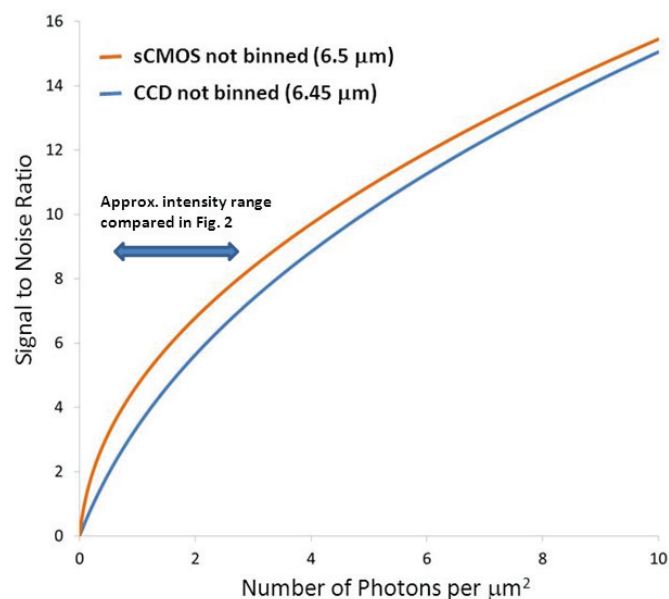


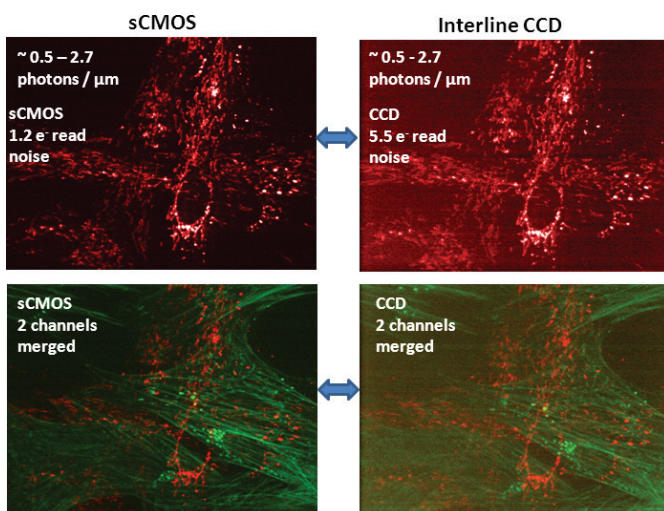
# New sCMOS vs. Current Microscopy Cameras

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Since the launch in late 2010 of imaging cameras that are based on a new 5.5 megapixel scientific CMOS (sCMOS) sensor, there has been much speculation about whether or not sCMOS will be seen as a technology replacement for interline CCD and electron multiplying CCD (EMCCD) cameras – which, in many ways, can be considered the current gold standards for low light fluorescence microscopy and bio-imaging in general. Coming from the unique market position of manufacturing all of the aforementioned camera types, we provide here an analysis of how these sensitive imaging technologies compare.



**Figure 1:** Plots comparing SNR of sCMOS vs Interline CCD cameras (neither with pixel binning) as a function of incident photons per square micron on the sensor. sCMOS provides higher SNR throughout the intensity range due to a significantly lower read noise floor. The specifications for sCMOS are based on the Andor Neo camera, operated in rolling shutter mode with 560MHz readout speed and fan cooled to -30 0C. The CCD interline performance is based on the specifications of the Andor Clara camera @ 20MHz readout speed, fan cooled to -55 0C. Exposures of 1 sec are assumed, such that the darksignal contribution from each cooled camera is relatively small.



**Figure 2:** Comparative low light images taken with Andor Neo sCMOS (1.2 electrons read noise @ 400 MHz) vs Andor Clara interline CCD (5.5 electrons read noise @ 20MHz) of a fluorescently labelled fixed cell using a CSU-X spinning disk confocal microscope (x60 oil objective), each 100ms exposure, same laser power per channel, displayed with same relative intensity scaling. Note, the field of view is limited by the aperture size of the CSU-X spinning disk unit, which is matched to the 1.4 megapixel interline sensor.

