

To See or Not to See...

Can Non-Cooled sCMOS Cameras Do the Job?

Gerhard Holst

In the years since their first appearance in 2010, scientific cameras based on scientific CMOS image sensors have had a large impact on numerous new technologies and methods in microscopy. Practically all camera based methods employing super resolution and nanoscopy have made use of the ideal combination, of low readout noise, high quantum efficiency and high frame rates associated with a very convenient pixel pitch of 6.5 μm , which fit perfectly to many microscope applications.

sCMOS Image Sensors Are Improving

Improvements in general manufacturing technology of CMOS image sensors such as higher integration densities, buried channel technology and wafer scale reverse side thinning, in association with new pixel architectures which use the technology improvements, as well as new ideas coming from the older CCD technology also enabled significant improvements in sCMOS image sensors. The first thing to mention was an improvement step of appr. 10% more quantum efficiency by advances in the image sensor manufacturing process, this means that sCMOS image sensors such as the BAE Fairchild CIS2020A and the GPixel GSENSE2020e (tab. 1) are now able to offer quantum efficiencies with peak values higher than 80%, a value which has not been seen in front illuminated image sensors before. Further, the wafer scale reverse side thinning has now been matured and is becoming a standard process, which has also made the first backside thinned sCMOS image sensors like the GPixel sensors GSENSE400BSI and GSENSE2020BSI available. These are now making things difficult for the remaining emCCD image sensor cameras, because they offer the same peak quantum efficiency > 90%, higher resolution and higher frame rates at lower readout noise, only missing the gain. The advantage of emCCD cameras with their inbuilt emCCD multiplication, which enables them to image single photons is still valid, but in most of the imaging applications even in single molecule fluorescence imaging, there are a couple of photons involved and the signal level is not the only criterion.

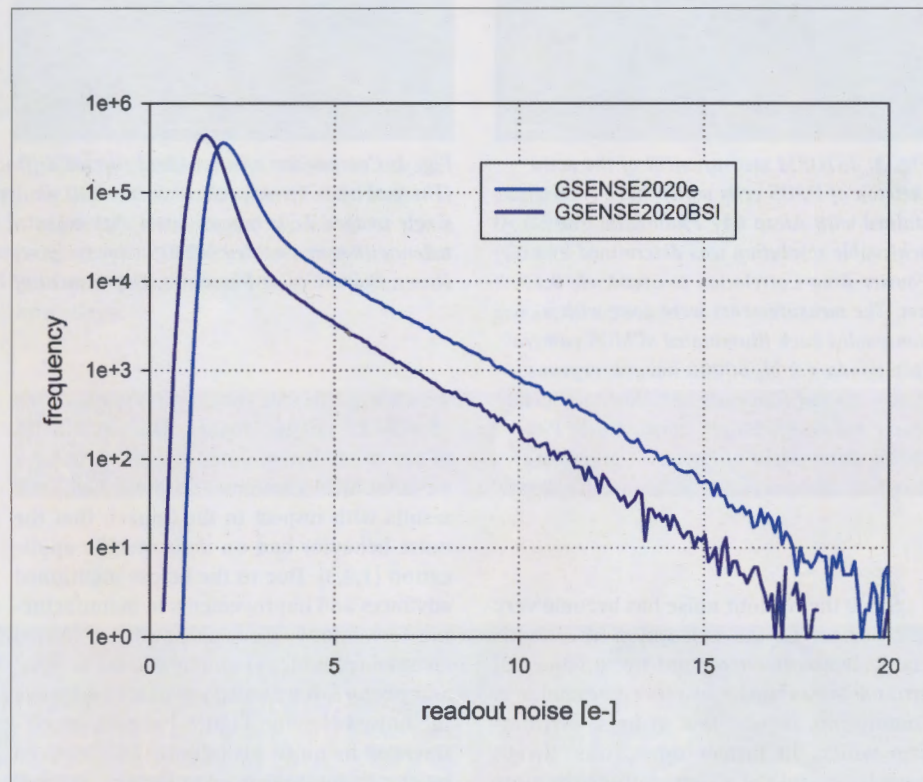


Fig. 1: Comparison of noise histograms of the two sCMOS image sensors GSENSE2020e (front illuminated) and GSENSE2020BSI (back illuminated) measured at 35°C image sensor temperature (data source: GPixel Inc, Changchun, China).

