User Guide to the SPOT Vision Camera

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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.

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LIMITED WARRANTY: Diagnostic Instruments, Inc. (henceforth referred to as Diagnostic) warrants to the original purchaser, that the camera will be free of defects in material and workmanship for a period of one year from the date of purchase. Diagnostic shall, at its choice, either repair or replace, free of charge, any product that, upon inspection by Diagnostic, is found to be defective. Abuse, misuse, or improper maintenance and/or repair during the one year period invalidates this warranty. This warranty does not cover defects caused by ordinary wear and tear.

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About This Manual

Introduction

The manual contains instructions for the SPOT Vision cameras (Color and B/W) and version 3.5 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**
Vision B/W	***Vision B/W*** Vision B/W notes indicate specific instructions for users with the monochrome version of the SPOT Vision camera. In most cases, these notes indicate that a color related option is unavailable.
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 Vision Camera* is structured as shown in the table below.

Chapter	Description
About This Manual	Describes document conventions, manual structure.
Installation Guidelines	Describes hardware/software installation.
Quick Start	Steps for immediately capturing your first image.
Ch. 1 – Navigating the Program	Details the program environment.
Ch. 2 – Preparing to Take a Picture	Describes the different procedures involved in setting up the software for image captures.
Ch. 3 – Taking a Picture	Describes the image capture process.
Ch. 4 – Viewing, Editing, and Annotating Images	Describes the image editing and annotation features in the software.
Ch. 5 – Reporting	Describes the reporting functionality in the software.
Ch. 6 – Printing Images	Describes the procedures for printing images from the SPOT program.
Ch. 7 – Using Other Windows Imaging Applications	Describes how to capture images (via a TWAIN interface) with the SPOT camera using other imaging programs such as Photoshop or Image-Pro Plus.
Ch. 8 – Using Other Mac Imaging Applications	Describes how to capture images (via a 3rd Party Interface) with the SPOT camera using other imaging programs such as Photoshop or Image-Pro Plus.
Appendix A: Electronic Imaging Theory and SPOT Cameras	An introduction to the theory behind SPOT cameras.
Appendix B: Specifications	Technical specifications for the SPOT hardware and software.
Appendix C: Keyboard and Toolbar Button Commands	A table of menu commands and their keystroke and button equivalents.
Appendix D: Selected Error Messages	A table of errors, causes, and solutions

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. 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' web site – **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 to do so and obtain the user name and password.

4.   Click **OK**.

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Installation Guidelines

QUICK REFERENCE:

- ➲ Compare the minimal and optimal hardware requirements for using the SPOT Vision camera… pp. x – xiv

- ➲ Set up an Epson printer for use with the SPOT software… p. xiv

- ➲ Adjust the video card settings in Windows…p. xv

- ➲ Install the PCI board… p. xviii

- ➲ Connect the SPOT Vision camera to your microscope… p. xix

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

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

Introduction

The following sections detail the system and installation guidelines for using the SPOT Vision camera and v 3.5 of the SPOT software. They are as follows:

- System Requirements
- What is in the Box
- Installing the Software
- Installing the Hardware
- Using SPOT's On-line Documentation

System Requirements

The following sections details the hardware and software requirements needed for the SPOT Vision camera/software. Minimum and optimal system recommendations are detailed for each item.

Equipment Standards - Windows

	Minimum	**Optimal System Recommendations**
Processor	Pentium, 166 MHz	***Dell Dimension 4100*** with 1 GHz Pentium III Processor In addition to the fact that this is a fast and reliable machine, there are many good reasons to order from Dell, including: • Easy to use web site • On-line ordering and product selection • One week shipping time • Excellent technical support
Video Card	A card that supports 24 bit, true color at the desired resolution with a refresh rate above 70 Hz.	***32 Mb Nvidia TNT2 M64 AGP Graphics Card*** This is the standard card offered on the aforementioned Dell Dimension 4100. For customers who want to videotape their live image output, Diagnostic Instruments recommends the following cards: • **Matrox G400 Dual Head video card** • **ATI Radeon 64 Mb VIVO card** Both of these cards have video outputs for connection to a standard VCR. All of the above cards offer refresh rates of 85 MHz or more at 1600 x 1200 resolution. All of these cards provide displays with sharp resolution, superb color quality, and clean, artifact-free images.

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	64 Mb	***256 Mb*** of RAM is recommended for optimal smoothness of operation.
PCI Slot		
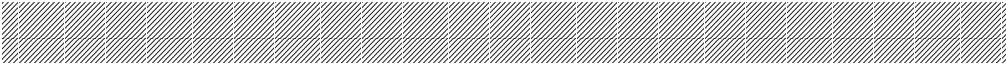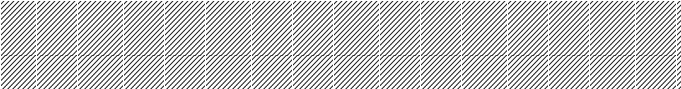
Equipment Standards – Windows (cont.)

	Minimum	Optimal System Recommendations
Monitor	Any color monitor with at least 800 x 600 pixel resolution at 24 bit RGB, and a refresh rate over 70 Hz.	*Hitachi CM 815 Plus* This is Hitachi's latest 21" monitor, the replacement for the 814. It has perfect focus from the center to the corners of the screen, very fine resolution, and consistent color rendition. A very bright monitor, the 815 can display brilliant images even in high ambient light environments. It is also compact by comparison to other 21" monitors.
Operating System	Windows 95/98/NT/ 2000/ME/XP	*Windows XP* Microsoft intends to replace all versions of the Windows operating system with Windows XP. Previous versions of the Spot software experienced significantly slower Live image frame rates when operating under Windows NT/2000 than when operating under Windows 95/98/ME. This situation has been rectified. With v3.5 of the Spot software, live image frame rates are equally fast with ALL versions of Windows, including XP.
Printer		*Epson Stylus Photo 750* This printer produces high resolution, true color, 8.5 x 11 glossy prints that look better than those produced by many dye sub printers. It is perfect for printing reports that contain a mixture of images and text. It sells for an incredible $298. Refer to the "Epson Printer Settings" section below for details on how to optimize print quality.

Additional Hardware and Software

Web Browser

Internet Explorer 4.0 or greater.

This requirement applies only to Windows 95 users. Current releases of Internet Explorer are available as free downloads from **www.microsoft.com/downloads.**

Coupler

A digital camera coupler designed to fit a camera with a C mount.

Diagnostic Instruments makes couplers for most microscopes. They are available from the dealer where the camera was purchased. Diagnostic Instruments suggest the **D10XXC – 1x C-Mount Coupler**, which works with most microscopes. If the D10 series will not work with your microscope, Diagnostic Instruments suggests the following alternatives:

- **HRU060-CMT coupler (0.60x)** and the appropriate bottom clamp.

- **HRD100-CMT coupler (1x)** and the appropriate bottom clamp.

Lens

C- Mount lens for 1" format CCD

Light Source

Because the SPOT Vision 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 is problematic, you can use the Minimum Exposure option on the Image Settings window to avoid flicker and give you correct brightness and color balance readings. Refer to Ch. 2 – Preparing to Take a Picture (see – Exposure: Auto or Manual > More (additional exposure and orientation settings) for more information.

Equipment Standards - Mac

|	**Minimum**	**Optimal System Recommendations**
Processor	PowerPC computer with a PCI bus	***G4 with a Hitachi CM815 Plus monitor*** (Note that the Apple 21" Studio Display has been discontinued) Note that the G4 and "Blue and White" G3 computers have new 64 bit PCI bus connectors that are larger than the standard 32 bit PCI edge card connector used on the SPOT PCI bus plug-in card. Despite the apparent size discrepancy, the SPOT cards work when plugged in – the small slot on the SPOT edge connector lines up with the bar on the PCI bus connector.
✏️	*Note: Apple Power Macintosh models 6100, 7100, and 8100 are PowerPCs, but they do not have a PCI bus, and thus cannot be used with the SPOT camera. The iMac does not have a PCI bus and cannot be used.*	
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	The ***ATI Rage 128*** plug-in card that is standard on G4 and "Blue and White" G3 computers works well. The ***ATI Radeon*** card is recommended for 19" and larger monitors used with Macs other than the G4 and "Blue and White" G3 models.
RAM	64 Mb	***256 Mb*** of RAM is recommended for optimal smoothness of operation.
PCI Slot	/////////////////////////////////	/////////////////////////////////
Monitor	Any color monitor with at least 800 x 600 pixel resolution at 24 bit RGB, and a refresh rate over 70 Hz.	***Hitachi CM 815 Plus*** This is Hitachi's latest 21" monitor, the replacement for the 814. It has perfect focus from the center to the corners of the screen, very fine resolution, and consistent color rendition. A very bright monitor, the 815 can display brilliant images even in high ambient light environments. It is also compact by comparison to other 21" monitors. Note that the 21" Apple Studio Display has been discontinued.
Operating System	Mac OS 8.6 through OSX	/////////////////////////////////
Printer		***Epson Stylus Photo 750*** This printer produces high resolution, true color, 8.5 x 11 glossy prints that look better than those produced by many dye sub printers. It is perfect for printing reports that contain a mixture of images and text. It sells for an incredible $298.

Equipment Standards – Mac (cont.)

Additional Hardware and Software

Coupler   A digital camera coupler designed to fit a camera with a C mount.

Diagnostic Instruments makes couplers for most microscopes. They are available from the dealer where the camera was purchased. Diagnostic Instruments suggest the **D10XXC – 1x C-Mount Coupler**, which works with most microscopes. If the D10 series will not work with your microscope, Diagnostic Instruments suggests the following alternatives:

- **HRU060-CMT coupler (0.60x)** and the appropriate bottom clamp.

- **HRD100-CMT coupler (1x)** and the appropriate bottom clamp.

Lens   C- Mount lens for 1" format CCD

Light Source   Because the SPOT Vision 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 is problematic, you can use the Minimum Exposure option on the Image Settings window to avoid flicker and give you correct brightness and color balance readings. Refer to Ch. 2 – Preparing to Take a Picture [see **Exposure: Auto or Manual > More (additional exposure and orientation settings)**] for more information.

Epson Printer Settings (Windows)

To optimize print quality when printing images with an Epson Photo Stylus 750 printer on a PC running Windows 98, do the following:

- Use photo quality, glossy paper.

- Set the Windows printer preferences as follows (after you have installed the printer):

  a) From the Start menu, select **Settings > Printers**. The Printers window displays all of the printers that are available to you.

  b) Right click on the Epson Stylus Photo 750 icon, and select the **Properties** option. The properties window for the printer appears.

  c) Click on the **Main** tab. The properties window should now appear as shown at right.

  d) Set the **Mode** to **Advanced** (i.e., click on the Advanced button).

  e) Click on **More Settings**, and set the following parameters:

    ♦ Print Quality – *Super Fine 1440 dpi*
    ♦ Media Type – *Photo Paper*
    ♦ Ink – *Color*
    ♦ MicroWeave – Ensure that Super is *not* checked.
    ♦ Flip Horizontal – Ensure that this option is *not* checked.
    ♦ Color - *ICM*

  f) Click on OK. The **Current Settings** under the **Main** tab on the properties window should appear as you defined them.

  g) Click on **Apply** and then click on **OK** to close the window.
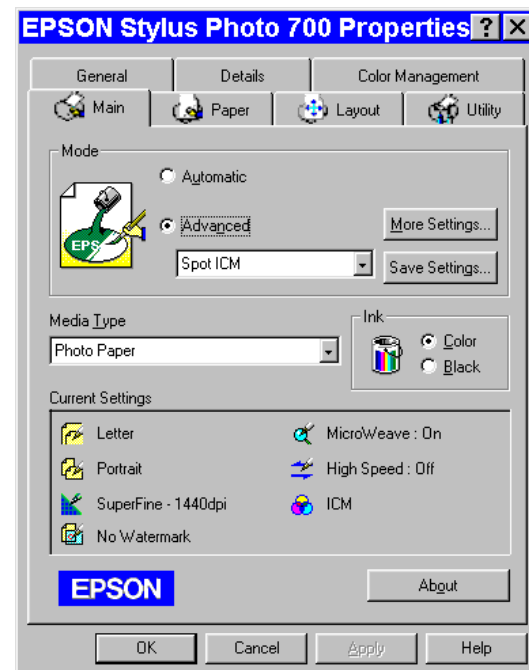
What is in the Box

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
- 50 pin data 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 Vision Camera.
  – The Adobe Acrobat Reader software that is required to read and/or print the user's manual. Refer to "Using SPOT's On-line Documentation" below for instructions on installing the Reader on your computer.

