A Guide to CCD Camera Parameters: Applications for Gel and Membrane Imaging

Introduction

Whether you're investing in your first digital imaging system for gel and membrane imaging, upgrading existing equipment, or just going with what's already installed in your laboratory, you'll need to understand key technical specifications to get the most out of your imager. Camera resolution and bit depth, such as 8, 10, 12, 14 or 16 bit, are commonly considered factors that influence the linear dynamic range and quality of digital images. However, lesser-known factors can also significantly contribute to the real data dynamic range and image quality obtained by a digital imaging system.

ProteinSimple (formerly Alpha Innotech and Cell Biosciences) has been developing and innovating charge-coupled device (CCD) imaging systems for over 20 years, and our in-house team in CCD technology has an expert understanding of the gel-, plate- and membrane-based biological assays for which imaging products are used.

In this technical note, we discuss the features and terminology of CCD technology relevant to biological imaging, enabling you to understand how the components of the entire system can contribute to performance.

CCD TECHNOLOGY SPECIFICATIONS THAT AFFECT PERFORMANCE

Introduction to CCD Technology

A CCD sensor (**Figure 1**) consists of a silicon chip containing photon-collecting regions called pixels. Each pixel acts as a well or bucket, collecting photons and converting the photons into an electrical signal.

Pixel resolution and size of a CCD sensor

Each CCD sensor has a vertical and horizontal array of pixels that determine the overall resolution achievable. The CCD sensor size is reported as the diagonal measurement of the sensor, typically referred to as 1/2", 1/3", 2/3", 1" or larger chip size formats. Fundamentally, increasing pixel number for a given sensor size results in higher image resolution, assuming appropriate optics are used for the size of the CCD sensor. For a given pixel size, increasing the pixel number will result in a larger chip format. Assuming appropriate optics, the image resolution is only related to the number of total pixels in the CCD array. Image resolution is important for applications in which bands or spots of interest are spatially close to each other. The size of the pixel itself, usually expressed in microns, can affect overall dynamic range. Reducing pixel size can significantly reduce the well capacity of a pixel. ProteinSimple's FluorChem™ CCD sensors have pixel sizes up to 7.4 µm x 7.4 µm.

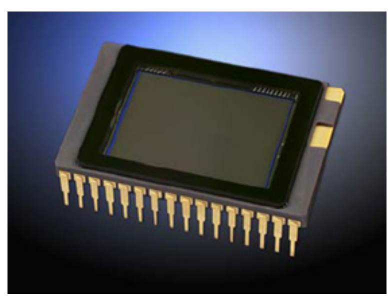

FIGURE 1. A CCD chip from a digital imaging system. This chip contains 8,328,304 pixels. Within the pixels, accumulated photons are converted to an electric signal, which is carried to the analog-to-digital converter (not shown) by electronics built into the chip.

Pixel well capacity

Photons are converted into electrons through interaction with the crystalline silicon structure, and those electrons accumulate in the pixel, a localized region defined by voltage profiles generated by the controlling electronics. Well capacity is the limit on the number of electrons a pixel can hold, and the well capacity value is set by pixel geometry and controlling electronic structures. When excess electrons accumulate and overwhelm the capacity of the voltage profile, the excess electrons spill into neighboring pixels and cause a smearing, or blooming, artifact. Well capacity is expressed in units of electrons and is a significant contributor to the total dynamic range of the CCD sensor. Typically, larger pixels have larger well capacities.

Quantum efficiency curve of the CCD sensor

Every CCD sensor has a quantum efficiency (QE) curve that characterizes the ability of photons to generate the electrons necessary to produce a detectable signal. QE is expressed as the percentage of photons converted into the electron signal. The QE is usually expressed in CCD literature as one fixed percentage. QE values are actually unique at every wavelength, and, therefore, are better expressed as a range of values. QE values represent the percentage of light that hits the CCD sensor and is converted to signal; to obtain optimal QE values, it is important to select the correct lens technology that will transmit the most light to the CCD chip.

Microlens technology

Microlens technology serves to focus and concentrate light onto the photodiode surface of the pixel instead of allowing it to fall on nonphotosensitive areas of the CCD (**Figure 2**). Incident photons that strike the microlens are directed onto the photodiode by refraction through the glass or polymer microlens. A photodiode without a microlens collects a significantly lower portion of incoming photons. FluorChem CCD chips employ this microlens technology.

