Application Note

Fluorescence and DIC

For many years, biologists have been using fluorescence microscopy and differential interference contrast (DIC) imaging in applications where it is desired to correlate the fluorescence emission from a probe with specific cell structure, especially when the cell structure is morphologically correlated to a biological process of the cell.[1] The use of simultaneous fluorescence and DIC imaging can provide information about physiological parameters of different cell types, including intracellular-signaling mechanisms, activities of specific ion-transport pathways, and changes in intracellular environments.

It is of significant interest to researchers to utilize both modes of imaging with the same instrumentation to allow multi-parameter determinations in single living cells. In the past, simultaneous-imaging configurations using dual-detector systems have been used to acquire fluorescence and DIC images (Foskett, 1988). While these configurations are well suited to live-cell imaging applications, precise image registration is very difficult to achieve because the two separate imaging arms use different cameras and different optical components, resulting in each image having slightly different magnifications and other image distortions.[2] Sequential imaging techniques have also been implemented to acquire fluorescence and DIC images (Spring, 1990). While this approach results in the precise registration of the images, the time delay between the fluorescence and DIC images is problematic in dynamic imaging situations such as live-cell imaging.

The Photometrics® DV2™ and QV2™ provide excellent solutions for these applications because they allow simultaneous fluorescence and DIC imaging with a single detector (see **Figures 1** and **2**). Thus, simultaneous images are acquired, which is crucial for live-cell imaging, and these images can be precisely registered.

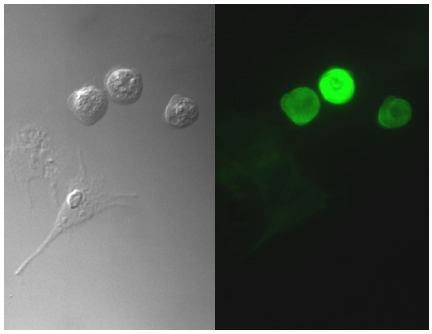
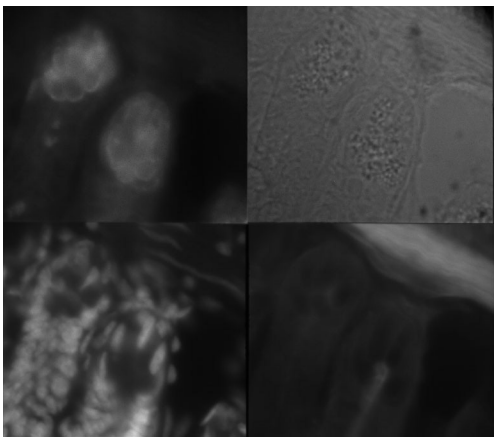


Figure 1. Simultaneous GFP fluorescence (right) and DIC image (left) of cells acquired with the DV2 system.

a.



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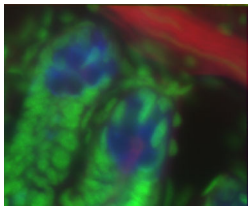

c.

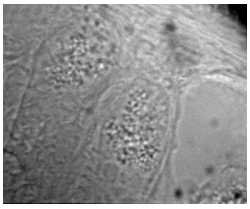

Figure 2. Simultaneous three-channel fluorescence and DIC images acquired with the QV2 system. The sample being imaged was obtained from Molecular Probes (FluoCells® prepared slide #4 – mouse intestine section with Alexa Fluor® 350 WGA, SYTOX® Green, Alexa Fluor 568 phalloidin). (a) Raw image. (b) Color overlay of blue, green, and red fluorescence channels. (c) NIR DIC image.

System Configuration

The Photometrics DV2 and QV2 have been used with success in various fluorescence imaging applications for microscopy. In most applications, these systems are fitted with dichroic filters and spectral emission filters for simultaneous imaging of multiple fluorescent labels. When used with a DIC-fluorescence microscope, the DV2 and QV2 systems can also be used for simultaneous fluorescence and DIC imaging.

The DV2 is fitted with a standard dichroic module that contains a dichroic filter and a fixed fold mirror. The emission filter holder is fitted with a spectral emission/barrier filter for the fluorescence channel and an analyzer and near-IR filter for the DIC channel. A near-infrared bandpass filter (750-nm center with a 10-nm bandpass) is inserted into the brightfield channel somewhere in the illumination path (i.e., right after the lamp in the lamp housing, or right after the condenser lens, but before the sample). The operation of this system is shown conceptually in **Figure 3**. When imaging shorter fluorescence wavelengths (blue-yellow), a deep-red visible filter (660 nm) can be used for the DIC channel instead of the NIR filter.

The Photometrics QV2 allows the simultaneous visualization of three fluorescence images and one DIC image on a single detector. In this scenario, each image appears in a different quadrant of the detector. As an example, this configuration can be used to view DAPI, FITC, Texas Red, and DIC simultaneously.

Key Advantages

- The system design provides significant flexibility in the selection of the DIC illumination wavelength, ranging from deep-red visible to near-infrared wavelengths.
- The use of deep-red visible or near-infrared wavelengths allows DIC imaging without fluorescence, photobleaching, or heating of the sample due to this illumination.
- The analyzer is no longer in the fluorescence emission path as in standard DIC, so the efficiency of the fluorescence is not affected by DIC imaging.
- The intensity in the DIC channel can be independently adjusted using the manual adjustment knob for the brightfield lamp intensity. Low-light fluorescence can be imaged at the same time as the DIC without adjusting the gain of the camera differently for the DIC image versus the fluorescence image.

Citations

1. J. Kevin Foskett, "DIC and Quantitative Low-Light Fluorescence Video Imaging," *Optical Microscopy*, eds. Brian Herman, John LeMasters (New York: Academic Press, ©1993) pg. 259.

2. J. Kevin Foskett, "DIC and Quantitative Low-Light Fluorescence Video Imaging," *Optical Microscopy*, eds. Brian Herman, John LeMasters (New York: Academic Press, ©1993) pg. 255.

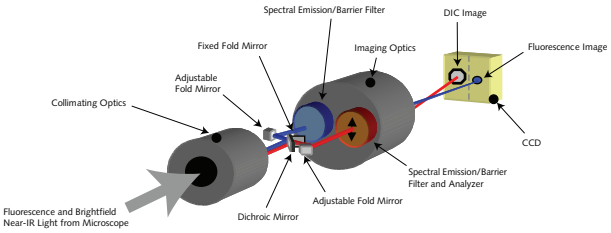

Figure 3. Operation of the DV2 fluorescence/DIC imaging system.

Resources

To learn more about the Photometrics DV2 and QV2, visit **www.photomet.com/products/multichannel**.

To learn more about high-performance CCD cameras, visit **www.photomet.com** and **www.qimaging.com**.