- A registration card
- 1 year limited warranty

Installing the Software

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

- Windows 95/98/NT/2000/ME/XP
- Mac OS 8.6 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.*

Windows Installation

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

2. If it is open, close the MS Office 95/97/2000 Tool Bar.

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

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

5. 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**.

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

7. 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?**

8. Select **Yes**. The installation program runs.

9. When the installation is complete, shut down your computer, and install the plug-in card, as described in the **Installing the Hardware** section of this chapter. At this point, if you plan to use multiple SPOT cameras with the computer, you can install all of the PCI cards.

10. Re-start your computer. Following the restart you should see a shortcut to the SPOT program on your desktop.

Video Card Related Problems (Windows)

Diagnostic Instruments has found that the certain video cards can cause the SPOT program to crash when using the Live Image feature. 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**.

10. Restart your computer before using the SPOT program.

Windows 95 Related Errors

If, after installing the PCI board you cannot access Windows 95, or if the camera will not communicate with the PC, one of the following situations might be the cause:

•   The slot that you selected for the SPOT PCI board might have been occupied (previously) by a board from another device.

•   The PCI slot s in the shared position (the PCI slot immediately adjacent to an ISA slot).

These are areas that Diagnostics Instruments has found to be problematic on *some* installations. This is due to the fact that Windows 95 is not fully plug and play compliant. To resolve the problem, try plugging the board into another PCI slot.

Windows NT Related Errors

If, when using Windows NT, your computer freezes during an image capture process (i.e., Get Image, Live Image viewing. Compute Exposure, etc…), it is may be due to a problem with a setting in your computer's BIOS.   To correct this problem, follow these steps:

1. Access the BIOS settings. The method for accessing BIOS settings varies from computer to computer. If you are unsure of how to do this, contact your MIS department or an IT professional.

2. Go to the **Advanced Setup** area. This is the typical location, but it can vary according to the make of your computer.

3. Access the **PCI Bus Mastering** setting. The heading that you will see depends on the make of your computer.

4. Change the setting to **ENABLED.**

5. Restart the computer.

♠WARNING♠

DO NOT MODIFY OTHER BIOS SETTINGS. ALTERING OTHER SETTINGS COULD CAUSE SYSTEM INSTABILITY OR RENDER THE SYSTEM UNUSABLE.

Mac Installation

The CD-R disk provided with your camera contains the SPOT software, which can be used with Mac OS 8.6 or higher. *If you are installing the SPOT software on a system with **Mac OSX**, 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. The SPOT window appears.

2. Double click on the installer icon.

3. Click on **Continue**. The Spot 3.5 Installer window appears.

4. Select the installation location for the program.

5. Click on **Install**. The Installing progress indicator appears. When the installation is complete, you will be prompted with one of two messages, depending on the version of the Mac OS that you are using:

    –   *If you are using Mac OS 8.6 – 9.x*, the installation is complete. The program should indicate that the installation was successful. Quit the installer program, and restart the computer.

    –   *If you are using Mac OSX*, the **Apple Package Installer** program, which installs the device driver for the SPOT camera, launches and immediately prompts you to enter an **Administrator** password, as shown at right.
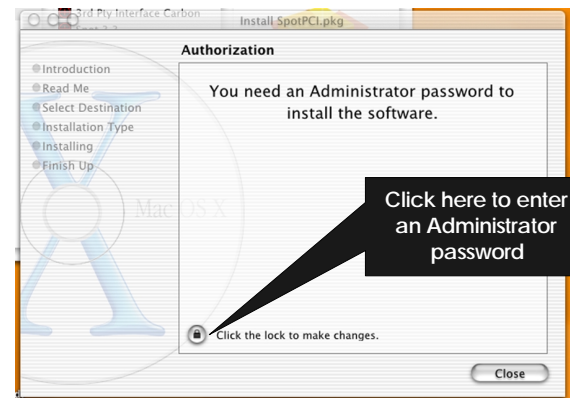
♦☀WARNING♦☀

DO NOT CLICK ON THE CLOSE BUTTON. IF YOU DO, THE DEVICE DRIVER WILL NOT INSTALL, AND YOU WILL NOT BE ABLE TO OPERATE THE CAMERA.

6. Enter an administrator password. To enter the password, click on the lock icon. ("**Click on the lock to make changes**"). After you have entered a password, the SPOTPCI.pkg welcome screen appears.

7. Click on **Continue**. An informational screen appears.

8. Click on **Continue**. The program prompts you to select an installation destination, as shown at right.

9. Select the **Mac OSX** boot drive as the installation destination. The driver must be installed in this location.

10. Click on **Continue**.

11. When the installation is complete, restart the computer.
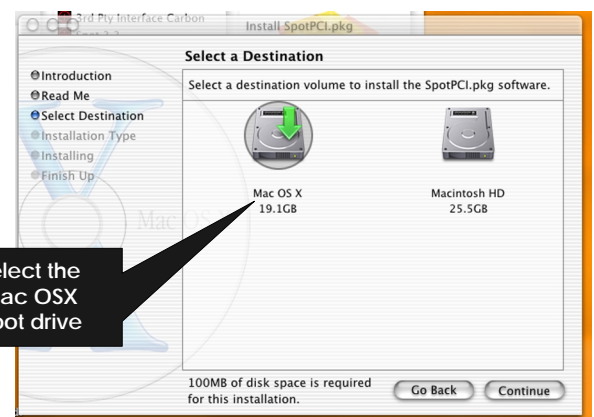
Mac OS 8.6 - 9.0 Installation Problems

If you are having difficulty installing the SPOT software with OS 8.6 – 9.0 systems, you will need to download and install the latest CarbonLib Apple Extension. This is a standard extension included with the Mac OS, and is offered as a free download/upgrade from the Apple support website – www.apple.com/support. After upgrading your CarbonLib extension you should be able to install the SPOT software.

Installing the Hardware

There are two main steps to installing the hardware:

- Installing the PCI board
- Connecting the camera to the microscope

✎ *Note: Although the SPOT software can be installed either before or after installing the PCI card, 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 Board

To install the PCI 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 mother board 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.

    – **If you have Windows 95**, the computer should initially indicate that it has found a new device. The wording of the messages varies according to which version of Windows 95 you are using.

    – Windows 95 then prompts you to reboot the computer to complete the installation. Click on **Yes** to reboot your computer.

    – **If you have Windows NT**, you will not see any messages of this type because NT automatically updates its registry with the new information.

Connecting the Camera to the Microscope

After you have installed the PCI board, you can connect the SPOT camera to your microscope. All models of the **SPOT Vision** camera require a C Mount coupler. C-mount versions of Diagnostic Instruments' **D10 (1x)** series couplers work with most microscopes. If your microscope will not work with a D10 series coupler, the following options are available:

- **HRU060-CMT coupler (0.60x)** and the appropriate bottom clamp.

- **HRD100-CMT coupler (1x)** and the appropriate bottom clamp.

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

1. Attach the bottom clamp to the coupler.

2. Screw the Vision 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.

5. Connect the camera to your computer as follows:

☛ WARNING ☛

MAKE SURE THAT BOTH THE COMPUTER AND THE SPOT POWER SUPPLY ARE TURNED OFF BEFORE CONNECTING THE CABLES. FAILURE TO DO SO CAN RESULT IN DAMAGE TO ELECTRONIC COMPONENTS IN THE CAMERA.

    a) Attach one end of the of the 50 pin (10 ft.) cable to the PCI board on the back of the computer, and attach the other end to the camera head.

☛ WARNING ☛

THE 50 PIN CABLE USES THE SAME CONNECTOR AS A PC SCSI PORT. PLUGGING THE CABLE INTO A SCSI PORT WILL DAMAGE BOTH YOUR CAMERA AND SCSI CARD.

    b) Attach the power supply/cable to the camera head.

    c) 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.   The process for installing multiple cameras is simple and applies to both Windows and Mac users. Any combination of SPOT, RT, Insight or Vision cameras can be used.

To install multiple cameras on one machine, follow these steps:

1. Install the software, as specified in **Installing the Software** (see above).

2.   Shut down the computer and install ALL of the PCI cards for the cameras that you want to connect to the computer, as described in **Installing the Hardware** (see above).

3. Re-start the computer. When you open the SPOT program, you are prompted to select the camera that you want to use.

Using SPOT's 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.

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

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 Program .**
   From the desktop click on the SPOT 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 to **Move to Clear Area of Slide**.

b) Move the slide so that the light is going through a clear part of the slide.

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
- **Gamma** – 1.00
- **Quality/Speed** – Quality (left-most position)
- **Chip Imaging Area** – Full Chip
- **Live Image Auto Brightness** - Selected
- **Flatfield** – unselected
- **Color Enhancement** – selected

More Image Settings:
- **Image Flip** – as needed
- **Rotate 90°** – as needed
- **Minimum Exposure** – unselected
- **Live Image Maximum** – default (10 sec)
- **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. 2 – Preparing to Take a Picture (see – **Profile**).
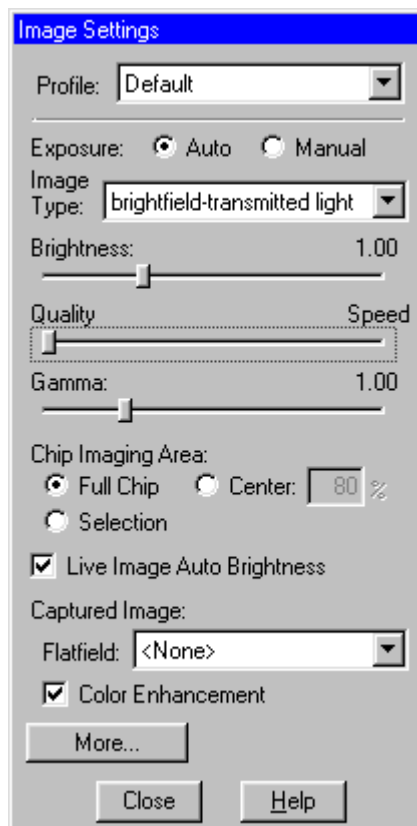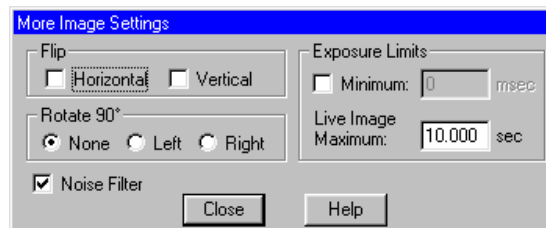
6. Click on the Get Image toolbar button.
The Get Image dialog box displays the status of the capture as it is downloaded to your screen.

Because Get Image uses the values from the live image view, the image capture skips the initial exposure computation sequence.

Ch. 1 – Navigating the Program

QUICK REFERENCE:

➲ Display thumbnails of selected images…p. 26

➲   Create an sequence of images… p. 27

➲ Define a calibration setup… p. 28

➲ Define program preferences – pp. 29 –30

Introduction

The following sections are an introduction to the SPOT Vision's 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 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:

<div align="center">

File
Camera
Setup
Help

</div>

If you have either captured a new image or opened an existing image, the following menus are available:

<div align="center">

File
Edit
View
Camera
Setup
Window
Help

</div>

- 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 Ch. 5 - 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 Ch. 4 - Viewing, Editing, and Annotating Images (below) for details.

- 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 Ch. 4 - 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. The following diagram illustrates the Toolbar, as it appears for an open image:
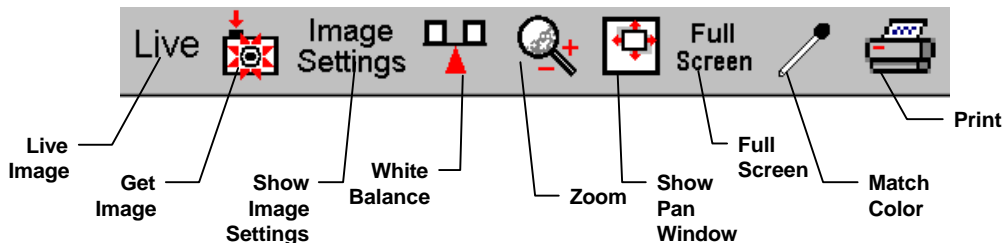

Figure 3- 1: SPOT Toolbar

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:
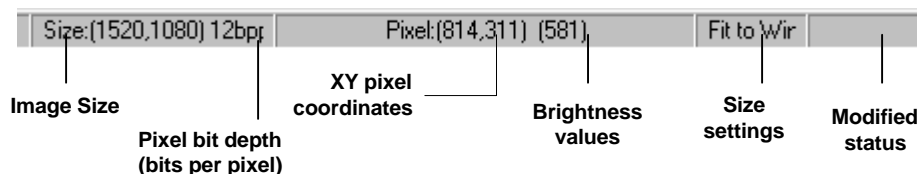

Figure 3- 2: Status Bar

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

- **Image size** – (Columns, rows) in pixels [e.g., (243, 257)].

