degrees.
- Select **Left** to rotate the image counterclockwise 90 degrees.

Exposure Limits – Minimum

Because SPOT RT cameras are capable of extremely short exposure times, light sources with intensities that vary with the waveform of the 60 Hz line supply (e.g., fluorescent lamps, 120 volt incandescent bulbs, and 6 volt 20 watt halogen lamps) can cause the Live Image window to flicker and result in incorrect brightness and color balance readings in captured images. This option allows you to correct for these problems by limiting the camera to a minimum exposure time that is greater than the pulse interval of the light source. Consider the illustration to the right. In

Setting a Minimum Exposure to Accommodate Varied Light Sources

this case, the pulse interval of the fluorescent light source is 8.3 ms. Entering a Minimum Exposure of 25 ms ensures that the camera's exposure time is greater than the pulse interval of the light source and that any error is minimized. The greater the value above 25 ms, the more the error is reduced. Alternatively, you can enter any multiple of 8.333 ms. To enter a minimum exposure, check the **Minimum** box, and enter a value in milliseconds.

Exposure Limits – Live Image Maximum

Because The Live Image Maximum option helps you to avoid unnecessarily long exposures when viewing a live image. Consider the following situation: When you change from a lower resolution objective to a higher resolution objective, the camera detects a brief interval of blackness. When the Auto-Brightness option (see above) is enabled, it compensates by modifying the exposure time to maintain a constant brightness level. This results in you having to wait for the camera to compute a lengthy and incorrect exposure for the black interval. Using the **Live Image Maximum** option, you can limit this exposure time. For most cases, Diagnostic Instruments recommends a value of 0.5 seconds.

Similarly, the Live Image Maximum option can also be used in cases where the microscope's light path selector is incorrectly set.

Filter Color (RT 3-Shot/Slider)

The Filter Color option specifies the color or colors that will be exposed in the live image preview/image capture.

- **Red** – Preview/capture a monochrome preview of your specimen using the red state of the LCD filter.
- **Green** – Preview/capture a monochrome preview of your specimen using the green state of the LCD filter.
- **Blue** - Preview/capture a monochrome preview of your specimen using the blue state of the LCD filter.
- **Clear** – Preview/capture a monochrome preview of your specimen using the clear state of the LCD filter.

Display Images in Grayscale (RT 3-Shot/Slider)

To counter the difficulty of viewing, focusing, and adjusting monochrome images in live mode, the **Display Images in Grayscale** option enables you to view a monochrome image in grayscale, as opposed to with the red, green, or blue filter. This option applies only when the **Filter Color** (see above) is set to Red, Green, or Blue.

Binning (RT 3-Shot/Slider)

Binning combines adjacent pixels on the CCD chip, thereby reducing the number of pixels used in the image capture. Binning is useful when you have dim images and want to increase sensitivity and reduce download time. This gives you higher frame rates and less noisy images. But, as binning level increases, image resolution decreases. All of these factors should be considered when you select a binning option. There are four binning options:

- None   1 pixel   = 1 pixel
- 2 x 2   4 pixels = 1 pixel
- 3 x 3   9 pixels = 1 pixel
- 4 x 4   16 pixels = 1 pixel

Noise Filter

The Noise Filter corrects for the effects of electrical or thermal noise. The Noise Filter checks each pixel on the CCD chip as described below:

1. The program compares the value of each red pixel with the values of the eight neighboring red pixels.

2. If none of the eight neighboring red pixels has a value within ±15 % (i.e., the threshold value that the SPOT program uses) of the value for the red pixel being checked, the value for the red pixel being checked is replaced with the average value of the eight neighboring red pixels.

3. The program repeats this procedure for the values of the green and blue pixels being checked.

To activate the Noise Filter, check the Noise Filter box. The program runs the noise filter each time an image is captured using the **Preview** or **Acquire** options.

Device

Clicking on the device button allows you to select the camera that you would like to use (or switch to) for the next image capture. This window is identical to the device selection window that appears when you open the SPOT software program.

Taking a Picture

From the SPOT TWAIN Interface, you can capture images in different ways:

- **Preview -** You can capture a **Preview** image. Preview images remain available in the TWAIN window for basic editing and serve as the basis for the final image capture. When you click on the **Preview** button, the **Get Image** dialog displays the capture status as it is captured, and the captured image then replaces in the Live Image in the window. Note that the status bar indicated that you are viewing a Preview image.

- **Transfer** - If you like the Preview image, you can **Transfer** it image directly to the third party application that you are using. The Transfer button is active only for Preview image captures.

- **Acquire -** Capture an image and have it automatically transfer to (open in) the third party imaging application.

Ch. 16 – Using Other Mac Imaging Applications

Introduction

The SPOT 3rd Party Interface is a stand-alone mini-application that is designed for Mac users who want to use the SPOT camera to capture images, and then edit the images in a third party imaging application (e.g., Photoshop, Image-Pro). Using the 3rd Party Interface, you can:

- View a live image preview
- Create and/or modify a SPOT image setup
- Capture an image and have it automatically open in another application
- Capture an image and save it to file (i.e., the image is not associated with a specific application)
- Transfer captured images into the image editing software

You *cannot*, however, use the 3rd Party Interface to open images from file.

Preparing to Take a Picture

Before taking a picture via the 3rd Party Interface, you can set the following parameters as needed:

- The SPOT 3rd Party Interface File and Save Options
- The SPOT 3rd Party Interface live image viewing preferences
- The Image Setup, which defines how the camera will take pictures

Setting File and Save Options

Before using the 3rd Party Interface program, you can specify file format, open, and save options. This includes the following information:

- File format for captured images
- Imaging application to edit captured images
- Location for saved image files
- File naming conventions
- File opening conventions

To specify this information, follow these steps:

1. Open the 3rd Party Interface. The **SPOT Camera** window appears, as shown at right.

2. Click on the **Setup** button. The **Preferences** window appears.

3. Specify the **File Type**. Click on the File Type selector and select one of the following options:

    – Windows Bitmap
    – Mac PICT (pct, pic, pict)
    – TIFF Uncompressed (tif, tiff)

    ✎   *Note: To capture images at pixel bit depth settings other than 8 or 24 bpp (e.g., 12 or 36 bpp), you should select the* **TIFF/Uncompressed** *file type.*

4. If you use the 12 bpp or 36 bpp settings, and you use an imaging application that saves files in 16 bpp and 48 bpp format (e.g., Photoshop), ensure that the **Save 12/36 bit images in 16/48 bit files (best for Photoshop)** box is checked.

5. Click on the **Set Application** button to specify the imaging application (e.g., Photoshop) that you will use with the SPOT camera and the 3rd Party Interface.
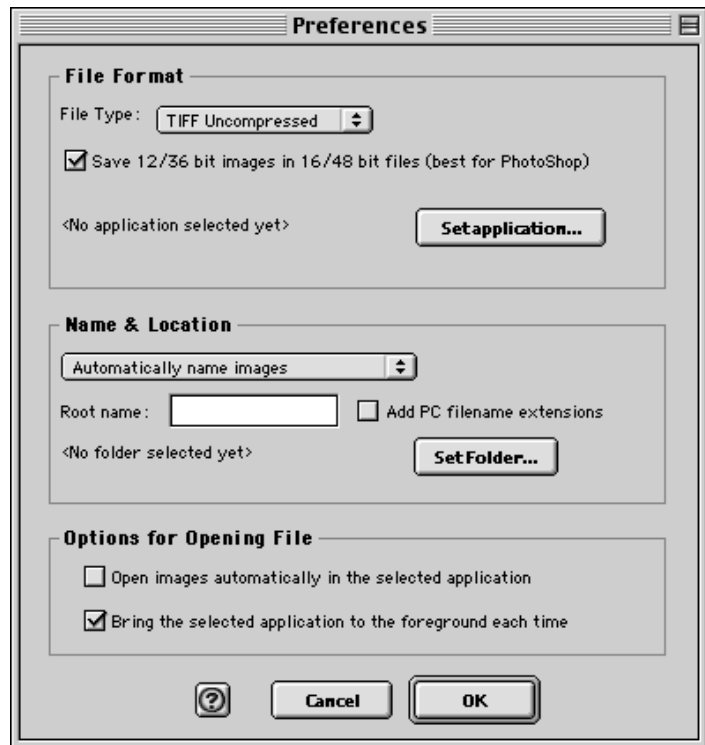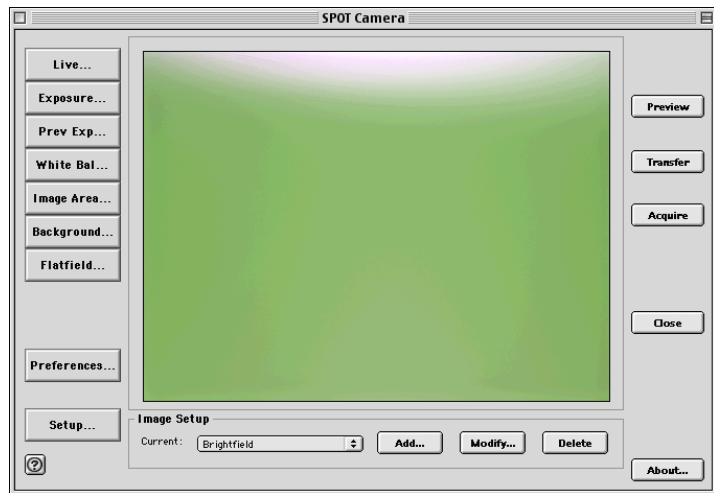
6. Specify how files are named and where they are saved. There are two options.
   - **Automatically name images** – The program automatically names and saves images according to the parameters that you specify.
   - **Ask for filename each time - Have** the program prompt you to enter the name and file location each time.

   If you choose the **Automatically name images** option, specify the **Root name** for the image files and the folder that they will be saved to.
   - Enter a name in the **Root name** box. The Root name is the first part of the file name. If the root name is **test**, for example, the first image that you save will be **test0001**, the second image **test0002**, etc… If you do not specify a Root name, the files are saved with the numerical names (e.g., 0001, 0002, etc…).
   - To display the PC file type extension for image files, check the Add **PC filename extensions** box.
   - Click on the **Set Folder** button to specify where you will save the file. If you do not specify a location, the 3rd Party Interface program creates a folder on the desktop called SPOT Images; captured images are automatically saved to this location.

7. Specify the file open options. You can select one, both, or neither of the two options:
   - **Open images automatically in selected application** – When you Acquire or Transfer images, the images open automatically in the application that you specified in step 4 above.
   - **Bring the selected image to the foreground each time** – When you Acquire or Transfer images, the imaging application window opens in front of the SPOT Camera window, which is sent to the background.

