

## **ELECTRON MULTIPLYING CCD TECHNOLOGY AND ITS IMPACT ON ULTRA LOW-LIGHT MICROSCOPY**

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Current trends towards lower concentration and even single molecule measurements in rapid microscopy approaches are heralding a new era of challenging biophysical analysis, whereby optimal Signal-to-Noise standards must be achieved at significantly lower photon input levels, and demands on instrument performance are very much towards pushing sensitivity to new heights.

The EMCCD is an imaging sensor with single photon detection ability.<sup>1</sup> Signal amplification is achieved by way of a unique electron multiplying structure built into the silicon, rather than the incorporation of an image intensifier tube. The EMCCD is not limited by the highly restricted Quantum Efficiency (QE), otherwise imposed by intensifier tubes of ICCD cameras, enabling the combined advantages of both single photon amplification and the unimpaired harnessing of the higher/broader QE curves of the CCD silicon. A recent prolific advancement in this area has been to combine EMCCD technology with Back-illuminated CCD sensors, uniting single photon sensitivity with highest possible QE, affording ultra-sensitivity even beyond that which is offered by an avalanche photodiode detector (APD), but in a fast readout CCD-array format.

Ultrasensitive detection of extremely low photon fluxes in live-cell confocal imaging microscopy has been performed using Electron Multiplying Charge Coupled Devices (EMCCDs).<sup>2</sup> Front and back-illuminated versions of the technology were compared to ICCD cameras, and the degree of reliable detection ability under extreme ultra low-light conditions was determined for each. Photon flux levels were of a level which is undetectable under 'normal' CCD operation, and were reduced by: (a) using shorter exposure times, facilitating more dynamic experiments; (b) using lower excitation power, reducing rate of photodecomposition; (c) using higher magnifications; (d) using lower fluorophore concentrations.

[1] D. J. Denvir and Emer Conroy, "Electron Multiplying CCD Technology: The New ICCD", *Proc. of SPIE*, Vol 4796, 164-174 (2002).

[2] C. G. Coates, D. J. Denvir, M. A. Hollywood et al., "Ultra-sensitivity, Speed and Resolution: Optimising Low-light Microscopy with the Back-illuminated Electron Multiplying CCD", *Proc. of SPIE*, Vol 5139, 56-66 (2003).