Imaging at LightSpeed™

Increase EMCCD Camera Acquisition Rates for Imaging Highly Dynamic Cellular Events

Imaging at extremely high frame rates presents a variety of challenges that need to be overcome to produce functional image data. The first and foremost issue is simply this: the faster the data is being collected, the smaller the amount of time allowed to expose and readout. Due to this, when imaging dim, dynamic cellular events such as neuronal activity, the sensitivity of the camera becomes paramount.

EMCCD cameras have been able to provide the required sensitivity through near-perfect 90% photon conversion to signal electrons, coupled with the ability to amplify and detect individual photoelectrons. However, with regards to the frame rate, the camera often is incapable of reaching the desired rates.

In the quest for speed, field of view is the first sacrifice made, with smaller regions of interest (ROIs) being used to achieve the required faster frame rates. EMCCD cameras – typically provided with a 512x512 sensor array, are used with smaller regions such as 128x128 to achieve frame rates greater than 240frames/second. For those who need even more speed, cameras specifically designed for ultimate speed and sensitivity were developed – the Evolve™ 128 being the perfect example. Equipped with a 128x128 pixel array and delivering over 530 frames/second, it became the preferred solution for high speed imaging. Using the smaller 128x128 sensor ensured no time was lost discarding unused pixel data.

EMCCD Cameras

An EMCCD camera reads data from the sensor by shifting one row of collected data into the serial register and then reading the single row out of the serial register one pixel at a time. This is repeated for every row until all the data has been transferred. There are two main delays associated with this implementation:

1. How fast can rows be moved into the serial register?
2. How fast can pixels be transferred out of the serial register?

The rate at which one row can be moved into the next is known as the parallel-shift rate, and for the majority of EMCCD cameras, this is in the range of 300-1000ns/row. Transferring pixels out of the serial register is the specification normally cited for the speed of a camera, such as 10MHz or 20MHz. For example, with a 512x512 camera running at 20MHz, such as the Evolve 512 Delta, the theoretical maximum frame rate achievable is about 76 frames/second. Including the delay due to shifting rows plus other overhead due to properly running the camera, the achieved frame rate is 67 frames/second.

When using a 128x128 ROI, fewer pixels to digitize results in an increase in the Evolve 512 Delta frame rate to 243 frames/second. However, when a camera is set up to use an ROI to increase the frame rate, the portions of the sensor outside the ROI are no longer being used for imaging and simply contribute overhead. With some unique methods of operating the sensor, Photometrics is able to overcome this overhead to deliver significant improvements in frame rate.

LightSpeed Mode

LightSpeed mode enables significantly higher frame rates when using ROIs for imaging with a two-step approach:

1. Pack multiple rows into the serial register before transfer
2. Increase the parallel shift rate

By placing multiple rows of data into the serial register for each transfer, the efficiency of data transferred per read out is maximized and the total rate of data transfer is increased.

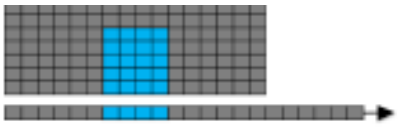

Figure 1. Single Row per Read Out

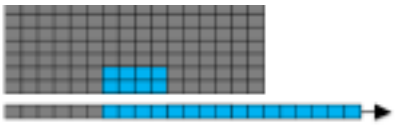

Figure 2. Multiple Rows per Read Out

Increasing the parallel shift rate also provides improvements in frame rate by reducing the time required to readout an image. The parallel shift rate of the Evolve 512 Delta has been increased to achieve a shift time of 200 ns/row, a 33% improvement from the previous fastest EMCCD cameras available.

Previously, where a 128x128 array was imaging at 243 frames/second, in LightSpeed mode the camera is able to achieve 794 frames/second – an improvement of over 300%.