   If neither option is selected, the image file is saved to the location that you specify.

8. Click on OK. The file type and application that you specified appear next to the Change button on the SPOT Camera window.

Defining Live Image Preferences

The 3rd Party Interface window includes a **Preferences** button, which allows you to define live image viewing and exposure preferences. This includes the following options:

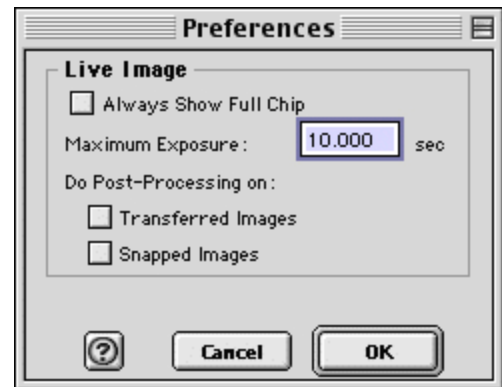- Always Show Full Chip
- Maximum Exposure
- Do Post Processing On

Each option is discussed below.

Always Show Full Chip

Always display the full chip in live image mode. If you select a region, it will be displayed in a rectangular outline within the full-chip view.

Maximum Exposure

The Maximum Exposure option helps you to avoid unnecessarily long exposures when viewing a live image. Consider the following situation: When you change from a lower resolution objective to a higher resolution objective, the camera detects a brief interval of blackness and compensates by modifying the exposure time to maintain a constant brightness level. This results in you having to wait for the camera to compute a lengthy and incorrect exposure for the black interval. Using the **Maximum Exposure** option,

you can limit this exposure time. For most cases, Diagnostic Instruments recommends a value of 0.5 seconds. Similarly, the Live Image Maximum option can also be used in cases where the microscope's light path selector is incorrectly set.

Post Processing

Specify whether to apply the Post Processing options for the current Image Setup to images captured from the live image view.

Transferred Images

- *If the Transferred Images box is selected*, the Transfer option applies the Noise Filter, Color Enhancement, and Image Size Double options as specified for the current Image Setup.

- *If the Transferred Images box is unselected*, the Transfer option ignores the Post Processing options for the current Image Setup, with one exception: if the Chip Defect Correction option is selected for the current image setup (Enhanced and RT cameras only), the corrections will appear in the image capture, regardless of the Transferred Images preference.

Snapped Images

- *If the Snapped Images box is selected*, the Snap option uses all of the Post Processing options from the current Image Setup, except for Background Subtraction and Flatfield Correction.

- *If the Snapped Images box is unselected*, the Snap option ignores the Post Processing options for the current Image Setup, with one exception: if the Chip Defect Correction option is selected for the current image setup (RT and Enhanced cameras only), the corrections will appear in the image capture, regardless of the Snapped Images preference.

# Palettes (RT B/W, RT 3-Shot/Slider)

The Palettes option allows you to define new palettes and edit existing palettes.

SPOT color palettes allow you to apply specific hue and saturation values to monochrome (8 bit or 12 bit) images. Palettes are useful in fluorescence applications where a sample is fluorescing at a single wavelength, and you want the acquired image to have the correct color. For such a case, you could create a palette to represent the color of the fluorescent dye and then apply that palette to the captured image. There are two ways to apply a palette to an image:

- You can associate a palette with an Image Setup, as discussed in Ch. 5 - Preparing to Take a Picture.

- You can apply a palette to a captured image by selecting the **Set Palette** option (Advanced mode) from the **Edit** menu. Refer to Ch. 8 – Viewing, Editing, and Annotating Images for more details.

To define a new palette, follow these steps:

1. From the **Setup** menu, select **Palettes**.

2. Click on **Add**. The Color Palette dialog appears as shown here:

3. Either use the **Hue** and **Saturation** selection lists or move the cross-hairs on the color wheel to enter the values

    – **Hue** is the designation for the actual color, as distinguished from others in the color spectrum. Hue is derived from the color wheel and is expressed in the color's angular location (in degrees) on the wheel. (e.g., 0° to 360°).

    – **Saturation** is the proportion of perceived pure hue in the color. Saturation (in the SPOT software) is measured on a numerical scale of 0 to 100, where zero is pure gray and 100 is pure hue (e.g., day glow).

4. Click on **OK**.
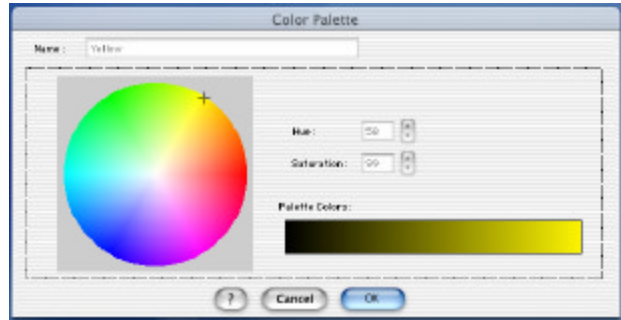
Defining an Image Setup

An image setup serves two crucial purposes:

- It defines the way a picture will be taken.
- It defines the way the program automatically modifies a picture after it is taken.

Image setups are defined to optimize the picture taking process for different types of images or microscopy techniques. They can be created, saved, and recalled as needed. To define a new image setup, you must also select one of 11 pre-defined image types as a template for defining the new setup. The 11 pre-defined image types (in addition to the Factory Defaults setting) correspond to commonly used microscopy techniques. Refer to Ch. 5- Preparing to Take a Picture for a table of the different image types. Brackets around the title indicate pre-defined image types, while the user defined image setups appear without brackets.

- To modify the current image setup, click on the **Modify** button.

- To delete an image setup, select the image setup from the list, and click on the **Delete** button.

- To define a new image setup, click on the **Add** button. The New Image Setup window appears with a list of pre-defined and user-defined templates (image types). Brackets indicate pre-defined image types < >. Select a template, and click on OK. The New Image Setup window appears, as shown at right:
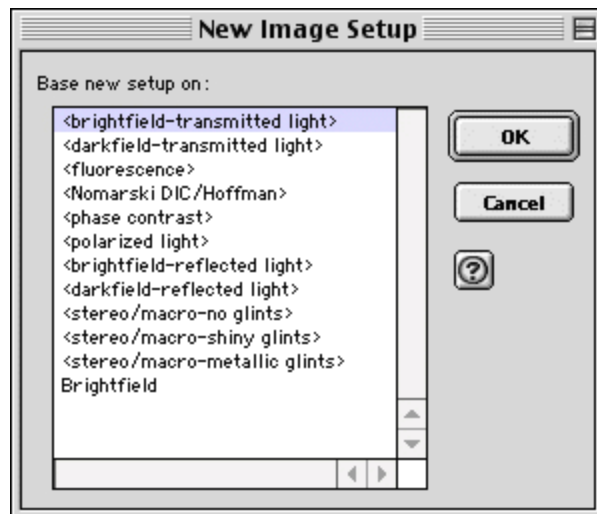
RT B/W	RT B/W camera users will not see the fluorescence – red, fluorescence – green, and fluorescence – blue Image Type options.

When you add a new image setup or modify an existing setup, the Image Setup window appears, as shown here.
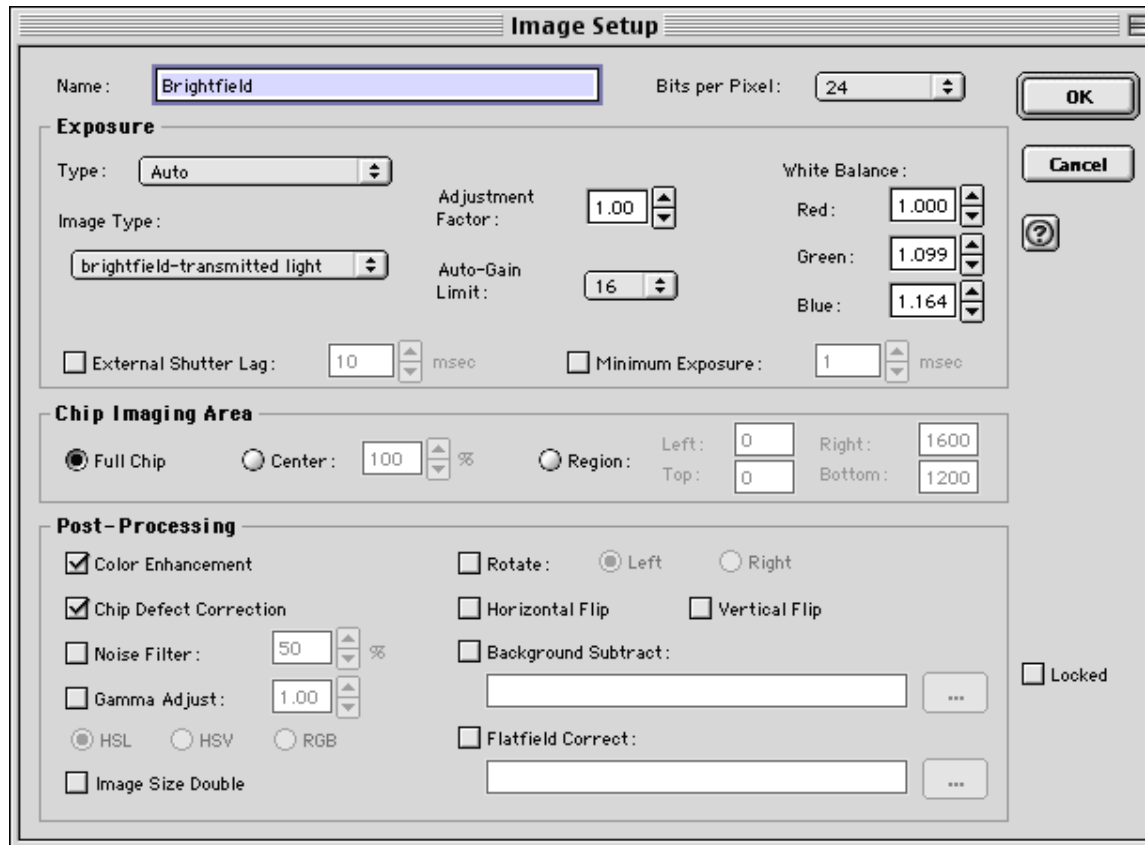

Refer to Ch. 5 – Preparing to Take a Picture for a detailed explanation of the Image Setup options.

Taking a Picture

Using the 3rd Party Interface, you can perform both initial image captures (e.g., for computing exposure, white balance, etc…) and final image captures.