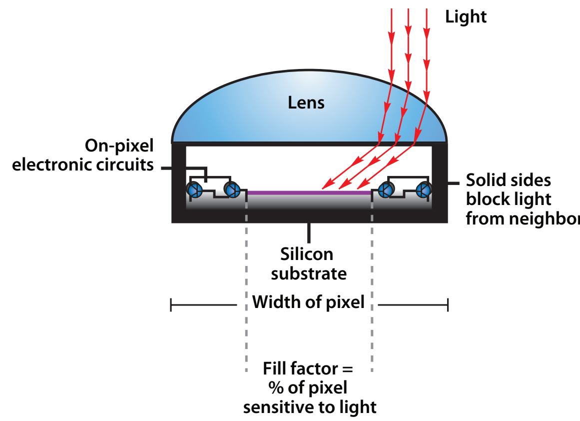

FIGURE 2. Increased efficiency of light collection with microlens technology. The microlens, which is a component of the CCD chip, is placed over the pixel and covers the non-light reactive surface surrounding the pixel as well. Light that would normally fall on the non-reactive surface is directed into the pixel where it is detected.

Pixel binning

The mechanism by which the electron charge is transferred from a CCD lends itself to a process called on-chip pixel binning. Most CCDs can combine multiple pixel charges in both the horizontal and vertical direction to form a single larger charge or superpixel. This super pixel represents the area of all the individual pixels contributing to the charge. This is referred to as binning. A binning of 1x1 means that the signal arises from an individual pixel. A binning of 2x2 means that four adjacent pixels have been combined into one larger pixel, increasing the area and the sensitivity to light by a factor of four (**Figure 3**). While this can reduce exposure times and increase sensitivity, the resolution of the image will decrease. **Figure 4** shows how speed increases and resolution decreases as a product of binning.

Pixel binning can be thought of as a trade-off of resolution for the speed of image acquisition. Because pixel binning reduces resolution, it is important to start with a high-resolution camera, so even when binning, the images produced are of high enough resolution for publication.

Binning is an optimal tool for low-light applications like chemiluminescence, which typically require long exposure times. All members of the FluorChem family of instruments are equipped with several different binning options, allowing you to determine the optimal speed and resolution for the image, thus saving time by increasing the sensitivity of the camera.

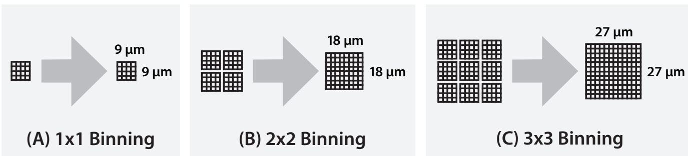

FIGURE 3. A schematic depicting the mechanism of pixel binning. In 1x1 binning, one pixel is treated as one pixel (A). In 2x2 binning, four pixels are treated as one pixel in order to increase sensitivity and decrease the time for image acquisition, while resolution decreases by a factor of four (B). In 3x3 binning, nine pixels are combined to make one pixel, and resolution decrease by a factor of nine (C).

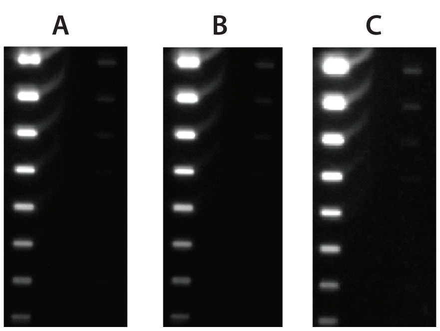

FIGURE 4. The effect of binning on speed and resolution. All three images are of the same slot blot, and all three have been contrasted to the same black and white levels. A five-minute exposure at 1x1 binning (A), a 30-second image at 3x3 binning (B) and a 10-second exposure at 8x8 binning (C). Note the similarity in sensitivity with a substantial decrease in imaging time afforded by increased binning. The slight pixelation visible in C demonstrates the reduced resolution that results from higher binning modes.

Error and noise

The dynamic range of the CCD imaging system is determined by the ratio of signal-to-noise. Undesirable signal components found in every electronic system are called noise. Different types of noise can affect a CCD imaging system and reduce its total dynamic range. A high signal-to-noise ratio is particularly important in applications requiring precise light measurements.

When calculating the overall signal-to-noise ratio, all noise sources must be considered. The three primary sources of noise in a CCD imaging system are photon noise, read noise and dark noise.

Photon statistics

The noise associated with counting photons is proportional to the square root of the number of photons counted. This is a physical property of the nature of light and cannot be overcome by any amount of advanced electronics. It has been called a variety of names like photon shot noise, photon noise or photon statistics, but regardless of the name, the results are the same—counting photons is noisy. This places a physical limit on the real data dynamic range for a given pixel.

Read noise of the CCD sensor

The act of reading the voltage present at the output node has an associated noise level independent of the actual electron load present, including when there are no electrons present. This is called the read noise and is a fundamental limit on the dynamic range of the CCD. Because read noise is a camera-specific noise source, most camera manufacturers specify the dynamic range of a camera with respect to this read noise. The read noise is the minimal noise level that a CCD camera can achieve, and the signal must be higher than the read noise for it to be detected.

