White Paper: FieldBrite™ XT² Optical System Within the Odyssey® Fc Imaging System

Introduction

Digital imaging with a CCD can often pose problems that require data manipulation for optimal image correction and quality. Methods that are commonly used for data manipulation in CCD systems include:

- Image Stacking
- Flat Fielding
- Binning

From a research perspective, it would be advantageous to have a system that provides highly accurate quantitation, without the need for post-imaging data correction.

Image Saturation and Stacking

The signal level a CCD generates is the rate of light that hits the detector (photon flux) multiplied by the exposure length (Fig.1). With a fixed photon flux, changing the exposure time can control the signal level a CCD generates.

Selecting the appropriate exposure length is critical for obtaining a quality image with quantifiable data. In order for the generated signal to be reliably measured, it needs to be strong enough to overcome the noise, but not stronger than the saturation level of the CCD. However, without knowing the level of light being emitted from a given sample, it can be difficult for the user to initially select the correct exposure time. With many CCD systems on the market, the user will generally need to acquire more than one image, at different exposure lengths, to finally obtain a high quality image. Some systems need to have the option to "stack" multiple images at a set of exposure times.

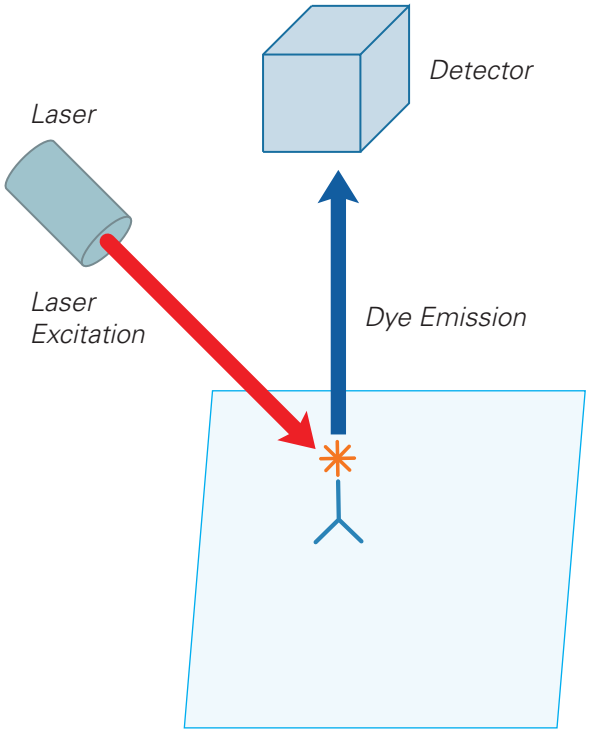


Figure 1. *The amount of light reaching a detector (photon flux) is a critical component of imaging sensitivity.*

This method will select the best image from the "stack" of images, or it will combine the frames in the "stack" into a single image through software manipulation.

Some imaging systems have image "auto exposure" features, an iterative approach to finding an exposure length that achieves a predefined maximum intensity level without saturation. In this case, image saturation can be avoided but the exposure still may not be optimal for the signal of interest.

The optimal method for digital imaging with CCD detection would include a system to allow for a single, accurate exposure.

Flat Fielding

Some CCD-based imaging systems offer a flat fielding technology that corrects for image nonuniformity within the initial image capture. This nonuniformity may be due to the lens, and for fluorescence imaging, the illumination, or the filters. Ideally, an instrument would be designed to give a reproducible imaging experience with low coefficients of variation (CV) without data manipulation (Fig. 2).

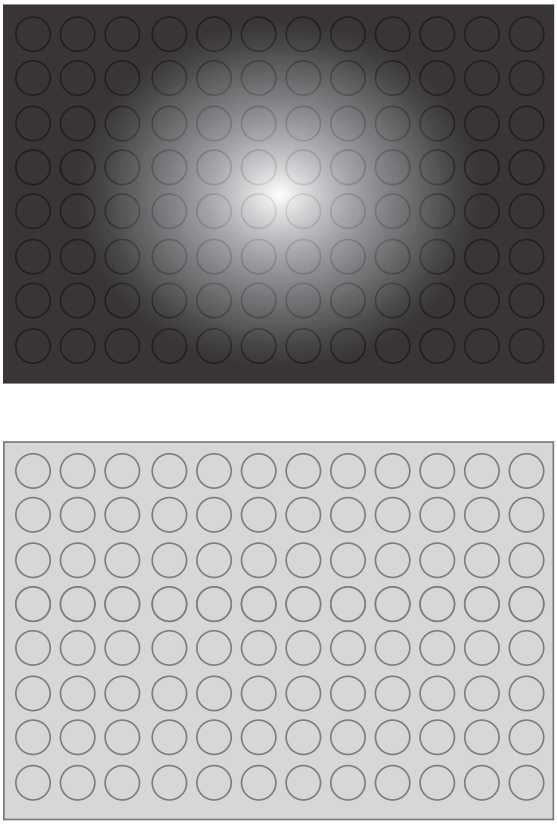
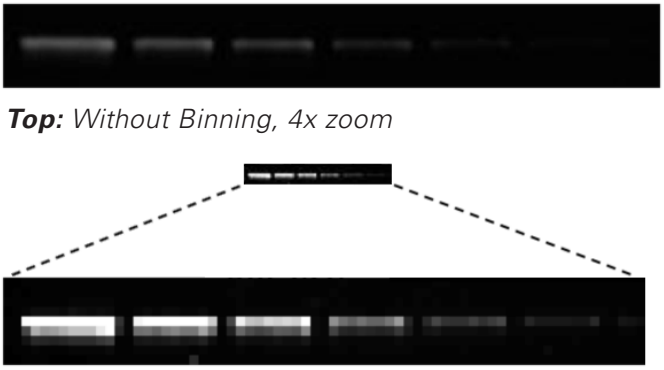


