Scientific CMOS

Neo and Zyla sCMOS Cameras Widen Your Expectations

Scientific CMOS (sCMOS) technology overview

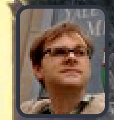
Scientific CMOS (sCMOS) is a breakthrough technology that offers an advanced set of performance features that render it ideal to high fidelity, quantitative scientific measurement.

Scientific CMOS can be considered unique in its ability to simultaneously deliver on many key performance parameters, overcoming the 'mutual exclusivity' associated with current scientific imaging technology standards, and eradicating the performance drawbacks traditionally associated with CMOS imagers.

sCMOS is uniquely capable of simultaneously delivering:

- Extremely low noise
- Rapid frame rates
- Wide dynamic range
- High resolution
- Large field of view
- High Quantum Efficiency (QE)
- Rolling and Global (Snapshot) exposure modes

"Neo cameras will literally allow one to see cells in a new light with ultra sensitive imaging at speeds never achieved before - as we have seen in our tests of vesicle trafficking. These scientific CMOS cameras are not a small step, but a quantum leap, that will open up new possibilities of what can be studied in fast cellular processes, rapid screening, and super-resolution imaging."



Derek Toomre, PhD., Associate Professor, Department of Cell Biology, Yale University School of Medicine, USA

See page 40 for 'Comparing sCMOS with other detectors' technical note

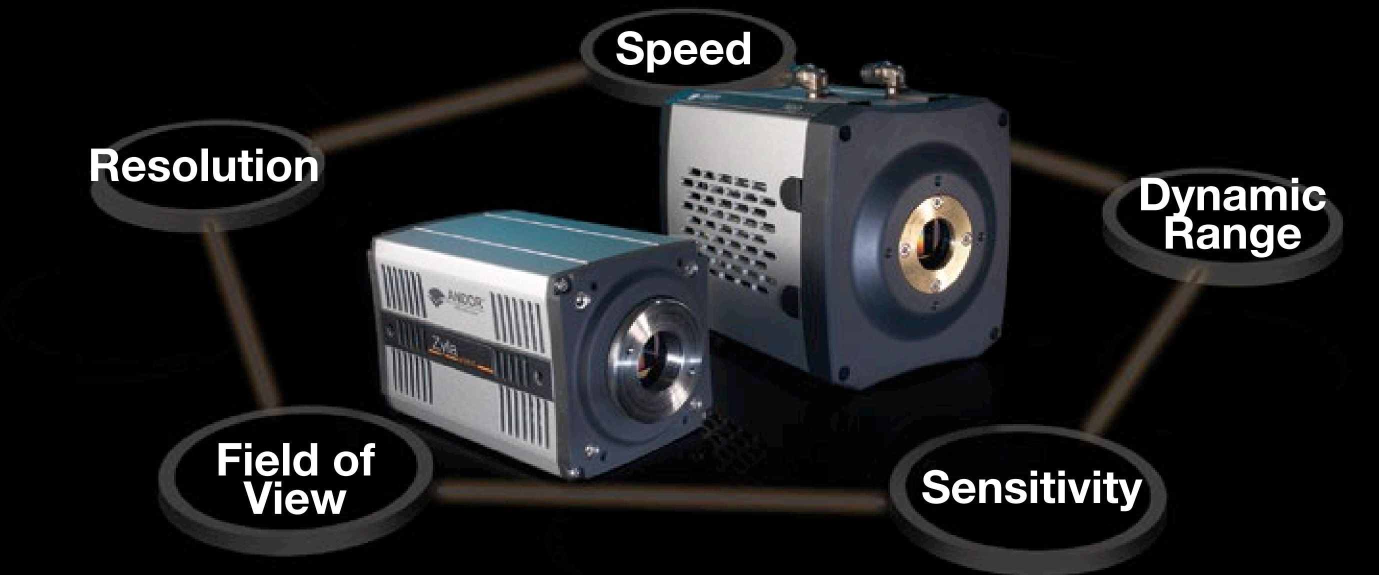
sCMOS - Imaging without compromise

The multi-megapixel sensors offer a large field of view and high resolution, without compromising read noise, dynamic range or frame rate. Rolling and Global (Snapshot) shutter readout ensure maximum application flexibility.

Read noise is exceptional, even when compared to the highest performance 'slow-scan' CCDs. The fact that an sCMOS device can achieve 1 electron rms read noise while reading out up to 5.5 megapixels at 30 fps renders it truly extraordinary in the market. Furthermore, the technology is capable of achieving 100 full fps with a read noise 1.3 electrons rms. By way of comparison, the lowest noise Interline CCD, reading out only 1.4 megapixels at ~16 fps would do so with ~10 electrons read noise.

The low noise readout is complemented by up to 33,000:1 dynamic range. Usually, for CCDs or EMCCDs to reach their highest dynamic range values, there needs to be a significant compromise in readout speed, yet sCMOS can achieve this value while delivering high frame rates. The unique dual amplifier architecture of sCMOS allows for high dynamic range by offering a large well depth, despite the relatively small 6.5 µm pixel size, alongside lowest noise. A 1.4 megapixel Interline CCD with similarly small pixels achieves only ~1,800:1 dynamic range at 16 fps.

Parameter	sCMOS (Zyla 4.2)	Interline CCD	EMCCD
Sensor Format	4.2 megapixel	1.4 to 4 megapixel	0.25 to 1 megapixel
Pixel Size	6.5 µm	6.45 to 7.4 µm	8 to 16 µm
Read Noise	0.9 e- @ 30 fps 1.1 e- @ 100 fps	4 - 10 e-	< 1e- (with EM gain)
Full Frame Rate (max.)	Sustained: 100 fps full frame	3 to 16 fps	~30 fps
Quantum Efficiency (max.)	72%	60%	90% 'back-illuminated'
Dynamic Range	33,000:1 (@ 30 fps)	~3,000:1 (@ 11 fps)	8,500:1 (@ 30 fps with low EM gain)
Multiplicative Noise	None	None	1.41x with EM gain



Neo 5.5
sCMOS

Andor's Neo sCMOS vacuum cooled camera platform has been engineered from the ground up, specifically to realize the absolute highest sensitivity from this exciting new sensor technology.

Neo 5.5 offers an exceptionally low dark current and read noise floor detection limit, maintained even under longer acquisition times, alongside a wide dynamic range of 30,000:1. Speeds of 30 fps (full frame) can be maintained over extended kinetic series acquisitions, with 100 fps achievable in burst mode.

Neo 5.5 offers an advanced set of unique performance features and innovations, including deep vacuum TE cooling to -40°C, extensive 'on-head' FPGA data processing capability, a 4 GB memory buffer and a Data Flow Monitor. Andor's UltraVac™ vacuum process has been implemented to offer not only the necessary deep cooling capability, but also complete protection of the sensor. These capabilities have been conceptualized to drive the best possible performance, image quality and longevity from sCMOS technology.

Neo 5.5 offers both Rolling and *true* Global (also known as 'Snapshot') shutter exposure mechanisms. Snapshot mode provides an exposure sequence that is analogous to that of an Interline CCD, whereby all pixels begin the exposure simultaneously and end the exposure simultaneously.

Key Features

-40°C vacuum cooling	30 fps / 100 fps burst
Rolling and true Global shutter modes	30,000:1 dynamic range
Vacuum longevity	Superb image quality
Blemish minimization	Quantitative stability
4 GB on-head memory	Vibration free fan off mode
5.5 megapixel	Fast exposure switching
1 e- noise	Data Flow Monitor