## Technology Overview

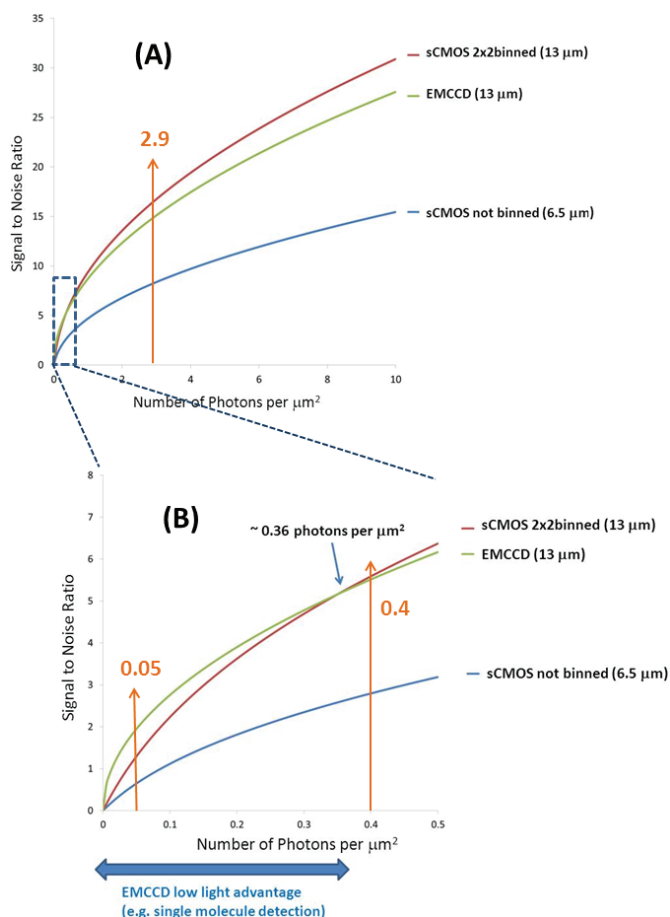
The small pixel interline CCD has dominated bio-imaging for more than a decade. For cell microscopy, the leading sensor type has been a 1.4 megapixel format from Sony, offering 5 to 6 electrons read noise at approximately 11 frames/sec (down to 2.4 e- at 1 frame/sec). The 6.45 μm pixel size represents a distinct “sweet spot,” an ideal balance between the photon collection area per pixel and the ability to oversample the diffraction limit for better resolution of fine intracellular detail.

The electron multiplying CCD camera was introduced into the scientific market by Andor in 2000 and represents a significant leap forward in combining ultra-sensitivity with speed. EMCCD cameras employ an on-chip amplification mechanism called “impact ionization” that multiplies even single photon events well above the read noise floor. Importantly, this renders the EMCCD capable of single photon sensitivity at fast frame rates. This attribute has rapidly gained recognition for EMCCD technology in demanding ultra-low light measurements, such as single molecule detection and photon counting experiments. While a well optimized EMCCD is close to a “zero noise floor” detector, the down side is that the signal amplification mechanism carries an additional noise source called multiplicative noise which effectively increases the shot noise (or poisson noise) of the signal by a factor of x1.41, manifest as an increase in the pixel to pixel and frame to frame variability of signals. Furthermore, the popular back-illuminated EMCCD sensors are limited to 13 μm smallest pixel size, which while offering good photon collection area, tends to limit the ability to resolve fine intracellular detail.

Scientific CMOS (sCMOS) technology is based on a new generation of CMOS design and process technology. This device type carries an advanced set of performance features that renders it entirely suited to high fidelity, quantitative scientific measurement. sCMOS can be considered unique in its ability to simultaneously deliver on many key performance parameters, whilst also overcoming the performance drawbacks that have traditionally been associated with conventional CMOS imagers.

The 5.5 megapixel sensor offers a very large field of view and high resolution, without compromising read noise or frame rate and a 6.5 μm pixel size is again ideally suited to cell microscopy. The read noise is exceptionally low, even when compared to the highest performance slow CCDs, but not as low as the effective read noise of EMCCDs. The sCMOS device can achieve down to 1 electrons RMS read noise, without amplification, while reading out 5.5 megapixels at 30 frames/sec. Furthermore, the sensor is capable of achieving 100 full frames/sec with a read noise of 1.4 electrons RMS.

