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Bio Focus
Application Notes for Scientific Imaging & Analysis

CCD Cameras for Molecular Research

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Have you ever had questions about CCD cameras and been more confused by the answers than before you asked the questions? Don't feel alone! Many researchers are confused about camera technology and the application of CCD's in the laboratory. We hear from them daily and it is not surprising why. Scientific cameras have become more sophisticated than just a few short years ago and are being installed on everything! From gel documentation systems and chemiluminescent detection systems to microscopes, microarray scanners and in-vivo imaging systems, CCD cameras are sprouting up in every life science laboratory. This detailed, but "down to the basics" reference paper should help everyone better understand CCD cameras.

Analog versus Digital

All CCD chips are analog devices. If the signal is digitized off the camera and in a board installed in a PC, then the camera is considered to be an "analog" camera, even though the image produced in the PC is "digital". Digital cameras actually have the digitizer installed in the camera directly off the CCD in order to minimize electrical, or read-out noise. This improves the signal-to-noise ratio of the camera, increasing its dynamic range and maximum attainable gray scales.

Signal-to-Noise Ratio

Signal-to-noise ratio, or SNR, is the true test of a CCD camera's detection capability. All scientific CCD camera manufacturers attempt to maximize the signal (the number of available full well electrons) and minimize the noise (electrical and thermal) in order improve the camera's performance. This is simple math based on the following equation:

$$\text{SNR} = \frac{\text{Full Well Electrons}}{\text{Noise Electrons}}$$

$$= \text{Dynamic Range}$$

$$= \text{Maximum Gray Scales}$$

$$= 2^n\text{, where } n = \text{CCD bits}$$

CCD Chips

The majority of the CCD chips manufactured for use in scientific cameras are made by Sony or Kodak. Complete data, including performance curves, quantum efficiency and specifications, are available from most system suppliers, but are also available from the chip manufacturer's web site (see references). By getting the full well and noise electron data, the user can calculate the signal-to-noise ratio, dynamic range and maximum number of gray scales of the camera or the actual maximum bit depth of the camera.

CCD Noise

CCD cameras have come a very long way in last five years. Thermal, or dark current, noise has basically been eliminated with deeply-cooled thermoelectric peltier devices installed in cooled CCD cameras. CCD chips heat-up as exposure times exceed 5-10 seconds. Without cooling, "hot", or white, pixels begin to emerge as noise and start to cloud images beyond 10 seconds of integration. Cameras cooled 25 degrees C from ambient can overcome exposure times greater than 5 minutes without significant hot pixels appearing in the image. Cameras cooled to -35 degrees C can typically integrate, or acquire light signal without significant noise interference, in excess of one hour.

Additional improvements in the clocking, sampling and digitizing methods of CCD’s has reduced electrical read-out noise down to 4 to 5 electrons per photodiode, or pixel. This is nearing the practical limit of minimizing electrical noise and is very expensive to accomplish reliably. Therefore, in order increase signal-to-noise ratios, designers then look to the chip manufacturers to produce CCD’s with very high electron count pixels at an affordable price. The problem is ... more electrons equals more surface area on the chip equals more silicon equals much more expensive CCD chips. Therefore most scientific CCD's are either 1/2", 2/3" or 1" as these are cost effective sizes. This "size" is the diagonal measurement of the rectangular chip.

Optical Lenses

Another limitation on CCD chip size is the availability of optical lenses. Standard zoom and fixed CCTV lenses with c-mount adapters are only available up to 1" formats. This is not coincidental. They are made in these sizes to accommodate the available sizes of CCD chips and for economic reasons. Larger lenses increase in cost exponentially with size, very similar to CCD chips. In addition, lens manufacturers are becoming much more focused on the retail digital camera market and have retooled their production lines to mass produce these smaller lenses.

Pixel Size

Pixel size (or full well size) is also an issue on CCD chip selection for a camera. Bigger pixels are more sensitive to light because they can hold more electrons and produce more signal. This is very similar to a bucket and its ability to collect water. The larger the opening on the bucket, the more water it will collect. Bigger pixels, however, reduce the maximum number of pixels on 1/2", 2/3" or 1” chips, and affects the spatial resolution of the camera. This is the huge trade off ... large pixels for increased sensitivity or smaller pixels for maximum resolution.

16-Bit Cameras

True 16-bit cameras (those CCD's actually able to detect 65,536 levels of gray scale, or 2 to the 16th power) typically have very large pixels (10-25µm squared) with over 50,000 full well electrons and are usually deeply cooled (below -60°C). These cameras, true 16-bit cameras, are very expensive and are not required to detect chemiluminescent signals on membrane blots or surface fluorescence in gels, plates or live specimens. But true 16-bit cameras are required for detection of bioluminescent signals and fluorescent labels in plants and underneath the skin and in organs, glands and tumors of small animals.