Binning	Single Channel				Dual Channel		
	256 x 256	128 x 128	64 x 64	32 x 32	256 x 128	128 x 64	64 x 32
1 x 1	225	794	1820	2745	424	1300	2428
2 x 2	421	1291	2371	3065	755	1906	2870
4 x 4	748	1862	2787	3217	1237	2552	3118

(Frames per second)

Note: Frame rates are preliminary. For the latest published frame rates visit www.photometrics.com

Figure 3. LightSpeed mode frame rates

LightSpeed mode achieves this improvement in frame rate with superb flexibility – placing no restrictions in the location of ROIs, and allowing the flexibility of being used in conjunction with binning. LightSpeed automatically evaluates the position of your ROI to determine if a horizontal adjustment of the ROI will place a larger number of rows in the serial register. If an adjustment will provide another row into the serial register, the ROI will be adjusted appropriately and increase imaging speed. No ROI adjustment is typically more than ±4 columns.

To use LightSpeed mode, a physical mask of the pixels not actively being used for imaging must be in place. The mask is only required on the left and right of the selected ROI, simplifying the masking requirements. If any light is collected in the pixels to the left or right of the ROI, there will be contamination the pixels which are used to pipeline the rows during read out, affecting the measured intensities. A masking device such as the Photometrics DualView Λ™ is recommended.

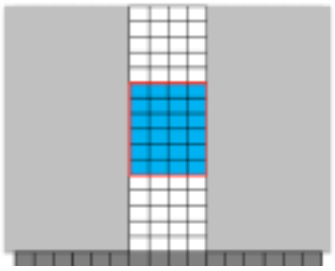

Figure 4. Physical masking is required only on the sides of the ROI

Dual-Channel Imaging

The flexibility of ROI placement and size afforded with LightSpeed mode enables high speed dual-channel imaging as well. When used with a wavelength-splitting device such as the DualView Λ, it is possible to image two 128x128 regions simultaneously, using a 256x128 ROI at a frame rate of 428 frames/second.

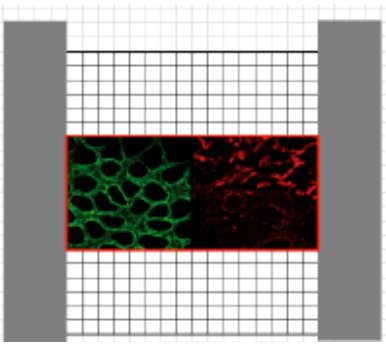

Figure 5. Dual-Channel Imaging is possible with LightSpeed mode

Applications

LightSpeed mode lends itself extremely well to demanding applications which require achieving the absolute best possible frame rates, with maximum sensitivity. Applications can range from Neuronal Imaging and Calcium Flux or Voltage Sensitive Dye imaging to Super Resolution applications such as STORM and PALM.

The Evolve 512 Delta with LightSpeed mode increases the possible frame rates while providing the highest levels of sensitivity for these applications.

Examples of such applications requiring such high frame rates and sensitivity are listed below.

Progesterone modulates SERCA2a expression and function in rabbit cardiomyocytes
Karni S Moshal, Zhe Zheng, Karim Roder, Tae Yun Kim, Leroy Cooper, Bogdan Patedakis Litvinov, Yichun Lu, Vishal Reddy, Dmitry Terentyev, Bum-Rak Choi, Gideon Koren
American Journal of Physiology - Cell Physiology, 24 September 2014
http://ajpcell.physiology.org/content/307/11/C1050

Physiological Characterisation of Human iPS-Derived Dopaminergic Neurons
Elizabeth M. Hartfield, Michiko Yamasaki-Mann, Hugo J. Ribeiro Fernandes, Jane Vowles, William S. James, Sally A. Cowley, Richard Wade-Martins,
PloS one, February 21, 2014
http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0087388

Live Intracellular Super-Resolution Imaging Using Site-Specific Stains
L Carlini, S Manley, *ACS chemical biology,* 2013
http://pubs.acs.org/doi/abs/10.1021/cb400467x

Summary

Scientific EMCCD cameras provide the required levels of sensitivity for many demanding research applications. However, they have been limited with providing the needed higher speed image acquisition capability for these applications.

The Evolve 512 Delta EMCCD camera enables the ability to obtain image data at high acquisition rates with its new LightSpeed mode. Along with unprecedented speed, users have complete control in selecting a targeted ROI when imaging with LightSpeed. Additionally, when used with a wavelength splitting-device such as the DualView Λ, it is possible to image two channels simultaneously for even more efficient imaging and data collection.

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