- **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 captured images 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 [e.g., RGB: (255, 34, 194)].

- **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 percentage – e.g., 100 %, 300 %, 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.

Opening and Saving Files

All of the file open and save options 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 95/98/NT/2000 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 Ch. 3 - Taking a Picture **(see – Get Sequential Images)** for information on capturing an image sequence file.

•   Refer to the Ch. 4 - Viewing, Editing, and Annotating Images **(see – Working with Image Sequences)** 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. 4 for more details.

New Report

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

Open Report

Open a 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 6 – Printing Images for information on standard Windows and SPOT custom printing features.

Printer Setup

Define the page setup parameters. Refer to Ch. 6 – Printing Images for information on SPOT's custom printing features.

Exit (Windows) Quit (Mac)

Exit the SPOT program.

Setup

The Setup menu options are as follows:

- Show Image Settings
- Calibration Setup
- Preferences

Show Image Settings

Use this option to display the **Image Settings** window. 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 **Ch. 2 - Preparing to Take a Picture** (see – **Profile**) **f**or 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. Refer to Ch. 4, 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. 2 – Taking a Picture (see **Viewing a Live Image**), 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. 3 – 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.
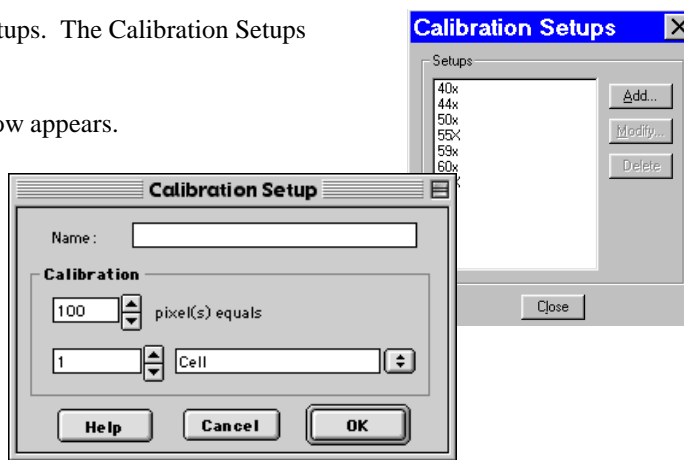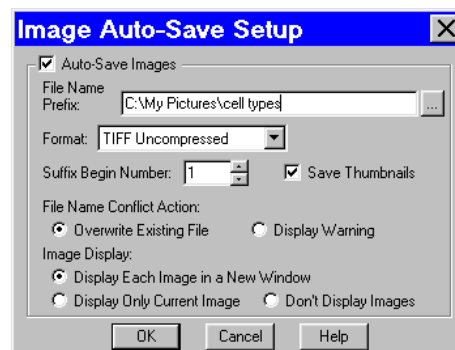
    To draw the line, follow these steps:

    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.
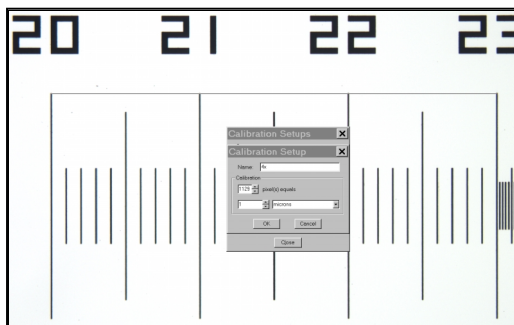
9.   Enter the length and units of the line that you drew (e.g., 3 mm).
10. Click on OK. The 4x objective is now calibrated to perform measurements. Repeat steps one through ten for each objective.

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.

Preferences

Use the Preferences option to specify:

- Sequential imaging memory settings

- Specify thumbnail appearance.

- Live image viewing settings

- Fit to window view settings

- File type extension setting (*Macs only*).

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

Image Sequences   <u>Memory Usage Limit</u>
Specify how much memory is allocated for image acquisition. Note that the entry parameters are system dependent.

<u>Cache Images During Play</u>

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

Thumbnail Button Size

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

Maximum Thumbnail Resolution

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 = 20 pixels
  Maximum resolution = 200 pixels

Always Show Full Chip in Live Image

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 more details.

Open Images in Fit to Window Mode

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.

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, tif, 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.

INTENTIONALLY BLANK
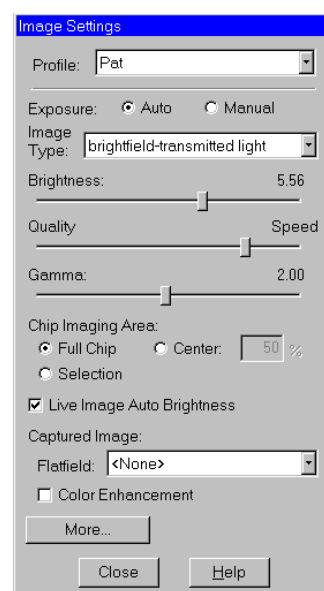
Ch. 2 – Preparing to Take a Picture

QUICK REFERENCE:

➲ Define image capture settings…p. 32

➲ Define automated exposure settings… p. 34

➲ Define manual exposure settings… p. 36

➲ Define the area of the chip that will be used in the image capture… p. 36

➲ Define live image viewing preferences… p. 38

Introduction

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 **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 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.

Understanding Gain and Auto-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 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.

Auto-Gain Limit (see the **Quality/Speed** section below) allows you to set an upper limit on the gain options that will be available during the Auto-Exposure process.

✏ *Note: If you are using* **Manual** *exposure, you can set an exact gain for an image capture.*

For example, with an Auto-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 Auto-Gain Limit to four. When the Auto-Gain Limit option is set to four, the camera is limited to the lower three gain options: 1, 2, or 4.

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:

Auto-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

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
Nomarksi 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.
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 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 Auto-Gain Limit is 32, which allows the camera to select from the all six gain options. This shortens exposure times, but decreases image quality.

Refer to the **"Understanding Gain and Auto-Gain Limit "** section above for more details on 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. 2 – Preparing to Take a Picture for more details on gamma adjustment.

Manual Exposure Options

Exposure Time

Use the slider-bar to specify an exposure time 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. 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 six gain options: **1**, **2**, **4**, **8**, **16**, or **32**. The gain value displays to the right of the slider-bar. Refer to the **"Understanding Gain and Auto-Gain Limit "** section above for more details on gain and exposure.

Gamma

The manual Gamma adjustment is identical to the auto Gamma adjustment (see above.)

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., vignetting).

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

For all other SPOT camera lines, 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. Flatfield can still be used for this purpose with SPOT Vision cameras.   But, because SPOT Vision 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.*

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>SPOT 3.x Prefs>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.
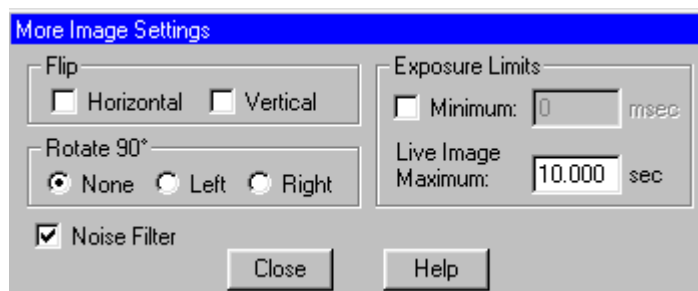
Flip

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 Vision 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 the fluorescent light source is 8.3 ms. Entering a Minimum Exposure of 10 ms ensures that the camera's exposure time is greater than the pulse interval of the light source. To enter a minimum exposure, check the Minimum Exposure box, and enter a value in milliseconds.
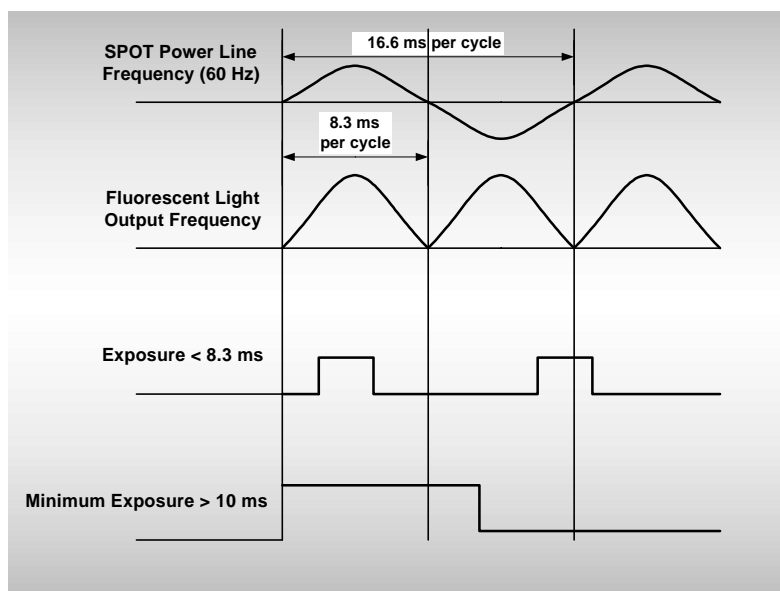
Figure 3- 1: Setting a Minimum Exposure to Accommodate Varied Light Sources

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.

Noise Filter

The Noise Filter corrects for the effects of electrical or thermal noise. When capturing dim images with lengthy exposure times, the noise filter acts as a "virtual cooler" for the Vision camera, eliminating "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.

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Ch. 3 – Taking a Picture

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.

Image captures 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 **Ch. 2 - Preparing to Take a Picture** for more details
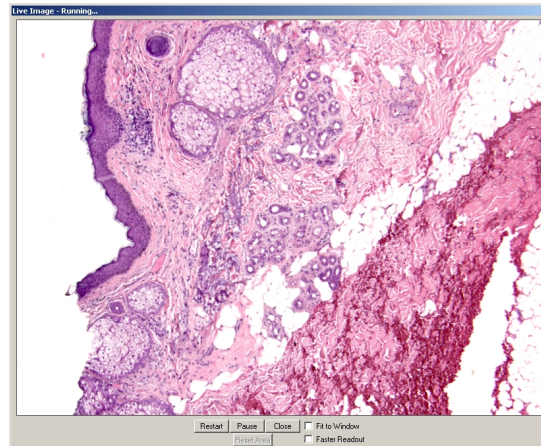
> *Note: With some video cards, you might experience system crashes or display problems when working with live images. To correct these problems, lower the Graphics Acceleration setting in Windows, as described in the* Video Card Related Problems *section of the* Installation Guidelines *chapter.*

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 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. 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 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 (Vision Color)

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 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)
Nomarksi 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 (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, either click on the Get Image button, 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. Getting Image – The camera is downloading the image to the computer.

3. Shutter open 0:07 – 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.

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

The newly captured image appears on screen.

Capturing a Sequence of Images (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. 4 – 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 on the following page:
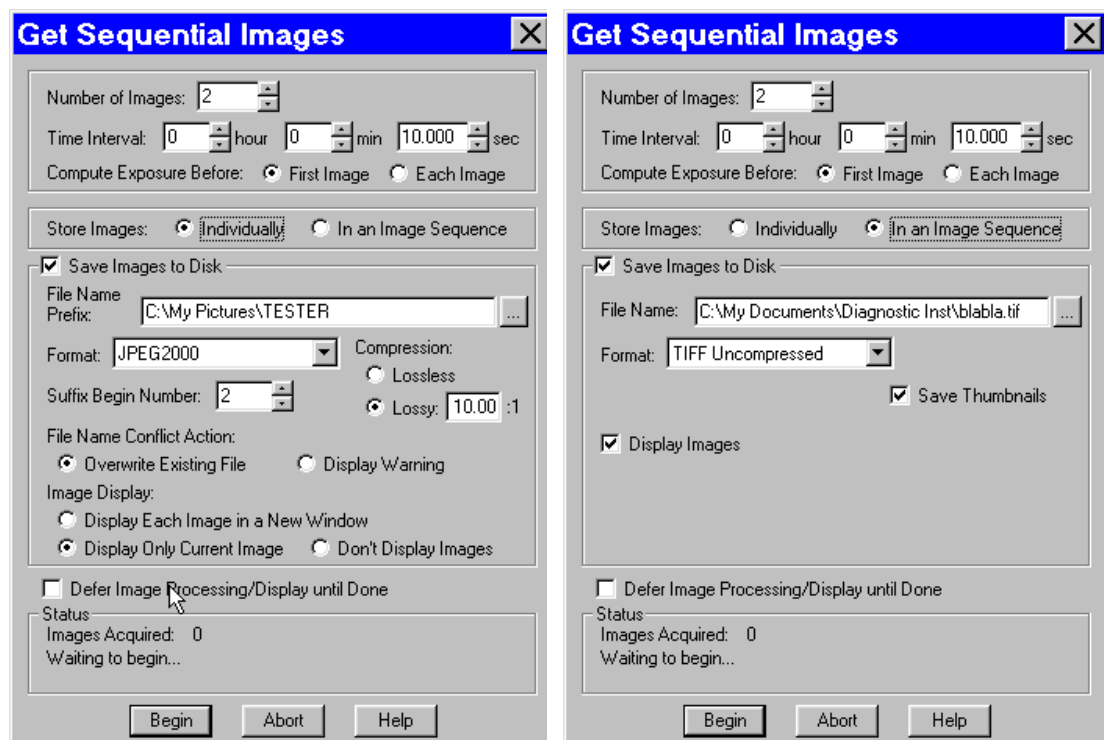
Save and name options – individual files   **Save and name options – image sequence**

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.
Compute Exposure Before	Select either **First Image** or **Each Image**: • First Image – The program computes the exposure value for the first capture only. • Each Image - The program computes a new exposure value for each image capture in the sequence.