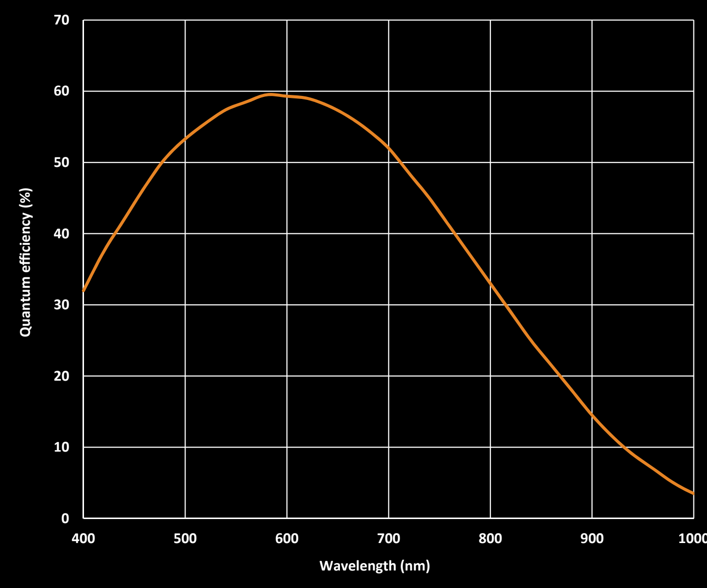
Features and Benefits

TE cooling to -40°C	Minimization of dark current to maintain low noise advantage under all exposure conditions. Minimization of hot pixel blemishes meaning more useful pixels. Fan-off mode for vibration sensitive set-ups.
Rolling and *true* Global (Snapshot) shutter	Maximum exposure and readout flexibility across all applications. Snapshot for 'Interline CCD' exposure capability.
1 e^- read noise	Offers lower detection limit than any CCD.
5.5 megapixel sensor format and 6.5 µm pixels	Delivers extremely sharp resolution over a 22 mm diagonal field of view: ideal for cell microscopy, digital pathology, high content screening and astronomy.
Dark Noise Suppression (DNS) technology	Extremely competitive low dark current of 0.14 e^-/pix/sec with fan cooling. Maintains low noise advantage across range of exposure conditions.
Rapid frame rates	>30 fps over extended kinetic series. Burst to memory at 100 fps full frame.
UltraVac™	Sustained vacuum integrity and unequalled cooling with 5 year warranty; complete sensor protection.
Dual-Gain amplifiers	Maximum well depth and lowest noise simultaneously, affording extended dynamic range of 30,000:1.
4 GB on-head image buffer	Enables bursts of 100 fps @ full dynamic range. Capture extended kinetic series faster than PC write speed, avoiding prohibitively expensive PCs.
Sub-microsecond inter-frame gap	Global Shutter offers down to 100 ns inter-frame gap, ideal for PIV applications.
Extensive FPGA on-head data processing	Essential to ensure best image quality and quantitative fidelity from sCMOS technology.
Hardware timestamp	FPGA generated timestamp with 25 ns accuracy.
Dynamic baseline clamp	Essential to ensure quantitative accuracy across the image area and between successive images of a kinetic series.
Spurious noise filter	Realtime FPGA filter that identifies and compensates for spurious high noise pixels.
Data flow monitor	Innovatively manage acquisition capture rates vs data bandwidth limitations.
iCam	Market leading fast exposure switching with minimal overheads.
Comprehensive trigger modes and I/O	Communication and synchronization within intricate experimental set-ups.

Key Specifications

Active Pixels	2560 x 2160
Pixel Size (W x H; µm)	6.5 x 6.5
Sensor size (mm)	16.6 x 14
Read Noise (median, e^-)	1 @ 200 MHz 1.3 @ 560 MHz
Sensor Cooling	-40°C
Pixel Well Depth (e^-)	30,000
Max Readout Rate (MHz)	560 MHz (280 MHz x 2 outputs)
Max Frame Rates (fps)	Sustained: 30 fps full frame Burst: 100 fps full frame
QE max	60%

Neo 5.5 QE curve



Zyla 5.5 sCMOS

Andor's Zyla 5.5 sCMOS camera offers high speed, high sensitivity imaging in a remarkably light and compact design. Both Rolling and true Global shutter modes offer extensive application flexibility. Global shutter is ideally suited to fast multi-dimensional microscopy, offering tight synchronization to 'moving' peripheral devices such as z-stage or light source.

Featuring Industry Fastest USB 3.0
40 fps @ 5.5 megapixel

Zyla is ideally suited to many cutting edge applications that push the boundaries of speed, offering sustained frame rate performance of up to 100 fps (faster with ROI). A highly cost-effective USB 3.0 version is also available, offering an unparalleled 40 fps (full frame) and 1.2 e^- rms read noise, representing an ideal low light 'workhorse' camera solution for both microscopy and physical science applications, in either research or OEM environments.

Rolling and Global (Snapshot) shutter readout ensures maximum application flexibility. Global shutter in particular provides an important 'freeze frame' exposure mechanism that emulates that of an Interline CCD, overcoming the transient readout nature of Rolling shutter mode.

'Conceptualized to dramatically outperform Interline CCD technology within a 'mid-range' price bracket, Andor's Zyla 5.5 sCMOS is ideally placed to become the new gold standard workhorse imaging detector.'

Key Features

Compact and light	25,000:1 dynamic range
Rolling and Snapshot exposure	0°C cooling @ up to 35°C ambient
100 fps sustained	Superb image quality
1.2 e^- noise @ 30 fps	Quantitative stability
5.5 megapixel	Fast exposure switching
Cost effective	Market leading USB 3.0

Zyla sCMOS for OEM

The light and compact form factor coupled with design and mounting adaptability, board level or private labelling options, and unparalleled engineering support, renders the Zyla highly suited to OEM integration.

Please call Andor to discuss how Zyla can be made to work for you.

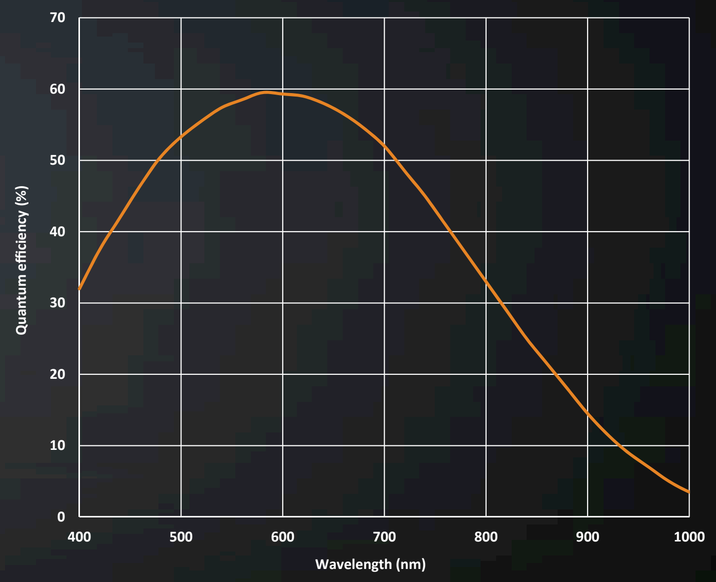
Features and Benefits

Compact and light	Highest QE sCMOS sensor, providing optimal signal to noise in low light applications.
Rolling and *true* Global (Snapshot) shutter	Maximum exposure and readout flexibility across all applications. Snapshot for 'Interline CCD mode' freeze frame capture of fast moving/changing events.
Industry fastest frame rates	100 fps sustained via Camera Link (full frame). Industry fastest USB 3.0 frame rates.
NEW Ultra low fan vibration	Designed with vibration sensitive experiments in mind, such as super-resolution microscopy.
1.2 e^- read noise	Offers lower detection limit than any CCD.
5.5 megapixel sensor format and 6.5 µm pixels	Delivers extremely sharp resolution over a 22 mm diagonal field of view: Ideal for cell microscopy, digital pathology, high content screening and astronomy.
Dual-gain amplifiers	Maximum well depth and lowest noise simultaneously, affording extended dynamic range of 25,000:1.
12-bit and 16-bit modes	12-bit for smaller file size and absolute fastest frame rates through USB 3.0; 16-bit for full dynamic range.
TE cooling to 0°C in 35°C ambient	Ideal for OEM integration into enclosed systems.
Dark Noise Suppression (DNS) technology	Extremely competitive low dark current of 0.14 e^-/pix/sec with fan cooling. Maintains low noise advantage across range of exposure conditions.
Sub-microsecond inter-frame gap	Global Shutter offers down to 100 ns inter-frame gap, ideal for PIV applications.
Water cooled option	Access lowest possible vibration and -10°C cooling.
High Quantum Efficiency	Optimized for popular green / red emitting fluorophores.
Extensive FPGA on-head data processing	Essential to ensure best image quality and quantitative fidelity from sCMOS technology.
Hardware timestamp	FPGA generated timestamp with 25 ns accuracy.
Dynamic baseline clamp	Essential to ensure quantitative accuracy across the image area and between successive images of a kinetic series.
Spurious noise filter	Real time FPGA filter that identifies and compensates for spurious high noise pixels.
Data flow monitor	Innovatively manage acquisition capture rates vs data bandwidth limitations.
iCam	Market leading fast exposure switching with minimal overheads.

Key Specifications

Active Pixels	2560 x 2160
Pixel Size (W x H; µm)	6.5 x 6.5
Sensor size (mm)	16.6 x 14
Read Noise (median, e^-)	1.2 @ 200 MHz 1.45 @ 560 MHz
Sensor Cooling	0°C (up to +30°C ambient)
Pixel Well Depth (e^-)	30,000
Max Readout Rate (MHz)	560 MHz (280 MHz x 2 outputs)
Max Frame Rates (fps)	Camera Link: 100 USB 3.0: 40
QE max	60%

Zyla 5.5 QE curve



Zyla 4.2 sCMOS

NEW

The newest addition to the Andor sCMOS camera portfolio, the Zyla 4.2 utilizes a high Quantum Efficiency (QE), low noise sensor variant, yielding frame rates up to 100 fps (faster from region of interest). The Zyla 4.2 is ideal for applications that benefit from optimal sensitivity, speed and low vibration, such as calcium imaging and super-resolution microscopy.

