Making Sense of Ultra-Sensitivity:

The Back-Illuminated Electron Multiplying CCD

The trend in instrument performance across a wide range of scientific CCD-based imaging applications is moving very much towards higher sensitivity at faster speed, enabling lower concentrations of emitting molecules to be detected with shorter exposure times and lower excitation powers. The true nature of detector sensitivity and its relationship to Signal to Noise (S/N) is often misunderstood or misrepresented. Dr Colin Coates, Andor Technology's Application Specialist for Low Light Imaging breaks down the concept of sensitivity to it's fundamental parameters and describes how recent revolutionary developments in CCD technology are influencing these parameters, culminating with the latest prolific advancement, the Back-illuminated Electron Multiplying CCD. The combination of technologies incorporated into this camera range enable, literally, the world's most sensitive detectors.

Understanding Sensitivity…

Two parameters fundamentally influence the sensitivity of detectors – "Quantum Efficiency" (QE) and "System Noise". QE is a measure of a camera's ability to capture valuable photons. A high QE results in more photons being converted to photoelectrons within the CCD pixels. Once converted, the photoelectrons in a given pixel have to overcome the "detection limit" or "noise floor" of the camera, which is set by the system noise. By maximising QE and minimising noise, the weakest of signals may be detected.

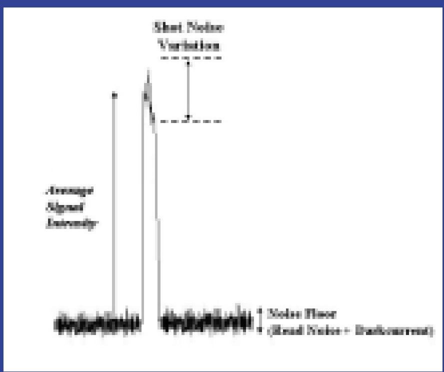

Fig.1: 2D intensity profile illustrating primary CCD noise sources.

System Noise – CCD system noise is composed of two primary sources – dark-current noise and read noise. Through enhanced thermoelectric cooling, dark-current noise can be virtually eliminated. This renders the camera's read noise as the true detection limit, particularly in fast cameras – faster readout speed (multi-MHz) results in much higher read noise, e. g. > 40 electrons rms @ 5 MHz compared to values down to 2 electrons rms @ < 1 MHz.

QE and Shot Noise – Shot noise is the quantitative uncertainty of a signal and measures the extent of "variation" of a signal from its mean value. Shot noise experienced by the detector is in fact related to both the intensity of light (density of incoming photons) and the QE of the detector. Back-illuminated sensors with higher QE yields a better Signal/Shot Noise ratio. There is a simple intuitive explanation for this – shot noise, given by the square root of the mean signal, must be calculated from the signal represented by the mean number of photoelectrons in the sensor, not simply from the number of incoming photons. Therefore a detector with higher QE will yield not just a more intense signal relative to the noise floor, but also a better Signal/Shot Noise ratio.

When comparing overall S/N figures, the nature of the relative individual noise contributions must always be borne in mind, to get a realistic idea of how detectable your signal really is! The most important aspect is that the signal should be well clear of the noise floor. These primary noise sources are represented pictorially in Fig. 1 as a 2D line intensity profile through a weak signal.

Back-illuminated EMCCD – Tackling the Fundamentals of Sensitivity…

EMCCD technology, an innovation first introduced to the digital scientific imaging community by Andor Technology in 2001, is built around E2V's patented L3 Vision sensor technology, and is sometimes known as "on-chip multiplication". (Download the pioneering papers on this technology at:

http://www.andor-tech.com/products/mspapers.cfm?marketSegment=1). Essentially, the EMCCD is an image sensor that is capable of detecting single photon events without an image intensifier, achievable by way of a unique electron multiplying structure built into the chip, therefore avoiding the acute QE and resolution limitations of intensifier tubes. EM gain can be increased to a degree, readily tuneable in real time through the software, where extremely weak signals

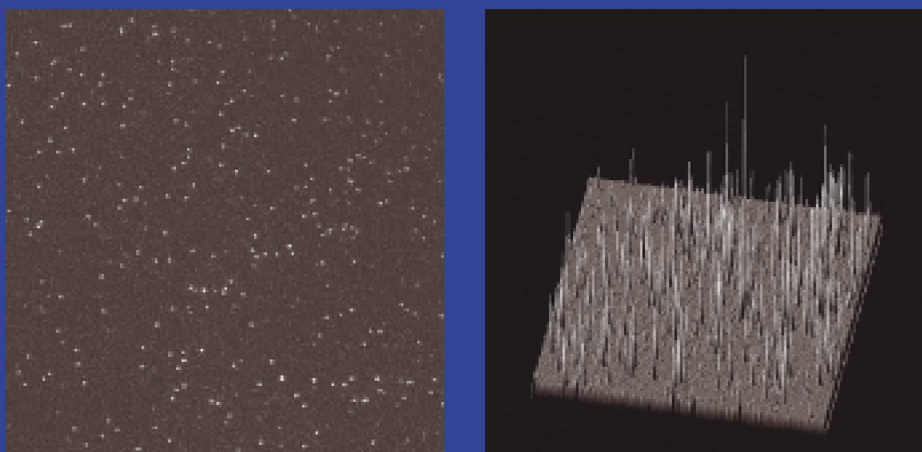

Fig. 2: (a) Single-molecule image of Cy-3 dye on a glass slide, recorded using the EMCCD in a total internal, reflectance microscopy set-up, 58 ms exposure time. (b) Corresponding 3D surface plot of the image.

may be detected above the read noise of the camera, at any readout speed. This is an important point because the traditional problem with combining sensitivity with speed in standard CCDs is that the two are mutually exclusive – i. e. greater read noise detection limits result from faster pixel readout.