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 five options: **Windows Bitmap**, **JPEG**, **Mac PICT**, **TIFF Uncompressed** or **TIFF JPEG**.

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

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 or TIFF JPEG 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 (image sequences)   Select this box to open an image sequence immediately after the capture. To save the 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. 4 – Viewing, Editing, and Annotating Images for details on viewing and modifying image sequence files

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Ch. 4 – Viewing, Editing, and Annotating Images

QUICK REFERENCE:

➲ Show pixel borders for a magnified image… p. 53

➲ Navigate around a magnified image without having to zoom in and out… p. 53

➲ View the date and time associated with an image capture…p. 54

➲ View and edit image sequence files… pp. 54-56

➲ Crop an image…p. 58

➲ Adjust image brightness… p. 59 - 60

➲ Adjust the contrast of an image… pp. 59-60

➲ Lighten the dark areas of an image without burning out the light areas… p. 60

➲ Add a calibration mark to an image… p. 61

➲ Measure an image… p. 62

➲ Annotate an image… p. 63

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

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 five options – **Fit to Window**, **Zoom In/Out**, **Zoom Level, Show Grid Lines**, **Show Pan Window**, **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.

Zoom In/Out

1. Either select Zoom In/Out 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 In/Out** option enables you to view zoomed images. However, unlike the Zoom In/Out 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 In/Out option, the 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.

This tedious process is replaced by the pan window. 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.*

The pan window appears, superimposed upon the image.
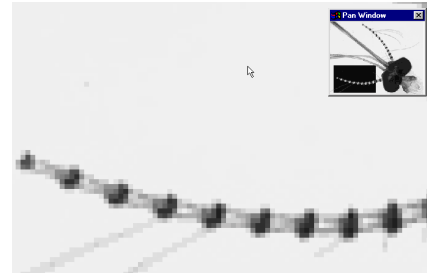
2. Position the cursor on the image in the pan window: it changes from a magnifying glass to a four arrow pointer.

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.

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 (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, 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.

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] + O =**	Object (Report mode)
[Alt] + C =	Camera		**[Alt] + Q =**	Sequence
[Alt] + E =	Edit		**[Alt] + S =**	Setup
[Alt] + F =	File		**[Alt] + V =**	View
[Alt] + H =	Help		**[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
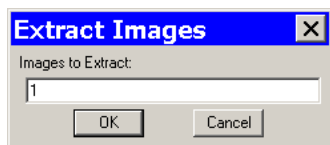
Extract an image or images from a sequence file. Extracting creates new image(s), leaving the original sequence intact. Click on **Extract Images** and enter the number(s) of the image(s) that you want to extract. To extract multiple images from a sequence, separate numbers with commas and/or dashes - i.e., **5, 6, 7** or **5-7** or **1 – 5, 6, 9**.

Insert Image
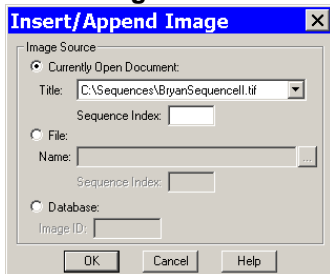
Add an image to the sequence. Using **Insert Image**, you can add an image to a sequence from one of three sources:

- **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.

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
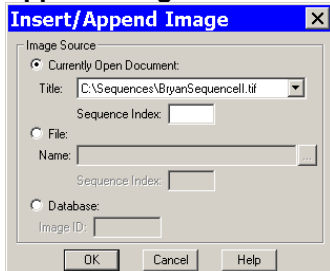
Add an image to the end of the sequence. Using **Append Image**, you can add an image to a sequence from one of three sources:

- **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.

Remove Images
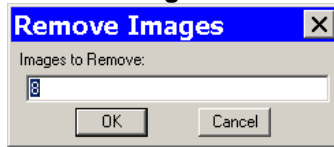
Remove an image or images from a sequence file. Removing an image deletes the image from the sequence, which is automatically reordered. Click on **Remove Images** and enter the number of the image(s) that you want to extract. To extract multiple images from a sequence, separate numbers with commas and/or dashes - i.e., **5, 6, 7** or **5-7** or **1 – 5, 6, 9**.

Move Image
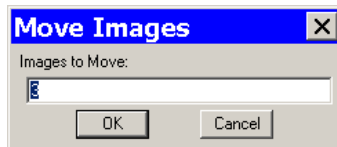
Move an image or image(s) to a different point in the sequence.   Click on **Move Image** and enter the number(s) of the image(s) that you want to move. To move multiple images from a sequence, separate numbers with commas and/or dashes - i.e., **5, 6, 7** or **5-7** or **1 – 5, 6, 9**.

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
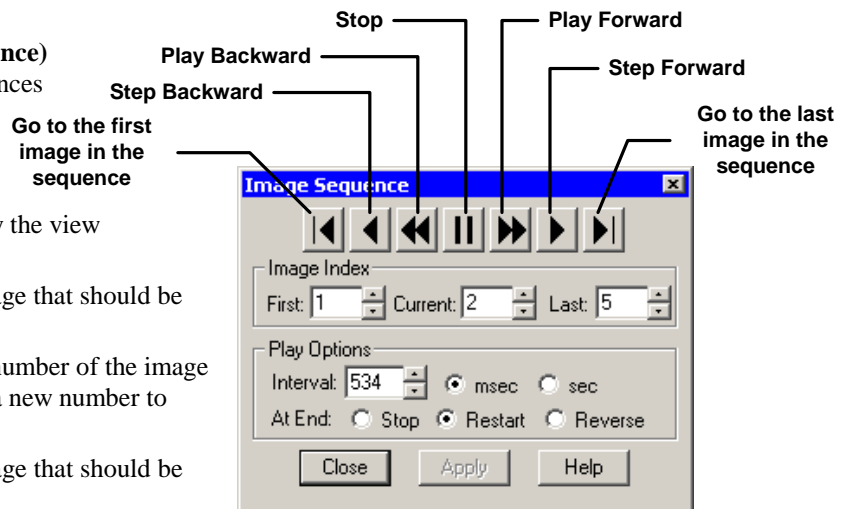
Play Options

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 Images** – 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**.
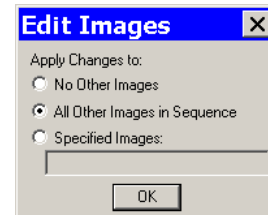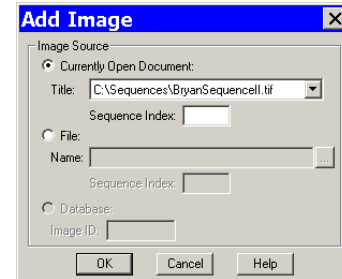
Edit

All of the editing options are accessed via the Edit menu. There are five editing categories:

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

The options associated with each category are described below.

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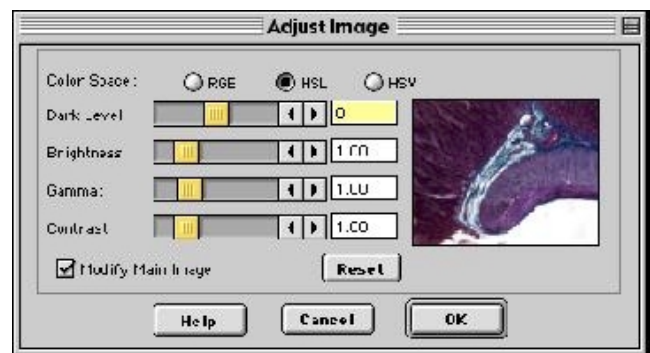
- **Color Space** – This option enables you to select the color space that is best suited to the type of image that you are editing:

  – **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

- **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 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 eye-dropper.

2. 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 255.

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.*
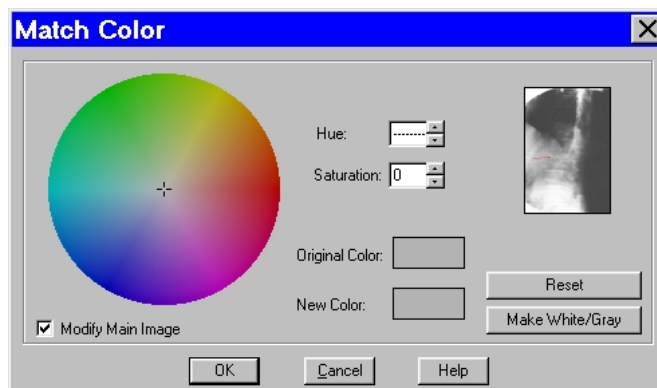
The window displays information about the pixel that you selected:

– **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.

Annotation – Calibration and Measurement

The Add Calibration Mark and Add Measurement options enable you to add calibration and measurement references to SPOT images.

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. 1 – Navigating the Program 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.*
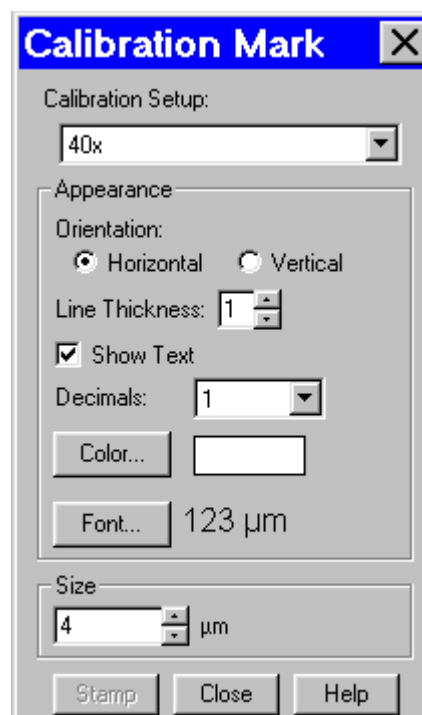
5. Using the on-screen mark as a reference, modify the Appearance options:

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** – Check this option to display the calibration number in the mark. If this option is deselected, the calibration mark is simply a 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.   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. 1 – Navigating the Program 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.   Select the type of measurement that you want.
   –   **Straight Line Length** – Point to point distance measurement
   –   **Curve Length**– Length of a freehand line
   –   **Rectangle Perimeter –** Perimeter length of a rectangle
   –   **Ellipse Perimeter –** Perimeter length of an ellipse
   –   **Region Area** - Area of a freehand outline
   –   **Rectangle Area** – Area of a rectangle
   –   **Ellipse Area –** Area of an ellipse
   –   **Angle** – Angle measurement

5.   Move the cross-hair cursor to the point in the image capture where you want to begin the measurement.
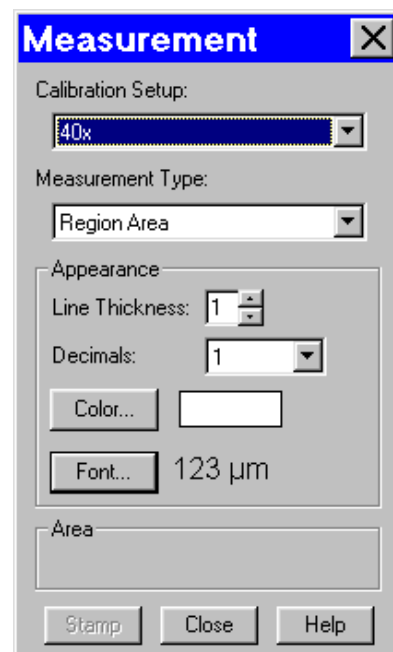
6. Single click to activate the drawing function. ***Do not hold the mouse button down between the measurement points***.