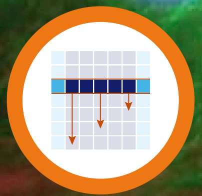
Industry Fastest USB 3.0 53 fps @ 4.2 megapixel

Zyla 4.2 delivers > 70% QE and down to 0.9 e- read noise, making it the most sensitive sCMOS camera available. The dark current at 0°C is extremely competitive, at only 0.14 e^-/pixel/sec. Under moderate to fast frame rate operation, the Zyla 4.2 excels. The Camera Link version offers a blistering 100 fps, whereas the more cost effective USB 3.0 version achieves market leading performance of 53 fps.

The Zyla 4.2 differs fundamentally from Zyla 5.5 in terms of shutter flexibility. Whereas Zyla 5.5 offers both Rolling and true Global shutter modes, the Zyla 4.2 operates in Rolling shutter mode. However, a mechanism called '*Simulated* Global Exposure' is available, whereby a TTL output from the camera can be used to activate a pulsed light source, emulating the Global shutter exposure condition, albeit with less efficiency.

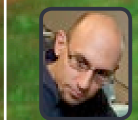
Key Features

> 70% QE	Cost effective
< 1 e- read noise	33,000:1 dynamic range
Dark Noise Suppression Technology	Superb image quality
100 fps sustained	Quantitative stability
Market leading USB 3.0	Fast exposure switching
4.2 megapixel	LightScan PLUS with FlexiScan and CycleMax



Featuring LightScan PLUS with FlexiScan and CycleMax. See page 30 for more information

"The Andor Zyla provides an impressive combination of field-of-view, sensitivity, resolution and speed. It is a highly versatile camera that can be used for many different applications."



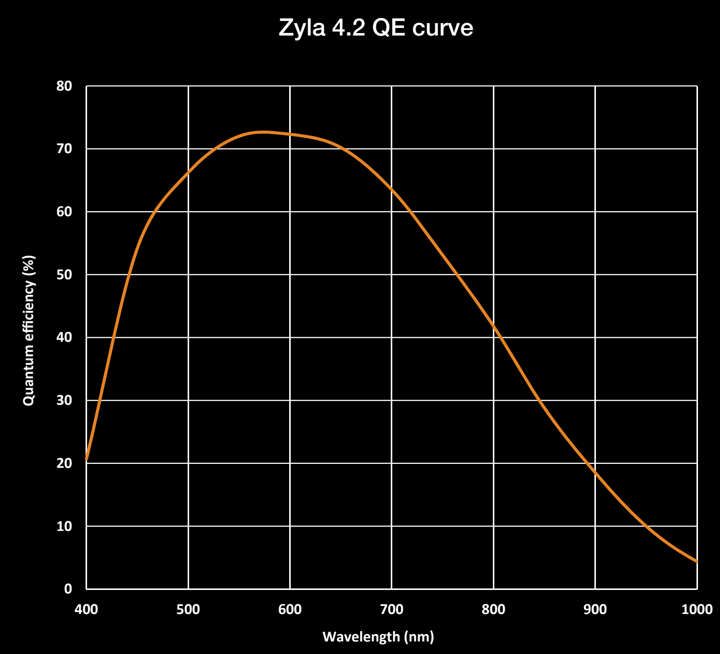
Kurt Thorn, Assistant Adjunct Professor, UCSF School of Medicine, California, USA

Features and Benefits

	> 70% QE	Ideal for integration into space restrictive set-ups. Ideal for OEM.
	0.9 e^- read noise	Lowest read noise sCMOS. Significantly lower than any CCD.
	Industry fastest frame rates	100 fps sustained via Camera Link (full frame). Industry fastest USB 3.0 frame rates.
NEW	Ultra low fan vibration	Designed with vibration sensitive experiments in mind, such as super-resolution microscopy.
NEW	LightScan PLUS with FlexiScan and CycleMax	Reduce background and improve contrast and resolution in scattering samples. Designed to allow users to maximise signal and confocality concurrently in applications such as Scanned Light Sheet Microscopy and Line Scanning Confocal Microscopy.
	4.2 megapixel sensor format and 6.5 µm pixels	Delivers extremely sharp resolution over a 18.8 mm diagonal field of view; ideal for cell microscopy and astronomy.
	Compact and light	Ideal for integration into space restrictive set-ups. Ideal for OEM.
	Rolling shutter and simulated Global Exposure mode	Rolling shutter mode optimizes read noise and frame rate. Employ simulated Global shutter method if possibility of Rolling shutter spatial distortion.
	Dual-gain amplifiers	Maximum well depth and lowest noise simultaneously, affording extended dynamic range of 33,000:1.
	TE cooling to 0°C in 27°C ambient	Ideal for OEM integration into enclosed systems.
	Dark Noise Suppression (DNS) technology	Extremely competitive low dark current of 0.14 e^-/pix/sec with fan cooling. Maintains low noise advantage across range of exposure conditions.
	Water cooled option	Access lowest possible vibration and -10°C cooling.
	Extensive FPGA on-head data processing	Essential to ensure best image quality and quantitative fidelity from sCMOS technology.
	Hardware timestamp	FPGA generated timestamp with 25 ns accuracy.
	Dynamic baseline clamp	Essential to ensure quantitative accuracy across the image area and between successive images of a kinetic series.
	Spurious noise filter	Real time FPGA filter that identifies and compensates for spurious high noise pixels.
	Data flow monitor	Innovatively manage acquisition capture rates vs data bandwidth limitations.
	iCam	Market leading exposure switching with minimal overheads.

Key Specifications

Active Pixels	2048 x 2048
Pixel Size (W x H; µm)	6.5 x 6.5
Sensor size (mm)	13.3 x 13.3
Read Noise (median, e^-)	0.9 @ 216 MHz 1.1 @ 540 MHz
Sensor Cooling	0°C (up to +27°C ambient)
Pixel Well Depth (e^-)	30,000
Max Readout Rate (MHz)	540 MHz
Max Frame Rates (fps)	Camera Link: 100 USB 3.0: 53
QE max	72%



Zyla - The Biologist's Choice

Zyla sCMOS has proven a superb camera choice for the biologist and microscopist. Many simply see the Zyla as an **amazing value**, superb price/performance 'workhorse' camera with which to replace their existing Interline CCD and **upgrade** the performance of their fluorescence microscope. Others are driven by distinct **application performance criteria** that only sCMOS can answer.

Quality, Throughput, Performance, Accessibility…

- **High Sensitivity and Wide Dynamic Range** – quantify very weak and very bright structures with one image.
- **Superb Image Quality** – high resolution and uniform backgrounds for publication-quality imaging.
- **Capture Everything** – the larger field of view matches that of modern microscopes. Achieve better statistics and higher throughput in high content experiments.
- **Blazingly Fast** – more and more studies of cell processes require greater temporal resolution.
- **Ease of use** – designed to get you up and imaging with minimal fuss.
- **Flexible** – fast or slow, big or small, weak or bright … Zyla is adaptable all of your imaging challenges.

Example Areas of Application

Light Sheet Microscopy

LightScan PLUS with FlexiScan and CycleMax, a new feature set available on Zyla 4.2 has the following benefits for Light Sheet Microscopy applications:

- Independent control of pixel row height, scan speed and exposure time and multiple read-out options
- Optimize signal to noise AND confocality concurrently
- CycleMax – Maximum frame rates with reduced dead-time, no need to reset light sheet for each alternate frame

TIRF Microscopy

The Zyla's fine pixel resolution, great sensitivity, large field of view and fast imaging speed offers a superb choice of platform for following/tracking fast processes at the cell membrane. Multi-wavelength TIRF may benefit from Zyla 5.5 in Global shutter.

High Content Screening

Zyla sCMOS yields markedly improved throughput and statistical validity of data in high content analysis. For example, a larger field of view results in analysis of more cells per image; wider dynamic range means a field of variable intensity cells can be quantified in only one acquisition; and higher sensitivity results in reduced acquisition times.