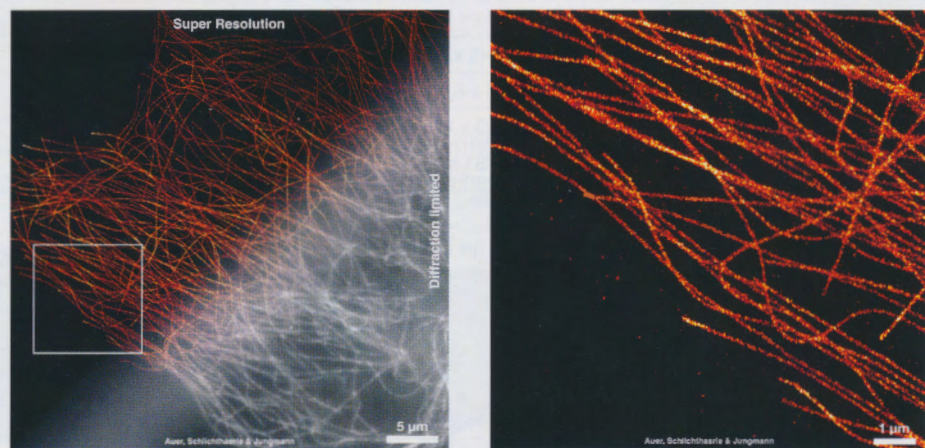


Fig. 2: Left: Comparison of a standard diffraction limited fluorescence image of COS7 cells with α -tubulin immunostaining with a superresolution image based on DNA-PAINT [4] in a TIRFset-up and an image recording with a non-cooled sCMOS camera (pco.panda 4.2)- right: zoomed version of the image area within the white frame in the left image, the localization precision as determined by a nearest neighbor based analysis was 4.5 nm resulting in a resolution of 10.5 nm. (Courtesy of R. Jungmann, A. Auer et al. [manuscript in submission], Max Planck Institute of Biochemistry, Martinsried, Germany).

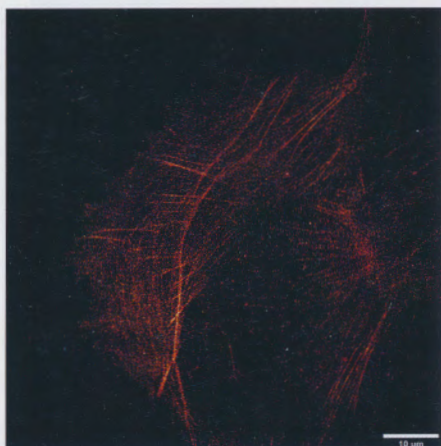


Fig. 3: dSTORM measurement of the actin skeleton of U2OS cells which have been stained with Alexa 647 Phalloidin. The achievable resolution was determined by a Fourier-Ring-Correlation to 68.04 ± 9.17 nm. The measurements were done with a non-cooled back illuminated sCMOS camera (pco.panda 4.2 bi, 50000 images, exposure time = 20 ms) (Courtesy of S. Bergmann, T. Huser and G. Wiebusch, Biomolecular Photonics, Department of Physics, University of Bielefeld, Germany).