    – For a **Straight Line Length**, 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 Length**, 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 the **area** and **perimeter** type measurements, use the cross-hair cursor to trace an 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 (for the **Region Area** option), 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

    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.

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.

- 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 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 on the grid. *Refer to* **Adding Annotation Objects to SPOT Images.**

- 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**

- The **Attributes** menu appears with options for specifying annotation qualities.
  Refer to **Defining Annotation Attributes.**

- 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 SPOTprogram 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 at right, 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.*
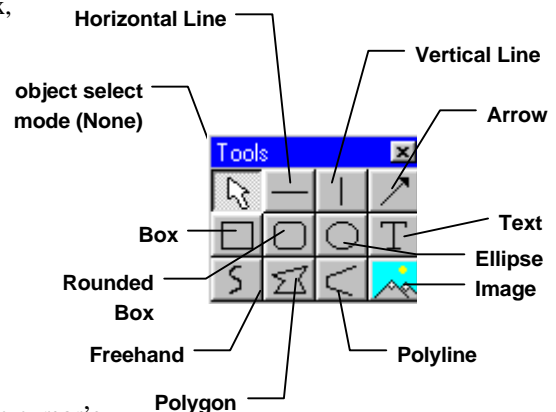
1. From the Object menu, select the type of item (i.e., Lines, Connected Lines, etc…) that you want to add to the image. The cursor's functionality varies according to whether a report object type (i.e., line, text) is selected:.
   - If a report object type is selected, the cursor changers to a cross-hair 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, box, or other shape, hold down the mouse button and drag the object to the desired size.
- – If you selected any 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.
- – 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 arrow head 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   **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.

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

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.

                   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. 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 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.

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.

<table>
<tr>
<td>Line Thickness

</td>
<td>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.</td>
</tr>
<tr>
<td>Colors

</td>
<td>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.</td>
</tr>
</table>
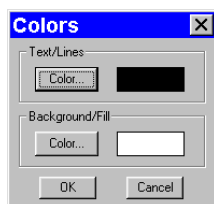
> ✎ *Note: In order to define a background/fill color for a Box or a Rounded Box, a fill must be selected. Similarly, in order to define a background/fill color for a text box, the background (see Text Border, Justification, and Background below) must be set to Opaque.*
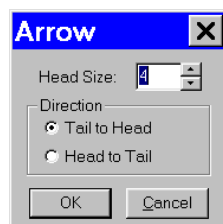
<table>
<tr>
<td>Arrow Size and Direction

</td>
<td>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.

The Arrow Size and Direction attribute is not available for Mac users. For Mac users, arrows are always drawn Tail to Head.</td>
</tr>
</table>

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.

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
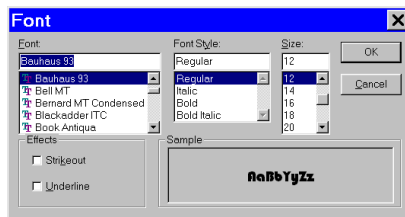
Define the appearance of the text for the **Text**, **Image Date**, **Image Time**, **Image Title**, **Image Memo** objects.

1. Select a **Font**, a **Font Style**, and a point **Size**. The Sample box displays a preview of the font.

2. Define any Strikeout or 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.

Stamp Image
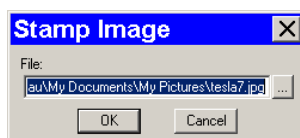
Use the Stamp Image option to select the image that you want to use for the Stamp option. To select the image, choose Stamp Image. The Stamp Image dialog box prompts you to enter a file name and path:

•   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.

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 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.

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.
Cut	Remove the selected annotation and place it on the clipboard.
Copy	Copy the selected annotation clipboard.
Paste	Place the cut or copied annotation from the clipboard into the image.
Clear (Mac)	Delete the selected annotation object(s).
Select All	Select all annotation objects.
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.
Delete (Windows)	Delete the selected annotation object(s).
Delete All	Delete all of the annotations on the image.

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 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 the to smallest or largest object's in pixels.
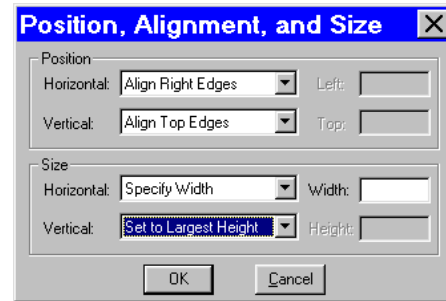
Duplicate Image

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

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.*

Ch. 5 - Reporting

QUICK REFERENCE:

➲ Define the style for your report... p. 75

➲ Design a layout for a new SPOT report... p. 76

➲ Edit report objects... p. 77 - 79

➲ Save a report... p. 79k

➲ Distribute a report electronically to a user who does not have the SPOT program... p. 80

Introduction

The SPOT software includes a built-in reporting functionality that can be used 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:

- Creating a Report
- Saving a Report
- Printing and Distributing Reports

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 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-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.
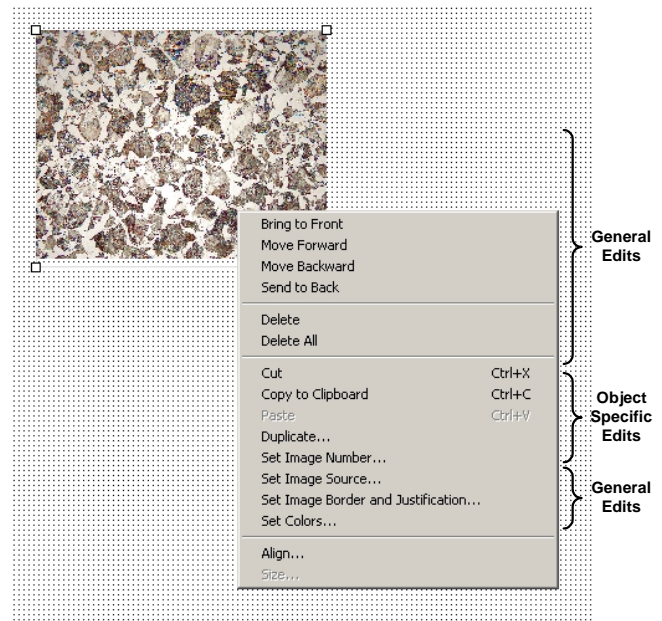
Refer to the **Defining Report Attributes** and **Editing Report Objects** sections for more details.

Defining View Options

The **View** menu provides you with eight 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.

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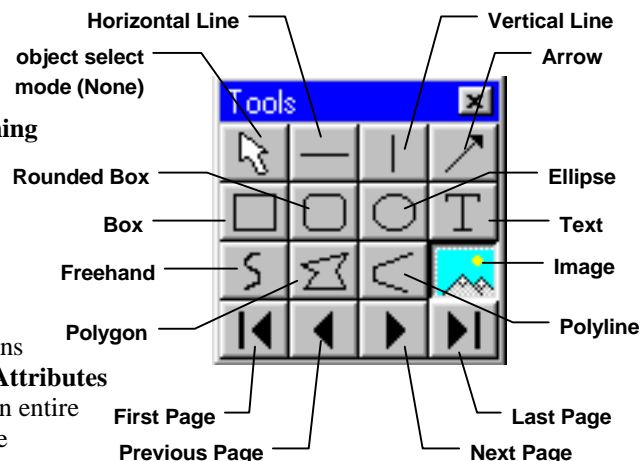
Colors
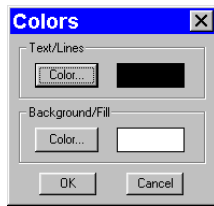
Colors Define the foreground and background colors for all objects. Follow these steps:

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.*

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, 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 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 arrow head 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.
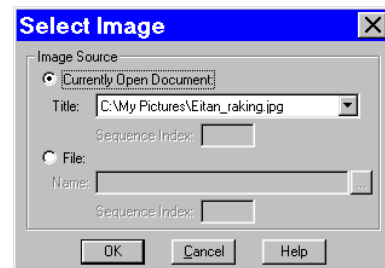
Text **Text**

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 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.
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.
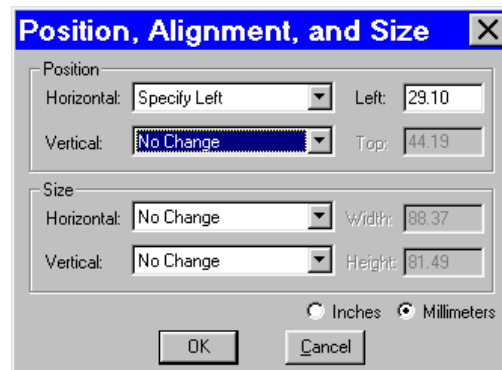
Add Page

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 a Report

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 .*
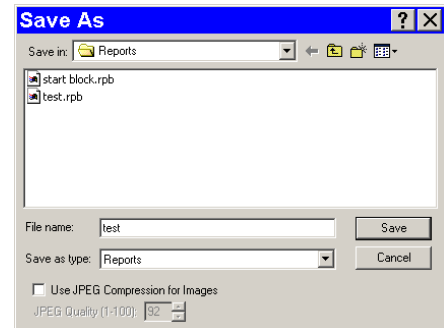
3. Enter a file name for the report.

4. Define the JPEG Compression for the image(s) in the report. The **Use JPEG Compression for Images** box is checked as a default. To turn off JPEG compression, deselect the 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 PDFWriter** 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.
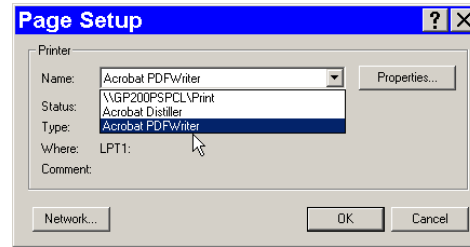
If you intend to distribute SPOT image reports to people who do not have the SPOT program, we
recommend that you purchase Adobe Acrobat. Using Acrobat, you can convert the SPOT report (.rpb) files
to PDF files, which can be distributed to users who do not have the SPOT software. They can view the
reports 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.

Ch. 6 - Printing Images

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.

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.
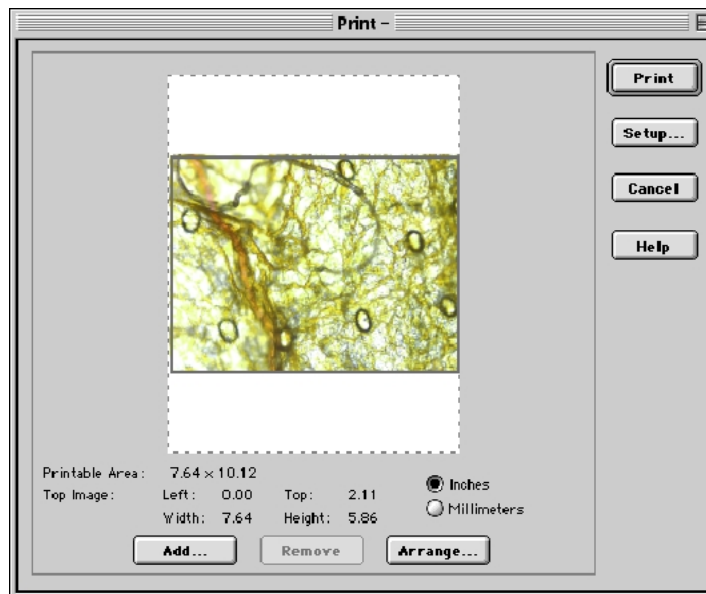
   ♦ **Top -** The distance between the top of the printable area and the top of the image.

   ♦ **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.

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 two 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.
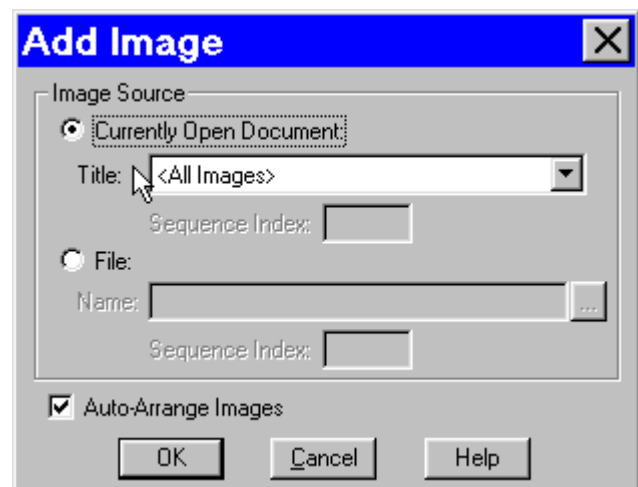
    c) Set the auto-arrange option. If you check the **Auto-Arrange Images** box, the SPOT program automatically sizes and positions the images to fit on the print page.