Super Resolution Microscopy

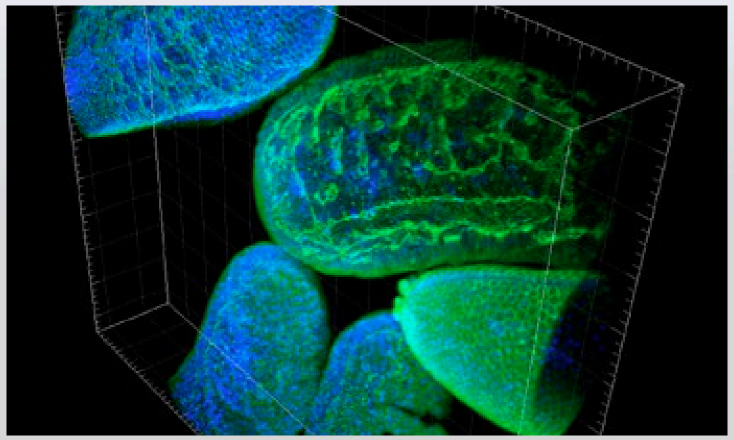
The low vibration, high QE, low noise and speed capability of Zyla 4.2 (USB 3.0 and CameraLink) is well suited to the particular detection criteria of single molecule based 'STORM / PALM' approaches, and is used by some as an alternative to EMCCDs for this purpose. Note, this should be considered distinct from the general needs of single molecule microscopy, which are best served by back-illuminated EMCCD cameras (see Andor iXon EMCCD range). There is the capability to switch off interpolative filtering and the provision of custom blemish maps.

Physiology / Ion Imaging

The fast frame rate and excellent sensitivity of Zyla is ideally suited to the particular needs of ion signalling microscopy. Zyla 4.2 offers superlative sensitivity at speed, but electrophysiology may require the Global shutter exposure mode of Zyla 5.5 to ensure temporal correlation across the whole image.

Cell Motility

The motile cell is captured extremely well by the speed and resolution of the Zyla. Generally, the Rolling shutter of Zyla 4.2 is suited, but care must be taken of distortive effects if the cell is moving particularly fast. For example, it has been noted that the Zyla 5.5 in Global shutter mode was required to image motile sperm cells.



Other biological applications include:
Neuroscience, Vesicle Transport, Parasitology, Blood Flow, Ophthalmology

Zyla - The Physicist's Choice

Zyla sCMOS has become a well established detector amongst physicists, biophysicists and astronomers; the advanced combination of speed, sensitivity and dynamic range enabling new ground to be broken.

Performance and Adaptability

- **Dual Amplifier** – novel pixel architecture means you don't need to pre-select gain. Access lowest read noise and full well depth simultaneously.
- **1000 fps** – access extremely fast frame rates through user definable Region of Interest control, suited to many applications within the physical sciences.
- **Global shutter** – Zyla 5.5 offers this important mode that completely avoids spatial distortion, and ensures temporal correlation across all regions of the sensor. Sub-microsecond inter-frame gap, ideal for PIV applications.
- **Low dark current** – low read noise is complimented by extremely competitive dark current, also ensuring minimized hot pixel blemishes.
- **Cooling options** – standard camera air cools to 0°C up to +35°C ambient. Water cooled option available on request.
- **Blemish correction maps and advanced control** – upon request, Andor provides bespoke capability to turn off/on blemish correction, for those who prefer to perform this themselves. Blemish maps can be provided.
- **Compact and Light** – the extremely small volume footprint of Zyla renders it adaptable to intricate optical set-ups.

Example Areas of Application

Lucky / Speckle Imaging

Zyla's fast frame rate and large field of view are ideal for this resolution enhancing technique.

Particle Imaging Velocimetry (PIV)

The true Global Shutter mode of Zyla 5.5 facilitates an inter-frame gap of down to 100 ns.

Solar Astronomy

Fast frame rates, wide dynamic range and great linearity present a very formidable solution to the specific detector needs of next generation large solar telescopes.

Fluorescence Correlation Spectroscopy

Superb temporal resolution from small ROIs are excellent for accurately measuring diffusion coefficients.

Adaptive Optics

Accessing > 1000 fps using ROIs renders the Zyla an ideal Wavefront detector. Use with a data splitter to enable direct data access.

X-Ray / Neutron Tomography

The Zyla can be readily lens coupled to scintillators and phosphors, presenting a high resolution, sensitive and fast solution for tomography.*

Bose Einstein Condensation

The QE profile of Zyla is very good in the red/NIR region, ideal for BEC of Rb.

sCMOS image courtesy of Jin Ma, Xinglong Observatory, National Astronomical Observatory of Chinese Academy of Sciences

***Fiber-Optic coupled Zyla**
Please enquire for details on Andor's new Fiber-Optic coupled Zyla, superb for fast indirect X-Ray applications such as tomography.

Upgrade Your Microscope's Performance

Zyla sCMOS - Disruptive Technology, Familiar Price

What was the benchmark imaging detector?

Interline CCD technology has been the dominant 'workhorse' detector type for fluorescence cell microscopy for almost 15 years, the benchmark sensor being a 1.4 megapixel, 6.45 µm pixel size device, offering typical read noise floor between 5 and 8 e- rms at modest frame rates of 11-12 fps.

Upgrade your microscope using the imaging superiority of Zyla sCMOS:

Fundamentally, sCMOS technology has been conceptualized as a vastly superior alternative to Interline CCDs. Indeed, Andor's Zyla sCMOS offers dramatically higher performance, yet remains within the same price bracket as Interline cameras, and is ideally placed to become the new gold standard 'workhorse' laboratory detector. Importantly, Zyla uniquely comes with both Rolling and true Global (Snapshot) shutter.

In particular, Global shutter offers a simple Snapshot imaging capability, directly analogous to that of Interline CCDs, offering zero image distortion and perfect for synchronizing to peripheral devices.

Benefits of Upgrading

- 4x more pixels
- 5x more sensitive
- 10x more dynamic range
- 16x faster

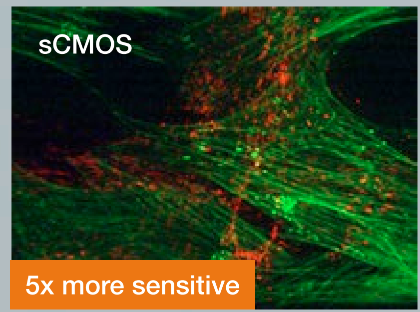
The table below compares some typical performance specifications of a 1.4MP Interline CCD to those of the new Zyla sCMOS, outlining the approximate factor improvement that is available.

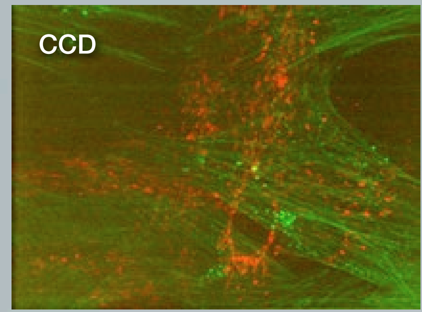
Parameter	Typical Interline CCD Specifications	Zyla 5.5 sCMOS Specifications	sCMOS Factor Improvement (approx.)
Read Noise	$6\ e^-$	$1.2\ e^-$	5x more sensitive
Sustained Frame Rate	12 fps @ 1.4 MP	100 fps @ 5.5 MP* 200 fps @ 1.4MP ROI*	16x faster
Dynamic Range	2,250:1	25,000:1	10x more dynamic range
Sensor Format	1.4 megapixel	5.5 megapixel	4x more pixels

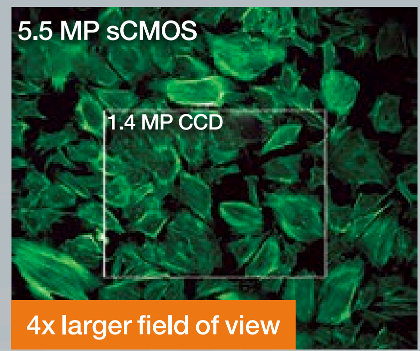
* Frame rates provided are for the '10-tap' Cameralink model. An even more affordable USB 3.0 model is available, in the case of the Zyla 5.5 offering 40 fps sustained at full 5.5 megapixel resolution.

Why is Zyla such an impressive all-round imager?

- **Superior performance** – vastly superior to Interline across key performance parameters.
- **Rolling and Global exposures (Zyla 5.5)** – Zyla is unique in offering both these exposure modes in one camera. Global shutter (Snapshot) is directly analogous to the Interline exposure mechanism.
- **Image quality** – a huge amount of effort and on-camera (FPGA) intelligence has gone into optimizing image quality in Zyla.
- **Flexibility** – fast, slow, weak, bright, pixel binned, region of interest, Rolling shutter, Snapshot shutter… Zyla is adaptable to a broad gamut of application requirements.
- **Affordable price** – with so many superb features, we have endeavoured to make the Zyla accessible to every lab. Request a quote, you'll be pleasantly surprised!
- **USB 3.0** - 'Plug and play' interface with industry fastest frame rates.







