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A CCD Sensor Optimized for Chemiluminescence, Fluorescence, and Brightfield Applications

by John Pizzonia

Complete multimodal imaging for applications in chemiluminescence, fluorescence, and brightfield imaging is achieved with the LAS-3000 image analysis system (**Fujifilm Life Science USA,** Stamford, CT). At the core of the system is Super CCD technology[1] (**Fujifilm**). The octagonal-shaped pixels in the M3833 processor (**Fujifilm**) (*Figure 1*) are situated on a 45° angle, creating a pattern similar to the layout of the optical sensing neuron of the human eye. This results in increased sensitivity, improved signal-to-noise, and a much wider dynamic range. The 3.2-M pixel charge-coupled device (CCD) (1536 × 2048) is laid out on a 1-in. chip, resulting in well sizes capable of producing true 16-bit depth image data. This standard resolution can be increased to 6.3 M pixels (3072 × 2048) using proprietary interpolation algorithms.

The LAS-3000 uses a two-stage Peltier system with an additional fan to cool the Super CCD to –30 °C, and provides ultrahigh binning states to maximize sensitivity. The CCD coupled to a F0.85 Fujinon lens (**Fujifilm**) makes the image analysis system one of the fastest for chemiluminescence imaging.

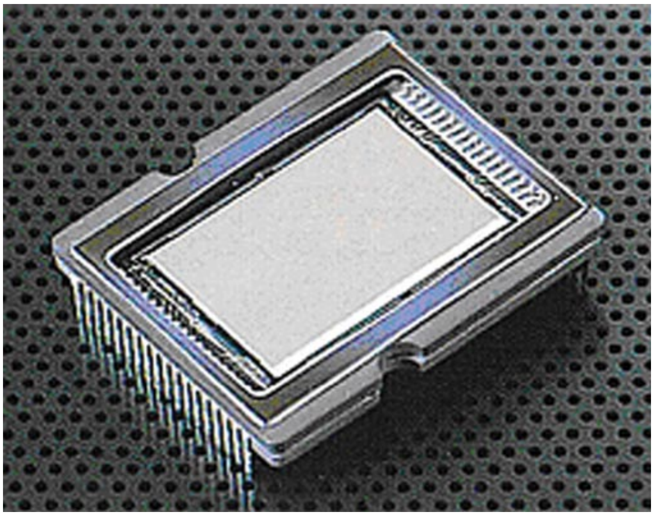

Figure 1 M3833 *processor*.

CCD layout

While a conventional CCD has rectangular pixels arranged in columns and rows (*Figure 2a*), the Super CCD has octagonal pixels approximately 10.75 µm × 10.75 µm in a honeycomb configuration (Figure 2*b*). By rotating the pixels 45° to form this interwoven layout, the CCD's pixel pitch in the horizontal and vertical directions is narrower than in the diagonal direction. This gives a larger relative area of the pixels per total size of the CCD than possible with the conventional CCD structure, and contributes to the high sensitivity and high resolution attainable with the Super CCD. In the high-resolution mode, virtual pixels are created within the spatially interleaved real pixels (Figure 2*c*) to effectively double the output and provide high resolving capability.

CCD calibration

Under normal CCD operating conditions, noise arises as a result of both thermal and electronic factors. The thermal noise is reduced by cooling the CCD to –30 °C. The residual thermal contribution, along with the contribution of electronic noise (which results from moving charges around on the CCD), become a function of the exposure time. In the LAS-3000 system, this residual noise inherent in the Super CCD is corrected for by taking a series of dark frame shots with the camera shutter closed. Short exposures (1/100, 1/60, 1/30, 1/15, 1/8, 1/4, 1/2, 1, 2, 3, 4, 5, 6, 7, and 8 sec) are applied directly, while the average of 16 × 9 sec is used for exposure from 9 sec to 2 hr. A single 2-hr dark frame is used for exposure times over 2 hr. Dark frames are subtracted to perform the correction.

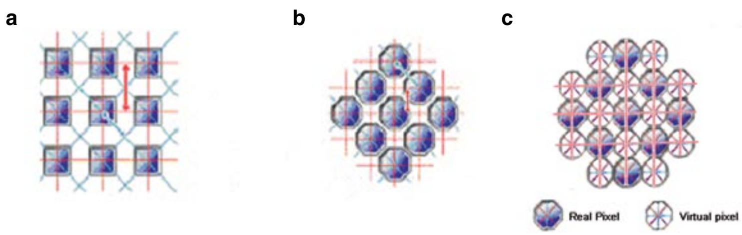

Figure 2 *Standard architecture of a) conventional CCD, b) Super CCD, and c) interpolated pixel resolution*.

