It should be kept in mind, however, that imaging instrumentation rarely has just a single function in a laboratory. Thus, the hardware selected should not limit the efficacy of applications that may be encountered over time.

For instance, with the maturation of techniques such as spectral imaging, many researchers are now enhancing their FISH experiment capabilities by using a far greater number of fluorescent probes at one time. Spectral imaging enables the identification of probes based on their spectral curves, allowing differentiation of closely overlapping fluorophores. When cameras are utilized for spectral imaging with purpose-built systems such as the Optical Insights Spectral-DV™, a specimen’s fluorescent emission can be split into component wavelengths. As a result, excellent camera performance and detector sensitivity become critical.

CoolSNAP cf Color Camera
One high-performance camera solution for FISH imaging is the Photometrics® CoolSNAP cf color camera system, which employs an interline-transfer CCD with a Bayer color mask. The color mask set has two green filters, one blue filter, and one red filter in each pixel quartet. A full-color image is produced using standard interpolation methods that preserve the high-resolution nature of the data.

While sequential acquisition with separate filter sets represents the best solution for mFISH, this reliable CoolSNAP™ camera can be used for two- or three-color FISH preparations in conjunction with a multi-pass filter cube (multiple excitation, dichroic, and emission windows). The 4.65-micron square pixels help compensate for the slight loss of resolution from interpolation, yielding clear color images (see Figure 1).

First and foremost, of course, it is important to match the camera to the FISH technique being used. Many researchers utilize two- to four-color FISH analysis on fixed samples. The main requirement for imaging of this kind is an extremely high-resolution detector that allows all of the spatial information to be preserved under high magnification. Since the preparations most often contain fixed cells, more intense illumination can be used to produce signals that are relatively strong, a fact that suggests that a medium-performance CCD camera could be used for this imaging application.

Figure 1. Individual chromosomes are viewed in metaphase using triple-wavelength fluorescence illumination and detection. FISH image taken with a CoolSNAP cf camera.
**CoolSNAP**<sup>HQ</sup><sup>2</sup> Camera

The CoolSNAP**<sub>HQ</sub><sup>2</sup> camera system utilizes a CCD that has greater than 1.44 million 6.45-micron square pixels, allowing collection of images with very high spatial resolution (see Figure 2). In fact, this pixel format helped make the original CoolSNAP**<sub>HQ</sub> the most highly published camera for this application area.

The new CoolSNAP**<sub>HQ</sub><sup>2</sup> camera system comes in a compact housing and provides the industry’s lowest-noise readout, software-selectable gains, and a comprehensive set of subregion readout and binning controls. It also has an additional visual gain setting to enable optimal visual display and a controllable fan to minimize vibration. The quantum efficiency (QE) of the CCD is best for wavelengths ranging from 500 to 800 nm. This detector is particularly well suited for “very long wavelength” probes (near IR).

**Cascade II:512 Camera**

The Photometrics Cascade II:512 camera system utilizes a back-illuminated electron-multiplying CCD (EMCCD) that offers unparalleled sensitivity for bio-imaging. Back-thinning the device results in >92% QE, making it one of the most efficient light-gathering detectors available to bio-researchers. This Cascade<sup>®</sup> camera’s electron-multiplying capability, coupled with deep cooling (-80˚C), facilitates extremely low-light imaging.

The performance of the Cascade II:512 lends itself to spectral imaging for FISH by minimizing integration times and noise factors while maximizing sensitivity. Additionally, more than 2.62 thousand 16-micron square pixels allow the camera to collect images with good spatial resolution.

When the Cascade II:512 is coupled to a spectral imaging system like the Optical Insights Spectral-DV, the detector’s x axis, usually used for spatial resolution, instead correlates to spectral sampling ability. The resultant sampling frequency, up to 1.07 nm, provides highly accurate spectral curves. The Spectral-DV allows emission light to be spread across the EMCCD to yield a three-dimensional spectral cube (see Figure 3a) in which each image pixel has an associated spectral curve (see Figure 3b). Researchers can also specify their spectral sampling rate via the asymmetric binning function of the Cascade II:512. These key features let researchers use and decipher multiple overlapping fluorophores, helping extend FISH studies so that many genes — easily up to eight — can be examined within a single sample.

The ultrahigh QE and electron-multiplying capability of the Cascade II:512 allow unsurpassed spectral-imaging data collection. For the utmost in application versatility, the camera system can even be operated in a traditional CCD mode (in which electrons are not multiplied) that delivers high-QE, low-read-noise performance when photons are not as scarce!

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**Figure 2.** GFP actin and β-actin FISH images taken at specific wavelengths with a CoolSNAP**<sub>HQ</sub><sup>2</sup> camera to show expression of actin in a mouse myoblast (C2C12) cell. Image courtesy of Alex Rodriguez, Robert Singer Lab, Albert Einstein College of Medicine, New York.
**Fluorescence In Situ Hybridization (FISH) Imaging**

**Figure 3a.** When the Cascade II:512 is coupled to the Spectral-DV, emission light is spread across the EMCCD to yield a three-dimensional spectral cube.

- High QE (>92%)
- Selectable spectral sampling via asymmetric binning
- Series of acquired images allows construction of x-y-λ spectral cube
- Electron-multiplication gain for extreme low-light sensitivity
- Variable electron-multiplication gain to suit sample imaging requirements
- -80°C cooling to minimize dark current

**Figure 3b.** When used in conjunction with the Spectral-DV, each pixel in the Cascade II:512 detector array has an associated spectral curve.