PHOTOMETRICS

HIGH-PERFORMANCE EMCCD & CCD CAMERAS FOR LIFE SCIENCES

Technical Note

Evolve EMCCD for Spinning Disk Microscopy

Confocal microscopy is based on a sophisticated collection of approaches that are capable of producing data with a remarkable degree of 3-D spatial resolution and superior contrast to classical microscopy using thick samples. There are two major approaches to confocal microscopy: single point scanning methods and multi-point scanning methods. Single point methods are conceptually simpler; such methods assemble images by acquiring a single point in 3-D space at a time. Multipoint scanning methods take this basic approach a step further and acquire multiple points in a 2-D array simultaneously. The most popular multipoint scanning method in use is commonly referred to as Nipkow spinning disk microscopy.

The Nipkow disk was conceived by Paul Nipkow in 1884 and was used in early mechanical scanning television technology of the 1920's. The basic paradigm of using a carefully designed spinning disk with pinholes for producing a raster scan at a high rate of speed was further adapted and applied to the practice of confocal microscopy in 1967 by Egger and Petran, since then the idea has evolved into different implementations. The primary advantage of multipoint spinning disk microscopy is parallel acquisition of multiple points, this drastically decreases the amount of time required to capture an entire 2D image at a given resolution. Spinning disk confocals may be engineered to have 20,000 pinholes arranged in a symmetrical spiral pattern and scanning occurs at a very high rate of speed, much faster than the human eye flicker response. Thus the sample may be viewed in real-time by a human observer or very fast camera sensor.

A variety of light sources may be used with spinning disk confocal systems; arc lamps, LED's and lasers have all been adapted to spinning disk confocal technology. Optimizations such as microlensing can pick up the quantum efficiency of excitation through a spinning disk by an order of magnitude.

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 spinning disk 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.

Spinning disk confocal technology coupled to the Photometrics Evolve offers several advantages for dynamic 3D microscopy. Foremost, 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. 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). The Evolve sensor technology offers extremely high (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. Typically the quantum efficiency for a photomulitiplier tube is on the order of 30-40%, the quantum efficiency for a good interline camera is generally limited to 60-70%.

The Photometrics Evolve has been optimized to have very high dynamic range. The dynamic range is a measure of the ability to quantify very small signals along side very intense signals without reaching the limits of the detector. 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. The Evolve has a vast dynamic range by virtue of the large pixel well capacity (total number of electrons that the pixel can hold) and the very low noise levels (determines the smallest signal that can be reliably quantified). To adequately sample the entire dynamic range into brightness levels, the camera must be digitized at 16-bits, or roughly 65 thousand different brightness levels. However, the dynamic range of the sample may differ significantly from that available to the camera, for this reason the Evolve incorporates an innovative ability to change the bit-depth to better match the dynamic range of the sample. Digitizing at a lower bit-depth means that more electrons are accumulated to transition to a higher brightness level, this decreases the contribution of shot noise to variation in brightness levels, makes detection of brightness transitions and boundaries more discrete, and makes the data smaller and easier to manipulate. There are also provisions to reduce the dynamic range being recorded to a subset of the available dynamic range. This can be optionally used to make data easier to interpret by reducing the distraction of extraneous information.

Finally, the relationship between the number of photons integrated by any single pixel on the array and the electronic signal that is subsequently quantified is engineered to be 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 camera 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 technology offers superior benefits where the quantitation of intensity levels is of major concern.

Fast Readout

The Evolve uses an advanced implementation of electron multiplication (EM) gain technology. Such cameras are commonly referred to as EMCCD cameras. EM gain technology multiplies the photon-generated charge from each pixel on a CCD array to a level above the read noise; this permits signal detection at low light levels and high pixel readout rates, a regime that would not be possible using conventional CCD technology because the read-noise would dominate the image and cause excessive uncertainty in the data. This signal boosting process occurs before the charge reaches the on-chip readout amplifier, effectively reducing the read-noise by the gain multiplication factor. Despite clear advantages where high-speed, low-light imaging is concerned, EMCCD technology has some additional considerations that must be taken into account to ensure integrity in signal quantification.

