PHOTOMETRICS

HIGH-PERFORMANCE EMCCD & CCD CAMERAS FOR LIFE SCIENCES

Technical Note

Evolve EMCCD for Super-Resolution Microscopy

There is an extremely rich variety of applications that fall under the topical heading of quantitative fluorescence microscopy. A number of factors have contributed to this recent explosion in the popularity of fluorescence imaging. The development of molecular technique and cloning technology has provided the prerequisite knowledge base for deciphering the information content that drives living systems. The development of genetically encoded fluorescent proteins that can be used in living cells to provide a marker for genetic expression has revolutionized biology. Also significant are the recent extension of 3-D imaging technologies to new frontiers of super-spatial resolution.

Variables that can be quantified using super-resolution fluorescence microscopy include spatial localization in 3-dimensions, as well as changes in fluorescent signal intensity. In some methods, probes may be multiplexed to track numerous intracellular variables in space and time.

Advantages of the Evolve EMCCD for Dynamic 3D Quantitative Fluorescence

The primary classes of detector commonly used in 3D fluorescence microscopy fall into 2 major categories: charge-coupled device (CCD and EMCCD) and photomultiplier tubes. EMCCD detectors such as the Photometrics Evolve are preferred for low light dynamic imaging applications because they are array detectors and can capture images of many points within the field of view simultaneously; they furthermore carefully amplify the signal above read noise levels to enable unprecendented frame rates for dynamic imaging.

The advantages offered by the Evolve EMCCD technology for dynamic 3D fluorescence imaging are several: 1.) Because the data are collected in parallel, more photons are integrated for each pixel in the time taken to acquire a single frame than for a point-scanning confocal system; this generally yields a higher signal/noise ratio for a given exposure time. As a general rule, because of the increased integration time for each pixel provided by parallel capture, much lower illumination intensities can be used to acquire an image in a given amount of time. This is of enormous importance to studies involving single molecule dynamics in vitro or in living cells (where phototoxicity and bleaching are major concerns). 2.) EMCCD technology offers comparatively large (Evolve > 90% across the visible spectrum) quantum efficiency as compared to either PMT technology or conventional interline cameras; again, this can serve to reduce the exposure time for greater temporal resolution and for reduced phototoxicity/photobleaching. 3.) EMCCDs have been developed for quantitative microscopy that have very high dynamic range. High dynamic range increases the range of brightness values that can be quantified, and the Evolve leads the industry in reducing noise levels to increase dynamic range and detect small signals. 4.) the relationship between the number of photons integrated by any single pixel on the array and the electronic signal that is subsequently quantified is inherently very linear; the Evolve EMCCD takes this a step further by providing integral system calibrations that permit unambiguous expression of brightness levels in absolute units of electrons.

The Evolve EMCCD technology has reached a high state of refinement and permits accurate and repeatable quantitation of absolute signal levels for demanding classical applications such as live cell imaging, single molecule imaging and real-time optical sectioning using spinning disk microscopy. It can be demonstrated that the Evolve EMCCD technology offers superior benefits where the quantitation of intensity levels is of major concern.

Next Generation Fluorescence: Super-Resolution Methods

Classical methods in fluorescence microscopy, which involve a wide range of approaches such as laser-scanning confocal, spinning disk confocal, conventional fluorescence microscopy, multiphoton microscopy, widefield deconvolution, and classical structured illumination are all united by a common limitation. The smallest resolvable details are all determined by the diffraction limit at the wavelength of light being used. Until very recently, it was thought that visualization of spatial localizations beyond this resolution limit was insurmountable because it is a physical property of light. Until recently, the only recourse to achieve higher spatial resolution was to resort to electron microscopy or scanned probe microscopy.

Several compelling and innovative approaches have been introduced that leverage new technology and break the conventional resolution limit of light microscopy. The appearance of methods such as TIRF, PALM, STORM and OMX provide greater spatial resolution than ever before thought possible; such technologies depend on the advantages provided by the latest generation of EMCCD camera technologies.

Photoactivation Localization Microscopy (PALM)

PALM and the related technique of STORM (stochastic optical reconstruction microscopy) leverages the unique properties of photoactivatable chromophores. Such fluorescent labels may be genetically encoded, such as Dronpa. These methods leverage the statistical probability of switching individual fluorochrome molecules on with a flash of light. The basic idea is to label structures of interest with dark fluorophores that can be activated by a flash of light at a particular wavelength. Because this activation phenomenon is stochastic, a distribution of well separated molecules is 'switched on' and the centroid of the single molecule localization can be calculated to well below the resolution limit. After these molecules photobleach, another flash of light activates another round. This process is repeated in turn until an image of the structure of interest with very high structural resolution is assembled by combining the data from each round of activation. Eric Betzig, Xiaowei Zhuang of the Howard Hughes Medical Institute, Harvard and Sam Hess of the University of Maine have contributed to the development of this exciting technique (2006).