Since the readout noise has become very low - most of the existing sCMOS camera systems offer temporal noise values of around 1 electron - another parameter is coming into focus - that of noise distribution which, in former times, was always assumed to follow a Gaussian distribution,

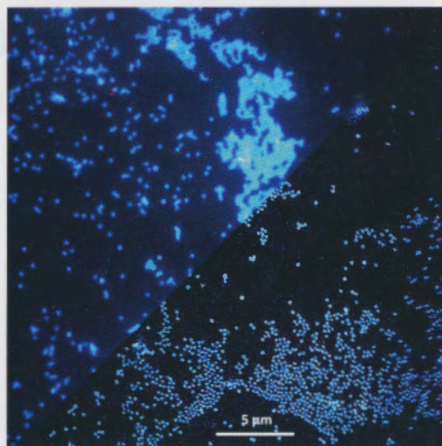
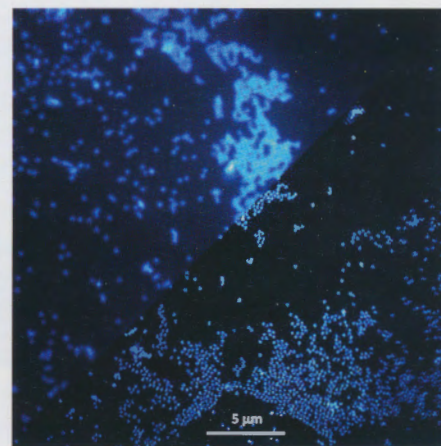


Fig. 4: Comparison of a standard filtered diffraction limited fluorescence image of microspheres (ThermoFisher TetraSpeck, diameter 200 nm, emission at 515 nm) to a SIM image based on 9 single images. Left: images taken with a cooled sCMOS camera (pco.edge 4.2), right: images taken with a non-cooled sCMOS camera (pco.panda 4.2 bi) (Courtesy of A. Markwirth, and T. Huser, Biomolecular Photonics, Department of Physics, University of Bielefeld, Germany).



which was not true for the first generations of sCMOS image sensors. Therefore a lot of scientists investigated the noise behavior of sCMOS cameras and published their results with respect to the impact, that the noise behavior had on their specific application [1,2,3]. Due to the before mentioned advances and improvements in manufacturing technology (e.g. application of buried channel technology) and new ideas in pixel architecture, it was also possible to improve the noise behavior. Figure 1 shows the differences in noise histograms as measured by the image sensor manufacturer GPixel,

illustrating that the improvements of the back illuminated image sensor architecture also show a better, lower, noise behavior, despite the fact that the dark current has become larger.

The noise distribution measurements were taken at sensor temperatures of 35°C while the test system was at room temperature. The median readout noise values of the GSENSE2020e and the GSENSE2020BSI were 2.1 [e⁻] and 1.76 [e⁻] respectively. This triggered the idea of making sCMOS cameras without an active cooling of the image sensor. Up to this point, all sCMOS cameras being applied in microscopy, were cooled sCMOS cameras because the low readout noise values could be kept and they enabled measurements with exposure times in the range of a few seconds, to keep the temperature dependent dark current reasonably low. But many of the relevant new methods in microscopy whether it be localization microscopy (PALM, STORM, dSTORM, GSDIM, DNA-PAINT), structured illumination microscopy or light sheet fluorescence microscopy, rely on high frame rates and short exposure times.

First Applications of Non-Cooled sCMOS Cameras

So now, in 2017/2018, the first non-cooled sCMOS cameras have been developed based on the image sensors whose noise histogram are shown in figure 2. They share the same compact housing of $65 \times 65 \times 65 \text{ mm}^3$ and they are powered via their USB 3.1 interface cable. Both the front illuminated camera and the back illuminated camera have been tested in real appli-