Key Biological Applications

- Live cell imaging
- Widefield fluorescence microscopy
- Developmental biology
- Embryo studies
- Physiology / Ion Imaging
- Neuroscience / Vesicle Transport
- Parasitology
- Cell Motility

Performance and Innovations

Extended Dynamic Range

The Andor Neo and Zyla cameras are designed to make use of the innovative dual ‘column-level’ amplifier design of the sensors.

Traditionally, sensors require that the user must select up-front between high or low amplifier gain (i.e. sensitivity) settings, depending on whether they want to optimize for low noise or maximum well depth. The dual amplifier architecture of the sCMOS sensor circumvents this need, in that signal can be sampled simultaneously by both high and low gain amplifiers. As such, the lowest noise of the chip can be harnessed alongside the maximum well depth, affording widest possible dynamic range of up to 33,000:1.

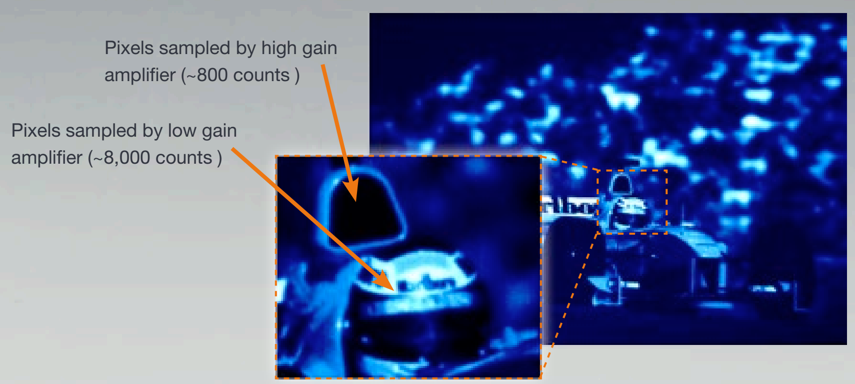
Dual Amplifier Architecture:

Each column within each half of the sensor is equipped with dual column level amplifiers and dual analog-to-digital converters (ADC).

This architecture was designed to simultaneously minimize read noise and maximize dynamic range. The dual column level amplifier/ADC pairs have independent gain settings, and the final image is reconstructed by combining pixel readings from both the high gain and low gain readout channels to achieve an unprecedented intra-scene dynamic range from the relatively small 6.5 µm pixel pitch.

See page 34 for
‘Dual Amplifier Dynamic Range’
technical note

High contrast image recorded with dual amplifier 16-bit mode of Neo



Zyla uniquely offers both 12-bit and 16-bit modes. 12-bit for smaller file size and absolute fastest frame rates through USB 3.0; 16-bit for full dynamic range.

Lowest Noise Floor

Andor’s ultra sensitive sCMOS cameras have broken new ground in offering down to 0.9 electron rms read noise, without signal amplification technology.

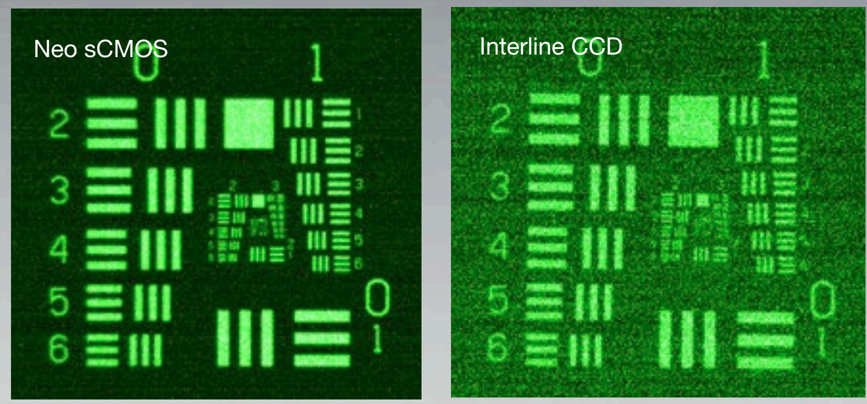
What is truly extraordinary is that this performance level is achievable at 30 fps, representing 200 MHz pixel readout speed. Furthermore, even at full readout speed, the read noise floor is negligibly compromised, maintaining down to 1.3 e^- rms at 100 fps. For the best CCD cameras to even approach 2 electrons noise, a readout speed of 1 MHz or slower is required. This minimal detection limit renders Andor’s sCMOS cameras suitable for a wide variety of challenging low light imaging applications.

Readout Speed (MHz)	Neo 5.5 Readout Noise (e^-)	
	Rolling Shutter	Global Shutter
200	1	2.3
560	1.3	2.5

Readout Speed (MHz)	Zyla 4.2 Readout Noise (e^-)
	Rolling Shutter
200	0.9
540	1.1

See page 49 for
‘Understanding Read Noise’
technical note

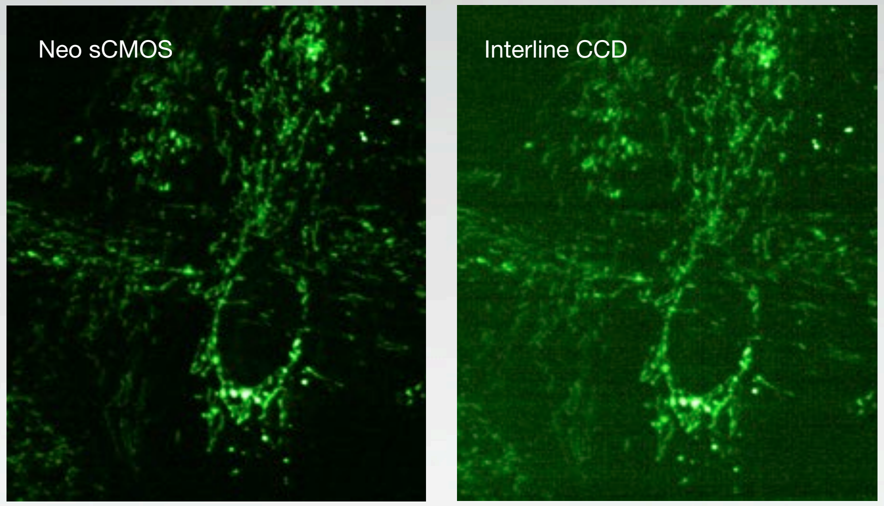
(a)



Comparative low light images taken with Neo sCMOS (1.3 electrons read noise @ 560 MHz) vs. Interline CCD (5 electrons read noise @ 20 MHz), displayed with same relative intensity scaling.

(a) LED signal in a light-tight imaging enclosure, intensity ~30 photons/pixel;
(b) Fluorescently labelled fixed cell using a CSU-X spinning disk confocal microscope (x60 oil objective), each 100 ms exposure, same laser power,

(b)



Spurious Noise Filter

Neo and Zyla platforms both come equipped with an optional in-built FPGA filter that operates in realtime to reduce the frequency of the occurrence of high noise pixels that would otherwise appear as spurious ‘salt and pepper’ noise spikes in the image background.

Performance and Innovations

Rapid Frame Rates

The parallel readout nature of sCMOS means it is capable of reaching very rapid frame rates of up to 100 full frames per second, and much faster with region of interest.

Distinctively, this is accomplished without significantly sacrificing read noise performance, markedly distinguishing the technology from CCDs. Andor's sCMOS cameras are uniquely designed to harness this speed potential.

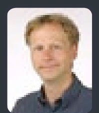
Array Size	USB 3.0		Camera Link 10-tap	
	Rolling shutter	Global shutter	Rolling shutter	Global shutter
2560 x 2160 (full frame)	40	40	100	49
2064 x 2048	53	52	105	52
1392 x 1040	107	98	199	97
512 x 512	419	201	419	201
128 x 128	1,639	716	1,639	716

Maximum frame rates achievable from the Zyla 5.5 sCMOS USB 3.0 and 10-tap Camera Link versions

NEW Market Leading USB 3.0 Performance

Zyla's speed optimized USB 3.0 interface delivers an unparalleled 40 fps from 16-bit mode and 53 fps from Zyla's unique 12-bit mode (4.2 megapixel array).

> Our experiments with Andor's new sCMOS camera have been highly encouraging. The combination of very low noise sensitivity at rapid frame rates, coupled with high pixel resolution and large dynamic range, will enable us to investigate single molecules at timescales which were previously not accessible.



Prof Stefan Diez, Heisenberg Professorship for BioNanoTools, Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany

iCam fast exposure switching

Neo and Zyla benefit from Andor's iCam technology, an innovation that ensures minimal overheads associated with fast exposure switching.

