

Advanced Feature Set for Photometrics Evolve[®] EMCCD Cameras



Photometrics continues to develop new camera performance capabilities which streamline user workflows while enabling researchers to concentrate directly on the image data that is important to their studies. These advanced functions enhance the quantitative nature of the camera while simultaneously allowing scientists to analyze very specific data.

All of the Advanced Features found in this document are available with every Evolve camera. Evolve provides versatility by giving researchers the option to turn these features on or off, pending their application. To use the Evolve in a more traditional manner, a standard-type setup can be achieved by turning off all of the Advanced Features.

Advanced Evolve features include:

- **Quant-View[®]**
- **Rapid-Cal[®]**
- **Background Event Reduction Technology[®]**
- **Black-Lock[®] / Top-Lock[®]**
- **Vari-Bit[®]**

This document contains a description of each of these features – what they actually do and how they can be utilized by the research community to make their data much more quantitative.

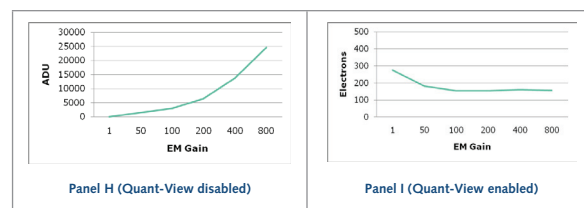
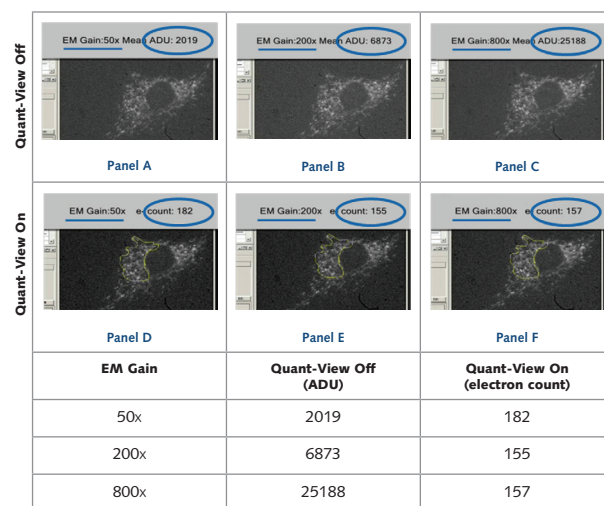
Quant-View

A CCD counts photons by generating electrons when incident photons hit the device's array. These electrons are counted and converted into an analog-to-digital unit (ADU or sometimes referred to as grey-scale) value. Knowing the conversion factor would allow interpreting image data in terms of photons which hit the CCD array. Measuring this conversion factor (also sometimes referred to as the system gain) is a complex process for most users to perform. In EMCCD cameras, measurement of this conversion factor is further complicated by the electron multiplication gain register.

The Evolve EMCCD cameras are the world's first to provide real time accurate and quantified data in terms of photo-electrons and effectively performs gain factor calculations and conversions in real-time. The camera reads out the pixel values in electrons generated at the imaging array. This number, along with the exposure time, can then be used to calculate the number of electrons generated at any pixel per unit measure of time – effectively providing an electron flux measurement.

In Figure 1 below, the difference between performance with and without Quant-View is demonstrated. With Quant-View disabled, as the EM gain increases, so does the ADU count (Fig.1 panel A through C, panel G column 2 and panel H). However, with Quant-View on (where the pixel value is converted into a photo-electron count in real time) the value of the sample remains constant (Fig.1 panel D through F, panel G column 3 and panel I).

Figure 1: Comparative image data with Quant-View enabled and disabled at various EM Gain settings



Low-light cell sample with immuno-fluorescent stained golgi apparatus. EM Gain applied at: panel A 50X, panel B 200X and panel C 800X with Quant-View disabled. Identical images were acquired with Quant-View enabled panels D through F. Region pixel data from panels A through F are tabulated in panel G. Results of average region pixel values with Quant-View disabled is plotted in panel H and comparative results using identical images with Quant-View enabled are plotted in panel I.

With standard EM cameras increasing EM Gain on an image results in ADU increase. This results in difficulty when doing direct comparisons between different studies due to arbitrary nature of pixel value. With Quant-View enabled, when increasing EM Gain the photo-electron count does not increase. The data output by the camera is expressed in electrons generated in the pixels via incident photons. This allows for consistent data regardless of the total system gain. It should be noted that when the EM Gain level applied is low, the photoelectron count is a little higher in Quant-View mode (Fig. 1 panel G column 3 and panel I). This is due to the fact that adequate EM gain has not yet been applied to effectively reduce the camera read noise to sub-electron levels. Thus at that EM Gain level, the pixel values includes the photo-electrons generated added to the effective read noise in electrons. However, when enough EM Gain is applied to ensure the effective read noise is less than 1 electron - the photo-electron count remains stable no matter how much more EM-Gain is applied. This feature also allows the user to empirically realize the optimal EM-gain settings required to obtain best signal to noise ratios. This feature also allows the user to extend the useful lifetime of the EMCCD. With EMCCD cameras that do not have this feature, the user will often set the EM gain at a level that is above this optimum setting. Prolonged high EM gain settings have the effect of prematurely aging the EMCCD.