Capturing Initial Images

The toolbar at the left of the SPOT Image Capture window enables you to quickly access the image capture functions that are used prior to the capture of the final image. The call-outs below page illustrate the toolbar buttons:
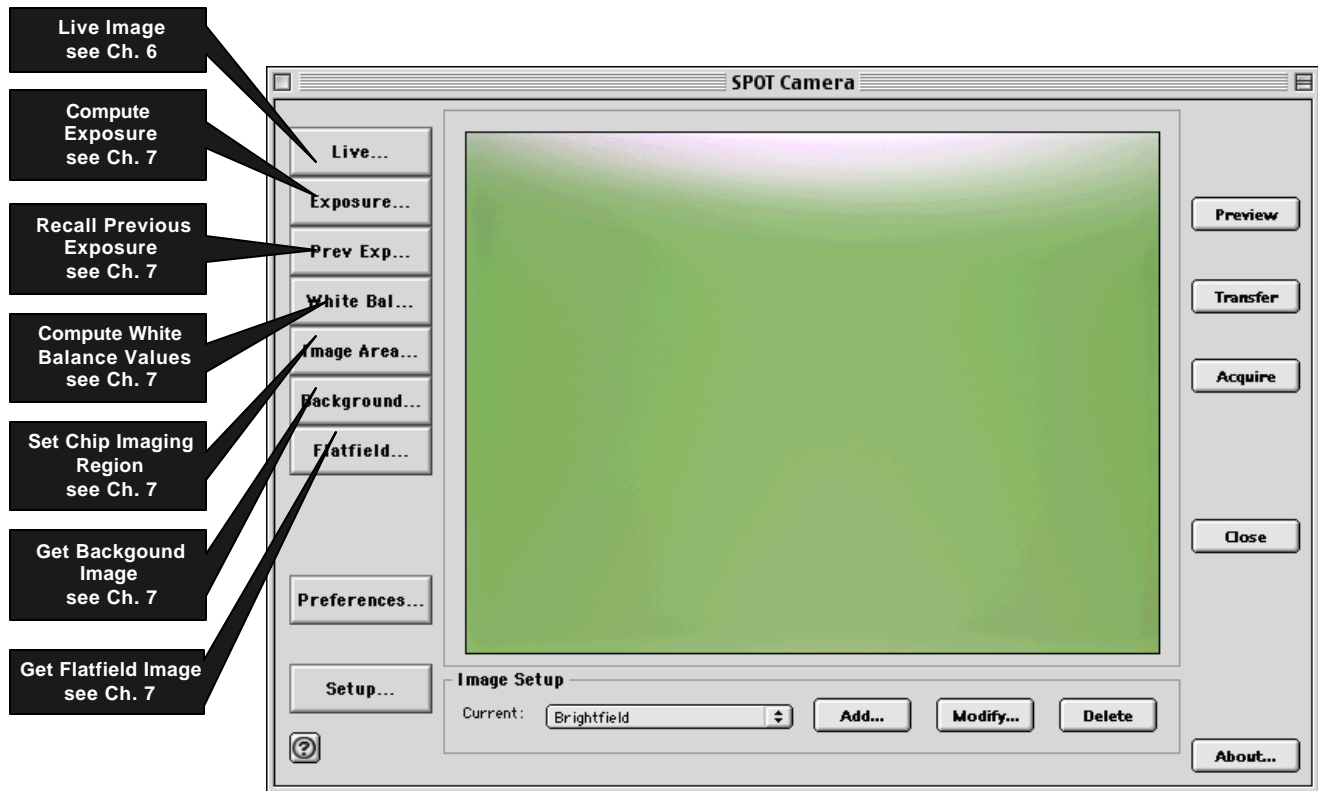
RT B/W	RT B/W camera users will not see the Compute White Balance Values toolbar button.

Capturing Final Images

From the 3rd Party Interface program, you can capture an image in two different ways. You can preview the image and then transfer it to another imaging application (e.g., Photoshop), or you can Acquire the image, which sends the captured image directly to the application.

- **Preview**– Capture an image and preview it within the SPOT Image Capture window.

- **Transfer** – Transfer a captured image to the imaging application.

- **Acquire -** Capture an image and have it open/save according to the parameters that you specified (see Setting File and Save Options). The one-step process of Acquiring an image is identical to previewing and then transferring an image

Ch. 17 – Using Other Mac Imaging Applications in Basic Mode

Introduction

The SPOT Basic 3rd Party Interface is a stand-alone mini-application that is designed for Mac users who want to use the SPOT camera to capture images, and then edit the images in a third party imaging application (e.g., Photoshop, Image-Pro). Using the 3rd Party Interface, you can:

- View a live image preview
- Create and/or modify a SPOT image setup
- Capture an image and have it automatically open in another application
- Capture an image and save it to file (i.e., the image is not associated with a specific application)
- Transfer captured images into the image editing software

You *cannot*, however, use the 3rd Party Interface to open images from file.

Working in the Basic 3rd Party Interface

The Basic 3rd Party interface is a one-stop point for previewing and capturing images. For example, using the Basic 3rd Party interface, a typical image capture session might involve the following steps, as illustrated below:

Preview your specimen

Use the live image as a point of reference to focus and position the image and to scan for areas of interest.
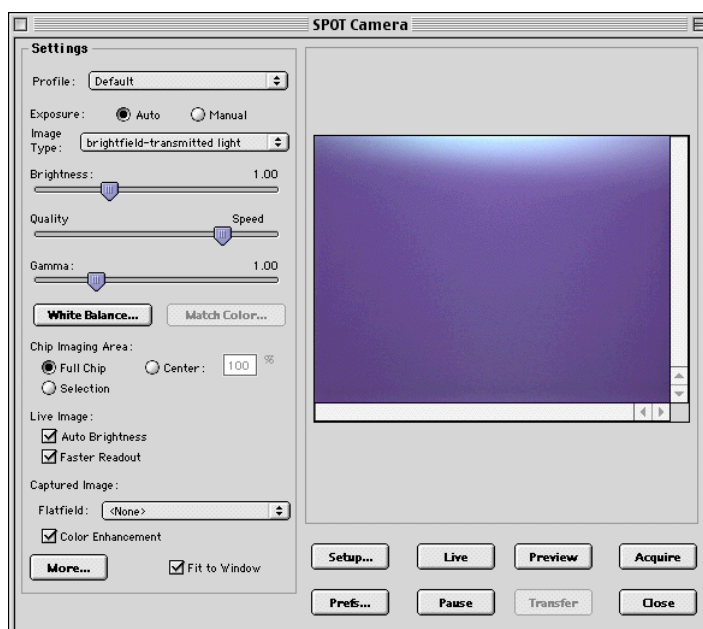
Create a Profile

Profiles allow you to create, modify, and reuse specific exposures settings that are tailored to your image capture needs. To create a new profile, **control-click** on the Profile box.
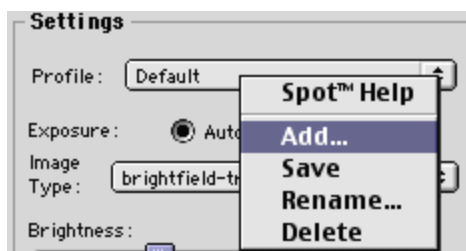
Define Settings

Define exposure settings for live and captured images. Each of these settings is discussed later in this chapter.
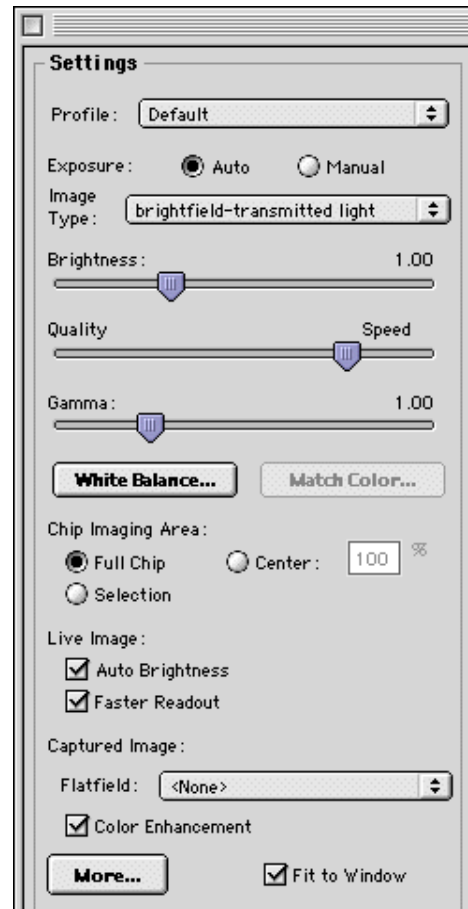
Capture an Image

All of the image capture options are located on the bottom portion of the window, as illustrated here.

Defining Preferences

In the Basic 3rd Party Interface, you can define three capture/view preferences.   Click on the **Prefs** button to open the **Preferences** window (see right). Each option is described below:

Captured Image Bit Depth
This option enables you to specify a different image bit depth when saving an image captured in the SPOT program.

- **8 Bits per Channel** – The 8 bits per channel option provides 256 gray levels per color:
    - With RT B/W cameras, captured images are 8 bits per pixel monochrome.
    - With RT Color cameras, captured images are 24 bits per pixel RGB color.

    This option results in smaller, more manageable image sizes, and is best for images that do not require extensive modification.
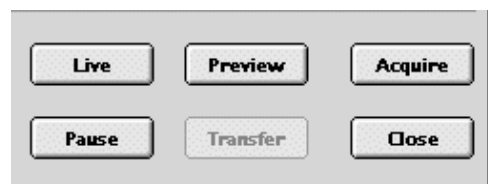
- **12 Bits per Channel** – The 12 Bits per channel option provides 4,096 gray levels per color.
    - With RT B/W cameras, captured images are 12 bits per pixel monochrome.
    - With RT Color cameras, captured images are 36 bits per pixel RGB color.

    This option reduces banding problems that can result with modified images, but also results in larger file sizes.

    When saving 12 bit per channel images, SPOT prompts you to select either 12 bits per channel or 16 bits per channel. If you use Adobe's Photoshop for image editing, you should select the 16 bit per channel option because Photoshop cannot read 12 bit per channel images.

> *Note: The SPOT RT camera captures all images using 10 bits per channel (color). The SPOT software immediately converts the captured image to either 8 bits per channel (color) or 12 bits per channel (color), depending on the* **Captured Image Bit Depth** *that you select.*

Always Show Full Chip in Live Mode
Checking this option ensures that **you** will always see the Full Chip view when viewing a live image. Keep in mind that the selection that you make here determines how the live image displays when viewing a region of the chip. Refer to the **Chip Imaging Area** section for more details.