This is particularly important during multi-color microscopy acquisition protocols, whereby it is necessary to repeatedly and rapidly flip between pre-set exposure times matched to the relative signal intensity of each fluorophore.

iCam offers market leading acquisition efficiency, whether software or externally triggered.

Data Flow Monitor

The sCMOS sensor in Neo and Zyla is capable of extremely fast data read rates, but this in itself imposes considerable challenges.

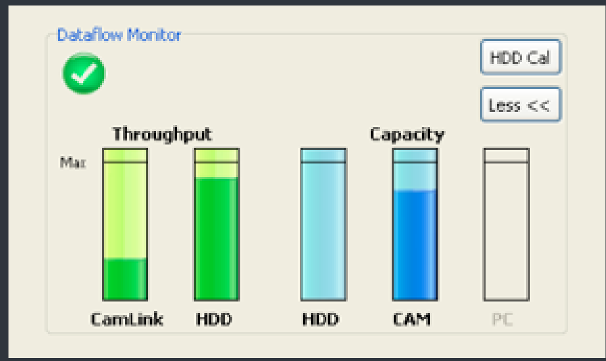
For sustained kinetic series measurements it is possible to be rate limited by:

(a) bandwidth of the Camera Link interface connecting the camera to the PC

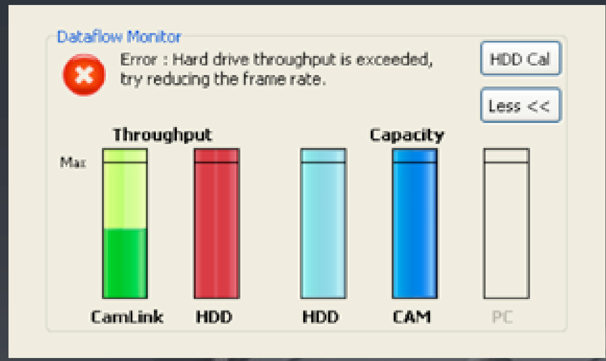
(b) hard drive write speed

In such circumstances the true frame rate threshold also depends on many set-up factors, including exposure time, ROI size, binning, pixel readout rate and choice of single or dual amplifier data.

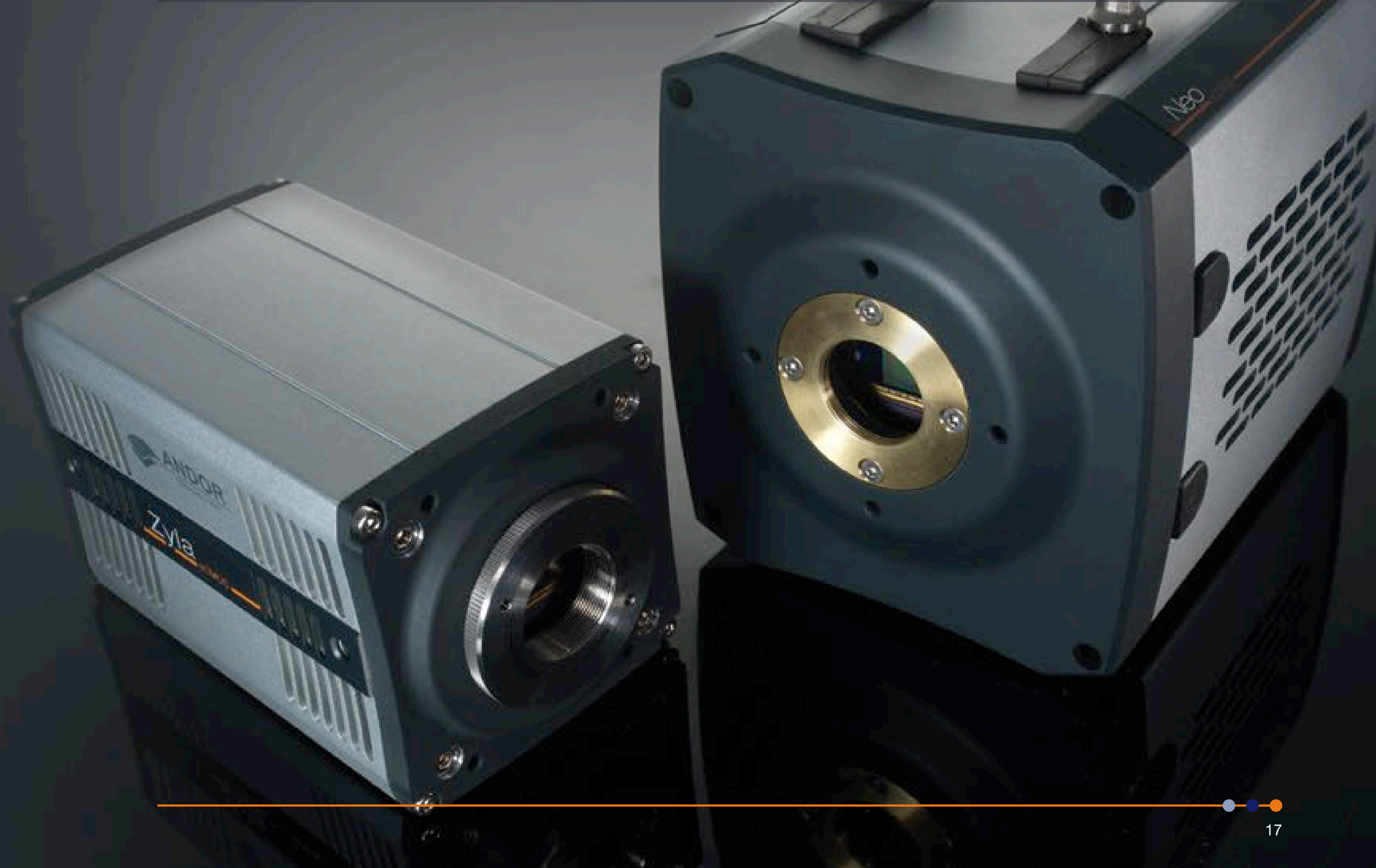
The Data Flow Monitor, available through Andor Solis acquisition and analysis software, has been innovated to provide a simple visual tool that enables you to instantly ascertain if your acquisition parameters will result in a rate of data transfer that is too fast for either interface or hard drive. It will also determine if the kinetic series size is within the capacity of camera memory, hard drive space or PC RAM.



e.g. 1 - Requested kinetic series within capability of Camera Link data transfer bandwidth and Hard Disk Drive write speed.



e.g. 2 - Hard Disk Drive will not write data fast enough for the requested kinetic series. Advised to first reduce data rate.

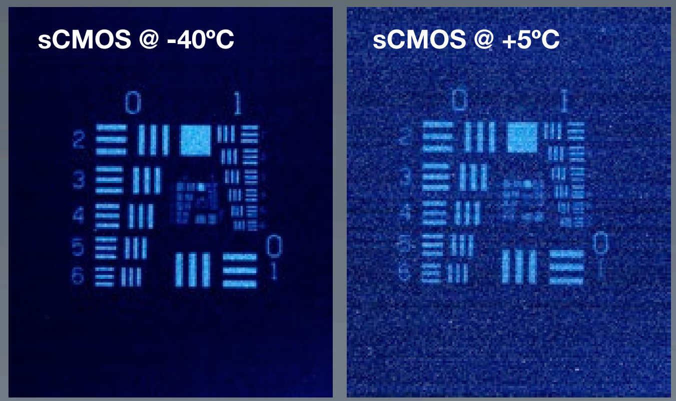


Performance and Innovations

Deep Thermoelectric Cooling

Andor's Neo offers the deepest sensor cooling available from any CMOS imaging camera on the market, minimizing both dark current and hot pixel blemishes. Additionally, through the use of water cooling, the fan can be switched off in the software to minimize camera vibration; ideal for set-ups that are particularly vibration sensitive.

Neo Cooling Temperature	Dark current (e⁻/pix/sec)
-30°C (fan cooling)	0.015
-40°C (10°C liquid)	0.007



Thermal noise can sacrifice the sCMOS low detection limit. Low light images recorded with a Neo sCMOS camera at +5°C and -40°C sensor cooling temperatures; 50 sec exposure time; 200 MHz readout giving 1 electron read noise.

Deep TE cooling is useful for a number of reasons:

Minimization of dark current

sCMOS cannot be considered a truly flexible, workhorse camera unless dark current contribution has been minimized. Deep cooling means the low noise advantage can be maintained under all exposure conditions.

Minimization of hot pixel blemishes

Hot pixels are spurious pixels with significantly higher dark current than the average and can be problematic even under relatively short exposure times. Cooling has a major influence in minimizing the occurrence of such events, offering both an aesthetically cleaner image and a greater number of unfiltered, usable pixels.

