PHOTOMETRICS®

Application Note

Green Fluorescent Protein (GFP) Imaging in Living Cells

The Green Fluorescent Protein (GFP) is a 27-kilodalton protein isolated from the jellyfish *Aequorea victoria* that fluoresces in the green upon illumination with UV light. The cDNA has been cloned and placed into high-level expression vectors optimized for use in organisms ranging from plants to mammalian cells. With the availability of this cDNA, GFP can be introduced easily into virtually any cell type using standard transfection and selection methods, producing a stable cell lineage with maintained GFP expression.

The fusion of GFP with other proteins generates labeled proteins that can be constitutively expressed *in situ* for long-term studies. These chimeric proteins often maintain normal function when GFP is added to the NH_2 or COOH terminus of the fusion partner. Hence, GFP is an ideal tool for labeling cellular proteins to follow their spatial and temporal localization in live-cell preparations (see **Figures 1** and **2**).

In addition, red-shifted spectral variants of GFP have been produced using mutagenesis to alter sequences in the region of the chromophore. With multiple spectral variants of GFP available, it is possible to perform simultaneous monitoring of multiple labels within a single cell/tissue sample. By using optical-filtering devices such as the MAG Biosystems™ Dual-View™ or Quad-View™ (which can be easily coupled to any Photometrics® camera), multiple emission channels can be observed *simultaneously* in order to reveal the movement and/or interactions of different proteins in living cells.

Imaging GFP Chimeras

Illumination of living cells with light in the UV range is highly detrimental to the cells (the amount of damage is dependent on cumulative exposure time). Even with 488-nm laser line illumination of GFP there can be a disruption of normal cellular events such as mitosis. Thus, to obtain physiologically relevant data, the excitation light must be kept to an absolute minimum.

The primary consideration when selecting a camera to image GFP chimeras in living cells, therefore, is sensitivity. It is important to choose a low-noise camera that maximizes signal-to-noise ratio. Additionally, to perform precise localization of the labeled structures within the cell, a medium- to high-resolution detector is preferred.

Both quantum efficiency (QE) and readout noise are factors in determining the sensitivity limit of a detector. For instance, a typical back-illuminated CCD has a QE of 92% at 520 nm, which is more than three times higher than the QE of a standard front-illuminated CCD. When a back-illuminated CCD is read out at a slow rate, the noise of readout can reach levels that are three times lower than CCDs running at higher speeds.

The major limitations of older-style back-illuminated CCDs are large pixel size (typically 24 x 24 microns for the SITe 502B) and high readout noise. Large pixel size lowers the spatial resolution of the detector for a given magnification and high readout noise limits the speed at which the detector can be operated.

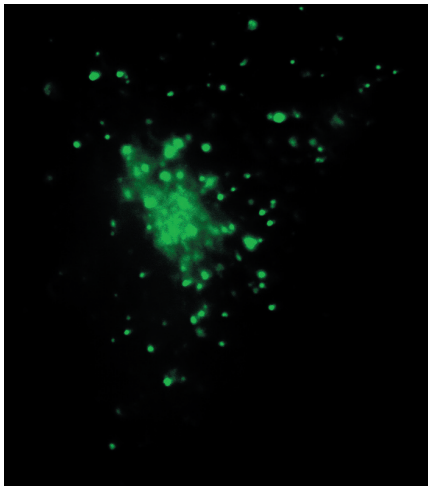

Figure 1. GFP wild-type Rab4. Image courtesy of Photometrics.

Photometrics has addressed these problems by working with leading CCD manufacturers to produce a series of the industry's lowest-noise CCDs with QEs optimized for the visible region. These CCDs also have smaller pixels, allowing biologists to maintain maximal spatial resolution while imaging their fluorescent proteins. They can be run at speeds up to 20 megapixels per second and are available in a variety of sizes in two distinct camera formats (the CoolSNAP™ and the Cascade®).

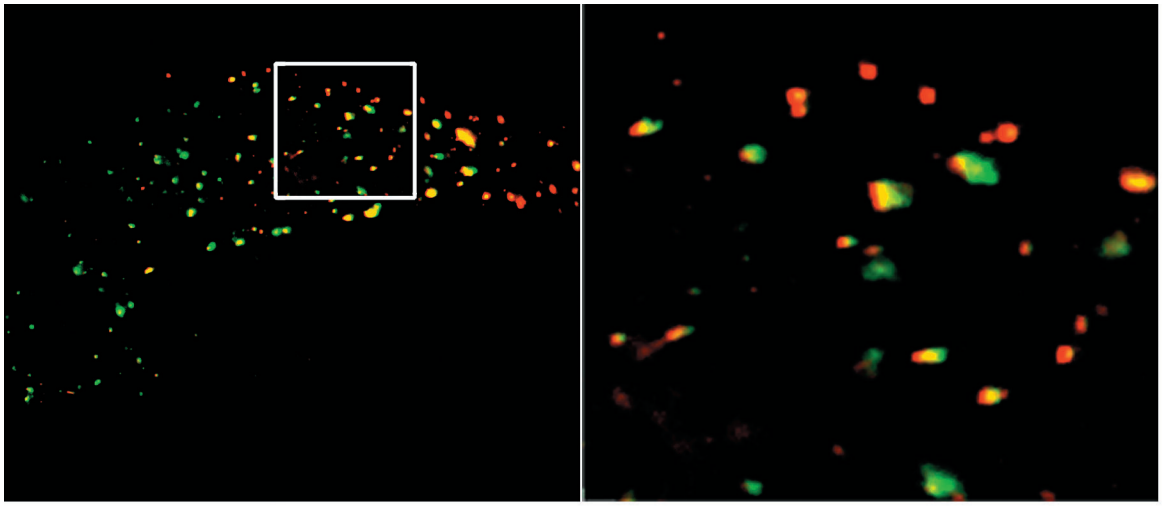

Figure 2. Peripheral region of a HeLa cell transiently transfected with GFP wild-type Rab5 and labeled with AlexaFluor® 594 (Molecular Probes) cholera toxin. The GFP compartments label the early endosomes, in which can be seen some of the cholera toxin. Image courtesy of Photometrics.