How will Quant-View help?

By actually measuring the electrons generated, users quantitatively know what the camera is measuring in actual electrons. There are many examples where such data will be important for bio-research. One example would be when normalizing for transfection levels. Often, fluorescent proteins are transfected at different levels and results are taken and measured. These results are often just reported relative to "control cells". Utilizing Quant-View measurements will enable researchers to discern if they are looking at cells that are transfected to similar levels, day to day and even year to year. Over-expression artifacts and results could be identified in this manner, leading to higher-quality research.

The potential benefits of Quant-View for the research community are vast. The standardization of units of measurements for bio-research imaging is required and will allow for more comprehensive data collection and efficacy. Scientific research will become more directly comparable when imaging experiment data is presented with an established standard. This will allow for a wider range of studies – even between different people and labs – to be directly compared and should result in higher productivity for the bio-research imaging research community.

NOTE FOR QUANT-VIEW FUNCTIONALITY:

Electrons-per-gray-level selector

This allows users to select how many electrons will cause a single gray-level increase in the image data. If there are enough photons

to saturate the 16-bit data bus but there is still no saturation of the physical pixel of the EMCCD, then users can raise the number of electrons needed to increase the gray level to the next value. For example, if you have 1000 electrons, then you are able to decide if this should be represented by 1000 gray levels (1 electron per gray level selected), by 500 gray levels (2 electrons per gray level selected), or by 250 gray levels (4 electrons per gray level selected).

This enables users to utilize Quant-View while maintaining the entire dynamic range of the EMCCD. The camera's highest bit depth in this quantitative mode is 16 bits. This means that as many as 65,535 gray levels per pixel can be sent over the bus. However, for the CCD97 and CCD60 used in the Evolve 512 and Evolve 128 respectively, the total number of electrons possible in a single pixel can easily reach 200,000. Thus, if the camera was set at 1 electron per gray level, then the pixel would come across as saturated (it would max out at the 65,535 value). So, by selecting each gray level to be equivalent to 4 electrons, the camera will send over a value of 50,000 gray levels, which is equivalent to 200,000 electrons. This feature is part of to the Quant-View feature, ensuring that users are able to utilize the full capability of the EMCCD while remaining in Quant-View mode.

Quant-View in action

For further explanation, including a real-time demonstration of Quant-View, please visit <http://www.photometrics.com/products/emccdcams/evolve/quant-view.php>

Rapid-Cal

EMCCD cameras are subject to aging of the EMCCD register as a result of its usage. The Evolve EMCCD cameras have a simple calibration feature that performs the industry's most accurate EM calibration within 3 minutes. A simple turn of the camera's nose-piece closes a shutter and activates a light source which the detector uses to calibrate its EM gain. This ensures that users will receive the most accurate electron multiplication and the EM gain applied matches what the user requests. Simple software control will allow the user to use this feature as a manual shutter in order to block all light from the sensor in order to take dark reference frames if necessary.

How will Rapid-Cal help?

It is a necessary requirement that your electron multiplication be accurate if you wish to be quantify the images taken by an EMCCD camera. The Evolve camera's Rapid-Cal allows extremely accurate and rapid calibration of the EM-Gain ensuring your data can be quantitative all of the time. It is recommended that calibration be performed once a week although the requirements of calibration will vary depending on the usage of the camera.

Rapid-Cal in action

LED INDICATOR	FUNCTION
OFF	Calibrator off
FLASHING AMBER/ORANGE	Calibration waiting for CCD temperature lock in order to proceed
FLASHING GREEN	Calibrating
SOLID GREEN	Calibration complete
RED	Error

For further explanation, including a real-time demonstration of Rapid-Cal, please visit <http://www.photometrics.com/products/emccdcams/evolve/rapid-cal.php>

Background Event Reduction Technology

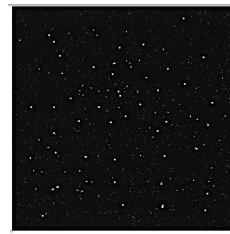
EMCCD cameras are actually capable of detecting single photons. However, the real detection limit of these cameras is set by the number of background events. These can arise from two things, Dark-current (which is thermal generation of an electron and is a temperature-dependent phenomenon) and also clock induced charge (CIC) electrons (also called spurious charge). Each can lead to the generation of non-photon derived electrons which are multiplied through the electron-multiplication register generating random high value pixels which are above the read noise. These are often referred to as "speckles" in the image. Photometrics has developed this real-time feature to help users identify such events and correct for them, in their images in real-time.