image sensor	CIS2020A	GSENSE2020e	GSENSE2020BSI	GSENSE400BSI
manufacturer	BAE Fairchild	GPixel Inc	GPixel Inc	GPixel Inc
resolution [pixel]	2048 x 2048	2048 x 2048	2048 x 2048	2048 x 2048
pixel pitch [μm^2]	6.5 x 6.5	6.5 x 6.5	6.5 x 6.5	11.0 x 11.0
active area [mm^2]	13.3 x 13.3	13.3 x 13.3	13.3 x 13.3	22.5 x 22.5
diagonal	18.83 mm	18.83 mm	18.83 mm	31.86 mm
readout noise	1e-	< 2e-	< 1.6e-	1.6e-
frame rate (full)	100 fps	47 fps @ HDR 94 fps @ single	94 fps @ HDR 234 fps @ single	48 fps
rolling shutter	X	X	X	X
global reset	-	X	X	-
global shutter	-	X	-	-
QE (peak)	> 80%	75 %	95%	95%
dynamic range	1 : 30000	1 : 22387	1 : 50119	1 : 44668
fullwell capacity	30000 e-	45000 e-	55000 e-	89000 e-
power consumption	1.5 W	0.8 W (RS)	0.85 W	0.65 W
monochrome	X	X	X	X
color	-	X	-	-

Tab. 1: Table of characteristic data of actual sCMOS image sensors.

cations with good results. Figure 2 shows a super resolution image of COS7 cells, labelled with an alpha tubulin immunostaining, using the DNA-PAINT [4] method in a TIRF set-up. The right image shows the zoomed in area within the white rectangle given in the left image. Clearly the high resolution of the fibers in the image compared to the 1 μm measuring gauge can be seen.

The second example shows the result of a dSTORM application with a non-cooled back illuminated sCMOS camera system. In figure 3 the actin skeleton of U2OS cells can be seen which have been stained with Alexa 647 Phalloidin. In this application the achievable resolution has been determined by a Fourier-Ring Correlation to 68.04 $\pm$ 9.17 nm.

Another method where non-cooled sCMOS cameras fit, is in structured illumination (SIM) microscopy where the addition of a known pattern into the excitation of the fluorophore labelled samples and additional image sampling, results in a removal of out of focus light and in an improvement of the diffraction limited res-

olution. There are a lot of special microscopy systems on the market which use the SIM principle and all of them use cooled sCMOS cameras. Therefore the group around Prof. T. Huser, University of Bielefeld, Germany has experimentally compared a non-cooled sCMOS camera (pco.panda 4.2 bi) to a cooled sCMOS camera (pco.edge 4.2) in their SIM microscopy set-up. For the first comparison they used fluorescence labelled microspheres (ThermoFisher TetraSpeck, diameter 200 nm). The results of the two cameras are shown in figure 4 in comparison to a similarly filtered wide field image.

This comparison (fig. 4) shows no significant difference in the quality and resolution of the images, which is a good indication, that SIM microscopy can also be done with non-cooled sCMOS cameras nowadays.

The Answer Is to See...

All these results show that meanwhile even high demanding microscope applications can be successfully done with non-cooled sCMOS camera systems, which might offer as well a more convenient price tag. But at the end of the day the final question in all of these microscope applications remains: To see or not to see? Clearly the answer is, to see - yes, because all presented results prove that non-cooled sCMOS cameras can do the job and offer the required performance for demanding microscopy applications like localization microscopy, and SIM microscopy.

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More information on camera technology: <http://bit.ly/IM-CT>