    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.

3. 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 print-out in inches or millimeters.

    d) Select **Apply to all pages** to make the arrangement applicable to every page of a multi-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.*

4. 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.
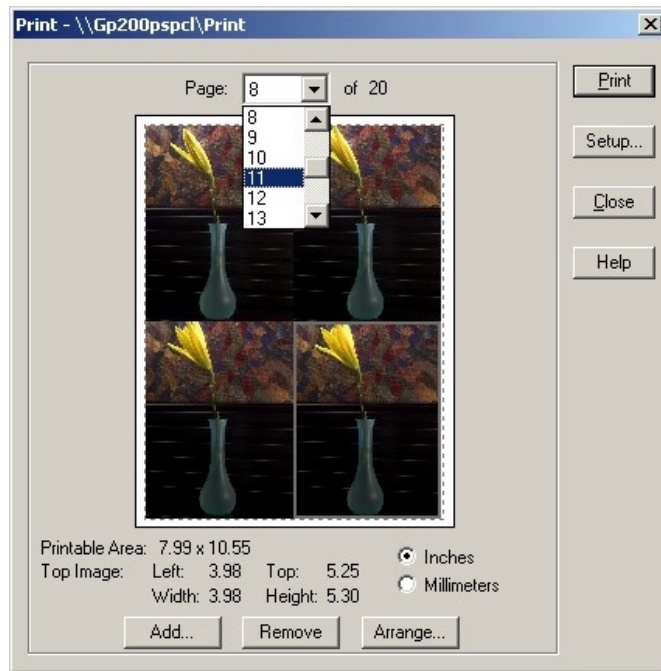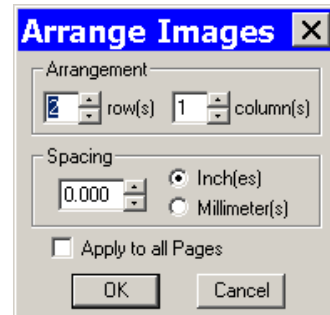
Printing Image Sequences

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. 7 – Using Other Windows Imaging Applications

QUICK REFERENCE:

➲ Set up the camera to work with another imaging application (define the TWAIN interface)…
p. 86

➲ Define image capture settings in the SPOT TWAIN interface… p. 89

➲ Perform a white balance on an image… p. 94

➲ View or capture a region of an image… p. 95

➲ Capture an image… p. 99

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.

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 Vision camera**. 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.
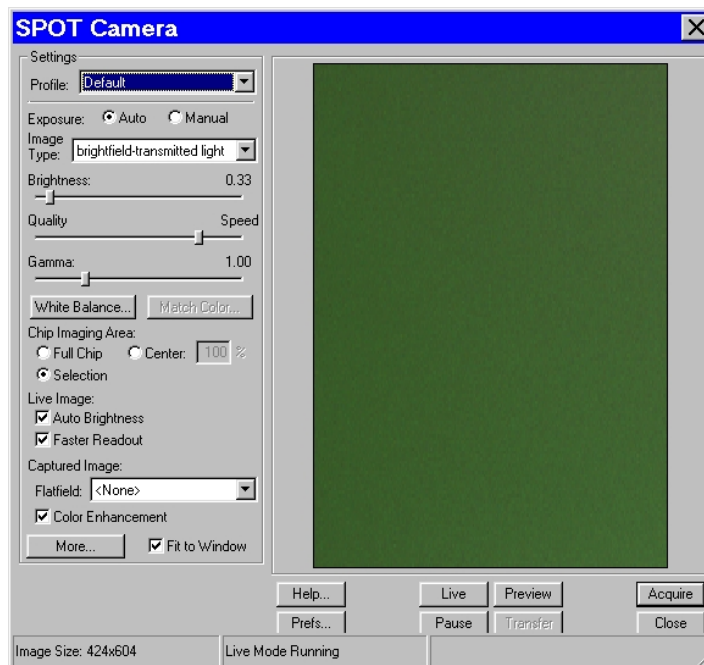
Working in the TWAIN Interface

The TWAIN interface is a one-stop point for previewing and capturing images. For example, using the 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.
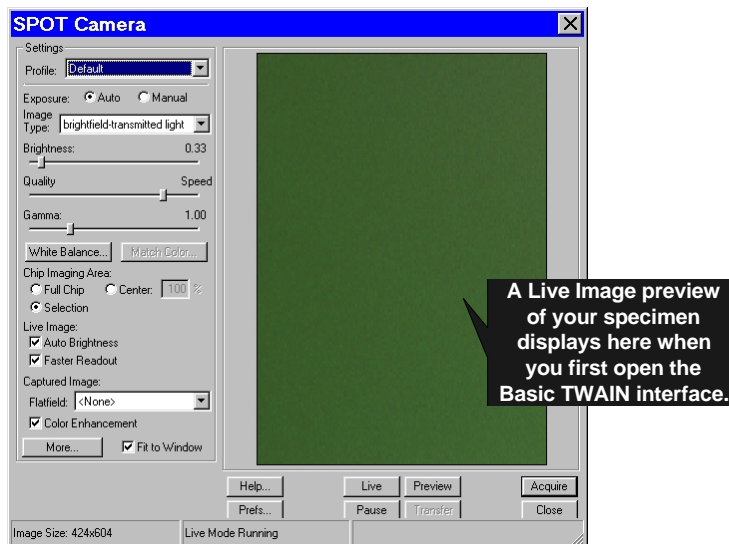
Create a Profile

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.
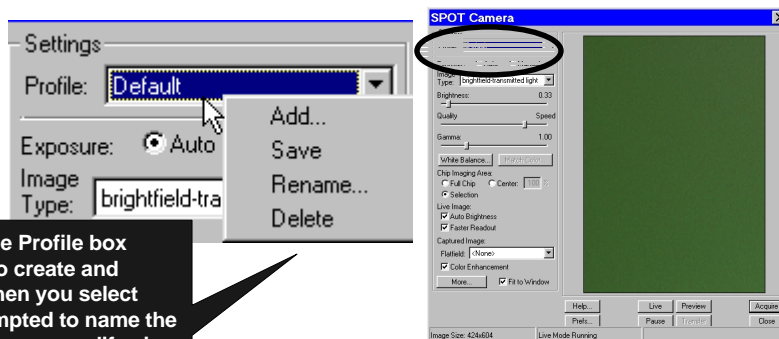
Define Settings

Define exposure settings for live and captured images. Each of these settings are discussed later in this chapter.
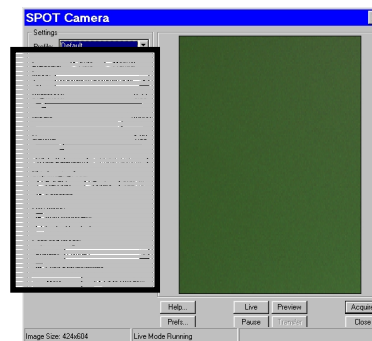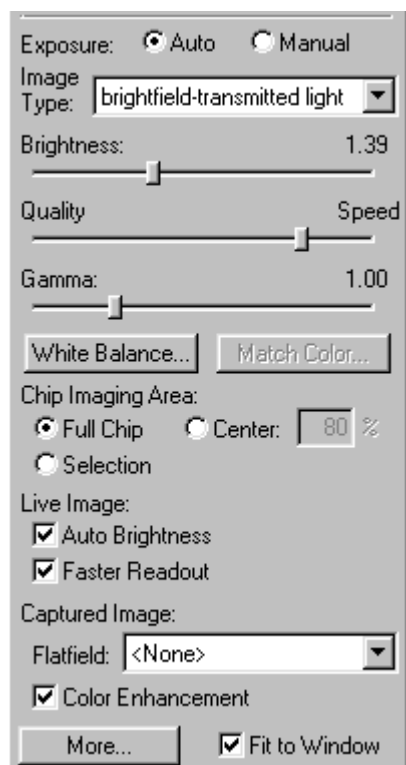
Capture an Image

All of the image capture options are located on the bottom portion of the window, as illustrated here
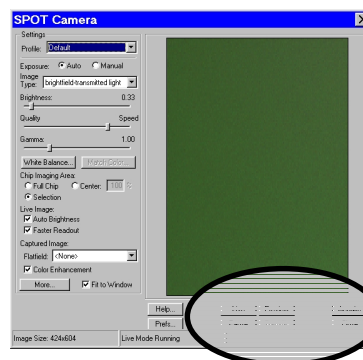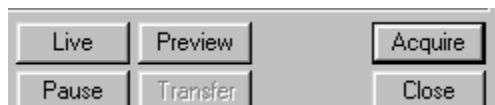
Defining Preferences

In the TWAIN Interface, you can define the following view preferences.   Click on the **Prefs** button to open the **Preferences** window. Each option is described below:

Keep TWAIN Window on Top

Checking this option ensures that the TWAIN window remains the top-most window when working in multiple applications. This 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 Camera window remains open. If the box is not checked, the window automatically closes after each image is transferred or acquired.

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 below for more details.

F-Keys

The following function keys are available as shortcuts in the 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 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 -** Deletea 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.

Understanding Gain and Auto-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 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.

Auto-Gain Limit (see the **Quality**/**Speed** section below) allows you to set an upper limit on the gain options that will be available during the Auto-Exposure process.

> ✎   *Note: If you are using* **Manual** *exposure, you can set an exact gain for an image capture.*

For example, with an Auto-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 Auto-Gain Limit to four. When the Auto-Gain Limit option is set to four, the camera is limited to the lower three gain options: 1, 2, or 4.

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:

Auto-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

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
Nomarksi 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.
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 **.25** and **32**.
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)

Gamma adjustment 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 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 Auto-Gain Limit is 32, which allows the camera to select from all six gain options. This shortens exposure times, but decreases image quality.

Refer to the **"Understanding Gain and Auto-Gain Limit "** section above for more details on 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. 2 – Preparing to Take a Picture for more details on gamma adjustment.

Manual Exposure Options

Exposure Time

Use the slider-bar to specify an exposure time between **.04 ms** and **526.746 sec**.
Gamma adjustment 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

Gamma adjustment Enter the precise gain that the camera will use for an image capture. Use the slider-bar to select one of the six gain options: **1**, **2**, **4**, **8**, **16**, or **32**. The gain value displays to the right of the slider-bar. Refer to the **"Understanding Gain and Auto-Gain Limit "** section above for more 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)
Nomarksi 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 (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. From the Edit menu, select **Match Color.** The cursor becomes an eye-dropper.

3. Using the mouse to move the eye-dropper, 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., vignetting).

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. Flatfield can still be used for this purpose with SPOT Vision cameras.   But, because SPOT Vision 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.*

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 **Defining Preferences** 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 (Additional Orientation and Exposure Settings)

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

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 Vision 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 10 ms ensures that the camera's exposure time is greater than the pulse interval of the light source. To enter a minimum exposure, check the **Minimum** box, and enter a value in milliseconds.

Setting a Minimum Exposure to Accommodate Varied Light Sources

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.

Noise Filter

The Noise Filter corrects for the effects of electrical or thermal noise. When capturing dim images with lengthy exposure times, the noise filter acts as a "virtual cooler" for the Vision camera, eliminating "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 using the **Preview** or **Acquire** options.

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.

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Ch. 8 – Using Other Mac Imaging Applications

QUICK REFERENCE:

➲ Define image capture settings in the SPOT 3rd Party interface… p. 105

➲ Define exposure options… pp. 105 - 113

➲ Perform a white balance on an image… p. 108

➲ View or capture a region of an image… p. 110

➲ Capture an image… p. 114

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.