PALMIRA (PALM + Independently Running Acquisition) has been developed by the group of Alexander Eggner and Stefan Hell (2007). PALMIRA is an extension of the photoactivation approach that improves the rate at which data can be acquired by a factor of 100.

The photoactivation methods are single molecule imaging approaches and the benefits of the very high quantum efficiency and state of the art low-noise optimizations of the Photometrics Evolve can be leveraged towards further advances of this approach. Precise molecular localization using these methods requires accurate photometric measurement of the number of photons (Gould, et al., 2009) and the quantitative calibration provisions of the Evolve permit access to such measurements with a level of convenience and control that is unparalleled by competing technologies.

3D SIM (structured illumination microscopy)–Optical Microscopy, Experimental (OMX)

OMX is a novel implementation of structured illumination that provides the advantage of being amenable to fast, multiwavelength, live-cell imaging. The OMX approach uses interference patterns of light to illuminate the sample, by so doing, the interaction of the sample with the interference patterns can be analyzed and computational methods allow reconstruction of the physical structures contributing to the raw data acquired on EMCCD cameras. The OMX approach was developed by John Sedat, Mats Gustafsson (2005) and David Agard at the University of California San Francisco.

This advanced structured illumination approach provides the ability for rapid, dynamic quantitative live cell imaging at multiple acquisition wavelengths. The interaction of the structured illumination pattern and the sample structures creates a fringe pattern that contains information about tiny low-frequency structures; the resolution that is achieved in this manner roughly halves the size of the smallest structures that can be discerned using classical methods.

The Photometrics Evolve is well suited to the demands of OMX microscopy, providing industry leading noise characteristics, high-dynamic range and quantitative considerations such as optimized clock induced charge characteristics and field uniformity. Furthermore, the linearized gain mapping, gain calibration and calibration to absolute units of incident photoelectrons permit confident use of this technology in a multiple camera instrument.

Total Internal Reflectance Microscopy (TIRF)

Total internal reflection microscopy (TIRF microscopy) is a powerful mode of fluorescence light microscopy in which a specialized illumination strategy is used to permit direct, time-resolved visualization of intracellular events including endocytosis and exocytosis, cytoskeletal dynamics and dynamic single molecule imaging.

TIRF microscopy is a relatively recent development in biological fluorescence microscopy that permits ultra-high (on the order of 100-nm) axial resolution at the sample/coverslip interface (Axelrod, 1981). The benefit of such high optical sectioning resolution at the cell/coverslip interface is that the signal/noise ratio for studies involving direct observation and recording of cell membrane function, vesicle fusion, membrane proteins is very high.

TIRF is based on the principles of refraction and reflection of light. When light strikes an interface between two materials of differing refractive index, the light rays are bent (refracted); the degree to which the light path is bent is dependent on the degree of difference of refractive index between the two materials (the refractive index may in turn vary as a function of the wavelength of the light itself). However, when the angle of the light incident on the interface between refractive indices becomes sufficiently great, then the light will begin to reflect rather than penetrate and refract.

The practical implication of this is that, using TIRF, only the portions of the sample within the first couple hundred nanometers is illuminated with enough intensity to provide a robust signal. For this reason, there is no contribution from background signal and/ or scattered illumination to the field of interest such as is typical on a conventional, far-field illumination setup.

The dynamic, low-light nature of TIRF studies frequently dictates that frame readout rates be kept high; in order to transcend the readout noise at short readout rates, EMCCD technology is favored. The Evolve EMCCD provides novel noise reduction optimizations such as background event reduction technology (BERT) and quantitative benefits for high fidelity TIRF experiments with super-resolution in the axial dimension.

Customer Assurance

In the interest of fueling rapid innovation and discovery, Photometrics Customer Assurance package has been developed to further assist researchers in meeting their research goals. The Performance Assurance Package provides training, rapid response to equipment maintenance, and loaner equipment in the event of service for the life of a research grant. This package provides maximum return on technology investment though tailored training on advanced operational use of imaging instrumentation in order to maximize data and also provides priority response in the event of instrument downtime.

For more information on the Performance Assurance Package for Photometrics cameras, please visit www.photomet.com/support.

To learn more about the ground-breaking high-performance EMCCD cameras from Photometrics, please visit www.evolve-emccd.com.

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