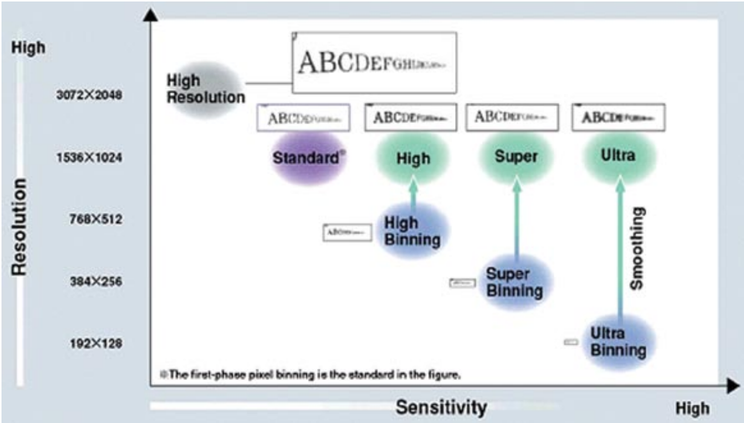

Figure 3 *Resolution for individual binning states with and without smoothing algorithm applied.*

Although only the highest-quality scientific-grade Super CCDs are used to build LAS-3000 systems, some pixels will exhibit hot (always on) and cold (always off) behavior. These pixels are identified and their data modified using nearest neighbor algorithms to correct for this. Finally, additional lens and flat frame corrections are performed within the system to address radial variability generated by the lens and the nonuniformity of the illumination fields used in certain applications.

Binning and image processing

Binning is a method of combining several pixels into one large pixel when reading out the electrons accumulated on the CCD after exposure. The light receiving area of a combined pixel increases to enhance sensitivity. The LAS-3000 offers standard binning (1 × 2 pixels), high binning (2 × 4 pixels), super binning (4 × 8 pixels), and ultra binning (8 × 16 pixels) modes. In the ultra mode, the exposure time is nearly 60 times faster than in the standard mode.

While binning can dramatically reduce exposure times and increase sensitivity, the downside is that is does so at the expense of resolution (see *Figure 3*). Thus, images become increasingly more pixilated as the binning is increased. The LAS-3000 is still able to produce visually palatable images, even in the ultra binning mode, using a smoothing feature that converts high, super, and ultra binning images to the same pixel size and number as the standard image. This result can be visualized in either the binned or smooth formats, and the smoothing function does not impact the analytical accuracy of the image data.

Quantum efficiency

All CCDs display variable efficiency in transducing photons of different wavelengths. A plot of this spectral response is known as a quantum efficiency curve. A representative curve for the M3833 processor is shown in *Figure 4*. In general, the highest efficiency is realized in the visible light range between 400 nm and 700 nm, while poor response is observed with photons in the UV or IR ranges. Peak response for the processor is observed in the green range, optimizing the LAS-3000 for chemiluminescent applications using either alkaline phosphatase (i.e., CDP-Star, **PerkinElmer**, Boston, MA) or any of the newer green shifted substrates for horseradish peroxidase (i.e., SuperSignal, **Pierce**, Rockford, IL). This property of the CCD also makes the image analysis system well suited for fluorescence applications using signals from the blue into the near-IR.

Dynamic range

Finally, one of the biggest advantages of the Super CCD over film and other video systems is the large dynamic range. Consistent measurement of system dynamic range has been problematic due to a lack of appropriate standards. Glowells™ (**LUX Biotechnology**, Edinburgh, U.K.) is a product that makes evaluation and ongoing system validation simple and accurate. Glowells are certified reference standards for calibration of optical equipment such as luminometers, microplate readers, and CCD-based bioimaging systems. The light output of the standard is generated using 70 millibecquerel (MBq) of gaseous tritium per unit and is calibrated and traceable to National Physical Laboratory (NPL, Middlesex, U.K.) standards. With a half-life of 12.3 years, the