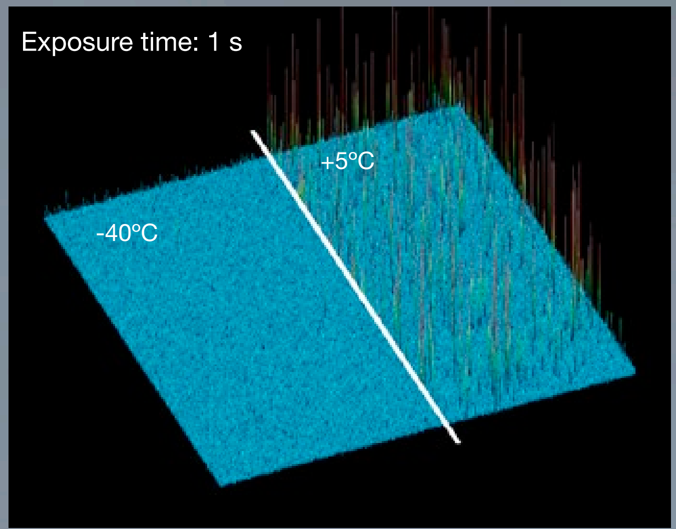
Minimization of vibration

Many optical configurations are sensitive to vibrations from the camera fan.

Andor's Neo offers:

(a) Two fan speeds

(b) The ability to turn off the fan, either temporarily or permanently if flowing liquid through the camera (the latter also allows the Neo to be stabilized at -40°C)



Hot pixel blemishes are significantly reduced at deeper cooling temperatures, requiring much reduced pixel correction. Uncorrected images are shown above for 1 sec exposure.

See page 36 for 'Importance of TE Cooling' technical note

Thermostatic Precision

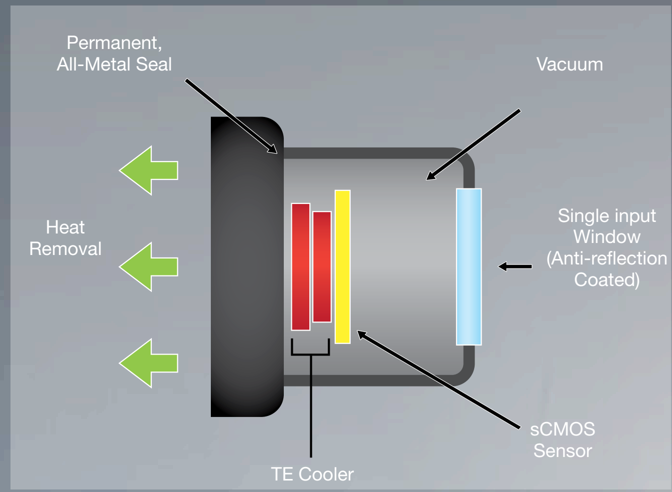
The temperature sensor in the Neo and Zyla sCMOS cameras measures with a thermostatic precision of 0.05°C

UltraVac™ (Neo only)

The Andor Neo is the only vacuum housed CMOS sensor available on the market, offering superior quality, performance and longevity.

Andor's proprietary UltraVac™ process has a proven track record of field reliability, accumulated over more than 15 years of shipping high-end vacuum cameras. Using a proprietary technique, we have adapted these process for use with the additional connections associated with the sCMOS sensor.

- Permanent hermetic vacuum seal
- Sustained deep TE cooling
- No maintenance / re-pumping
- No risk of condensation
- Minimize out-gassing



Schematic of the Neo sCMOS permanent vacuum head

5 Year Vacuum Warranty

Our faith in the unique sCMOS vacuum process used in Neo means that we are proud to offer an extensive 5 year warranty on the vacuum enclosure.

Performance and Innovations

Advanced FPGA on-head processing

Andor's Neo and Zyla cameras are each equipped with considerable FPGA processing power. This is essential in order to dynamically normalize data at the pixel level for minor variations in bias offset, thus eradicating fixed pattern noise associated with this CMOS phenomenon. This superior dynamic processing capability is also utilized to optionally filter the small percentage of spurious noise pixels from the image.

Pixel-level bias offset compensation

The advanced processing power and memory capacity permits implementation of bias offset compensation for **every pixel** in the array. This ultimately relates to a lower noise background.

Dynamic baseline clamp

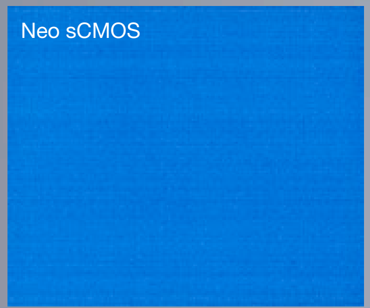
A real time algorithm that uses dark reference pixels on each row to stabilize the baseline (bias) offset. Necessary to ensure quantitative accuracy across each image and between successive images.

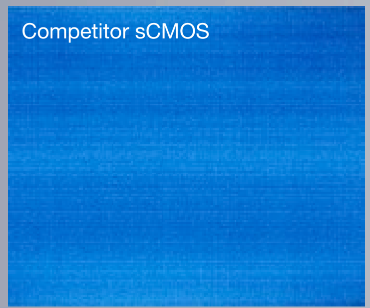
Spurious noise filter

An optional real time filter that identifies and compensates for 'spurious' high noise pixels that are greater than 5 electrons (< 1% of all pixels).

Andor offer the capability to switch off interpolative pixel filtering and provision of custom blemish maps, important for applications such as super-resolution microscopy or astronomy.

CMOS data requires compensation for fixed pattern variation. This is accomplished in real time for every pixel within the FPGA of Andor's sCMOS cameras, essentially eliminating this noise source from the image.





Dynamic Baseline Clamp affords superior background image quality (Dark image, Rolling shutter, 10 ms exposure)

6.5 µm pixel size combined with 30,000 electron well depth

The 6.5 µm pixel present in Neo and Zyla has been specifically designed to offer an optimal balance of optical resolution, photon collection area and well depth. This pixel size has been determined to provide ideal over-sampling of the diffraction limit in typical cell microscopy with x 60 and x 100 objectives.

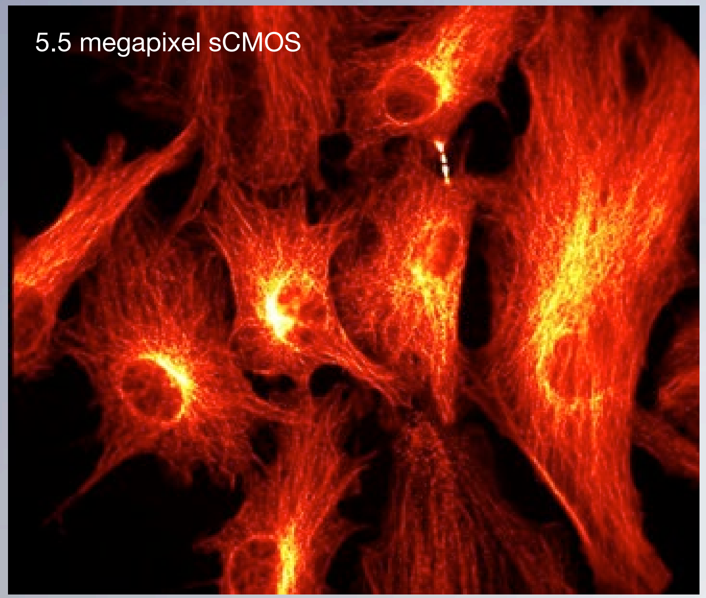
- Ideal balance of resolution, photon collection and well depth
- Superb 30,000 electron well depth
- No pixel binning required = no doubling of read noise
- No demagnification optics = no wasteful photon loss

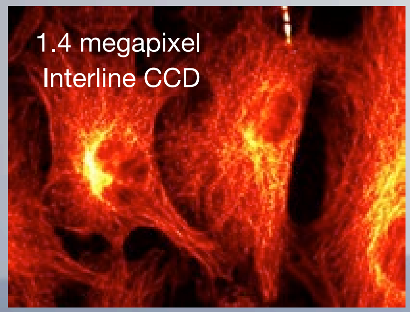
Large Field of View

The multi-megapixel sensors present in the Neo and Zyla offer an extended field of view, markedly exceeding the FOV available from alternative Interline CCD devices.

Flexibility is key however, and if a large FOV is not required for a particular application, Neo and Zyla offer a range of pre-selected ROI sizes at the click of a button, as well as user defined (with single pixel granularity).

