



Correction of CCD Image Imperfections

When you capture a digital image, there are imperfections in the image due to the practical limitations of the hardware used to capture the image. These imperfections become more significant the dimmer the images become. Scientific grade digital cameras reduce these imperfections through a combination of careful hardware design and software correction of known, reproducible image imperfections. Careful hardware design seeks to reduce imperfections at the source, by cooling the CCD, and by the use of advanced microchips and highly refined circuit designs. Software is then used to characterize the remaining image imperfections and remove them from the image. In this discussion we will explore how imperfections are scientifically accounted for and removed, with the goal of providing an image that is the closest match to the specimen.

Correlated Noise vs. Random Noise

The imperfections in the image data are often called noise. Noise is the difference between the measured data and what the actual data measurement should have been. There are two types of noise: random noise and correlated noise. Random noise cannot be predicted from one image to another. Correlated noise can be predicted and compensated for if the correlation factor can be identified, quantified, and tracked. The measured value for any pixel can be represented as the following equation:

$$\text{Measured value} = (\text{Conversion Factor}) * \text{Actual Intensity Value} + \text{Noise Value \#1} + \text{Noise Value \#2} + \dots$$

Fig. 1: Measured Value Equation

The Bias Correction: Noise Value #1

When a short exposure image is taken with the camera's lens cap on (note: any picture with the lens cap on is called a dark frame), one might expect a reading of zero at every pixel location. However, pixel values of zero are avoided in cameras used for scientific measurement. This is because, in addition to reading a zero pixel value as zero, digital cameras will read any negative pixel value as zero. The resulting uncertainty over what a zero value actually is makes a pixel value of zero not useful for scientific analysis. Instead, the offset (the zero level) of the camera is deliberately set to some number above zero. That way, even if random noise pushes a pixel value below the offset level, the camera still reads it as a known value above zero. To avoid this uncertainty over what a zero value is, scientific cameras start off with all pixel values above zero, even with no light reaching the CCD and even with short exposure times. This offset value is the same for all pixels.

In addition to this uniform offset value, there is another effect that happens on short exposure dark frames. The last pixel read off of the CCD will have a larger value than one that was read off first. This is because reading the pixels off of the CCD takes a certain amount of time. While the pixels are waiting to be read off of the CCD, they are accumulating dark current. Dark current is the accumulation of electrons in a pixel due to thermal action. The rate that each pixel accumulates electrons is constant, depending only on the temperature of the CCD and the impurities in the pixel. This effect, called "shading," makes the last portion of the CCD to be read appear "brighter" than the first, even though no light has reached the CCD.

When a researcher takes an image, bright areas in the image will cause electrons to accumulate in the corresponding pixels. As the CCD is read out, each pixel will contain a combination of electrons created by the bright parts of the image and thermal electrons that have accumulated while the pixel was waiting to be read off of the CCD. The final pixel value, after its electrons have been converted to a number, is a combination of three effects: image brightness, "shading" and camera offset value.

It is common for scientific researchers to subtract the effects added by the camera (shading and offset) from their images in order to isolate just the image portion of the data. They do this by subtracting an image consisting of the offset and shading only. This image is called a "bias frame." A bias frame is just a short exposure dark frame image.

The Dark Current Correction: Noise Value #2

Dark current is defined as the rate that electrons accumulate in each pixel due to thermal action. This rate depends on the temperature of the CCD and on the impurities in each pixel. Since dark current increases with CCD temperature, cameras designed for low light imaging will cool the CCD to minimize this effect. Unfortunately, the long exposures required to capture very dim images will cause enough electrons to accumulate in each pixel to significantly affect the image. Since each pixel has different impurities, each pixel will accumulate electrons at a different rate. This can cause a faint pattern to form on images taken with long exposure times.



Fig. 2 Accumulation of dark current on CCD (small area of 30 min. dark frame brightened 37 times for ease of viewing).

Even though this pattern is not due to light, it is difficult to determine which bright pixels were due to light and which were due to dark current by observing the final image. Luckily, for a given CCD temperature, the electrons due to dark current accumulate at a constant rate for each pixel. This means that the effect of dark current can be predicted and its effect subtracted from the captured image.

Researchers commonly use a “thermal frame” to subtract the effects of dark current from each captured image. To create a thermal frame, we first take a dark frame with an exposure time about as long as required for a typical dim image. This gives us an image that is a combination of the dark current effect plus the offset and shading effects that we talked about in the bias frame section. Subtracting a bias frame from our long exposure dark frame will leave us with an image consisting of just the effect of dark current. This is called the thermal frame. The value of each pixel in the thermal frame is a measure of its individual dark current. It is a measure of how many electrons accumulated in each pixel during the exposure time used for acquiring the thermal frame image. Since electrons accumulate in each pixel at a constant rate, the thermal frame can be scaled up or down to predict the number of electrons that will accumulate in each pixel for any exposure time. Subtracting a scaled thermal frame from any new image will remove the effects of dark current from that image.

