Improving microscopy and biological imaging with backside illuminated (bi) sCMOS sensors

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Biological sciences research depends heavily on microscopy, without which researchers would be unable to observe and collect crucial data. But researchers also need a way to capture visual phenomena for analysis, documentation, and reporting.

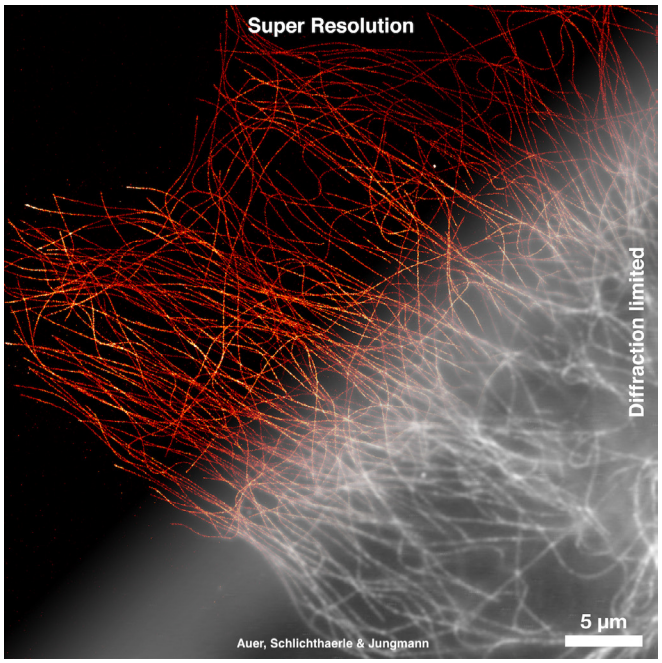
Until now, laboratories have typically opted for cameras with image sensors that employed either charge-coupled device (CCD) or relatively early complementary metal-oxide semiconductor (CMOS) technology. However, the introduction of scientific CMOS or sCMOS sensors has opened new possibilities for researchers to get better and more quantifiable image data out of biological samples.

To understand the advantages of sCMOS, it helps to appreciate the underlying differences between the technologies.
Traditional CCD sensor cameras can only be as good as the sensors at their core. A CCD sensor is a light-sensitive semiconductor divided into small spaces called pixels, typically arranged in rows that form a rectangle.

When photons—particles of light—strike the surface of the pixel, they generate electrons that are stored in a part of the pixel called the well. The number of electrons generated is proportionate to the number of photons that strike the pixel. The more light that strikes, the more electrons are stored. The wells are the electronic equivalent of dots of light of different intensity that, when viewed on a screen, look like a picture. But before the picture is available on the screen, the camera must pull it off the sensor.

The first type of camera sensor, and one still commonly used across many labs, is the CCD. To get the pixel data, the sensor shifts the rows of stored electric charge packages, one by one, to the edge of the sensor. Each row has to pass, pixel by pixel, through an analog-to-digital converter (ADC), which translates the number of electrons from each pixel into an equivalent level of brightness, or grey value, in the final digital image file.

The slow throughput nature of the digitization process means CCD sensors have major trade-offs in speed, resolution, and sensitivity. The increase in one will mean a decrease in another. However, many demanding biological imaging applications require a combination of high speed, high resolution, and high sensitivity. Because of their trade-offs, CCD-based cameras could not deliver all those requirements simultaneously at any given time. To attain the combination of high speed and high resolution, CCD cameras require greater levels of light to be directed on fragile specimens, in turn generating heat that negatively affects cell samples. Thus, researchers have had to use lower levels of light and settle for longer exposures with CCDs, limiting temporal resolution (amount of time available to observe and collect data) and limiting the ability to image significant biological processes.