Dark noise, bias and camera cooling

A CCD cannot distinguish electrons generated by photons from those generated by heat. A CCD always generates electrons from heat at a constant rate, called the dark current, and long exposure times allow more of these heat, or thermal, electrons to be captured in a pixel well. This adds an offset error—a systematic error—to the image, as well as some random noise to the signal itself. This dark noise can be minimized by cooling the CCD to very low temperatures (**Figure 5**). The amount of dark current depends strongly on the temperature, and for about every 6 °C the CCD is cooled, dark current is halved. When the CCD is cooled to at least -25 °C, much longer integration times can be applied. This enhances the sensitivity of the camera dramatically. The FluorChem E, M and R cameras are maintained at -30 °C, where the dark noise is reduced to less than one electron per pixel every ten seconds.

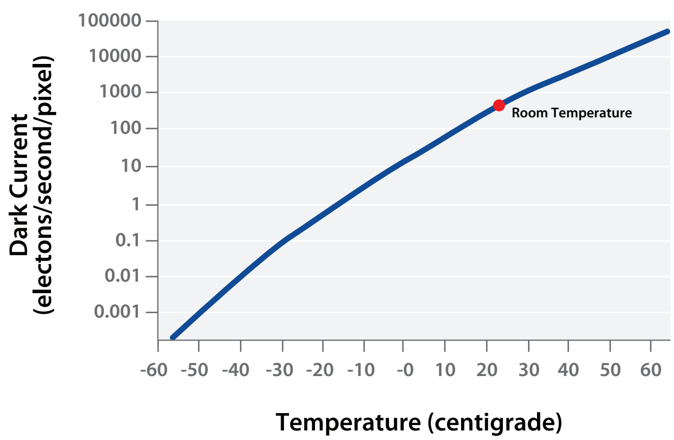

FIGURE 5. The effect of cooling on dark noise. For every 6 °C decrease in temperature, there is a resulting two-fold reduction in the dark noise.

While cooled cameras have reduced dark current, dark current is still present. The offset error generated by the dark current can be removed through dark field or dark master correction. These data-distorting signals are mapped and subtracted in the FluorChem imaging system using ProteinSimple's proprietary dark field or Dark Master file correction process. The Dark Master files, used for correcting the temperature noise, are created by conducting long exposures under dark conditions and at different binning settings, the results are then combined into a single image containing only the dark signal. Dark field images are then scaled according to the particular exposure time, subtracted from each image acquired with the FluorChem instrument. These images are a unique product of each FluorChem camera.

The bias level of a CCD frame is an artificially induced electronic offset, that ensures that the analog-to-digital (A/D) converter always receives a positive signal in order to reduce digitization errors. All CCD data have such an offset which must be removed if the data values are to be truly representative of the counts recorded per pixel. ProteinSimple's Bias Correction process, which runs in parallel with the Dark Master file correction process, corrects for the bias levels unique to every camera. Dark field and Bias field correction are possible due to the strict temperature regulation of the cooled CCD camera in the FluorChem instrument. Without precise maintenance of the temperature, dark field and bias corrections would not be accurate due to temperature fluctuations.

A/D converter and bit depth

Every CCD camera uses an A/D converter to transform inherent variable charges in the CCD into the binary data that represents the pixels. The higher the bit depth of the A/D converter (10 bit, 12 bit, 16 bit, etc.), the more accurate the analog-to-digital conversion will be because the analog signal will be sampled more frequently. This will make it easier to distinguish even slight differences in intensity values. While the bit depth of the A/D converter is frequently used as a description of the dynamic range of the camera, it really only describes the dynamic range of the A/D converter and can limit the dynamic range of the CCD chip itself if it has a smaller dynamic range than the CCD chip.

Total noise and dynamic range

The dynamic range of the CCD, defined as the signal-to-noise ratio, determines the range over which a camera can simultaneously record very low light signals and very bright signals. The dynamic range of a CCD is also the range over which the camera gives a linear response to signal (**Figure 6**).

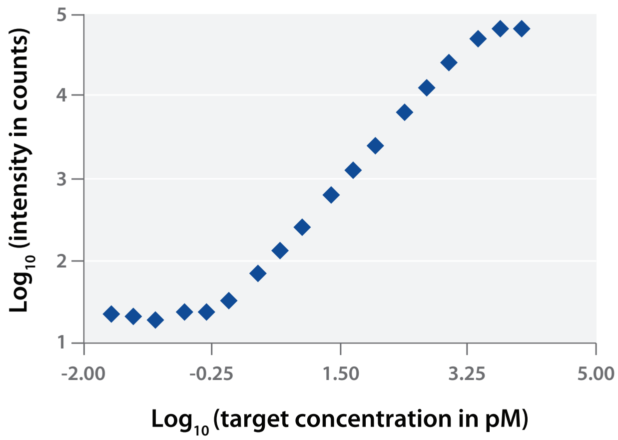

FIGURE 6. An illustration of the linear dynamic range of a digital camera. This plot depicts the response of the camera to signal. The response is linear over the range of 0.57 pM to 1800 pM of the target.