Users can set a threshold parameter that will measure the variation of a pixel value from all of its neighboring pixels and if the pixel value is unusually large such that it cannot come from real light through the microscope then the Evolve camera will realign the pixel value to a best approximation of where it should be – all in real-time. This serves to absolutely minimize the influence of clock induced charge and thermally induced amplified events on acquired data.

How will Background Event Reduction help?

This technology will help reduce the EMCCD induced artifact of spurious noise from entering your acquired data. Some may see this as a processing feature and claim that data is being altered. It is important to note that this feature (like all others) is completely controlled by the user and can be switched on or off. It should also be noted that the camera's major task is to most accurately represent the sample it is imaging. If the technology components used in the camera can cause the introduction of artifacts in the image then utilizing technology to minimize the influence of such artifacts is also just an extension of good technology design. The BERT function is an example of additional technology being used to enable the camera to more accurately represent the data being acquired.

Background Event Reduction in action



1000X EM Gain (no BERT)



1000X EM Gain (BERT=1.2)

For further explanation, including a demonstration of background event reduction technology please visit <http://www.photometrics.com/products/emccdcams/evolve/background-event-reduction.php>

Black-Lock / Top-Lock

Black-Lock

This advanced feature effectively functions as an intensity filter for the camera. It allows users to select the background level at which the EMCCD will make all pixels at or below the selected value equal to the value selected.

How will Black-Lock help?

This advanced feature allows users to define an effective floor for an image at a level which they select. Many users operate the camera in EM gain mode and apply EM gain at high levels. In such scenarios, EMCCD cameras can end up having multiple ADUs per electron. Traditional CCDs are set up to have electrons per ADU, which makes sense, as you can take the ADU measurement and convert it back to electrons. However, in the case of an EMCCD when EM gain is applied, the actual gain can increase such that you will end up with multiple ADUs being equivalent to a single electron.

For example, a traditional EMCCD camera with 3 electrons per ADU and 300x EM gain applied actually has 0.01 electrons per ADU. Thus, 100 ADUs is equivalent to a single electron of signal. For most bio-researchers, any signal less than 1 electron (equivalent to a single photon in a 100% QE system) does not represent meaningful data. With the Black-Lock feature from Photometrics, users can select at which point this value should be set. For instance, in the above example, one could set the Black Lock at 75 ADU above the image bias. As a result, the camera will read all pixels at or below 75 ADU as 75 ADU. Effectively, users are able to set the floor of the image and determine at which intensity levels data will be collected.

Top-Lock

This advanced feature effectively functions as an intensity filter for the camera. It allows users to select the background level at which the EMCCD will make all pixels at or above the selected value equal to the value selected.

How will Top-Lock help?

This advanced feature allows users to define an effective roof for an image at a level which they select. In many experimental conditions, there may be intense objects in the field of view that are actually of little or no interest to the experimenter. A simple example may be a piece of debris in the field of view that happens to be highly fluorescently labeled. If the debris is not of interest, a top intensity value of the image can be selected so that every single pixel in the image at or above the selected top intensity value will be displayed as the selected top value.

Another example where Top-Lock may be important is when researchers are looking for small, low-intensity-labeled features that are actually budding off or being released from a large feature. A specific example would be something like small vesicles being released from the Golgi apparatus in a cell. If the researcher is only interested in observing and tracking these features but the highly labeled Golgi is swamping the image display, then the camera can be set to lock all intensities above a certain level to that level. This will make the features the researcher is interested in much more visible, as well as scaled nicely within the display image for ready visualization.

Combining Black-Lock and Top-Lock

Utilizing Top-Lock and Black-Lock together allows users to narrow visualization rapidly and effectively into the intensity range of the image features they are most interested in.

Black-Lock and Top-Lock in action

For further explanation, including a real-time demonstration of black-lock and top-lock, please visit <http://www.photometrics.com/products/emccdcams/evolve/black-lock-top-lock.php>

Vari-Bit

This feature allows camera users to decide at which bit depth the digitization of the image should occur. Often with EMCCD imaging in biology, the intrascene dynamic range does not justify the use of high-bit-depth digitization (e.g., 16 bits) and the resultant image contains gray levels that represent fractions of photoelectrons. With this advanced feature, users can determine and select a more relevant digitization bit depth for their experiments.

How will Vari-Bit help?

By matching the digitization bit depth more closely to the actual intrascene dynamic range, the image quality will improve. Actual transitions between light levels from pixel to pixel will appear sharper to the eye; subsequently, users will be more certain of the results they are seeing. Using higher-bit-depth digitization does not make measurements less quantitative when measuring a scene with a low dynamic range; however, it does introduce many extra (or unnecessary) gray levels that do not represent actual physical photons.



For more information, visit <http://www.photometrics.com/products/emccdcams/evolve/vari-bit.php>