F-Keys

The following function keys are available as shortcuts in the Basic 3rd Party interface:

Function Key	Action	3rd Party Interface Option
[F 4]	Restart the live image view.	Live
[Alt] + [F 4]	Close the SPOT Camera window.	Close
[F 9]	Capture an image and automatically transfer it to the imaging application.	Acquire
[Ctrl] + [F 10]	Compute white balance values.	White Balance
[Alt] + [F 10]	Compute white balance values without displaying the informational dialog.	*Not available from the window*

Using the Live Image to Define Image Capture Settings

When you open the Basic mode 3rd Party Interface, the Live Image window is, by default, running, as indicated by the **Live Mode Running** message in the status bar. The settings options in the 3rd Party interface window enable you to optimize the live image view, and then capture an image using these settings. In the 3rd Party Interface, you can:

- Create profiles to use as pre-defined image capture settings.

- Modify exposure settings

- Define a specific portion of the chip to be used in the image capture.

- Modify the screen orientation of the specimen.

The following sections describe all of the Live Image/Image Capture Settings.

Profile

The **Profile** option allows you to create and re-use sets of live image/image capture settings. When you open the 3rd Party interface, the **Default** Profile is selected. When you make changes to any of the live image/image capture settings, these changes can be saved to a Profile – either the Default or one that you have defined. For example, if you gamma adjust a live image and then decide that the result is ideal for the type of specimen you are working with, you could create a new Profile with the gamma and exposure values and name it for that type of specimen – i.e., "Diseased Cells."

When you close the **SPOT Camera** window, the program prompts you to save changes. Carefully consider the effect of your choice:

- Clicking **Yes** modifies the Profile according to the adjustments that you made. Future image captures taken with that Profile will reflect these changes. For example, if you rotated a live image, and then clicked Yes after closing the window, all future captures taken with that Profile would be rotated in the same way.

- Clicking **No** retains the original values of the Profile. This allows you to maintain a unique set of image view and capture settings that can be re-applied to future captures of similar specimens.

To add, edit, or delete Profiles, **right-click** on the Profile box. Right clicking gives the following options:

- **Add –** Add a new Profile. When you add a new Profile, the program prompts you to name it.
- **Save –** Save a Profile that you have modified.
- **Rename –** Rename an existing Profile.
- **Delete -** Delete a Profile. This option appears only after you have defined a second Profile in addition to the Default Profile.

Exposure: Auto or Manual

The **Exposure** option gives you the choice of having the camera calculate exposure time (**Auto**), or setting the exposure on your own (**Manual**).   The exposure options will vary according to whether you select **Auto** or **Manual**, as illustrated below.

Auto   **Manual**

When you enter one of the above exposure settings, note that it applies **both** to the live image that you are viewing and the captured image.

Auto Exposure options

Image Type

An Image Type tells the SPOT program how to expose and process image captures. Each of the 11 available Image Types (see below) corresponds to a microscopy technique: select the Image Type that matches the technique you are using. The camera will capture the image accordingly. The table below describes typical situations for each image type.

Image Type	Description
Brightfield-transmitted light	Stained specimens on glass slides using transmitted light microscopes.
Darkfield-transmitted light	Bright specimens on dark backgrounds using transmitted light darkfield technique.
Fluorescence	Fluorescence specimens not fluorescing in a single red, green, or blue primary color
Nomarski DIC/Hoffman	Specimens being viewed with either Nomarski DIC or Hoffman techniques.
Phase contrast	Specimens being viewed using the phase contrast technique.
Polarized light	Specimens being viewed with polarized light techniques.
Brightfield-reflected light	Polished metallurgical specimens and semi-conductors using a microscope with a vertical illuminator.
Darkfield-reflected light	Bright areas of specimens being viewed with darkfield, reflected light techniques.

(cont.)

Image Type	Description
Stereo/macro–no glints	Matte specimens without bright glints.
Stereo/macro–shiny glints	Shiny specimens with bright glints.
Stereo/macro-metallic glints	Metallic specimens with intense glints.

Brightness

Enter a **Brightness** value between **.24** and **34**.
A brightness value of 1, the default value, gives you the correct image brightness for most images. The Brightness control allows you to overexpose or underexpose (darken or lighten) the image area. In many cases, an image has varying levels of brightness; to see a specific area better you might want to lighten or darken the image. When using the Brightness control, the SPOT camera uses an algorithm that combines gain and exposure time to provide the fastest possible image update rate (shutter speed) in live mode:

- When overexposing images to make them brighter (i.e., entering values greater than one), the camera first selects the highest possible gain before lengthening the exposure time. Because gain also brightens an image, less exposure time is needed.

- When underexposing images to make them darker (i.e., entering values less then one) the camera uses the lowest possible exposure time before lowering the gain.

Quality/Speed (Auto)

Use the slider to balance speed and quality when previewing dim images.

- Moving the slider toward the **Quality** side increases image quality and decreases frame rates.
- Moving the slider toward the **Speed** side increases frame rates and decreases image quality.

The Quality/Speed measurement corresponds to the Auto-Gain limit setting, which allows you to set an upper limit on the gain options that will be available during the Auto-Exposure process.

- When the slider is at the left-most point, the Auto-Gain Limit gives you higher quality pictures, but lengthier exposure times.

- When the slider is at the right-most point, the Auto-Gain Limit allows the camera to select from all available gain options. This shortens exposure times, but decreases image quality.

Refer to the "Auto-Gain Limit" section in Ch. 5 – Preparing to Take a Picture for a detailed explanation of gain and exposure.

Gamma

Enter a **Gamma** value between **.1** and **4**.
Gamma adjustment smoothly lightens darker areas of an image without burning out bright areas or lightening black areas. This is especially useful for capturing/editing images with a wide range of brightness values (dynamic range) such as fluorescence specimens or macroscopic objects with glints, (e.g., printed circuitry).

- Values between **1** and **4** lighten the darker areas of the image.
- Values between **.1** and **1** darken the mid-tones of the image.

Gamma adjustments on live images are made in the RGB color space, the only color space where adjustments can be made quickly enough to keep up with live imaging. Refer to Ch. 5 – Preparing to Take a Picture for more details on gamma adjustment and color spaces.

Manual Exposure Options

Exposure Time(s)

Use the slider-bar to specify an exposure time (or times) between **.04 ms** and **526.746 sec**.
When you specify an exposure time, the camera skips the automated exposure sequence that takes place when you open the live image view – i.e., the 3rd Party interface. This is very useful in cases where exposure times are lengthy, as with fluorescence samples.

Gain

Enter the precise gain that the camera will use for an image capture. Use the slider-bar to select one of the gain options: **1**, **2**, **4**, **8**, or **16**. The gain value displays to the right of the slider-bar. Refer to the "Auto-Gain Limit " and "Gain" sections of Ch. 5 – Preparing to Take a Picture for details on gain and exposure.

Gamma

The manual Gamma adjustment is identical to the auto Gamma adjustment (see above.)

White Balance

White balance is the ratio of red, green, and blue gains necessary to achieve the proper color rendition for an image. Many factors impact the coloration of an image capture, including lamp voltage, the coloration of the glass used in the slide, and the coloration of the glass used in the lenses of the microscope's objectives. To minimize the impact of these factors, use the White Balance feature.   When you perform a white balance calculation, SPOT samples the color of the light from your light source after it has passed through the slide and the objective, and then calculates the red, green, and blue gains needed to produce images where the white areas are white and the color areas are true.

You should perform a new white balance calculation when:

- Starting a new image capture session.
- Changing the lamp voltage.
- Switching to a different objective on the microscope.

To perform a white balance, follow these steps:

1. Set the illumination and the objective that you will use for the image capture.

2. If you have not done so, select your **Image Type** (see above). Each Image Type has its own set of white balance values. Any white balance values that you compute for an image type remain linked to that image type until you compute new values. When selecting a new image type, remember that you will be using the white balance values linked to that image type.

3. Click on the **White Balance** button.

4. Show the camera a sample of white light. This step varies according to microscopy technique and Image Type (sample), as shown in the table below:

Image Type	White Balance Technique
Brightfield-transmitted light	Position the slide so that the specimen is not in the field of view, but light is going through the slide near the specimen.
Darkfield-transmitted light	Perform the white balance using the brightfield mode on your microscope. See Brightfield – transmitted light above.
Fluorescence	Use the Match Color feature (see above)
Nomarski DIC/Hoffman	**For Nomarski DIC:** Adjust the Nomarski prism until the image is in either the "brightfield" or the "gray scale" view. **For Hoffman:** Perform the white balance directly on the Hoffman image.
Phase contrast	Position the slide so that the specimen is not in the field of view, but light is going through the slide near the specimen.
Polarized light	Pull all polarizers and compensators out of the light path and then position the slide so that the specimen is not in the field of view, but light is going through the slide near the specimen.
Brightfield-reflected light	Replace your sample with a mirror.
Darkfield-reflected light	Perform the white balance using the brightfield mode on your microscope. See Brightfield – reflected light above.
Stereo/Macro (all three options)	Using an EPI-illuminator (shining a light on the top of your specimen from off to one side), replace your sample with a white sheet of paper.

5. Click on **Begin** to start the calculation. The camera computes the white balance values, and they are automatically saved to the image settings for the image type that you selected.

Match Color

The Match Color feature is typically used in two situations:

- You want to change the colors of an image on the screen to match the way that you think the specimen looks.

- You want to set the white balance for pictures taken with a microscopic technique that never has white in its images. A prime example of this situation is fluorescence microscopy.

To use the Match Color feature, follow these steps:

1. Click on the **Preview** button to capture an image. The Match Color option remains inactive until a preview image is captured.

2. Select **Match Color.** The cursor becomes an eyedropper.

3. Using the mouse to move the eyedropper, position it on the pixel location of the color that you want to match. The R, G, and B values of the pixel that you select must all be less than 255.

4. Click on the pixel. The **Match Color** dialog appears with information about the pixel that you selected:
   - **Hue** – The actual color, as distinguished from others in the color spectrum. Hue is derived from a color wheel and is expressed in the color's angular location (e.g., 0 to 360 degrees).
   - **Saturation** - The proportion of perceived pure hue in the color. Saturation (in the SPOT software) is measured on a numerical scale of 0 to 100, where zero equals pure gray and 100 equals pure hue (e.g., "day glow").

5. Move the cross hairs to the desired color either by clicking on the new location or clicking and dragging.   As you move the cross hairs, the color in the image changes accordingly, allowing you to preview the color before you save the image. Alternatively, you can click on the **Make White/Grey** button to automatically white balance the image.

