The Prime 95B™ Scientific CMOS Camera for Super-Resolution Localization Microscopy

The most important consideration for improving localization accuracy in super-resolution localization microscopy is to increase the number of detected photons. When the point spread function (PSF) of a fluorophore is localized by fitting to a Gaussian function, the height and width of the peak are determined by the number of photons detected. A taller and thinner peak gives a better localization accuracy.

Aside from good fluorophore selection and sample preparation, the best way to increase the number of detected photons is with a sensitive camera. For this reason, super-resolution localization microscopy has historically been performed with electron-multiplying CCD (EMCCD) cameras. The high >90% quantum efficiency of the back illuminated EMCCD sensor, large pixels, and electron multiplication, combine to maximize photon detection and minimize read noise. However, some researchers are now choosing to use sCMOS devices to gain a considerable increase in speed and field of view but sacrificing sensitivity and localization accuracy.

This is an understandable sacrifice as higher speeds and a larger field of view allow researchers to increase throughput and image larger structures. The sensitivity of EMCCD cameras is also deceptive because the electron multiplication process introduces another source of noise into the system; excess noise factor. Excess noise factor multiplies the effect of all other noise sources by √2 (~1.4) due to uncertainty introduced as part of the electron multiplication process. CMOS cameras do not suffer from this drawback.

The world's first back-illuminated scientific CMOS camera, the Photometrics Prime 95B, offers localization microscopy researchers the best of both worlds. High >95% quantum efficiency delivers equivalent sensitivity to EMCCD cameras while maintaining the low read noise, large field of view and high speed of sCMOS cameras.

The high sensitivity of the Prime 95B isn't limited by excess noise factor, delivering higher signal to noise and therefore increased resolution accuracy than any EMCCD. Furthermore, the large 11μm pixels of the 95B perfectly reach Nyquist sampling with 100x magnification, eliminating the need for any additional lenses to correct for pixel size. Every additional lens in the light path reduces the number of photons by 3-4% so the 95B truly improves the number of photons detected.

We believe that the Prime 95B is the best choice of camera for localization microscopy. We've collected data in collaboration with some of our customers directly comparing the 95B with 1024x1024 EMCCDs and 512x512 EMCCDs on STORM and DNA-PAINT systems. In both cases, localization accuracy was increased on the 95B. We've also investigated our PrimeEnhance™ live denoising algorithm on this data and found that by optimizing the localization algorithm, localization accuracy could be further improved.

We encourage you to explore the data yourself by reading the Camera Comparison: Prime 95B Scientific CMOS and EMCCD Technical Note.