Figure 2. *The top image shows data that were imaged with CCD detection and resulted in image nonuniformity. The bottom image shows the same data after being altered with flat fielding technology. With proper optical design, the bottom image should be possible without image manipulation.*

Noise Reduction Techniques and Binning

Camera manufacturers and CCD users have found many techniques to reduce the noise of the camera relative to the signal the CCD generates. These techniques enable reliable detection of samples emitting low amounts of signal. As mentioned above, one technique is image "stacking", which includes the acquisition of multiple frames of the same data and combining them into one image.

Another common technique used to reduce noise is binning. Binning consists of the addition of signal from a group of pixels together as they are read out of the CCD to form a larger pixel. This can produce higher signal, relative to noise, and results in improved sensitivity. This technique of combining pixels, however, results in lower image resolution (Fig. 3).



Top: *Without Binning, 4x zoom*

Bottom: *With Binning, 4x zoom*

Figure 3. *An example of the effect of binning on a sample – it will produce a brighter image, but with a reduced resolution quality.*

FieldBrite™ XT² Technology

The FieldBrite XT² optical system within the Odyssey® Fc System has a highly advanced design that eliminates the need for post-image capture data correction.

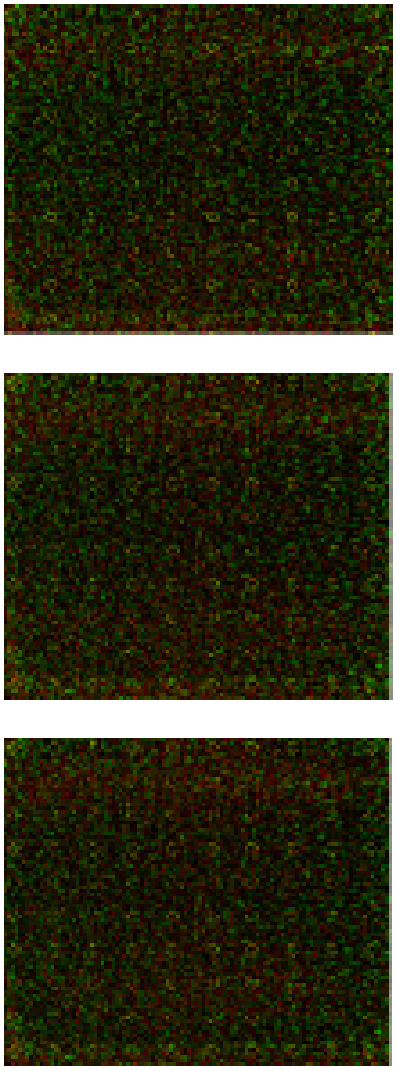
The FieldBrite™ XT² system also provides images with an exceptionally wide dynamic range without the need for the user to take multiple exposures or perform post-imaging data manipulation, such as flat fielding, noise reduction or binning.

The FieldBrite XT² optical system eliminates the need for image "stacking" and the worry of image saturation. The user selects only the total acquisition time before taking the one, and only, image necessary. This system provides images with a six-log dynamic range that produces a single image showing both strong and weak bands, without saturation. Because there is no need for software manipulation post-image capture, the FieldBrite XT² optical system consistently produces quantifiable data.

The FieldBrite XT² optical system provides uniformity across the entire field of view with a coefficient of variation of less than 3% (Fig. 4). Even laser illumination is superior to flat fielding technology, which is simply a post-image capture software manipulation, because it offers an image that is already flat – no additional software corrections are required by the user.

The even laser illumination, along with a dynamic range of greater than six logs, allows a FieldBrite XT² user to obtain a high quality image the first time, every time, without worrying about what exposure time to use in order to avoid saturation, or where to place the sample for best results. Because this technology does not require data manipulation, it is presented in its most accurate, quantifiable form. This feature also allows for superior data reproducibility.

In FieldBrite XT² technology, binning is not needed to increase sensitivity because of the low background resulting from its patented design. Because binning sacrifices resolution, this optical system has optimized resolution and sensitivity for high quality performance and image quality.



30 s
700 nm – 1.88% CV
800 nm – 1.36% CV

2 min
700 nm – 1.83% CV
800 nm – 1.42% CV

10 min
700 nm – 1.86% CV
800 nm – 1.41% CV

Figure 4. *The three images above were acquired using the Odyssey Fc Imaging System, with 100 spots analyzed per image across the entire field of view showing acquisition times with %CV per channel.*

The noise reduction techniques employed in the FieldBrite XT² optical system enable the detection of low light levels at the full resolution of the CCD. The only limitations in imaging with this technology almost always come from non-specific binding or optical background from the sample, not from the system itself.

Conclusion

Because FieldBrite™ XT² technology offers an optimized optical system, data images only need to be captured once, without the need for post-image data manipulation. The patented design includes a wide dynamic range that eliminates the need for image stacking and taking multiple exposures at different times. An even field of illumination means that there is no need for flat fielding manipulation – the user can be confident that, without software adjustments, the original image is already flat. The FieldBrite XT² optical system is sensitive with low background – there is no need to sacrifice resolution to gain sensitivity with this system.

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