6. Click on **Ok**.

Chip Imaging Area

SPOT allows you to use any rectangular portion of the CCD chip for image capture. Using a portion of the chip is helpful in cases where you want to:

- Keep file sizes small.
- Shorten download times.
- Avoid areas of the image with optical problems (e.g., vignette areas).

There are three image area options:

- **Full Chip** – Capture the entire area of the CCD chip.

- **Center** – Capture a rectangular part of the CCD chip's area that has the same height - to - width ratio as the full chip, and is centered on the center of the full chip. The percentage that you enter is the percentage of full chip width that the new area's width will be.

    To capture a centered proportion of an image, follow these steps:
    a) Check the Center option.
    b) Enter the percentage of the image that you want to capture. The minimum percentage is 10 and the maximum is 100.
    c) Capture the image.

- **Selection** –Capture a specific part of the CCD chip.

    a) Check the **Selection** option.
    b) Use the mouse to select a region for capture. Click and drag to define the selection. Note that the new pixel dimensions display in the status bar.
    c) Capture the image.

When you are viewing a live image **Selection**, how this selection is displayed, depends on two other settings: the **Fit to Window** option (see below) and the **Always Show Full Chip in Live Mode** preference (see above). The following table illustrates the possibilities:

Fit to Window Checked?	Always Show Full Chip in Live Mode Checked?	Selection Display	Live or Captured Image
YES	YES	When you select a portion of the chip, the selection is outlined with a **yellow rectangle** .	LIVE
YES	NO	When you select a portion of the chip the cursor is a **zoom tool**. To zoom in on a portion of a live or captured image, click and drag over the area that you want to zoom in on. To return to the non-zoomed image, click on the **Full Chip** option.	BOTH
NO	YES	When you select a portion of the chip, the selection is outlined with a yellow rectangle. However, because **Fit to Window** is not checked, you will not see the entire chip in the window.	LIVE
NO	NO	When you select a portion of the image, the cursor is a cropping tool. If you select a portion of an image, the program displays only the selected area and discards the rest of the image. To return to the non-cropped image, click on the **Full Chip** option.	BOTH

Auto-Brightness (Auto, Live Images)

Available only when you are using **Auto** exposure and viewing a live image, the **Auto-Brightness** option enables you to maintain a consistent brightness level for changing specimens.

Faster Readout (Live Images)

Faster Readout enables you to "speed up" the live image display (higher frame rates) in cases where you are willing to sacrifice some resolution for a higher frame rate.

Flatfield (Captured Images)

For all other SPOT camera lines, the Flatfield Correct option is used to correct for display problems associated with uneven intensity or coloration in illumination, or to correct for artifacts (e.g., dust) in the optical system.

> *Note: Flatfield correction with the SPOT camera refers only to the correction of uneven lighting. It should not be confused with the correction of optical field flatness.*

Flatfield correction works by taking a picture of the brightfield background without the specimen in the field of view. This image lets the SPOT software know, on a pixel-by pixel basis, which areas in the field of view are receiving less illumination than the brightest areas. When taking a picture that contains a specimen, the software uses this information to accurately increase the brightness of each pixel in the dimly illuminated areas. The resulting image gives the appearance of an even illumination.

To use the **Flatfield** option, follow these steps:

1. Ensure that the specimen is under the desired objective and in focus.

2. Control-click on the **Flatfield** drop-down list and click on the **Add** option. The **Get Flatfield** dialog appears.

3. Remove the slide from the field of view.

4. Click on Begin. When the program finishes computing the exposure times and acquiring the flatfield file, the **Flatfield** dialog prompts you to name the file:

   a) Enter a name. Because flatfield image files are different for each objective, you might want to name the file according to the objective's magnification (e.g., 10x).

   b) Click on OK. If you do not specify a location, the program automatically saves the flatfield file to the **c:\SpotCam\Fltflds** directory.

> *Note: The default file location varies, depending on where you installed the SPOT program.*

5. Move the specimen back into the field of view, and perform the image capture, using either the **Preview** or the **Acquire** option.

Color Enhancement (Captured Images)

The Color Enhancement option optimizes image captures by closely matching the final capture to the image as seen through the microscope. To optimize the final image, the SPOT program processes the raw image data to produce a final image that matches the view seen through the microscope.

If you want an image capture to match the live image view, you will want to leave Color Enhancement off. Color Enhancement applies only to captured images.

Fit to Window

In addition to automatically resizing images to fit within the live image window, the Fit to Window option also determines cursor behavior in the live image view.

- When the **Fit to Window** option is checked, the live image window **cursor** becomes a **zoom tool**. To zoom in on a portion of a live image, click and drag over the area that you want to zoom in on. To return to the non-zoomed image, click on the **Full Chip** option.

- When the **Fit to Window** option is not checked, the cursor is a cropping tool. If you select a portion of an image, the program displays only the selected area and discards the rest of the image. To return to the non-cropped image, click on the **Full Chip** option.

These points apply *only* when the **Always Show Full Chip in Live Mode** (see above) is *not selected*. For more details, refer back to the **Chip Imaging Area** section (see above).

More (Additional Orientation and Exposure Settings)

When you click on **More**, the program displays a dialog of additional orientation and exposure settings for the 3rd Party Interface, as shown at right.

| *RT 3-Shot* | RT 3-Shot users will also see *the* **Filter Color**, **Binning**, and **Display Images in Grayscale** options (see below). |

Flip

Specify whether images will be flipped in the live image view and during the image capture process.

- Select **Horizontal** to flip the image from left to right.
- Select **Vertical** to flip the image from top to bottom.
- Select both options to flip the image from left to right and from top to bottom.
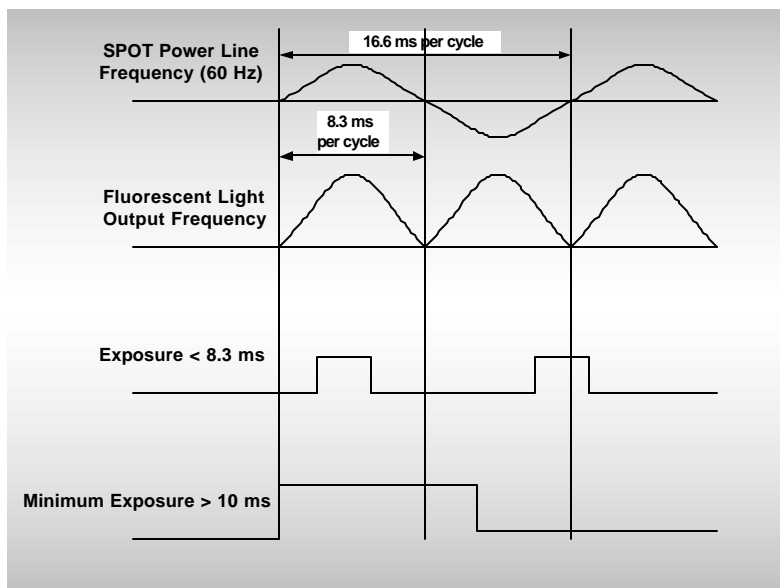
Rotate 90°

Specify whether images will be rotated in the live image view and during the image capture process.

- Select **None** if you do not want the image to be rotated.
- Select **Right** to rotate the image clockwise 90 degrees.
- Select **Left** to rotate the image counterclockwise 90 degrees.

Exposure Limits – Minimum

Because SPOT RT cameras are capable of extremely short exposure times, light sources with intensities that vary with the waveform of the 60 Hz line supply (e.g., fluorescent lamps, 120 volt incandescent bulbs, and 6 volt 20 watt halogen lamps) can cause the Live Image window to flicker and result in incorrect brightness and color balance readings in captured images. This option allows you to correct for these problems by limiting the camera to a minimum exposure time that is greater than the pulse interval of the light source. Consider the illustration to the right. In this case, the pulse interval of the fluorescent light source is 8.3 ms. Entering a Minimum Exposure of 25 ms ensures that the camera's exposure time is greater than the pulse interval of the light source and that any

**Setting a Minimum Exposure to
Accommodate Varied Light Sources**

error is minimized. The greater the value above 25 ms, the more the error is reduced. Alternatively, you can enter any multiple of 8.333 ms. To enter a minimum exposure, check the **Minimum** box, and enter a value in milliseconds.

Exposure Limits – Live Image Maximum

Because The Live Image Maximum option helps you to avoid unnecessarily long exposures when viewing a live image. Consider the following situation: When you change from a lower resolution objective to a higher resolution objective, the camera detects a brief interval of blackness. When the Auto-Brightness option (see above) is enabled, it compensates by modifying the exposure time to maintain a constant brightness level. This results in you having to wait for the camera to compute a lengthy and incorrect exposure for the black interval. Using the **Live Image Maximum** option, you can limit this exposure time. For most cases, Diagnostic Instruments recommends a value of 0.5 seconds.

Similarly, the Live Image Maximum option can also be used in cases where the microscope's light path selector is incorrectly set.

Filter Color (RT 3-Shot)

The Filter Color option specifies the color or colors that will be exposed in the live image preview/image capture.

RT Slider	RT Slider users can select **RGB** you to preview/capture live images in full color. If the filter slider on the camera is moved to the **B/W** position, the Filter Color setting should likewise be changed to one of the monochrome options (e.g., Clear, Red, Green, or Blue). The **Monitor Camera Color Filter Position** preference monitors the color filter position only for image captures– it does not monitor color filter position when viewing live images.

- **Red** – Preview/capture a monochrome preview of your specimen using the red state of the LCD filter.

- **Green** – Preview/capture a monochrome preview of your specimen using the green state of the LCD filter.

- **Blue** - Preview/capture a monochrome preview of your specimen using the blue state of the LCD filter.

- **Clear** – Preview/capture a monochrome preview of your specimen using the clear state of the LCD filter.

Display Images in Grayscale (RT 3-Shot)

To counter the difficulty of viewing, focusing, and adjusting monochrome images in live mode, the **Display Images in Grayscale** option enables you to view a monochrome image in grayscale, as opposed to with the red, green, or blue filter. This option applies only when the **Filter Color** (see above) is set to Red, Green, or Blue.

Binning (RT 3-Shot)

Binning combines adjacent pixels on the CCD chip, thereby reducing the number of pixels used in the image capture. Binning is useful when you have dim images and want to increase sensitivity and reduce download time. This gives you higher frame rates and less noisy images. But, as binning level increases, image resolution decreases. All of these factors should be considered when you select a binning option.