All references: <http://bit.ly/IM-Holst2>

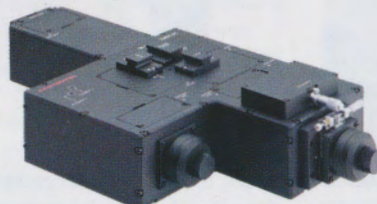
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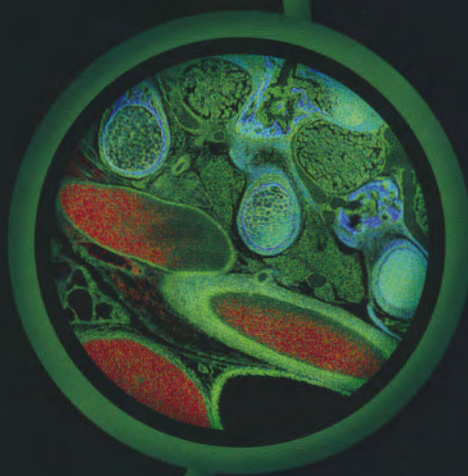
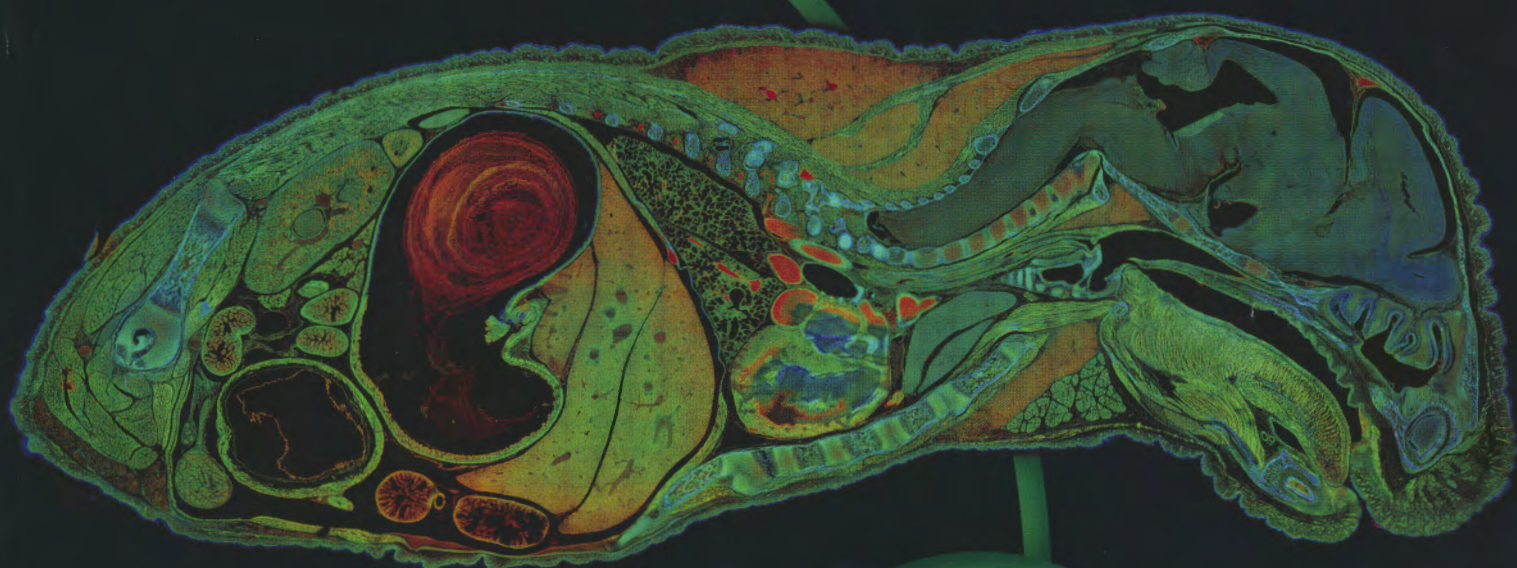
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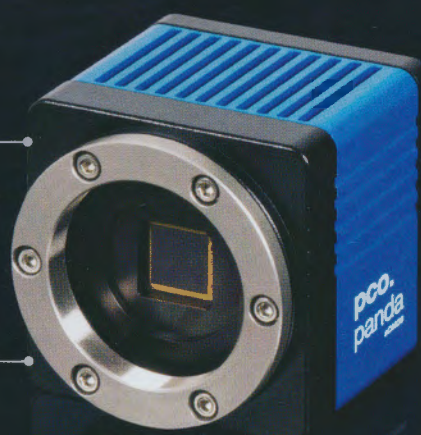
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