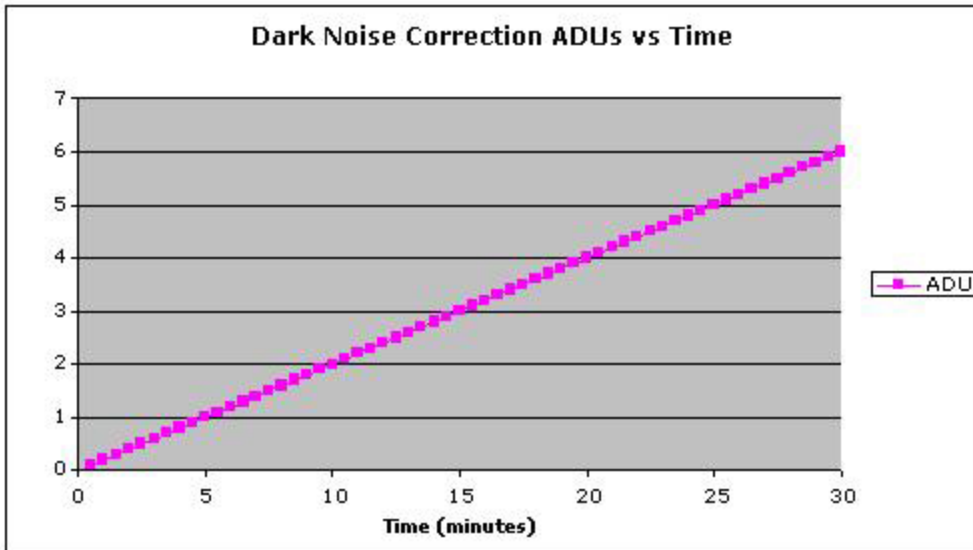


Fig. 3 Cooled quantitative camera dark noise correction factor vs. time

Diode Glow Correction: Noise Value #3

Some modern CCD's (namely Sony's ICX285) have on-chip circuitry that glows very faintly. This glow illuminates the pixels that are nearby, causing electrons to accumulate in each pixel at a constant and reproducible rate, dependent only on the location of the pixel in relation to the glowing circuit. The accumulation of electrons due to the glow is indistinguishable from the accumulation of electrons due to dark current. In fact, on these CCD's the combined effects of glow and dark current can be treated as one effect that can be corrected with one "glow + thermal" correction frame. This "glow + thermal" correction frame is acquired and applied to new images in the same manner as the thermal correction frame that was discussed in the previous (dark current correction) section.

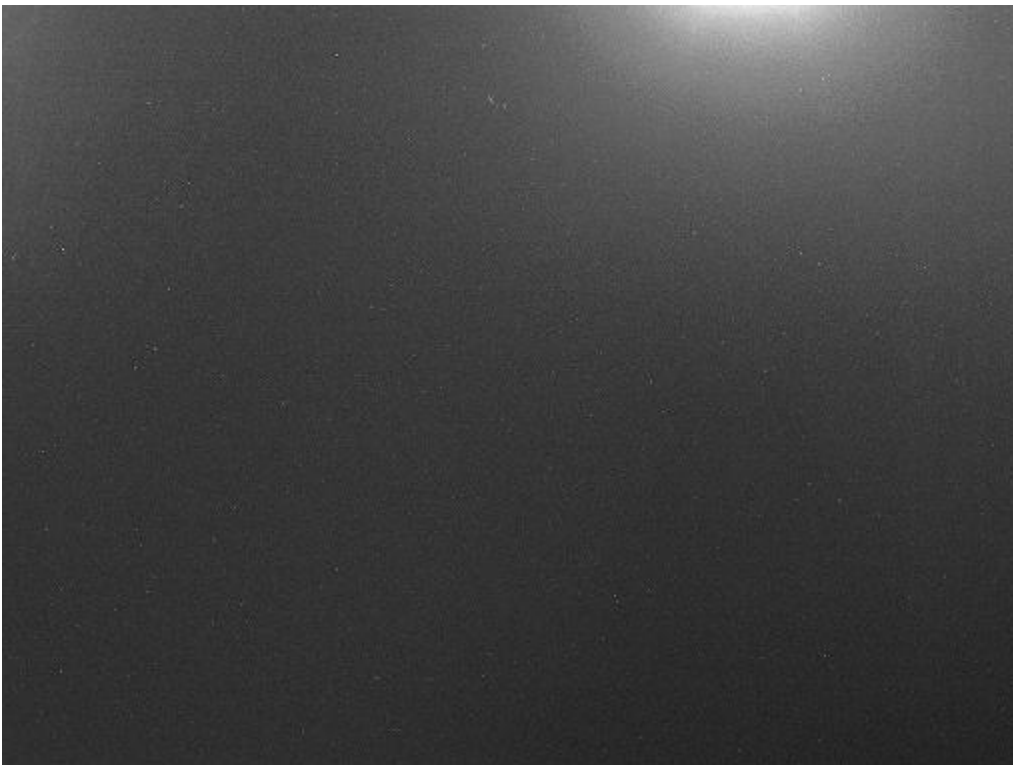
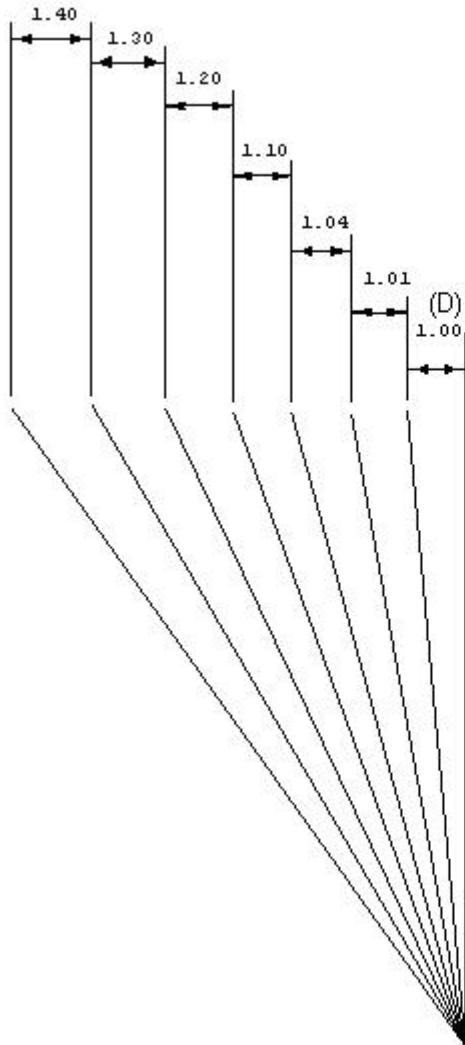