Quantitative Characteristics

The Evolve leverages Photometrics' established advanced clocking enhancement technology to enhance and optimize noise factors such as clock induced charge and charge transfer efficiency to the lowest levels yet achieved. This consideration provides for the highest data integrity at the fastest speed for spinning disk microscopy.

Important EMCCD specifications for dynamic spinning disk microscopy include: clock induced charge (CIC—data corruption resulting from imperfect clocking waveforms moving the signal through the chip), charge transfer efficiency (CTE—causes 'streaking' of signal as read from the chip), and field uniformity (does the entire chip respond exactly the same to exactly the same amount of incident signal? is there a 'fixed-pattern' noise contribution?)

Bias stability (stability of the offset of the signal with zero exposure time), and gain stability (stability of amplification) are also enormously important considerations in the context of cameras to be used for quantitative applications. The Evolve provides optimized (and ground-breaking) performance for each specification.

In the context of EMCCD cameras, the gain must be calibrated. Calibration ensures consistent and interpretable mapping of photoelectrons to grey levels. Without an understanding of the absolute units represented by a brightness value, there isn't really a quantitative measurement and comparison of measurements from different cameras (or at different camera settings) becomes ambiguous at best. As EMCCD chips age, the gain amplification characteristics change, and so it is of great importance to be able to reliably calibrate the gain amplification at regular intervals. The Evolve incorporates an innovative and convenient built-in light source to permit this calibration without the necessity of removing the camera from the microscope.

It is also important to linearize the gain mapping such that the investigator knows how much amplification is being applied (e.g. 20x gain amplifies the signal 20x, 100x gain amplifies the signal 100x). The Evolve linearizes the gain mapping with over 700 sample points all the way from 0x to 1000x. As a ground-breaking measure in quantitative EMCCD technology, the Evolve permits 'on the fly' presentation of brightness levels in terms of absolute units, in this case, electrons. In other words, because of the sophisticated built-in calibration and quantitative considerations, the mean number of photo-electrons contributing to a given pixel can be presented on the fly without arduous secondary calibrations and calculations. This technology brings the ability to compare images taken at different gain settings (a feature with a given quantum yield will appear at different brightness levels at different gain settings even though the number of electrons contributing to a pixel are the same) and on different cameras, in terms of absolute units. Experimental variability is therefore brought within the limits of variability due to noise, and the sources of noise have been carefully minimized to the current state-of-the-art.

Spurious charge is a phenomenon that contributes to variability in pixel values due to gain amplification conditions. These spurious 'hot-pixel' events are discrete, follow statistical probability, and can be seen as anomoulous bright pixels distributed randomly and changing position randomly from frame to frame. The Photometrics Evolve provides an optional on-the-fly noise reduction algorithm that can be optionally applied to greatly attenuate spurious charge speckling in each frame. This is a process distinct from a post-processing operation and the positive effect can be witnessed in real time, this makes the most of real time determination of focal planes in spinning disk microscopy. The parameters of this filtering operation can be adjusted interactively to reflect noise events that clearly fall outside the limitations of the optics and into the realm of noise artifact.

Deep Cooling

The Photometrics Evolve implmementation brings basic noise characteristics (dark noise and read noise) under conventional imaging conditions to the lowest levels presently available, thereby expanding the dynamic range and flexibility of the platform to a wide range of imaging conditions. The Evolve is peltier cooled to -85 degrees C (with the option of water cooling) to provide a dark current specification of 0.0001 e-/pixel/sec. This benefits longer exposures under non-EM gain conditions. The non-EM gain read noise specifications have been brought to an unprecedented level of performance on par with the most refined interline CCD cameras available.

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.

References

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