The latest exclusive approach to delivering the ultimate in sensitivity comes in the form of Back-illuminated EMCCD camera technology. We have already established that EMCCD technology eliminates the noise floor and renders the camera single-photon sensitive, indeed, front-illuminated EMCCD cameras already have markedly better QE performance than even GenIII+ ICCDs and are well worth evaluating for any ultra low-light application. However, the back-illuminated sensor affords the maximum possible QE across the visible and near IR wavelength range (> 90 % at peak) – UV coatings are also available. Thus, the majority of photons incident on the sensor will be captured and then amplified by the EM technology. This increases the signal intensity and directly improves the Signal to Shot Noise ratio, and means that the signal levels never before possible will now be detectable. It is noteworthy that back-illuminated EMCCD technology can have a profound influence, if the signal is so extraordinarily weak that if during a given exposure, even a Front-illuminated EMCCD can detect only a few single photon events from the area of the emitting signal. In such an instance, the extra photons (say a factor of 2 increase) converted and detected by the Back-illuminated EMCCD, may make the difference between confidently confirming the presence of the signal or not.

Figs. 2 and 3 show images recorded under ultra low-light microscopy conditions, the EMCCD camera incorporated into: Fig. 2 – a Total Internal Reflectance Fluorescence (TIRF) Microscopy set-up for single molecule measurements; Fig. 3 – a Nipkow spinning disk confocal microscopy set-up used for rapid live cell measurements. Each of these techniques are inherently low-light in nature, for example, the confocal approach yielding typically between 2 and 20 photons/pixel/sec. Without an amplification technology such as EMCCD, such signals would be entirely lost within the read noise floor. The benefit of combining back-illuminated technology with EMCCD technology can be visualised directly in Fig. 3, which shows a fluorescence cell image at the very limit of detection, recorded with both the

back-illuminated EMCCD and a GenIII+ ICCD under identical conditions of minimal excitation power and very short exposure time. (The full paper, entitled "Ultrasensitivity, speed and resolution: Optimizing low-light microscopy with the back-illuminated electron multiplying CCD" can be accessed at: http://www.andor-tech.com.). It is clear that the Back-illuminated EMCCD technology has both distinct sensitivity and resolution advantages over the ICCD camera at such photon fluxes. Such sensitivity enhancement is a direct result of the significantly higher QE of the back-illuminated EMCCD, affording a higher Signal to Shot Noise ratio.

The Back-illuminated Electron Multiplying Charge Coupled Device (EMCCD) camera stands to be one of the most revolutionary contributions ever to the burgeoning field of ultra low-light detection, combining extremely high photon conversion efficiency with the ability to virtually eliminate the instrumental detection limit. So much so, that EMCCD technology is being heralded as the future of high performance imaging and many future developments in CCD technology are likely to be focused in this area, with particular emphasis on faster frame rate capability (10 MHz pixel readout rate is currently standard). (The Andor iXon DV860 EMCCD camera is capable of > 400 full frames/sec.). Such performance will be of significant benefit to application areas such as cell motility, multi-dimensional live cell microscopy (4/5D), calcium flux microscopy, single molecule detection and chemi/bioluminescence detection, enabling employment of reduced laser powers, shorter exposure times, faster frame rates, greater magnifications and lower fluorophore concentrations.

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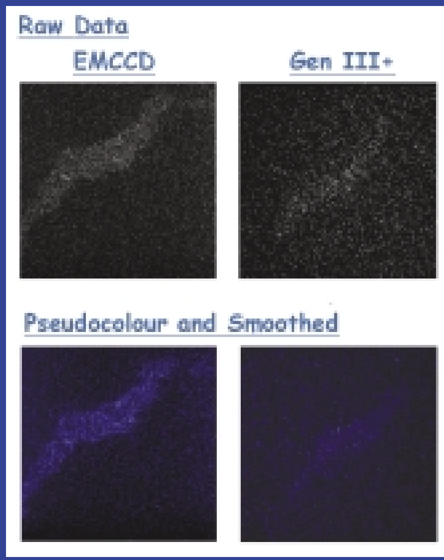
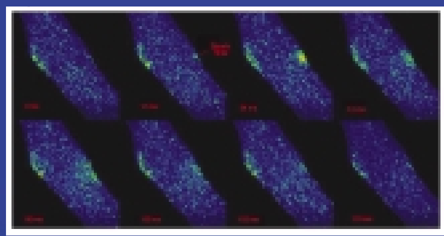
3 (a) Comparison of Technologies



3 (b) Ca $^{2+}$ Spark



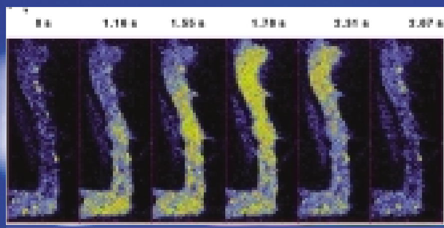


Fig. 3: (a) Comparative raw and smoothed ultra low-light fluorescent images recorded of a smooth muscle cell using the Back-illuminated EMCCD and the GenIII+ ICCD on a Nipkow disk confocal microscopy set-up, 33 ms exposure time, high EM and ICCD gain setting throughput. (b) Consecutive Back-illuminated EMCCD images from a 1500 frame kinetic series at 60 frames/sec showing the initiation and spread of a single Ca^{2+} spark in a smooth muscle cell. (c) Selected Back-illuminated EMCCD images from a 1500 kinetic series showing progression of a Ca^{2+} wave through a cell – waves were recorded with the same field of view at 30, 60 and 120 frames/sec.c

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