But be careful! Unfortunately, there are companies in the scientific imaging business selling systems for chemiluminescent detection that profess to have “true” 16-bit CCD cameras, but really don’t. They are using 10 or 12-bit CCD’s and capturing 10 or 12-bit images, but are using inexpensive 16-bit A/D (analog to digital) converters in the camera, or are automatically converting the image file to a 16-bit format in software. This gives the user the impression that the system is capturing a 16-bit image, when in fact, it is not.

It is easy to determine whether or not the camera you are using or plan to purchase is 16-bit or not. As discussed earlier, divide the published number of full well, unbinned electrons in each pixel by the read noise. This is the signal-to-noise ratio, SNR, of the camera expressed as the maximum number of gray scales or maximum dynamic range of the camera. Then take the natural log, LN, of this number and divide by the LN(2) or 0.693.

This is the true bit depth of the camera.

$$\text{SNR} = \frac{\text{Full Well Electrons}}{\text{Noise Electrons}}$$

$$\text{CCD Camera Bit Depth} = \frac{\text{LN (SNR)}}{\text{LN (2)}} = \frac{\text{LN (SNR)}}{0.693}$$

There is another way to determine whether or not you have purchased or are considering the purchase of a "true" 16-bit CCD camera … the price! True 16-bit cameras start around $25,000 and can be in excess of $100,000 depending how they are packaged and what features are included. Once these cameras are packaged into systems with light tight enclosures, high speed optics, filters, excitation sources and software, the entire 16-bit system would cost a minimum of $60,000. So if you are considering a "16-bit" imaging system that costs anything less than $60,000, do your homework, get the specifications and do the math. You may looking at a system that digitizes images to 16-bit files, but it does not use a "true" 16-bit CCD camera.

CCD Calculations

By using the following equation, anybody can calculate the size of the chip, the number of pixels on the chip or the chip size by knowing any of the other parameters.

In general, the area of a CCD chip, A, is equal to the size of each pixel, L x W (in µm), multiplied times the number of pixels, N:

$$A = L \times W \times N$$

Since L= W, as pixels are essentially square, and converting units from µm to inches, the equation can be simplified to solve for the diagonal length of the chip, or the chips format in inches:

$$Z = \frac{\sqrt{4L^2N}}{25400}$$

Where Z is the actual diagonal size of the CCD chip in inches, L is the square side of the pixel in µm and N is the total number of pixels.

Binning

Combining charges from neighboring pixels to increase the CCD's signal-to-noise ratio and enhance sensitivity is creating "super pixels". This, again, uses the "bucket of water" analogy, the larger the pixel, the more light it can detect. Super pixels are created in software in formats of 2x2, 4x4 or 8x8, allows the collection light with a bigger "bucket". Because read-out noise is not additive from the use of these super pixels, the net result is a huge increase in the sensitivity of the camera from these large pixels. When utilizing super pixels to increase the sensitivity of detection, it is important to remember that resolution is reduced by the same binning factor.

Following are calculations that detail the four different cameras used in UltraLum's Omega Systems:

Omega 10gD

$$\text{SNR} = \frac{\underline{\text{9700 (full well electrons)}}}{\text{15 (noise electrons)}} = \text{647, therefore, Bits} = \frac{\text{LN (SNR)}}{\text{LN (2)}} = \frac{\text{LN (647)}}{0.693} = \text{9.3 Bits}$$

Thus, this camera is digitized to 10 bits

Omega 12iC

$$\text{SNR} = \frac{\text{15,000 (full well electrons)}}{\text{6 (noise electrons)}} = \text{2500, therefore, Bits} = \frac{\text{LN (SNR)}}{\text{LN (2)}} = \frac{\text{LN (2500)}}{0.693} = \text{11.3 Bits}$$

Thus, this camera is digitized to 12 bits

Omega 14vR

$$\text{SNR} = \frac{\text{40,000 (full well electrons)}}{\text{10 (noise electrons)}} = \text{4000, therefore, Bits} = \frac{\text{LN (SNR)}}{\text{LN (2)}} = \frac{\text{LN (4000)}}{0.693} = \text{12.0 Bits}$$

Thus, this camera is digitized to 14 bits

Omega 16vS

$$\text{SNR} = \frac{\text{100,000 (full well electrons)}}{\text{2 (noise electrons)}} = \text{50000, therefore, Bits} = \frac{\text{LN (SNR)}}{\text{LN (2)}} = \frac{\text{LN (50000)}}{0.693} = \text{15.6 Bits}$$

Thus, this camera is digitized to 16 bits

References

Sony CCD Sensors

http://www.sony.net/Products/SC-HP/pro/image_senser/progressive_scan.html

Kodak CCD Sensors

http://www.kodak.com/US/en/dpq/site/SENSORS/name/ISSInterlineProductFamily