Significantly, under extremely low light conditions a sCMOS camera can be readily operated with pixel binning, thus creating larger



**Figure 3:** Plots comparing SNR of sCMOS (non-binned and 2x2 binned) and larger pixel EMCCD (non-binned) as a function of incident photons per square micron on the sensor. Two ranges are given, the expanded low light range (B) is where the ‘zero read noise’ operation of EMCCDs offers a distinct sensitivity advantage.

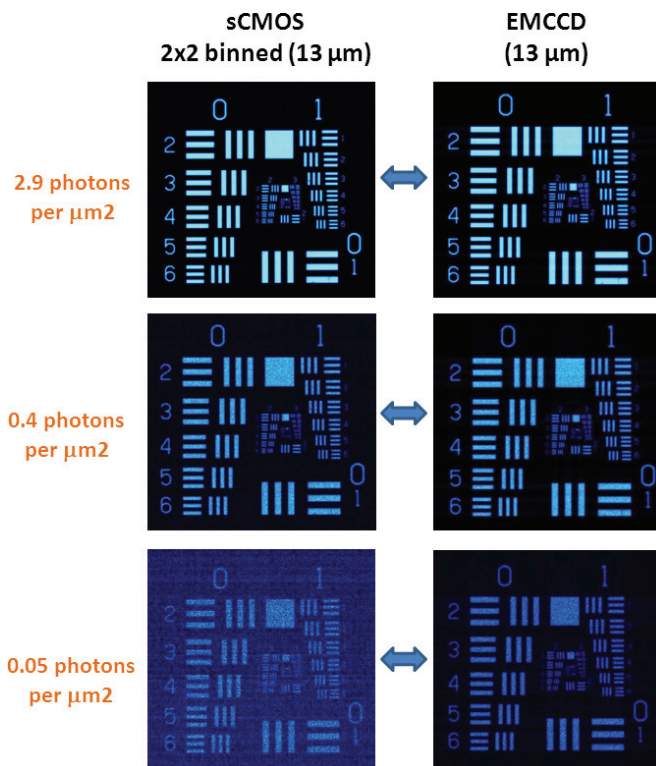
“super-pixels” for improved photon collection area when required. It is worth noting that, unlike with interline CCDs, under this 2x2 binning condition the read noise of sCMOS will double, i.e. 1 e-rms becomes 2 e-rms. This noise increase is indeed appropriately factored into comparative SNR plots shown in this article.

By way of a unique dual amplifier sensor architecture, the sCMOS camera offers much higher dynamic range than would be expected from a CCD with similarly small pixel size. This design circumvents the need to choose between high or low gain amplifiers, in that signal can be sampled simultaneously by both high and low gain amplifiers. As such, the lowest noise of the sensor can be harnessed alongside the maximum well depth, affording widest possible dynamic range.

The sCMOS can read out in both “rolling” and “global” (snapshot) shutter modes. Dark signal generated in each mode is very similar, but the absolute fastest frame rates and lowest read noise can be achieved in rolling shutter mode, which in reality will suit the vast majority of biological imaging applications since objects will typically move sub-pixel distances during the time taken for rolling readout to traverse them.

### Sensitivity Comparisons

Figure 1 shows a plot of SNR against number of photons per  $\mu\text{m}^2$  for sCMOS vs. interline CCD camera technologies. The x-axis is a representation of photon flux incident on the detector surface; a value of 1 equates to approximately 42 photons incident within a 6.5



**Figure 4:** Comparative low light images taken with Andor Neo sCMOS (1.4 electrons read noise @ 560MHz) vs Andor iXon3 888 back-illuminated EMCCD (< 1e- read noise), under a range of photon intensity conditions, indicated on the figure 3 plots. sCMOS was 2x2 binned in order to have the same effective pixel pitch (and light collection area per pixel) as the 13  $\mu\text{m}$  pixel of the EMCCD sensor. The superior sensitivity of back-illuminated EMCCD under extremely low light conditions is readily apparent.