Working in the 3ʳᵈ Party Interface

The 3ʳᵈ Party interface is a one-stop point for previewing and capturing images. For example, using the 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.
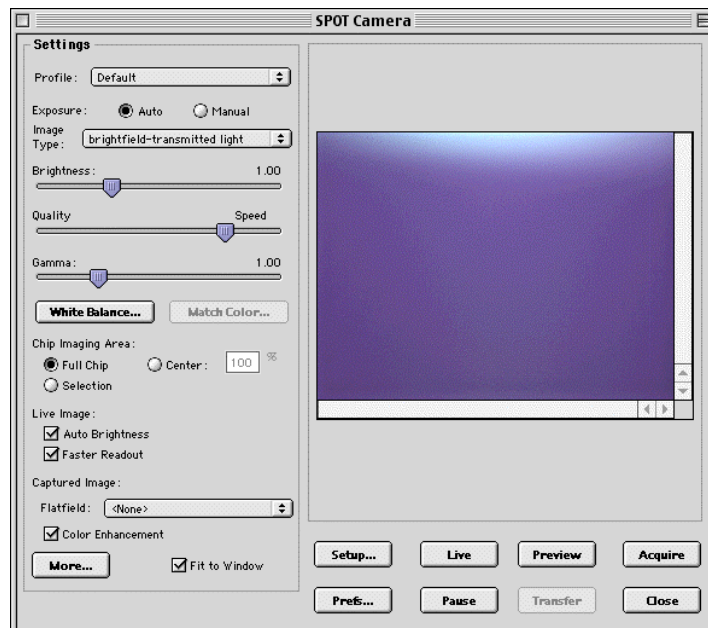
Create a Profile

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.
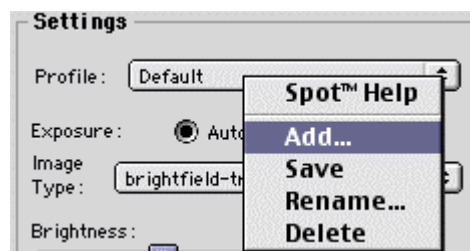
Define Settings

Define exposure settings for
live and captured images. Each
of these settings are discussed
later in this chapter.
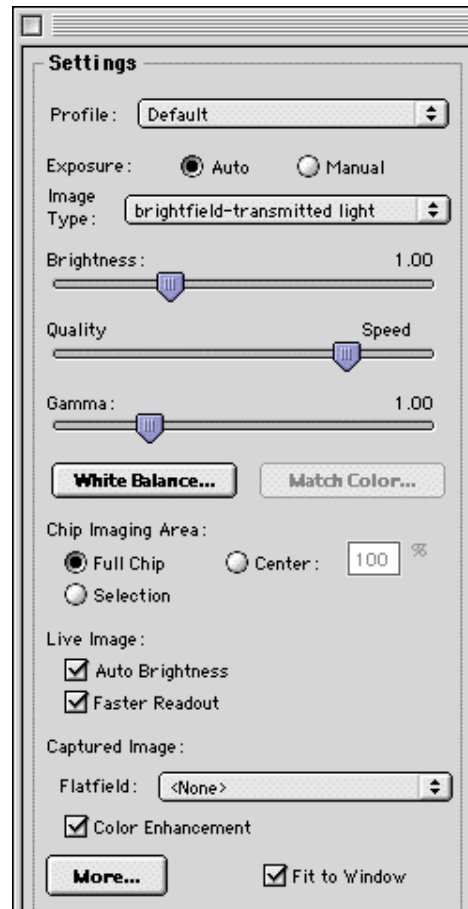
Capture an Image

All of the image capture options
are located on the bottom
portion of the window, as
illustrated here
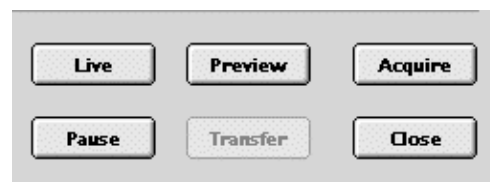
Defining Preferences

In the 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 Vision B/W cameras, captured images are 8 bits per pixel monochrome.
  - With Vision 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 Vision B/W cameras, captured images are 12 bits per pixel monochrome.
  - With Vision 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 Vision 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 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 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 -** Deletea 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
Nomarksi 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 **.25** and **32**.

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 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 Auto-Gain Limit is 32, which allows the camera to select from the all six gain options. This shortens exposure times, but decreases image quality.

Refer to the "Understanding Gain and Auto-Gain Limit" section in Ch. 2 – 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. 2 – Preparing to Take a Picture for more details on gamma adjustment and color spaces.

Manual Exposure Options

Exposure Time

Use the slider-bar to specify an exposure time 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 six gain options: **1**, **2**, **4**, **8**, **16**, or **32**. The gain value displays to the right of the slider-bar. Refer to the "Understanding Gain and Auto-Gain Limit " section of Ch. 2 – 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)
Nomarksi 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 (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 eye-dropper.

3. Using the mouse to move the eye-dropper, 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., vignetting).

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. Flatfield can still be used for this purpose with SPOT Vision cameras.   But, because SPOT Vision 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.*

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.

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 Vision 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 10 ms ensures that the camera's exposure time is greater than the pulse interval of the light source. To enter a minimum exposure, check the **Minimum** box, and enter a value in milliseconds.

Setting a Minimum Exposure to Accommodate Varied Light Sources

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.

Noise Filter

The Noise Filter corrects for the effects of electrical or thermal noise. When capturing dim images with lengthy exposure times, the noise filter acts as a "virtual cooler" for the Vision camera, eliminating "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 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 photo-sensitive 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 (SPOT Insight and Vision cameras)**
  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 beamsplitter**
  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) (SPOT and SPOT RT cameras)**
  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 reads the CCD chip and digitizes each pixel as it comes 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 Insight cameras use two methods of pixel digitization, one for live imaging, and one for image capture:

- **Live imaging (Insight, RT, and Vision)**
  To provide the fastest possible frame rate, SPOT Insight, RT, and Vision 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 models)**
  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. 2 – Preparing to Take a Picture.

Appendix B: Specifications

Camera and Filter
Vision Color – Color
Vision B/W – Monochrome

CCD Information
Vision Color – KAI 2000 Color Mosaic
Vision B/W – KAI 2000 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 - 31,500e

Vision Color Image Resolution
Live image – 800 x 600
Captured image – 1600 x1200

Vision B/W Image Resolution
Live image – 1600 x 1200
Captured image – 1600 x1200

Bit Depth – Vision Color
Sampled at 30 bit RGB
Saved file formats: 24 bit (RGB)

Bit Depth – Vision B/W
Sampled at 10 bit monochrome
Saved file formats: 8 bit (mono)

Pixel Readout Rate
Live images – 36 Mhz (2 channel x 18 Mhz)
Captured images – 18 Mhz (1 channel x 18 Mhz)

Live Image Frame Rate
733 PIII w/810e Graphics
Vision Color - 15 frames/s
Vision B/W – 15 frames/s

Acquisition Time to Monitor
(processor dependent)
Vision Color < 6 seconds
Vision B/W < 1 second

A/D Converter Full Scale Setting
31,500e (when gain = 1)

Signal to Noise Ratio
58 dB

Anti – Blooming Factor
300 times (nominal); 100 times (minimum)

Exposure
Automatic and User-Defined
Range – 1 ms – 536 seconds
(1 saturated hot pixel @ 5 sec. typical)

Lens Mount
"C" Mount
Flange to rear lens clearance - 0.46" (11.7 mm) max.

Computer Interface
PCI bus card (supplied)

External Shutter Control
BNC, TTL level output

Power Requirements
100 –240 VAC, 47 – 63 Hz

Weight
Camera - 1.5 lbs.
Power supply - 0.5 lbs.

Certifications
CE, FCC, Class A

Computer Requirements
PC - Pentium 166 or greater   **Mac** – Power PC w/PCI bus
      Windows 95/98/00/NT/ME/XP   Mac OS 8.6 or greater

RAM – 64 Mb RAM required, 256 Mb recommended.

Video card – Support of 24 bit RGB at the desired monitor resolution.

Software Features
• Color live image window
• Image capture window
• Pre-defined image types
• Auto exposure
• Auto white balance
• Flatfield correction
• Zoom and pan window
• Image enhancement tools
• Annotation
• Calibration mark
• Measurement – length, perimeter area, angle
• Print layout dialog
• On-line help menu
• Report generator
• Sequential imaging

Image File Formats
TIFF, TIFF-JPEG, JPEG, JPEG 2000, BMP, PICT

File Size
Vision Color	Vision B/W
24 bit - 5.49 MB	8 bit - 1.83 MB

Drivers
TWAIN for Windows

Couplers
D10XXC – 1x C-Mount Coupler (Diagnostic Instruments)
Alternatives made by Diagnostic Instruments:
• **HRU060-CMT coupler (0.60x)** and the appropriate bottom clamp.
• **HRD100-CMT coupler (1x)** and the appropriate bottom clamp.

Lens
C- Mount lens for 1" format CCD

Camera Dimensions

2.79"

5.11"

.65"

.30"
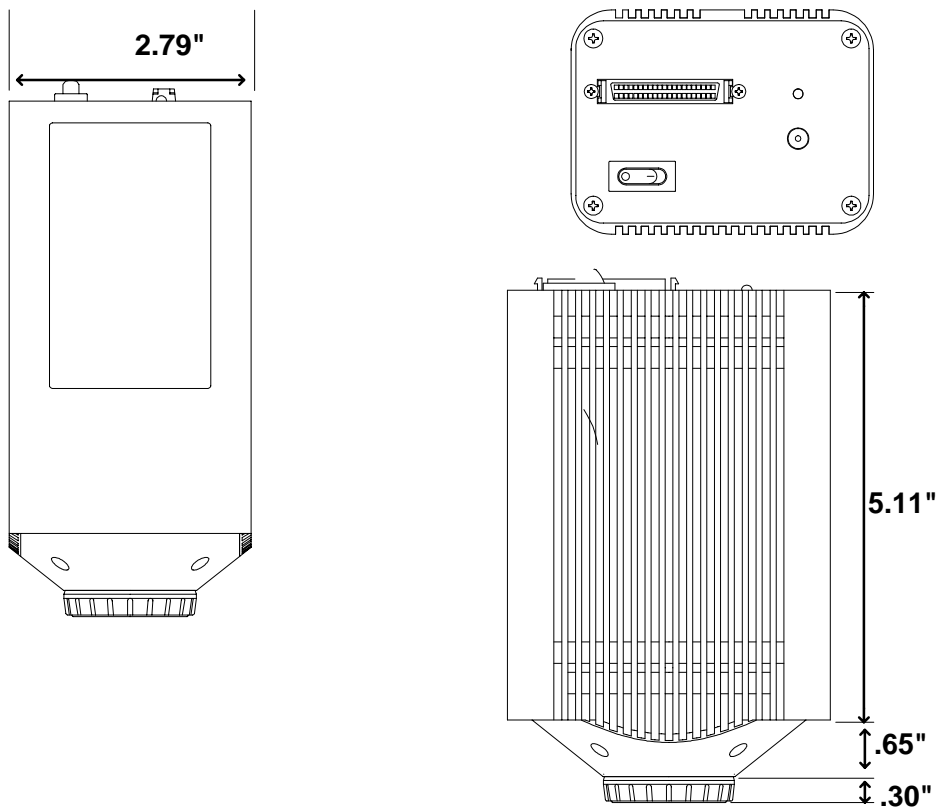
Power Supply Dimensions

3.350"

1.970"

1.300"
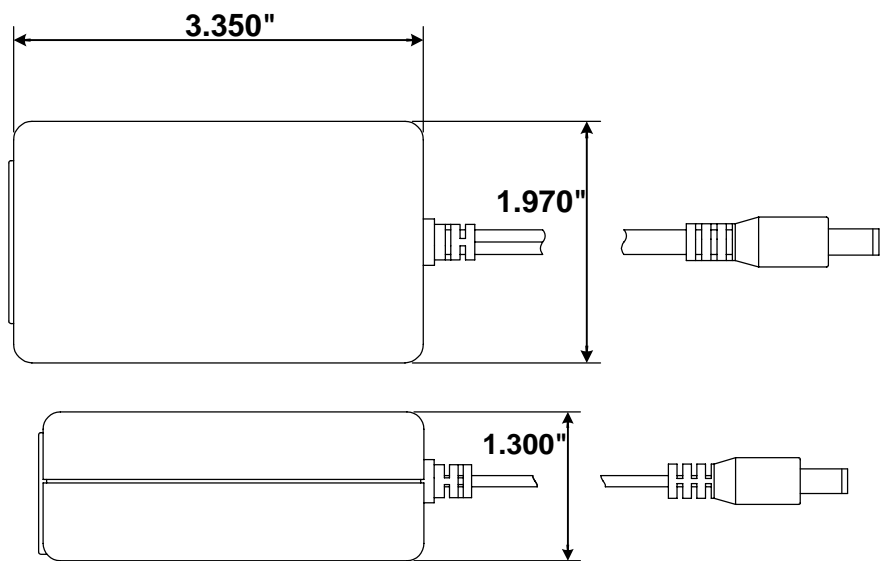
Vision Series Catalog Information

There are two cameras in the SPOT Vision series of cameras: Vision Color and B/W (monochrome).   The following table shows each camera with the standard and special order options:

Catalog Part Numbers	Model Number	Description	Stock/Special Order
IN820	8.2	SPOT Vision Color "C" mount camera *with IR filter*	Stock
IN810	8.0	SPOT Vision B/W (monochrome) "C" mount camera *with IR filter*	Stock
IN800	8.0	SPOT Vision B/W (monochrome) "C" mount camera *without IR filter*	Special Order

FCC "Class A" Product Warning
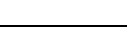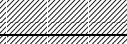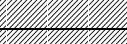
All SPOT cameras fall into the Class A category of the FCC (Federal Communications Commission) emission standard EN55022/CISPR22. The following is the FCC's standard warning for this type of device:

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.