There are four binning options:
- None   1 pixel   = 1 pixel
- 2 x 2   4 pixels = 1 pixel
- 3 x 3   9 pixels = 1 pixel
- 4 x 4   16 pixels = 1 pixel

Noise Filter

The Noise Filter corrects for the effects of electrical or thermal noise. The Noise Filter checks each pixel on the CCD chip as described below:

1. The program compares the value of each red pixel with the values of the eight neighboring red pixels.

2. If none of the eight neighboring red pixels has a value within ±15 % (i.e., the threshold value that the SPOT program uses) of the value for the red pixel being checked, the value for the red pixel being checked is replaced with the average value of the eight neighboring red pixels.

3. The program repeats this procedure for the values of the green and blue pixels being checked.

To activate the Noise Filter, check the Noise Filter box. The program runs the noise filter each time an image is captured using the **Preview** or **Acquire** options.

Taking a Picture

From the SPOT 3rd Party Interface, you can capture images in different ways:

- **Preview -** You can capture a **Preview** image. Preview images remain available in the 3rd Party window for basic editing and serve as the basis for the final image capture. When you click on the **Preview** button, the **Get Image** dialog displays the capture status as it is captured, and the captured image then replaces in the Live Image in the window. Note that the status bar indicated that you are viewing a Preview image.

- **Transfer** - If you like the Preview image, you can **Transfer** it image directly to the third party application that you are using. The Transfer button is active only for Preview image captures.

- **Acquire -** Capture an image and have it automatically transfer to (open in) the third party imaging application.

Appendix A: Electronic Imaging Theory and SPOT Cameras

Introduction

This appendix is a brief overview of the operational theory behind SPOT digital cameras. The next two sections, Digital Photography and Color Images, provide a short introduction to electronic imaging theory; the following section describes how this theory applies to the SPOT cameras.

Digital Photography

Modern electronic imaging is based on the *charged coupled device (CCD)*. All of today's digital cameras have a CCD chip or chips. In digital cameras, the CCD chip/sensor replaces the film of traditional cameras as the means by which the camera records the image. The surface of the CCD chip is composed of light-sensitive cells arranged in a checkerboard pattern. Each cell of the checkerboard is known as a picture element, or more commonly, a *pixel*. The following is a simplified description of what happens when you take a picture with a digital camera:

1. The camera's optical system forms images on the "checkerboard" of pixels.

2. The CCD is exposed to the image for a period of time.

   During this period, each photosensitive cell receives photons of light, converts the photons to electrons, and then stores the electrons in the cell. The process by which each cell accumulates electrons can be compared to a well filling with water. As more light hits a cell, the electron level in the well rises. The more electrons that are in the cell, the more voltage it will have when read by the digital camera.

3. Following the exposure, a digital camera does three things:
   a. It measures the voltage of each cell.
   b. It converts the voltage to a binary number.
   c. It transmits this number down a cable to your computer.

4. The computer reconstructs the image by assigning a brightness value to each pixel in the final image. Each brightness value is proportional to the voltage of the corresponding cell on the CCD chip.

Color Images

Because a CCD chip is inherently monochromatic (black and white), color filters must be used to extract the color information from the image. There are three basic design methodologies.

- **Single CCD design using color masked pixels (Insight Color Mosaic camera; RT KE Color Mosaic)**
  Digital cameras designed in this manner have one CCD chip with color filters physically bonded to each pixel on the chip. Some pixels get red filters, some pixels get green filters and some pixels get blue filters. Since each pixel is only able to measure the intensity of one color, the intensity of the missing two colors for this pixel must be estimated, based on nearby pixels that have precise measurements of the missing colors. For example, a pixel that has a green filter will have an exact measurement of the green color value, but the red and blue values must be estimated, based on the nearest red and blue pixels. This estimation is known as *interpolation*.

  Pros and Cons: A digital camera designed in this way is inexpensive and can freeze moving images with a single exposure. However, because they interpolate brightness values, such cameras cannot provide as high of a resolution as that provided from a non-filtered CCD chip.

- **Three CCD design using color beam splitter**
  This design uses three CCD chips. The incoming image first goes through a color beam splitter which directs the red light to one chip, the green light to a second chip and the blue light to a third chip. The chips are very accurately aligned so as to achieve near perfect registration between the three chips. The red, green, and blue values for each point on an image are measured by the corresponding pixel on each of the three chips.

  Pros and Cons: This design produces an image that retains the high resolution of the individual chips. This type of camera can also freeze moving images with a single exposure. However, because most of the cost in a high resolution CCD camera is in the CCD chip itself, three chip cameras are far more costly than their single chip counterparts.

- **Single CCD design that takes three pictures (three pass method) (RT 3-shot and Slider cameras, Enhanced Slider camera and Insight 3-Shot camera)**
  Cameras designed in this way expose a single CCD chip three times, once to red light, once to green light, and once to blue light. One variant in this type of camera is how the chip is exposed to the different colors of light. Some cameras switch individual red, green and blue glass filters in front of the chip for each exposure. Others use a liquid crystal filter that changes from red to green to blue as different voltages are applied to it. By exposing the chip three times, each cell on the CCD is able to measure all three color values.

  Pros and Cons: This technique attains the high resolution of the three CCD design without the high cost associated with using three chips. However, because these cameras require three exposures, they cannot freeze moving images.

SPOT Digital Cameras

In order to provide you with an understanding of what happens when you take a picture, the following sections highlight some of the basic operating principles of SPOT cameras.

Pixel Digitization

Following each exposure, SPOT camera systems read the CCD chip and digitizes each pixel data as it is transferred off of the CCD chip, and before it is sent through the cable into the computer. This technique minimizes noise and maintains the lack of distortion that is inherent with CCD chip geometry. The SPOT RT cameras use two methods of pixel digitization, one for live imaging, and one for image capture:

- **Live imaging (RT cameras and Insight cameras)**
  To provide the fastest possible frame rate, SPOT RT and Insight cameras use two separate circuits to digitize the left and right halves of the CCD simultaneously.
  Pros and Cons: This method provides an extremely fast display when using SPOT's Live Image feature. The challenge comes in achieving a good brightness match between the two image halves. Our current algorithm achieves a good match with bright images. However, with dim images, mismatches can occur, resulting in a "split" appearance, where one half of the image is brighter that the other half. A "split" Live Image view is **not** indicative of what your image capture will look like.

- **Image capture (all SPOT cameras)**
  To provide the most accurate image captures, SPOT cameras use a single circuit to digitize the entire CCD chip.
  Pros and Cons: Because a single circuit is used to digitize the entire CCD chip, all pixels are inherently matched. However, this method provides a slower download than the two circuit method used for live imaging.

Gain

Normally an auto exposure routine in a digital camera sets the exposure time so that the brightest pixel on the CCD chip is completely "filled" with electrons, or is "full well" (see the Digital Photography section). This simple technique works well for bright images, but, as an image gets dimmer, exposure times can get uncomfortably long.

With the SPOT camera you can shorten exposure times by filling the brightest pixel on the CCD chip to less than full well, and then using this lower level as *full scale*. The inverse fraction of full well that the brightest pixel on the CCD is filled to is known as the *gain*. For example, filling a pixel to half of full well cuts the exposure time in half. Since the exposure time is cut in half, we call this a gain of 2.

The following table illustrates the relationship between gain, the fraction of full well that the brightest pixel is filled to, and the effect of gain on exposure time:

Gain	Fraction of Full Well	Exposure Time (sample)
1	1	80 seconds
2	½	40 seconds
4	¼	20 seconds
8	1/8	10 seconds
16	1/16	5 seconds

An 80 second exposure at a gain of one turns into a 5 second exposure at gain 16. This is good in terms of the exposure time. Unfortunately, background noise is directly proportional to gain. It is 16 times higher at a gain of 16 is than at a gain of one.

An Automated Exposure Process

Normally, for any given image, trial and error would have to be used to determine the optimal combinations of gain and exposure time. In order to eliminate the need for trial and error experimentation, the SPOT camera automatically determines this information by sampling light levels before each exposure. Based on the light levels and the user-determined Auto-Gain Limit, SPOT determines the optimal gain setting and exposure time for the image. For details on using the Auto-Gain Limit feature, refer to Ch. 5 – Preparing to Take a Picture.

Appendix B: Specifications

RT-KE Series Cameras

Camera and Filter
RT-KE B/W – Monochrome with and w/o IR filter
RT-KE Color Mosaic – Color mosaic CCD
RT-KE 3-Shot Color – Fixed liquid crystal color filter
RT-KE Slider – Slide mounted liquid crystal color filter

CCD Information
RT-KE B/W – KAI 2001M Monochrome
RT-KE Color Mosaic– KAI 2001CM
RT-KE 3-Shot Color– KAI 2001M Monochrome
RT-KE Slider – KAI 2001M Monochrome

Imaging Area – 11.8mm x 8.9mm
Active Pixels – 1600 x 1200 (1.92 million)
7.4 µm square pixels
Full well charge capacity - 40,000e

RT-KE Color Mosaic Image Resolution
Live image – 800 x 600
Captured image – 1600 x1200

RT-KE 3-Shot Color Image Resolution
Live image – 1600 x 1200
Captured image – 1600 x1200

RT-KE B/W Image Resolution
Live image – 1600 x 1200
Captured image – 1600 x1200

Bit Depth – RT-KE Color Mosaic, RT KE 3-Shot Color
Sampled at 36 bit RGB
Saved file formats: 24 bit, 36 bit, or 48 bit (RGB)

Bit Depth – RT-KE B/W
Sampled at 12 bit monochrome
Saved file formats: 8 bit, 12 bit, or 16 bit (mono)

Cooling
-46 C from ambient via thermoelectric cooler

Pixel Readout Rate
Live images – 36 Mhz (2 channel x 18 Mhz)
Captured images – 6 Mhz (1 channel x 6 Mhz)

A/D Converter Full Scale Setting
31,500e (when gain = 1)

Anti – Blooming Factor
300 times (nominal); 100 times (minimum)

Exposure
Automatic and User-Defined
Range – 0.04 ms – 536 seconds

External Shutter Control
BNC, TTL level output

Weight

RT-SE Series Cameras

Camera and Filter
RT-SE 6 – Monochrome with and w/o IR filter
RT-SE 6 Slider – Slide mounted liquid crystal color filter
RT-SE 18 – Monochrome with and w/o IR filter

CCD Information
RT-SE6 B/W – ICX-285AL Monochrome
RT-SE6 Slider– ICX-285AL Monochrome
RT-SE18 B/W– ICX-285AL Monochrome

Imaging Area – 8.77mm x 6.60mm
Active Pixels – 1360 x 1024 (1.92 million)
6.45 µm square pixels

RT-SE6 Slider Image Resolution
Live image – 1600 x 1200
Captured image – 1600 x1200