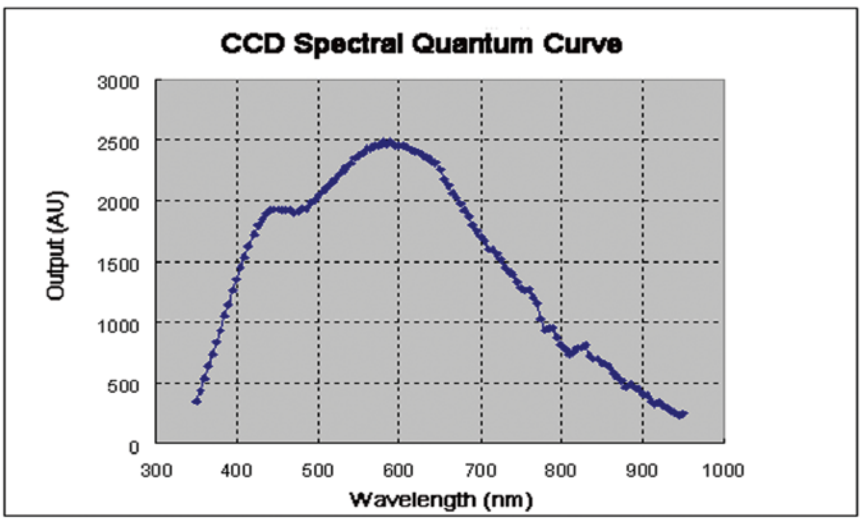

Figure 4 *Quantum efficiency curve for the M3833 processor.*

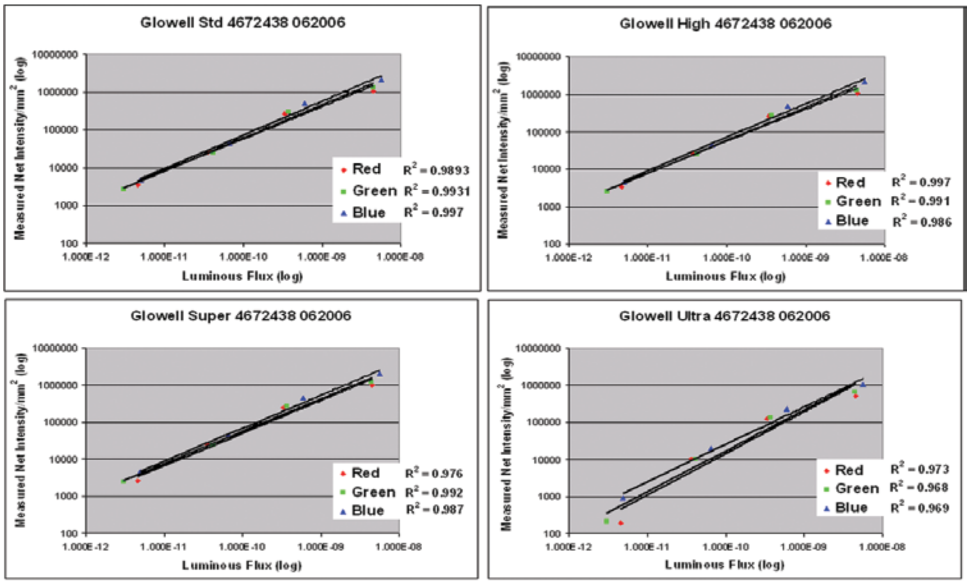

Figure 5 *Glowell standard data for LAS-3000 standard, high, super, and ultra binning states.*

standards remain virtually unchanged over the instrument lifetime. The Glowell low light imaging standard (LLIS) delivers light peaks in the blue (450 nm), green (525 nm), and red (650 nm), ranging over five orders of magnitude. Yellow (555 nm) and custom light outputs are also available upon request.

As shown in *Figure 5*, the M3833 processor in the LAS-3000 system is capable of delivering over four orders of magnitude of linear dynamic range in all four of the binning modes available. For this, analysis exposure times were adjusted to ensure that no regions reached saturation (i.e., intensity beyond 65,000 AUs). Even in the standard (slowest) binning mode, the exposure time to achieve four of the five light units did not exceed 5 sec. This performance almost doubles the typical 2.5 orders of linear dynamic range attainable with most film systems. The expanded dynamic range along with true 16-bit well depth greatly improves analytical accuracy, particularly when comparing intensities that are closely matched or are at the extremes of detection. In conclusion, the architecture of Super CCD, coupled with the optimized design of the LAS-3000 system, offer significant benefits to the researcher.

Reference

1. Yamada, T.; Ikeda, K.; Yong-Gwan, K.; Wakoh, H.; Toma, T.; Sakamoto, T.; Ogawa, K.; Okamoto, E.; Masukane, K.; Oda, K.; Masafumi, I. A progressive scan CCD image sensor for DSC applications. *IEEE J. Solid State Cir.* **2000**, *35*, 2044–54.

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