Fig. 4 Glow and accumulation of dark current on CCD (30 min. dark frame brightened 37 times for ease of viewing)

Flat Field Correction (Conversion Factor)

After the above corrections, you might expect that a captured image of an evenly illuminated field would have all values relatively equal (read noise and shot noise being the only variance). In fact, we would find that the center pixels would be brighter than the edge pixels, as well as, there is much more variance between adjacent pixels. What are the sources of these variances? The edge fall-off in brightness is a result of the optical properties of the illumination and lens systems. All lens systems have light ray spread that increases as the light rays are farther from the center. The light intensity (I) is inversely related to the area (D^2) the light rays occupy: $I \approx 1/D^2$. The diagram and table on the next page illustrates the increasing distance between equal 5° angle rays and the resulting calculation of light intensity fall off. It is remarkable that at the 35° angle ray the light intensity is 51% of the center ray. Note this assumes a plano first surface of the lens. Lens designs in the illuminator, condenser and objective will vary from this and are unique to each lens setup.



Light Intensity is related to the area and is proportional to the distance between rays: $I \propto 1/D^2$
Therefore at a distance D
the intensity is:

D	I
1.00	1.00
1.01	0.98
1.04	0.92
1.10	0.83
1.20	0.69
1.30	0.59
1.40	0.51

Fig. 5 Illustration of Light Ray Spread and the Resultant Light Intensity Fall-Off

What is the source of the adjacent pixel-to-pixel variation? Image sensors have inherent sensitivity variation from pixel to pixel equal up to $\pm 15\%$. These changes in intensity are proportional relationships; this means that the measured light intensity is just a percentage of the actual light intensity. It is the "Conversion Factor" in the "Measured Value" equation above. The conversion factor is measured for each pixel by taking a "Flat Field Correction Frame." For best results, a separate flat field frame should be taken for each objective magnification, since the optical setup changes with each magnification change.

Correction Frame Random Noise Component

Remember the random noise sources we mentioned at the start of this discussion. These noise sources result in unpredictable plus and minus offsets to the data from each pixel measured. To make our correction frames we have to capture data frames. If we subtract off a correction frame that has this random pixel to pixel offsets in it from a captured image with its own random pixel to pixel offsets, the random offsets in some instances will cancel and in some instances add to be twice as high or twice as low. We would find that the variation due to read noise and shot noise would be doubled in our images after the application of our correction frames. This is not a good result. So how can we predict values to compensate for them? We can't. Since they are random they should average to zero if multiple images are averaged together. That's why during the creation of these correction files you are asked how many images you want to use to create the file; the greater the number of images, the less the random noise component will be. The trade off is time during the creation of the correction frame versus the accuracy of the correction frame. As with any averaging process, at a certain number of images you reach a point of diminishing returns (see Chart).

Number of Frame Averages	Percentage of Original Random Noise ($1/\sqrt{n}$)
1	100.00%
2	70.70%
3	57.70%
4	50.00%
5	44.70%
10	31.60%
15	25.80%
20	22.40%
30	18.30%
40	15.80%
50	14.10%
75	11.50%
100	10.00%
200	7.10%
500	4.50%
1000	3.20%
10000	1.00%

Fig. 6 Random Noise Reduction Through Frame Averaging

Conclusion

From the discussion above, it can be seen that correction frames all share the same basic theory for correcting image data. Each correction frame is a measure of correlated noise signals. Each correction frame can be acquired and stored to allow future correction of the raw image data. Once the method and limitations of software correction factors are understood, individual researchers can confidently use these techniques to correct raw image data beyond the limits imposed by the camera hardware, providing a more accurate representation of the original image data.