RT-SE6 B/W, RT-SE18 B/W Image Resolution
Live image – 1600 x 1200
Captured image – 1600 x1200

Bit Depth – RT-SE6 Slider
Sampled at 36 bit RGB
Saved file formats: 24 bit, 36 bit, or 48 bit (RGB)

Bit Depth – RT-SE6 B/W
Sampled at 12 bit monochrome
Saved file formats: 8 bit, 12 bit, or 16 bit (mono)

Bit Depth – RT-SE18 B/W
Sampled at 10 bit monochrome
Saved file formats: 8 bit, 12 bit, or 16 bit (mono)

Cooling
-48 C from ambient via thermoelectric cooler

Pixel Readout Rate- RT-SE6 Slider, RT-SE6 B/W
Live images – 18 Mhz (1 channel x 18 Mhz)
Captured images – 6 Mhz (1 channel x 6 Mhz)

Pixel Readout Rate- RT-SE18 B/W
Live images – 18 Mhz (1 channel x 18 Mhz)
Captured images – 18 Mhz (1 channel x 6 Mhz)

A/D Converter Full Scale Setting
14,500e (when gain = 1)

Anti – Blooming Factor
300 times (nominal); 100 times (minimum)

Exposure
Automatic and User-Defined
Range – 0.04 ms – 536 seconds

External Shutter Control
BNC, TTL level output

Weight
RT-SE 6 Slider - Camera – 3.5 lbs., Power supply - 3.3 lbs.
RT-SE6 B/W, RT-SE18 - Camera - 1.4 lbs., Power supply –

RT-KE , Monochrome and Color Mosaic - Camera –
3.4 lbs., Power supply – 3.3 lbs.
RT-KE, 3-Shot, RT-KE Slider - Camera – 3.5 lbs.,
Power supply – 3.3 lbs.

Power Requirements
100 –240 VAC, 47 – 63 Hz

Certifications
CE, FCC, Class A, EN 60950

3.3lbs.

Power Requirements
100 –240 VAC, 47 – 63 Hz

Certifications
CE, FCC, Class A, EN 60950

Minimum Computer Requirements

PC* - Pentium 400 MHz, 128MB RAM, PCI interface, Windows 95/98/NT/2000/ME/XP

Mac* – Power PC 400 MHz G3, 256 MB RAM, PCI interface, Mac OS 10.1 or greater (Mac G5 computers do not support the PCI card)

Video card – Support of 24 bit RGB at the desired resolution.

Software Features
- Live image window
- Image capture window
- Pre-defined image types
- Auto exposure
- Auto white balance
- Bias Frame, Thermal Frame and Flatfield correction
- Zoom and pan window
- Image enhancement tools
- Annotation and Calibration mark
- Measurement – length, perimeter area, angle
- Print layout dialog
- On-line help menu
- Macro Scripting
- "Floating" Taskbar
- Image archiving database with export feature
- Report generator
- Sequential image capture and playback

Image File Formats
TIFF, TIFF-JPEG, JPEG, BMP, PICT, AVI, JPEG2000

File Size

RT-KE 3-Shot Color, Slider, Color Mosaic, B/W	RT-SE6 Slider, B/W, RT-SE18 B/W
24 bit - 5.49 MB/8 bit - 1.83 MB	24 bit – 3.99 MB/8 bit - 1.33 MB
36 bit - 8.24 MB/12 bit - 2.75 MB	36 bit – 6.00 MB/12 bit - 2.00 MB
48 bit – 10.99 MB/16 bit - 3.66 MB	48 bit – 7.98 MB/16 bit - 2.66 MB

Drivers
TWAIN for Windows supported Windows® Operating Systems;
AppleEvent for supported Mac® Operating Systems

Third Party Software
See Appendix E.

Couplers
RT-KE Monochrome, RT-KE Monochrome w/IR, RT-KE Color Mosaic: D10XXC (1.0x), HRU060-CMT (0.60x) and bottom clamp, HRD100-CMT (1.0x) and bottom clamp, all available from Diagnostic Instruments

RT-KE 3-Shot Color, RT-KE Slider: D10XXF (1.0x), HRU060-NIK (0.60x) and bottom clamp, HRD100-NIK (1.0x) and bottom clamp; all available from Diagnostic Instruments

RT-SE6 Monochrome, RT-SE6 Monochrome w/IR, RT-SE18 Monochrome, RT-SE18 Monochrome w/IR: D10XXC (1.0x), HRD076-CMT (0.76 x) and bottom clamp, all available from Diagnostic Instruments

RT-SE6 Slider: D10XXF (1.0x), HRD076-NIK (0.76x) and bottom clamp; all available from Diagnostic Instruments
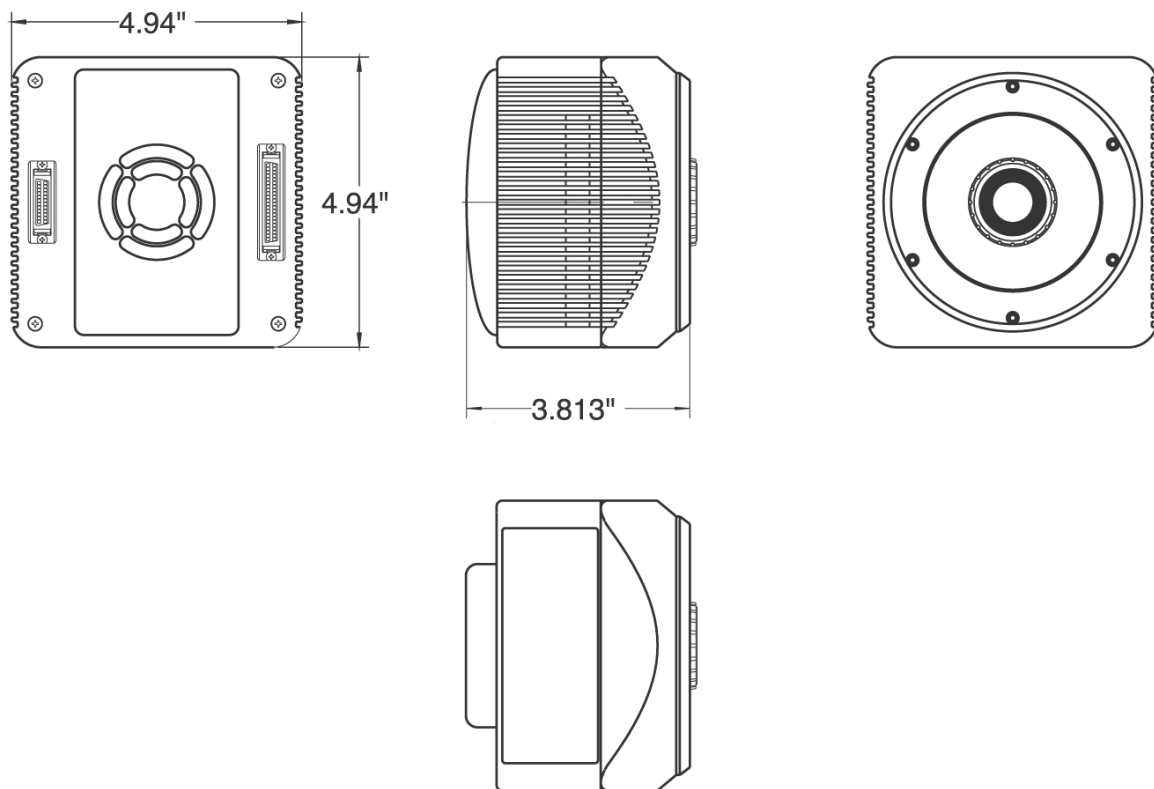
Lens & Mounting
RT Monochrome, Mono w/IR, Color Mosaic - C- Mount lens for 1" format CCD, Flange to rear lens clearance - 0.46" (11.7 mm) max.
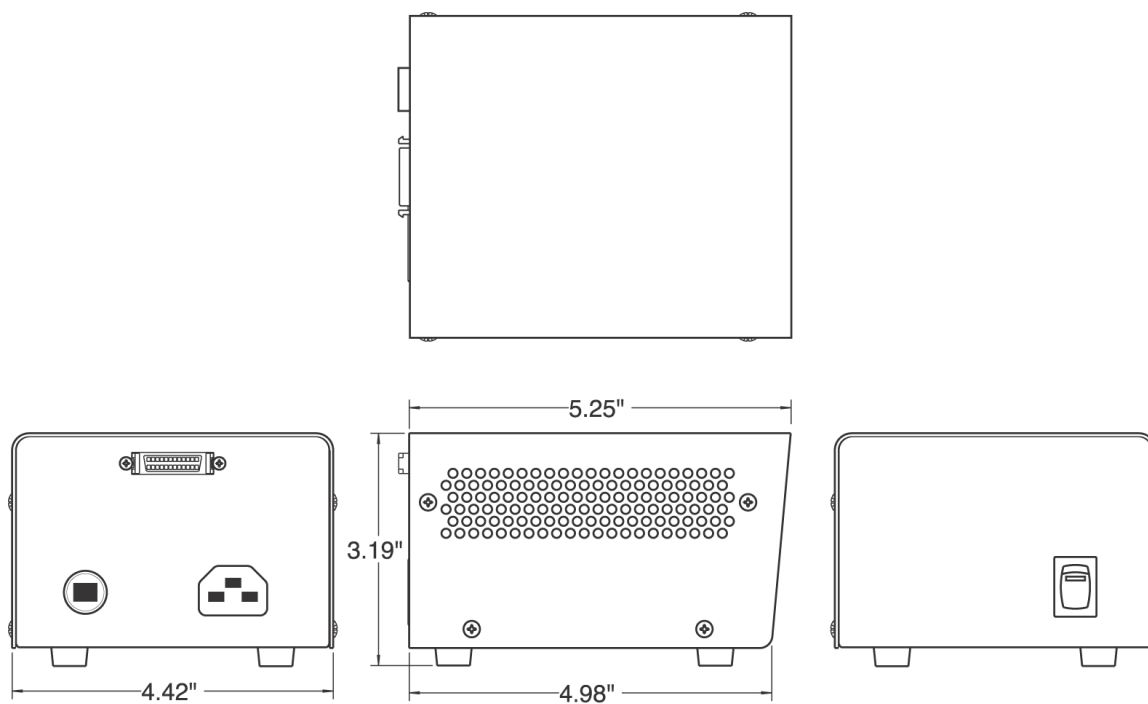
RT 3-Shot Color, RT Slider - F- Mount lens for 1" format CCD, Flange to rear lens clearance - 0.43" (10.8 mm) max.