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Appendix C: Keyboard and Toolbar Button Commands

Command	Keystroke or F-key	Toolbar Button	Windows and/or Mac
Open Image File	[F3]		Both
Show Image File Thumbnails	[F5]		Both
Save	[F2]		Both
Save As	[Ctrl] + [A]		Windows
Save All	[Ctrl] + [F3]		Windows
Close	[Ctrl] + [F4] ⌘ + W		Both
Print	[Ctrl] + [P] ⌘ + P		Both
Exit Quit	[Alt] + [F4] ⌘ + Q		Both
Edit menu			
Undo	[Alt] + Bksp ⌘ + Z		Both
Copy to Clipboard	[Ctrl] + [C] ⌘ + C		Both
Add Measurement	[Alt] + [M] ⌘ + M		Both
Sequence menu			
Play Forward	[Ctrl] + [Space] ⌘ + [Space]		Both
Play Reverse	[Ctrl] + [Bksp] ⌘ + [Bksp]		Both
Stop Play/Resume Play	[Enter]		Both
Step Forward	[Space]		Both
Step Back	[Backspace]		Both

(cont.)

Command	Keystroke or F-key	Toolbar Button	Windows and/or Mac
View menu			
Fit to Window	[Ctrl] + [F] ⌘ + F		Both
Zoom	[Ctrl] + [Z]		Both (Macs – button only)
Show Gridlines	[Ctrl] + [G] ⌘ + M		Both
Show Pan Window	[Ctrl] + [W]		Both (Macs – button only)
Image Date/Tme	[Ctrl] + [T] ⌘ + T		Both
Full Screen	[F 7]	Full Screen	Both
Camera menu			
Live Image	[F11]	Live	Both
Get Image	[F9]		Both
Compute White Balance Values	[Ctrl] + [F10]		Both
Setup menu			
Show Image Settings		Image Settings	Both

Appendix D: Selected Error Messages

In the following table, unless indicated, the error messages apply to all SPOT camera models and to both Windows and Mac operating systems.

Error Message	Cause(s) and Solution(s)
A file could not be found (Mac)	Something went wrong in a file operation, usually due to a disk error. *Reboot the computer.*
At Least One Exposure time must be specified	You defined your image setup as "User-Defined" (see Ch. 5), but did not enter exposure times. *Enter the exposure times or use the Compute Exposure option to have the camera compute exposure values for you. If you have captured an image previously, you can use the Recall Previous Exposure option.*
Camera Error	There is damage to the camera head or the shutter is malfunctioning. *Contact Diagnostic Instruments.*
Error deleting file (Mac)	Something went wrong in a file operation, usually due to a disk error. *Reboot the computer.*
Error modifying registry	Same as above
Error reading camera information (RT cameras only)	There is damage to the camera head, problems with the interface card, or a bad connection cable *Contact Diagnostic Instruments.*
Error reading file (Mac)	Something went wrong in a file operation, usually due to a disk error. *Reboot the computer.*
Error writing file (Mac)	Something went wrong in a file operation, usually due to a disk error. *Reboot the computer.*
Exposure time too short for camera	The brightness of the image requires an exposure time that is too short for the camera. *Add a neutral density filter to dim the image without changing the color balance.*
Flatfield file is incompatible with image setup	The pixel bit depth (bpp) of your image setup does not match the pixel bit depth of the flatfield file (e.g., the image setup is 8 bpp and the flatfield file is 24 bpp). *Modify either the image setup or the flatfield file so that the two match.*
Images have different bit depths	The images that you are trying to merge, add, or subtract have different pixel bit depths. *Change one of the images so that their pixel bit depths are the same.*

Images must have the same dimensions	The images that you are trying to merge, add, or subtract are two different sizes. This may be because you cropped or binned an image for viewing purposes. *Ensure that the two images have the same dimensions. You might simply use un-cropped/non-binned versions of the images.*
Image too dim	There is not enough light to capture the image. This error message appears for various reasons, so you might have to experiment to increase the light. Possible solutions include: • *Adjusting the microscope settings to let in more light* • *Binning the image (see Ch. 5) to increase camera sensitivity* • *For RT Slider users, sliding the color filter to the B/W position*
JPEG Quality value must be between 1 and 100.	When saving an image, or defining database (Ch. 9) or sequential image (Ch. 6) preferences, you entered a value less than one or greater than 100 (for a JPEG or TIFF JPEG format image). *Re-enter a value between one (highest compression, lowest quality) and 100 (lowest compression, highest quality).*
Memory is getting full. Please try to alleviate the problem by closing some documents. (Mac)	The system is running low on memory. *Increase the memory allocation for the SPOT program.*
No response from the camera. Make sure the camera is connected and the power is on ***(when you first try to open the SPOT program)***	This message appears for various reasons. The most typical are: • The power supply is not turned on. • The PCI board is not seated properly. • A cable is loose. *Turn on the power supply, ensure that the PCI card is properly seated, or ensure that the cables are firmly connected to the camera/computer/power supply. Refer to the* Installation Guidelines *section of the manual for details on installing the PCI card and connecting the camera to the microscope and computer.*
Not a supported bit depth image	You tried to open an image with a pixel bit depth that is not supported by the SPOT software. *Using another imaging program change the pixel bit depth of the image to one of the four depths that the SPOT program supports: 8, 12, 16, 24, 36, or 48. Note that while 16 and 48 bit per pixel images can be opened by the SPOT program, they are treated as 12 bit per channel images.*
Out of Memory (Mac)	The system is running low on memory. *Increase the memory allocation for the SPOT program.*

Place the color filter in the 'Color' position **Place the color filter in the 'B/W' position** *(RT Slider only)*	These messages appear when you are trying to capture an image and the position of the sliding color filter does not match the image setup filter definitions. For example, you will see this message if you selected the Use Color Filter option for the image setup, but the sliding filter is out (in the B/W position). Note that you will see this message only if the **Monitor Camera Color Filter Preferences** option is selected on the Preferences window.The camera will not allow you to capture an image without correcting this discrepancy. *Either move the filter to the correct position, or change the image setup definition to match the position of the color filter. Alternatively, you can deselect the Monitor Camera Color Filter Preferences option and the program will not prompt you with error messages.*
Printer Error	The printer software is not installed. In order for the SPOT program to work correctly, you must have a print driver installed on your system. The printer does not necessarily need to be attached to the computer, but the drivers need to be installed. *Install a printer driver – follow the manufacturer's instructions for installing the printer that you plan to use.*
Software is incompatible with camera hardware	The causes of and solutions to this error message, which appears when you try to run the SPOT program for the first time, vary according to the camera model: • For both RT and non-RT cameras, this message appears if, during the installation process, you specified the wrong interface card for the camera. *Reinstall the SPOT software and specify the correct interface card. Refer to the* Installing the Software *section of the Installation Guidelines chapter for more details.* • For non-RT cameras, this error message indicates that the chipinfo.dat file is from an earlier version of the SPOT program. *Contact Diagnostic Instruments and request an updated chipinfo.dat file.*
Spot cannot be launched due to a disk or permissions error (Mac)	The launcher failed to write a necessary file in the preferences folder. *Reboot the computer. If the problem persists, run Disk First Aid, located in the Utilities folder of the boot drive.*
Spot cannot be launched, because the application cannot be located. (Mac)	The SPOT application must be in the same folder as the copy of SPOT the user is trying to start. *Move the application file (SPOT 3_5) into the proper folder file.*
Spot cannot run in the Classic environment under OS X. (Mac)	This message appears if you try to run the OS 9 version of the SPOT v3.5 software (either the full application or the 3rd Party Interface) in the Mac OS X environment. SPOT closes immediately following the display of this error message. *Re-open the SPOT software in Mac OS 9.*
The Spot Prefs folder is missing. (Mac)	The folder, which is needed to run the program, is missing. *Run the SPOT 3.5 application (i.e., double-click on it) to create the folder.*

This version of Spot only runs on OS X. (Mac)	This message appears when you try to open the Carbon (OS X) version of the SPOT v3.5 software (either the full application or the 3rd Party Interface) in Mac OS 9. SPOT closes immediately following the display of this error message.
	Re-open the SPOT software in Mac OS X.

Video driver is not set to true color mode (millions of colors). Displayed images may not appear with the correct colors.	The video driver for your graphics card is not set to True Color mode.
	
	From the Windows Control Panel, change the graphics card settings to True Color (24 bit) or True Color (32 bit): Select
	        Start>Settings>Control Panel>Display>Settings>
	        Color Palette (Win 95/NT) or **Colors (Win 98)**
	and choose **True Color (Win NT only**), **True Color (24 bit)** or **True Color (32 bit)** *from the drop-down list.*

Index

Image Rotate, 112
Image Type, 106
Live Image Maximum, 113
Minimum Exposure, 113
Noise Filter, 113
Image Settings (TWAIN mode)
Brightness, 92
Color Enhancement, 97
Image Flip, 98
Live Image Maximum, 99
Noise Filter, 99
Image Settings (TWAINmode)
Exposure Time, 93
Gain, 93
Gamma, 93
Image Rotate, 98
Image Type, 92
Minimum Exposure, 98
Quality/Speed, 92
Image Type
selecting, 35
selecting (in the 3rd Party Interface), 106
selecting (in TWAIN mode), 92
images
annotating, 63
arranging for printing, 84
compressing, 79
flipping horizontally, 58
flipping vertically, 58
previewing in real time, 44
rotating, 58
saving automatically, 29
Initialize Camera, 43
installation
hardware, xviii
multiple SPOT cameras on one computer, xx
PCI board, xviii
SPOT software, xv
on a Mac, xvii
on Windows 95/98/NT/2000/ME, xv
interface
3rd Party, 102
TWAIN, 86

J

JPEG Quality, 79

L

light sources, xi, xiii, 40, 98, 113
lightening dark areas, 60
Live Image Controls
Exposure options
Exposure Time, 37, 108
Gain, 37, 108
Quality/Speed, 107
Live Image Maximum, 41, 90, 99, 105, 113
Live Image mode
and different light sources, 40, 98, 113
video card related problems, xv
live imaging, 44

different light sources, xi, xiii
low contrast image captures
halo effect on, 38, 96, 111

M

Mac OS
installing the SPOT software on, xvii
magnified images
navigating, 53
manual
introduction to, v
organization of, vi
Match Color, 60
3rd Party Interface, 109
TWAIN mode, 95
measurements
adding, 62
types of, 63
measuring specimens, 62
Memory Usage Limit for image sequences, 30
memos
associating with an image, 70
menus, 23
Edit, 57
Sequence, 55
View, 52
microscope
connecting the SPOT camera to, xix
microscopy techniques, 35, 92, 106
monitors, xi
Mac, xii
Move Backward
annotation mode, 69
Move Forward
annotation mode, 69

N

noise, electrical and thermal
reducing in images, 41, 99, 113

O

objects
report, 75
on-line documentation, xx
open
image files, 25
operating systems
Mac, xii
Windows, xi

P

Paste
annotation mode, 69
PCI board
installing, xviii
PDF documentation, xx
photography

Q

R

S

T

taking a picture, 43
  first time instructions, xxi
techniques
  microscopy, 35, 92, 106
  white balance, 45
  white balance (3rd Party Interface), 108
  white balance (TWAIN mode), 94
theory
  digital imaging, 115
thumbnails
  defining preferences for, 31
  displaying as previews, 25
time lapse photography, 47
toolbar, 24
TWAIN interface
  establishing, 86

U

Undo
  annotation mode, 69

V

video cards
  Mac, xii

problems with in Windows, xv
  Windows, x
view full screen, 54
View menu options, 52
viewing and editing window, 23

W

wells on CCD chip, 115
white balance
  techniques, 45, 94
  techniques (3rd Party Interface), 108
White Balance, 45, 94
  3rd Party Interface, 108
Windows 95 related errors, xvi
Windows 95/98/NT/2000/ME operating systems
  installing the SPOT software on, xv
Windows XP, xi

Z

zoom options
  Zoom In/Out, 52
  Zoom Level, 53
zoomed images
  navigating around, 53
zoomed images annotating, 53