DNA-PAINT imaging in a cellular environment. Microtubules are labeled with alpha tubulin primary antibodies and DNA-conjugated secondary antibodies. Imaging was performed in TIRF mode with an un-cooled sCMOS camera (pco.panda 4.2). (Courtesy of M. Auer, T. Schlichthaerle and R. Jungmann, Max- Planck-Institute of Biochemistry, Munich, Germany)

Still, CCD sensors have been dependable for many years and are well integrated into many major systems, which is why they still serve particular imaging needs in many labs. However, alternatives now exist that are better suited for many types of biological imaging.

sCMOS Sensors Bring Great Advantages

CMOS sensor architecture is inherently different than that of a traditional CCD. Instead of all the pixels eventually passing through a single A-to-D converter, each

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column of pixels has its own ADC. Thus, the image can be captured, digitized, and read far more rapidly.

Multiple types of CMOS sensors exist, but the newest version available for biologists is the sCMOS sensor. An sCMOS sensor is characterized as having lower inherent noise (that is, read noise, which is approximately 1 to 2 electrons) and higher quantum efficiency, or the percentage of photons striking the sensor that result in the generation of an electron. The combination offers greater sensitivity to the lower light levels that researchers need. In addition, sCMOS allow for a larger field of view, higher resolution, and greater dynamic range to improve the quality of the image and resulting analysis.

Building on the first-generation sCMOS sensors developed around 2009, the most recent and promising sensor enhancement is the backside illuminated (BI) sCMOS sensor. The first-generation sCMOS were known as front-illuminated sensors. They required light to pass through layers of electronic circuitry embedded in the sensor before striking the light-sensitive photodiodes of the pixels. The circuitry can block and scatter incoming light, reducing the number of photons reaching the photodiode, thereby limiting sensitivity. In contrast, using back-illumination, the sensor is effectively flipped around. The amount of bulk material is now thinner. The photons strike the light-sensitive material in the pixels (photodiodes) first, without passing through as many sensor layers as before, thereby increasing sensitivity.

With greater sensitivity, speed, resolution, and dynamic range, a shift to cameras with backside-illuminated sCMOS sensors can provide extensive benefits to researchers, all at prices similar to existing equipment.

The pco.edge 4.2 bi Camera

An example of a camera with a back-illuminated sCMOS sensor is the pco.edge 4.2 bi. The sensor provides 16-bit digitization, thus capturing the whole nearly 15-bit real dynamic range. The increased bit depth offers more than 65,000 possible levels for each pixel for a larger dynamic range and the ability to better distinguish subtle changes across the image.

The sensor has a quantum efficiency of up to 95 percent, which means the vast majority of the photons striking the sensor cause the creation of electrons, increasing sensitivity. A 2,048 by 2,048 pixel sensor with 6.5 micrometer pixel size provides high spatial resolution, which allows for a very large field of view.

An additional benefit of the new pco.edge 4.2 bi is the broader spectral range than previous types of cameras intended for use with microscopes. In the past, sensors were sensitive to visible light wavelengths from approximately 400 to 1,100 nanometers. Now, researchers can detect incoming light from 200 to 1,100 nanometers.

The pco.edge's exposure time, ranging from 10 microseconds to 20 seconds with a frame rate of up to 42 frames per second at full resolution, enables more flexibility for imaging across various applications. Faster exposure times mean better temporal resolution because the camera can acquire much more image data over a given amount of time, which is especially relevant for intracellular calcium and voltage studies.

The camera includes support for either air or liquid cooling down to -25 degrees Celsius to keep dark current associated noise—the temperature-dependent small amount of current present in photosensitive devices when not being struck by photons—at a very low level. The camera's electronic chassis is isolated, virtually eliminating dust and dirt contamination. Because there is no need for a fan with liquid cooling, vibrations are also minimized to further improve image quality. Improved image quality; faster frame rates; spatial, spectral, and temporal resolutions; and cleaner data mean more than doing a better job than before. Researchers can use the camera in areas that normally might be beyond the capability of a camera, like electrophysiology or piercing a cell with a needle. There is the flexibility to design better experiments, obtain cleaner data for better analysis, or even just expand the amount of observation time by controlling the conditions around a cell.

For more information about the pco.edge 4.2 bi camera and its applications in biology, visit www.pco.de/scientific-cameras/ and direct any inquiries to info@pco.de.

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