Camera Dimensions

5.51"   .285"   4.94"   4.96"

4.94"   4.94"   4.933"
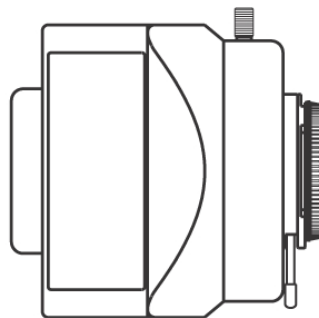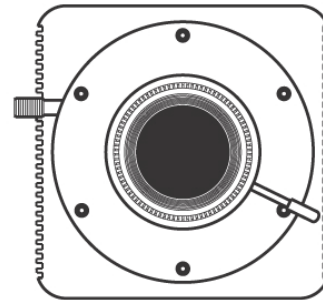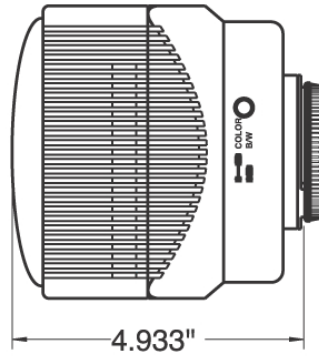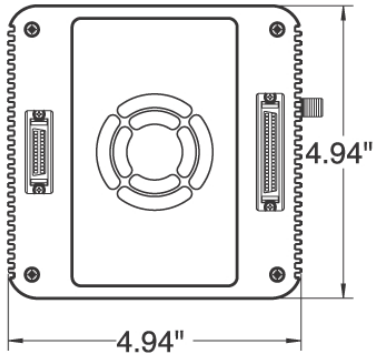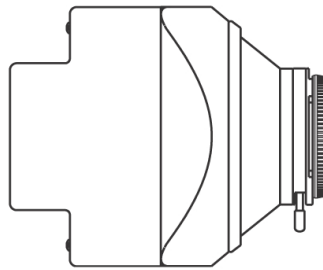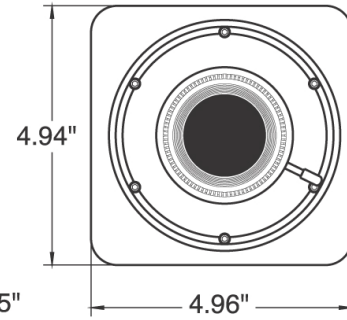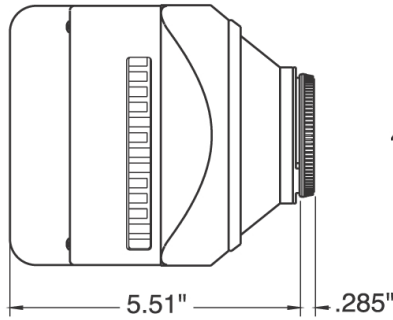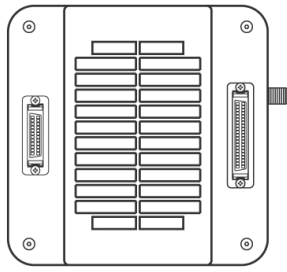
Power Supply Dimensions

RT Series Catalog Information

There are ten cameras in the SPOT RT series of cameras. The following table indicates each catalog number with the standard configuration:

Catalog Part Numbers	Description	Connectivity
RT700	SPOT RT-KE Monochrome "C" mount camera	PCI
RT710	SPOT RT-KE Monochrome "C" mount camera *with IR filter*	PCI
RT720	SPOT RT-KE Color Mosaic "C" mount camera	PCI
RT730	SPOT RT-KE 3-shot Color "F" mount camera	PCI
RT740	SPOT RT-KE Slider "F" mount camera	PCI
RT900	SPOT RT-SE6 Monochrome "C" mount camera	PCI
RT910	SPOT RT-SE6 Monochrome "C" mount camera *with IR filter*	PCI
RT940	SPOT RT-SE6 Slider "F" mount camera	PCI
RT1200	SPOT RT-SE18 Monochrome "C" mount camera	PCI
RT1210	SPOT RT-SE18 Monochrome "C" mount camera *with IR filter*	PCI

FCC "Class A" Product Warning

All SPOT cameras fall into the Class A category of the FCC (Federal Communications Commission) emission standard EN55022/CISPR22. The following is the FCC's standard warning for this type of device:

WARNING:
This is a Class A Product. In a domestic environment this product may cause radio interference in which case the user may be required to take adequate measures.

Appendix C: Keyboard and Toolbar Button Commands

Command	Keystroke or F-key	Toolbar Button	Basic and/or Adv. Mode	Windows and/or Mac
File Menu				
Open Image File	[F3]		Both	Both
Show Image File Thumbnails	[F5]		Both	Both
Run Report	[F6]		Advanced	Window s
Reload			Advanced	Windows
Save	[F2]		Both	Both
Save As	[Ctrl] + [A]		Both	Windows
Save to Database	[Ctrl] + [D]		Advanced	Windows
Save All	[Ctrl] + [F3]		Both	Windows
Close	[Ctrl] + [F4] ⌘ + W		Both	Both
Print	[Ctrl] + [P] ⌘ + P		Both	Both
Exit Quit	[Alt] + [F4] ⌘ + Q		Both	Both
Database menu				
Search			Advanced	Windows
Edit menu				
Undo	[Alt] + Backspace ⌘ + Z		Both	Both
Copy to Clipboard	[Ctrl] + [C] ⌘ + C		Advanced	Both
Add Measurement	[Alt] + [M] ⌘ + M		Advanced	Both

Command	Keystroke or F-key	Toolbar Button	Basic and/or Adv. Mode	Windows and/or Mac
Sequence menu				
Play Forward	[Ctrl] + [Space] ⌘ + [Space]		Advanced	Both
Play Reverse	[Ctrl] + [Backspace] ⌘ + [Backspace]		Advanced	Both
Stop Play/Resume Play	[Enter]		Advanced	Both
Step Forward	[Space]		Advanced	Both
Step Back	[Backspace]		Advanced	Both
View menu				
Fit to Window	[Ctrl] + [F] ⌘ + F		Both	Both
Zoom	[Ctrl] + [Z]		Both	Both (Macs – button only)
Show Gridlines	[Ctrl] + [G] ⌘ + M		Both	Both
Show Pan Window	[Ctrl] + [W]		Both	Both (Macs – button only)
Image Date/Time	[Ctrl] + [T] ⌘ + T		Both	Both
Full Screen	[F 7]		Both	Both
Show Image Settings			Basic	Both
Camera menu				
Live Image	[F11]		Both	Both
Get Image (exposure from setup)	[F9]		Both	Both
Get Image (last used exposure)	[F8]		Advanced	Both
Compute Exposure	[F10]		Advanced	Windows
Compute White Balance Values	[Ctrl] + [F10]		Both	Both
Non-menu driven commands				
Access the Image Setup window	[F12] [Ctrl] + [F 12] (OSX 10.1 and higher)		Advanced	Both

User Guide to the SPOT RT Camera

Appendix D: Converting Previous Version SPOT Databases

If you have SPOT databases that were created with an earlier version of the SPOT software (2.1 or earlier), these databases must be converted for use with the current version of the software (4.0). Follow these steps:

1. Access the files on the software CD that came with your camera. In addition to the documentation and program folders, you should see a Utilities folder.

2. Open the Utilities folder.

3. Double click on the **convdb.exe** icon. The program automatically converts your databases.

Appendix E: Third Party Software

The following table lists major third party imaging software systems and their compatibility levels with the SPOT Enhanced, SPOT RT, and SPOT Insight FireWire cameras.

Company	Software	System	Drivers are offered by the 3rd Party software manufacturer			Drivers are included with the SPOT software	
			Enhanced (1402ME & 0402ME)	RT (RTKE and RTSE)	Insight FireWire	TWAIN (Windows)	3rd Party Interface (Mac)
Adobe	Photoshop	PC/Mac	NO	NO	NO	YES	YES
Cerner	CoPathPlus	PC	NO	NO	NO	YES	NO
Clemex	Vision	PC	NO	YES	YES	YES	NO
Compix	C-Imaging	PC	YES	YES	NO	YES	N/A
Delta Sistemi	Biomed	PC	YES	YES	YES	YES	NO
Empix	Northern Eclipse	PC	YES	NO	NO	YES	N/A
IATIA	QPM	PC	NO	YES	YES	NO	NO
Improvision	Open Lab	Mac	YES	YES	YES	N/A	YES
Intelligent Imaging Innovations	3i	PC	YES	YES	NO	YES	NO
Media Cybernetics	Image-Pro (4.x or higher)	PC	YES	YES	YES	YES	N/A
Midwest Info. Systems	Paxit	PC	NO	NO	PENDING	YES	NO
NIH - Public Domain	NIH Image	PC/Mac	NO	NO	NO	NO	YES
Novovision	NovoPath	PC/Mac	NO	YES	YES	YES	YES
QED	Camera Plugin	PC/Mac	NO	YES	YES	NO	YES
Scanalytics	IP Labs	PC/Mac	YES	YES	YES	YES	YES
Soft Imaging System	AnalySIS	PC	NO	YES	YES	NO	NO
Universal Imaging	Metamorph	PC	YES	YES	YES	YES	NO

Appendix F: Enhanced Cameras, RT Cameras, and Polarized Light Microscopy

In polarized light microscopy, there are two polarizers in the microscope's light path. Because the color filter on Enhanced and RT cameras also uses a polarizer, the camera must have the correct rotational orientation in order to accurately render colors in image captures.

To set up a Enhanced or RT camera for use with a polarized light microscope, follow these steps:

1. Focus on the specimen and adjust the microscope's polarizers as needed.

2. Using a hex key, loosen the connection between the microscope coupler and its bottom clamp. It should be loosened enough so that a slight force is needed to rotate the camera. After you have loosened the connection, leave the hex key in the camera head so that you can quickly lock in the position.

3. From the SPOT program, either open the **Live Image** window (RT cameras) or click on the **Focus** button (Enhanced cameras). If you are using a Enhanced camera, ensure that the Focus speed is set to **Fast**.

4. Slowly rotate the camera until the window goes black. This is the extinction point.

5. Slowly rotate the camera back until the image reappears. Continue rotating the camera until the image reaches its brightest point (the highest quality image).

6. Rotate the camera halfway back toward the extinction point position.

7. Lock in this position (i.e., use the hex key to tighten the connection).

8. Capture the image.

The captured image serves as a reference point for tweaking the final position. You can use additional, small rotational adjustments to achieve a good match between the colors in the captured image and those seen through the microscope's eyepieces. More than one attempt may be necessary to find the optimal position.