$\mu\text{m}$  pixel. The read noise differences between the two technology types is reflected in the notable separation between the respective SNR curves across the intensity range shown. Figure 2 demonstrates clear distinction in low light signal contrast, arising from read noise differences between sCMOS and interline CCD cameras. The comparative images were recorded on a spinning disk confocal fluorescence microscopy set-up (an inherently low light modality), the signal intensity ranging between 0.5 to 2.7 photons incident per  $\mu\text{m}^2$  of the sensor.

Figure 3a shows a further plot of SNR against number of photons per  $\mu\text{m}^2$ , this time for sCMOS (non-binned and 2x2 binned) versus a 13  $\mu\text{m}$  back-illuminated EMCCD camera, for which the zero to 10 photons per  $\mu\text{m}^2$  range shown represents a relatively bright signal regime. Consideration of the curve for 2x2 binned sCMOS provides a keen perspective on just how impacting the pixel size becomes on the overall sensitivity performance. Across the range shown, the 2x2 binned sCMOS appears to exhibit a better SNR than the equivalent pixel size (non-binned) back-illuminated EMCCD camera. It is reasonable to ask why a “zero read noise,” back-illuminated EMCCD camera would exhibit lower SNR than a >2e- noise (when binned) front-illuminated sCMOS device. The answer to this lies in the additional multiplication noise imposed by the EMCCD signal amplification mechanism.

However, the light range shown does not emphasise the truly low light signal intensities and associated applications whereby EMCCD technology will provide a SNR advantage. Figure 3(b) shows the same data, but expanded on the low light intensity range between zero to 0.5 photons per  $\mu\text{m}^2$  sensor area (i.e. up to ~ 85 photons per 13  $\mu\text{m}$  pixel). Here we can see that at signal intensities below 0.36 photons per  $\mu\text{m}^2$ , the back-illuminated EMCCD will indeed offer an improved SNR compared to the 2x2 binned sCMOS. This in effect is

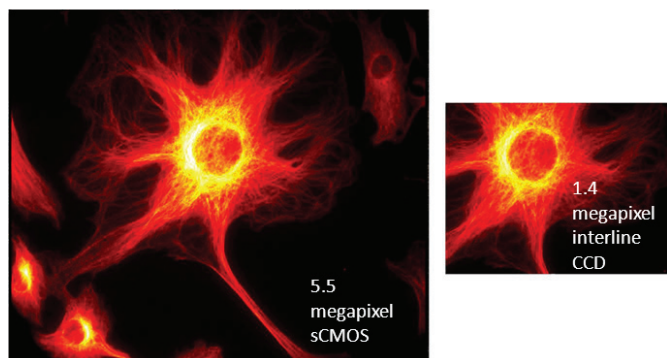


Figure 5: Field of view comparison of two technologies;  $\times 60$  magnification; 1.25 NA; 5.5 megapixel sCMOS vs 1.4 megapixel interline CCD (each have  $\sim 6.5 \mu\text{m}$  pixel pitch). sCMOS is capable of offering this larger field of view @ 100 frame/s with 1.4 e<sup>-</sup> read noise.

the region in which the “zero read noise” properties of an EMCCD outweigh the negative effect of multiplication noise.

By way of demonstration, figure 4 shows comparative images recorded of an LED illuminated resolution chart in a light tight imaging chamber at a series of light intensities, marked in the figure 3 graphs. The images taken in the very low light regime, i.e. below the “cross-over” point, clearly demonstrate the sensitivity advantage of the “zero read noise” EMCCD in this range.