Cascade II:512 Camera System

Highest Sensitivity, High Speed

The Cascade II:512 monochrome camera system is optimized for high-speed imaging experiments in live cells. The camera combines the sensitivity of a back-illuminated electron-multiplying CCD (EMCCD) with the high-speed imaging capability of a frame-transfer device. Its EMCCD is cooled to -80˚C via a Peltier device, minimizing noise attributable to dark current.

The combination of speed and sensitivity afforded by the Cascade II:512 is ideal for 3D imaging of live cells. Note that when collecting 4D (3D over time) data sets, the camera's high sensitivity becomes even more important.

GFP can be used to monitor a huge variety of processes in cells, tissues, or whole organisms. For example, in cell-trafficking experiments, fluorescent proteins can be tagged to proteins of interest and then monitored for intracellular localization and itinerary in living cells using real-time data acquisition. Detection of low-light-level fluorescent protein expression is vital for such studies, as it reduces the chances of artifacts due to over-expression. A camera like the Cascade II:512 is an excellent choice for this type of application. The detector's 16-micron square pixels enable subcellular structures labeled with GFP to be resolved quite easily.

In order to synchronize image collection with a fast wavelength-switching device or a piezo-driven objective, the Cascade II:512 provides various TTL triggers that reflect the exact status of the exposure or image readout. Additionally, the Cascade II:512 can acquire data continuously (the light-sensitive portion of the EMCCD array collects light while the stored image is being read out from underneath the permanent mask). When run in standard-mode operation at 10-MHz readout speed, the camera can acquire data at rates ranging from 29 frames per second (fps) at full resolution up to >300 fps on binned subregions of the EMCCD.

For applications where fast frame rates are not as critical, the Cascade II:512 offers an additional software-selectable readout speed of 5 MHz. The lower-noise readout performance at this slower speed enables higher signal-to-noise data collection.

Cascade:1K Camera System

High Resolution, High Sensitivity

The Cascade:1K monochrome camera system utilizes a front-illuminated EMCCD with a 1004 x 1002 format and 8-micron square pixels. The camera is cooled to -30˚C, reducing dark current to an almost non-measurable amount when using millisecond exposure times. Its 10-MHz A/D converter yields 16-bit data on single pixels. Compared to the Cascade II:512, the Cascade:1K has a slower frame rate and smaller pixels. These features make the Cascade:1K well suited for high-sensitivity imaging with high resolution.

CoolSNAP$_{HQ^2}$ Camera System

Highest Resolution

The CoolSNAP$_{HQ^2}$ monochrome camera system is a premier high-spatial-resolution GFP imaging device whose interline-transfer CCD delivers good QE across the full visible spectrum (>60% at 520 nm). The detector's 1392 x 1040 format and 6.45-micron square pixels provide ultra-high-resolution images. The camera also boasts an industry-leading read-noise specification, typically around 4.0 e- (at 10 MHz in gain state 3). Thermoelectric cooling to -30˚C minimizes dark current, enabling longer exposure times.

For greater application flexibility, the CoolSNAP$_{HQ^2}$ employs 14-bit digitization as well as both high-speed (20 MHz) and high-sensitivity (10 MHz) readout modes. The "live feel" of the camera's video output makes setting up and focusing extremely easy — without sacrificing low-noise performance. Camera vibration is eliminated via electronic shuttering, which also facilitates fast triggering.

The CoolSNAP$_{HQ^2}$ is best suited for GFP imaging that requires the absolute highest resolution possible at high frame rates. Under experimental conditions where resolution requirements are not as high, the camera can be run in binned mode to increase signal-to-noise ratio and further shorten readout times. The camera also allows programmable subregion readout, which reduces the digital data load while increasing frame rates for kinetic imaging applications.

With many of the same features and only a slight decrease in noise performance, another Photometrics camera, the CoolSNAP$_{ES^2}$, represents an economical, streamlined alternative to the CoolSNAP$_{HQ^2}$.

For assistance comparing the performance characteristics of the Cascade II:512, Cascade:1K, and CoolSNAP$_{HQ^2}$, consult **Table 1**.

	Cascade II:512	Cascade:1K	*CoolSNAP$_{HQ^2}$*
Format	512 x 512 pixels	1004 x 1002 pixels	1392 x 1040 pixels
Pixel size	16 x 16 microns	8 x 8 microns	6.45 x 6.45 microns
Diagonal	11.6 mm	11.3 mm	11.2 mm
Peak QE	92%	65%	62%
A/D converter	10, 5 MHz (16 bits)	10 MHz (16 bits)	20, 10 MHz (14 bits)
Read noise (fast/slow)	<1 e- with EM gain	<1 e- with EM gain	5.5 e- / 4.5 e-
Frame rates	29 to 331 fps	9 to 103 fps	11 to 104 fps
Regulated cooling	-80˚C	-30˚C	-30˚C
Summary	Higher-speed, live-cell, nonshuttered imaging	Lower-speed, live-cell, nonshuttered imaging	Fixed/live-cell, nonshuttered imaging

Table 1. Feature comparisons for Photometrics cameras.