



Focal Points

Application Note FP-114


Biolmaging Systems

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Understanding CCD Cameras

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 microscopes and spectrophotometers to gel documentation and chemiluminescent detection systems, CCD cameras are sprouting up in every life science laboratory. This detailed, but "down to the basics" article 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 to improve the camera's performance. This is simple math based on the following equation:

$$\begin{aligned} \text{SNR} &= \frac{\text{Full Well Electrons}}{\text{Noise Electrons}} \\ &= \text{Dynamic Range} \\ &= \text{Maximum Gray Scales} \\ &= 2^{(\text{CCD Bits})} \end{aligned}$$

CCD Chips

The majority of the CCD chips manufactured for use in scientific cameras are made by Sony, Kodak or SIT. Complete data, including performance curves 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 cloud images beyond 10 seconds of camera integration. Cameras cooled beyond -20 degrees C can overcome exposure times

greater than 5 minutes without significant hot pixels appearing in the image. Cameras cooled to -40 degrees C can integrate in excess of one hour.

Additional improvements in the clocking, sampling and digitizing methods of CCD's has reduced electrical read-out noise below 4 to 5 electrons per photodiode, or pixel. This may now be a ceiling, or point of diminishing return in terms of minimizing read-out noise. Therefore, in order to 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, means more surface area on the chip, means more silicon, means much more money. 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 available optical lenses. Standard zoom and fixed CCTV lenses with c-mount adapters are only available in 1/2", 2/3" and 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.

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 a 1/2", 2/3" or 1" chip 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 do have very large pixels (16-30µm squared). These cameras, however, are very expensive, extremely slow at processing image data and not required for capturing DNA or protein blots for genomic and proteomic research. 16-bit cameras have more practical application with professional astronomers for exploration of deep space. The reason is simple ... a true 16-bit CCD with 24µm pixels on a 1" format only has around 500,000 pixels. This is not enough resolution for detailed protein quantitation, microscopy or microarray detection.

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 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. These images may even appear to be high resolution images with millions of pixels. This is the first clue that the images are not 16-bit images and the system is not using a true 16-bit CCD camera.

CCD Calculations

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

$$Z = \frac{\sqrt{4LN}}{25400}$$

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.

Scan Rate

Scan rate of CCD cameras is also a very important consideration in selecting a camera for your application. This is the frame rate that a CCD is able to refresh an image. Most 8-bit video cameras are capable of 30 frames per second. This is considered "live video". The human eye can be trained to see the difference between 25 and 30 frames per second, but to many people, 20 frames per second still looks like live video.

Focusing any analog or digital camera at over 15 frames per second is generally considered acceptable. Focusing at less than 15 frames per second down to 2 or 3 frames per second can be very aggravating to most users. This is very common with 12, 14 and 16-bit cameras, as it is very difficult to refresh enormous amounts of digital data at a rate quick enough to resemble "live video". In addition, the faster the electronic charge of the chip is digitized, the more electronic read noise is generated. This reduces the signal-to-noise ratio of the camera and, generally results in very poor quality images. This is the "toll" that is paid for reading the stored charge to quickly. While everybody wants fast scan rates for focusing and fluorescent imaging, the moral is, faster is not necessarily better in digital camera technology.

Therefore, as a result of this limitation, some companies in the Gel Documentation Biolmaging business try to offer two different systems to accomplish fluorescence and chemiluminescence on the same lab bench. One system with an 8-bit analog CCD with live video for visible light and fluorescence and another system with a sensitive, but extremely slow CCD for chemiluminescence. Even though these may be offered at a very attractive price, this two system scenario may require two computers, two monitors, two image acquisition boards, two copies of software, two cameras and two enclosures. This requires a tremendous amount of bench space and a lot of electrical components and is not necessary.

BioChemi System

Systems do exist in this marketplace that have been designed to accomplish fluorescent and chemiluminescent imaging with one,

fast scanning, high resolution, extremely sensitive digital cooled CCD camera. One perfect example of this type of system is the BioChemi System from UVP. The BioChemi System was developed for fluorescent, chemiluminescent and visible light detection with a priority on maximizing signal-to-noise ratio, resolution, processing speed and economics. The BioChemi camera is a deeply cooled digital 2/3" CCD with a high resolution 1.3 million pixel array. It utilizes high frequency electronic data transfer for up to 30 frames per second scan rate. The BioChemi camera also allows 12-bit, 14-bit or 16-bit acquisition modes through the use of "super pixels". This enables the user to select the 16-bit high sensitivity mode for chemiluminescence detection, the 12-bit high resolution mode for detailed quantitation, or the 14-bit high performance mode for the best of both worlds.

Binning

Super pixels are an advanced binning technology that combines the charge from neighboring pixels to increase the CCD's signal-to-noise ratio and enhance sensitivity. This, again, uses the "bucket of water" analogy. Combining neighboring pixels into super pixel 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 three different acquisition modes for the BioChemi camera:

$$\text{Signal to Noise Ratio} = \frac{\text{Dynamic Range}}{\text{Full Well Electrons}} = \frac{\text{Max Gray Scales from CCD}}{\text{Noise Electrons}}$$

$$\text{CCD Camera Bit Depth} = \frac{\log(\text{Maximum Gray Scales from CCD})}{\log 2}$$

12-Bit Acquisition Mode

1.3M pixel cooled CCD Camera with 18,000 full well electrons, 4 electrons of read-out noise and binned 1x1

$$\text{SNR} = \frac{18,000}{4} = 4,500$$

CCD Bit Depth = log 4,500/log 2 = 12.1 = 12 bits of camera depth (at full CCD resolution or 1.3M pixels)

14-Bit Acquisition Mode

1.3M pixel cooled CCD Camera with 18,000 full well electrons and 4 electrons of read-out noise and binned 2x2

$$\text{SNR} = \frac{18,000 \times (2 \times 2)}{4} = 18,000$$

CCD Bit Depth = log 18,000/log 2 = 14.2 = 14 bits of camera depth (at 1/4 CCD resolution or 325K pixels)

16-Bit Acquisition Mode

1.3M pixel cooled CCD Camera with 18,000 full well electrons and 4 electrons of read-out noise and binned 4x4

$$\text{SNR} = \frac{18,000 \times (4 \times 4)}{4} = 72,000$$

CCD Bit Depth = log 72,000/log 2 = 16.2 = 16 bits of camera depth (at 1/16 CCD resolution or 81K pixels)

References

Sony CCD Sensors [Sony Semiconductor - Product Guide/CCD Area Sensor](#)
Kodak CCD Sensors [KODAK Product Catalog: Solid State Image Sensors](#)