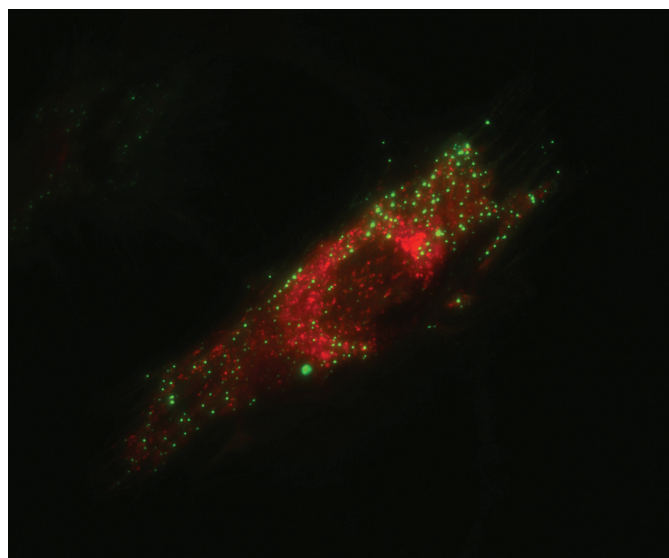


Figure 6. Mouse embryonic fibroblast (MEF) cells transfected with DNA plasmids, encoding for two fluorescent proteins: Clathrin, fused to green fluorescent protein (GFP-Clathrin), and Rab5, fused to the monomeric red fluorescent protein (mRFP-Rab5), imaged by total internal reflection fluorescence (TIRF) microscopy.

GFP-Clathrin localizes to vesicles that form on the plasma membrane of cells (visible as green spots). mRFP-Rab5 is found on vesicles located more internally, but still within the evanescent field generated by TIRF (red spots). Images courtesy of Dr Roberto Zoncu, Whitehead Institute for Biomedical Research, MIT.

### Application Decisions

Such raw sensitivity performance at extremely low light signal intensities means that EMCCD technology will still be the detector of choice for a number of demanding applications. For example,

the principal microscopy usage of EMCCDs to date has been in the field of single molecule biophysics, and this is unlikely to change significantly. An exception to this may become apparent in the area of super-resolution microscopy by single molecule localization techniques (e.g. PALM, STORM), in that since it is required to reach a threshold SNR in order to yield sufficient localization accuracy, then the threshold number of photons per exposure required may occur at or beyond the cross-over region between the two technology curves. While the majority of live cell microscopy experiments may eventually opt to utilize sCMOS technology, particularly to benefit from the  $6.5 \mu\text{m}$  pixel size combined with the larger field of view of the 5.5 megapixel sensor, there will be some exceptionally low light instances in which a back-illuminated EMCCD will remain indispensable, for example when measuring calcium flux from smooth muscle cells using the Nipkow spinning disk confocal modality.

Furthermore, since sCMOS are not single photon sensitive, EMCCD technology is still required for single photon counting experiments. From the consideration of the sCMOS vs interline evidence, it seems more likely that interline CCD technology will eventually be displaced by sCMOS technology, especially so in the field of cell microscopy, but also in other applications such as high throughput genome sequencing, high content screening and ophthalmology.

One exception remains, whereby a cooled interline CCD maintains an application advantage over a cooled sCMOS camera. This relates to long exposure luminescence detection, e.g. bioluminescence microscopy, chemiluminescence gel documentation or in-vivo bioluminescent imaging, in that exposures greater than 60 secs will yield a lower overall noise floor (read noise and dark noise combined) for the Sony interline CCD. There are two reasons for this; (a) at frame rates slower than 1 frame per sec, the Andor Clara interline readout can be reduced to 1MHz, which reduces the read noise to approximately 2.5 electrons (b) a cooled Sony interline sensor maintains an extremely low dark current performance relative to cooled sCMOS. Performance comparisons aside, the more general reality is that interline CCDs will most likely continue in the market for some time yet due to cost advantage.

Ultimately, however, it should be noted that we are witnessing the early stages of product entry and the ink is still wet regarding which applications will truly benefit from switching to sCMOS technology. However, the material presented here should at least prove beneficial in making an informed decision whether to evaluate any the detection solutions covered.

### Meet The Author

Dr Colin Coates is Product Manager at Andor Technology plc. in Belfast.

### References

1. M. Hollywood et al., Optimizing low-light microscopy with back-illuminated electron multiplying charge-coupled device: enhanced sensitivity, speed, and resolution, *Journal of Biomedical Optics* 9(6), 1–0 (November/December 2004)
2. B. Fowler et al., A 5.5Mpixel 100 frames/sec wide dynamic range low noise CMOS image sensor for scientific applications, *Proc. SPIE* 7536, 753607 (2010)