Capturing images within the dynamic range of the CCD is extremely important for downstream quantitation of the image. Quantitation cannot be performed on an image that is saturated, as there is not a linear response of the camera to light in the saturated range.

One common way to estimate the dynamic range of a camera is by dividing the well capacity, or signal, by the read noise, or noise. However, dynamic range is frequently described in other units. The following formulas can be used to convert one unit of dynamic range to another:

Signal to noise = number of grayscales

Bits = $\log_2$ grayscales

Optical density (OD) = $\log_{10}$ grayscales

Decibels (dB) = 20 x optical density

For a CCD camera with a full well capacity of 40,000 electrons and a read noise of eight electrons:

full well capacity/read noise = 40,000 e-/8 e- = 5,000 grayscales

bits = $\log_2$ 5,000 = 12.3 bits

OD = log 5,000 = 3.7 OD

dB = 20 x 3.7 = 74 dB

The most accurate method for determining the dynamic range of a camera requires the use of a calibrated light source to measure the range over which a camera gives a linear response to the signal. **Figure 7** shows an image capture of a calibrated light source. The camera is linear over an OD of 3.8 or a linear range of 6,200 to 1. If we estimate the dynamic range from the full well capacity and the read noise as calculated above, we would get a dynamic range of 3.7 OD.

Frame rate/scan rate and image refresh rate

One feature of the CCD camera often overlooked in the discussion of camera specifications is the image refresh rate. Several factors related to signal processing, camera electronics, and driving software architecture determine the frame rate and image refresh rate, commonly referred to as frames per second or FPS. Low FPS values mean there will be a lag between the image the camera captures and what is displayed on the computer screen. This value is especially important when focusing on an image. A slow refresh rate will increase the amount of time it takes to focus on an image. A minimum frame rate of 3 FPS is required for ease of use.

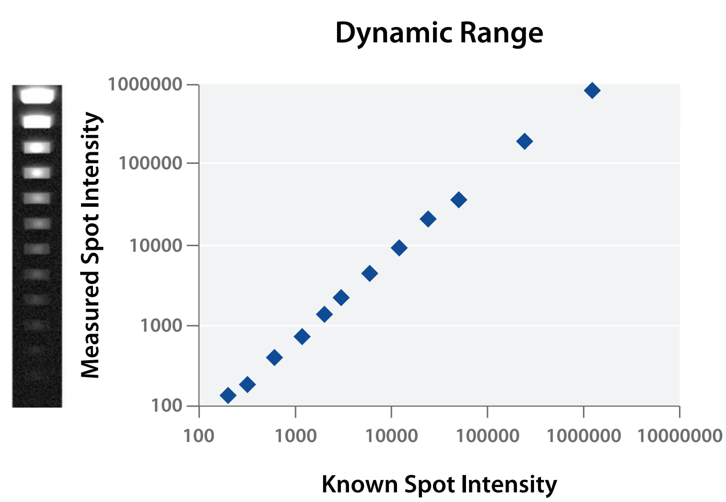

FIGURE 7. Use of a calibrated light source to measure the actual dynamic range of a CCD camera. The calculated dynamic range of this camera, based on pixel depth and read noise, is 3.7 OD. Measuring the response of the camera to a known, calibrated light source demonstrates that the camera has a dynamic range of 3.8 OD or 1 to 6,200.

High-quality, high-performance optics

Although not part of the CCD camera, optics are as important as the selection of the CCD sensor itself. The lens is responsible for focusing the image onto the CCD chip and transmitting the photons from the sample to the CCD. The CCD and the optics must be matched in several specifications for correct operation and reasonable performance.

Conclusion

Image quality with a CCD camera depends on the interaction of many parameters to produce high-quality images. When selecting a CCD camera for your research, it is important to avoid focusing on one specification alone, because, in isolation, that single parameter cannot guarantee quality optical imaging. For example, if a camera has a high QE but does not employ microlens technology, the CCD will lose the photon data that falls on the non-photoreactive areas of the pixels. If a camera has a high well capacity in addition to a high read noise, the dynamic range advantages of the larger well capacity will not be realized. If a camera is deeply cooled but does not regulate its cooling, any dark correction files made will most likely be inaccurate.

FluorChem imagers are developed with all of these factors in mind, resulting in systems that generate high-resolution, high-quality images to ensure you get the best data from all your applications.

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