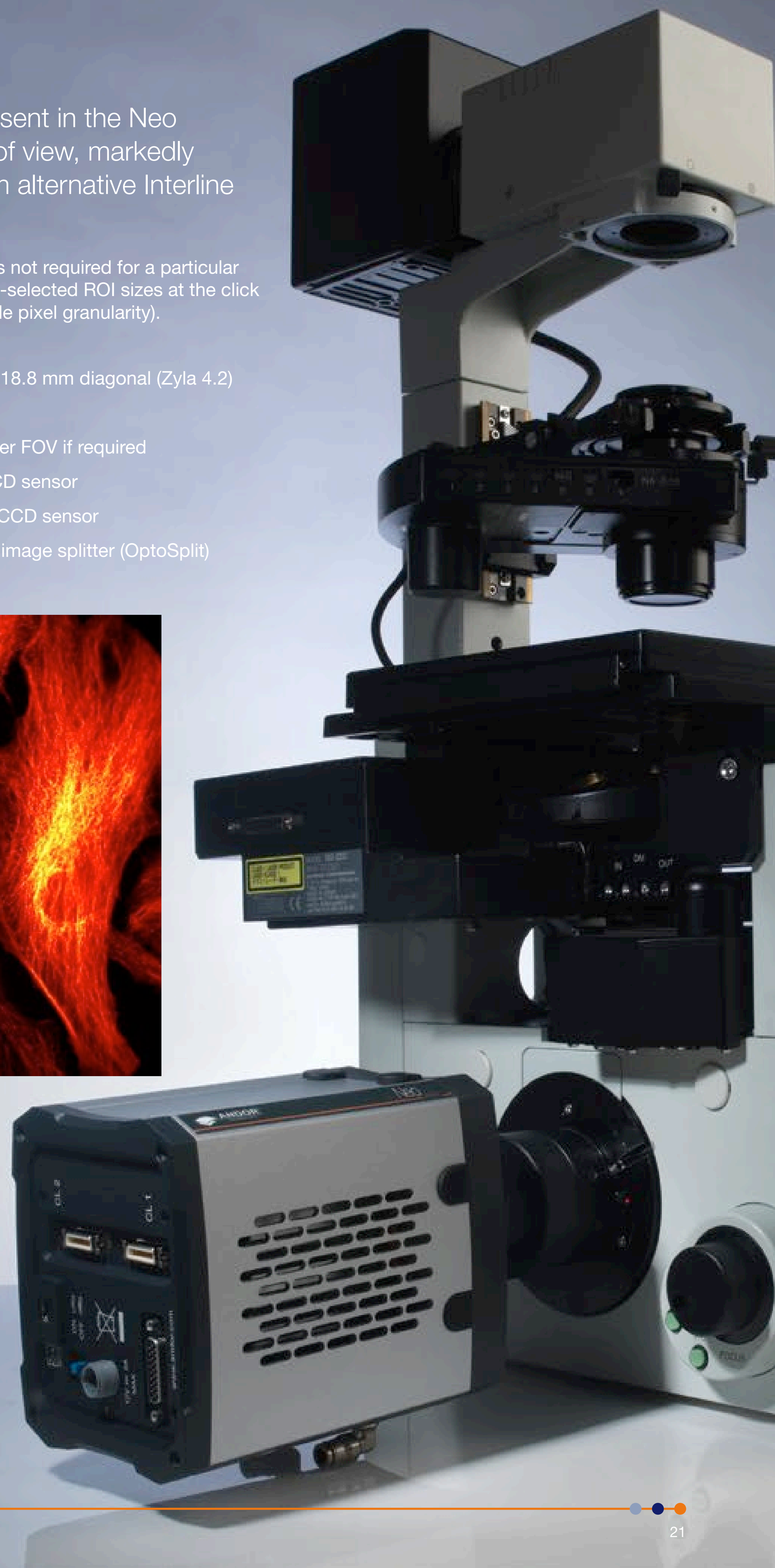
- 21.8 mm diagonal (Zyla 5.5 and Neo 5.5); 18.8 mm diagonal (Zyla 4.2)
- Closely matched to modern microscopes
- Pre-selected ROIs to quickly opt for smaller FOV if required
- x 3.5 larger than popular 512 x 512 EMCCD sensor
- x 3.9 larger than popular 1.4 MP Interline CCD sensor
- Combine large FOV with dual wavelength image splitter (OptoSplit)





Field of View Comparison

Neo 5.5 and Zyla 5.5 Field of View vs popular 1.4 megapixel Interline CCD



Performance and Innovations

Rolling and Global (Snapshot) Shutter Modes

Neo 5.5 and Zyla 5.5 are distinct in offering both Rolling shutter and ***true*** Global shutter modes from the same sensor, such that the most appropriate mode can be selected dependent on application requirements.

Uniquely benefit from a choice…

The 5.5 megapixel sCMOS sensor that is in Zyla 5.5 and Neo 5.5 sCMOS cameras uniquely offers both Rolling and Global exposure modes. This provides superior application and synchronization flexibility and the ability, through Global exposure, to closely emulate the familiar 'Snapshot' exposure mechanism of Interline CCDs.

Rolling and Global Shutter Mechanisms

Rolling and *true* Global shutter modes describe two distinct types of exposure and readout sequence.

In Rolling shutter, available in all Andor sCMOS cameras, different lines of the array are exposed at different times as the read out 'wave' sweeps through the sensor. 10 ms are required at the start to 'activate' the sensor to expose, and then 10 ms are required at the end to readout the sensor. Use when not synchronizing to peripheral devices and only when there is a minimal risk of spatial distortion from slow moving sample.

Global shutter exposure and readout (single scan)

Exposure start | Exposure | Exposure End

Rolling shutter exposure and readout (single scan)

Exposure start | Exposure | Readout

In *true* Global shutter, offered in both Neo 5.5 and Zyla 5.5 models, each pixel in the sensor begins the exposure simultaneously and ends the exposure simultaneously. This provides a true 'Snapshot' exposure capability for moving samples that is both 'photon-efficient' and easy to synchronize to, especially useful for 3D / 4D microscopy. Zyla 4.2, while utilizing a Rolling shutter sensor, offers a Simulated Global Exposure mechanism to overcome risk of spatial distortion. This mechanism is more elaborate and less photon/time efficient than *true* Global shutter. *True* Global Shutter is also essential for applications such as Particle Imaging Velocimetry (PIV), where sub-microsecond inter-frame gaps are required.

What should I be aware of as a buyer? Beware of 'Gen II' claims!

This topic carries particular relevance, as not all 'scientific CMOS' cameras on the market offer a choice of Rolling and true Global exposure. Most offer one or the other. In fact, a sensor that is currently being widely positioned in the market as "Gen II", is actually the sensor used in the Zyla 4.2. However, we would not go so far as to describe this as a 2nd generation sensor, as it achieves a higher Quantum Efficiency at the notable expense of true Global shutter capability.

See page 30 for **'Rolling and Global shutter'** technical note

The customer is highly advised to make an informed choice before purchasing, since in microscopy the more photon-efficient Global shutter approach can actually result in a higher signal to noise, faster synchronized frame rates and non-distorted images.

Key Benefits of *true* Global shutter (Zyla 5.5 and Neo 5.5)

Global shutter in particular is viewed as an important mode for the biologist, as its benefits are deeply synergistic with the core imaging requirements of live cell microscopy.

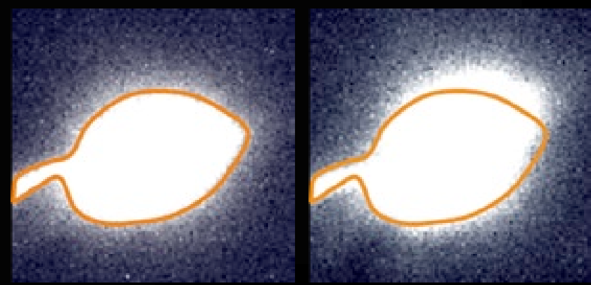
- **No spatial distortion** – avoiding the spatial distortion risk of Rolling exposure
- **3D / 4D microscopy** - recommended for synching to **peripheral switching devices**
- **Higher signal to noise** due to **reduced dead time** offering **higher 'effective' QE**
- **Simplicity** – all the benefits of an **'Interline exposure mode'**
- Compatible with **continuous or pulsed** light sources
- Sub-microsecond inter-frame gap, ideal for PIV applications

Global Exposure is Distortion Free

If light is falling on the sensor during the 'transient' phases (first 10 ms and final 10 ms) of the Rolling shutter exposure mechanism, and an object is moving during this time, then there is a chance of some degree of spatial distortion. The degree of distortion is dictated by the relative size, direction and speed of the object. Global shutter avoids spatial distortion since there are no 'transient' exposure phases.

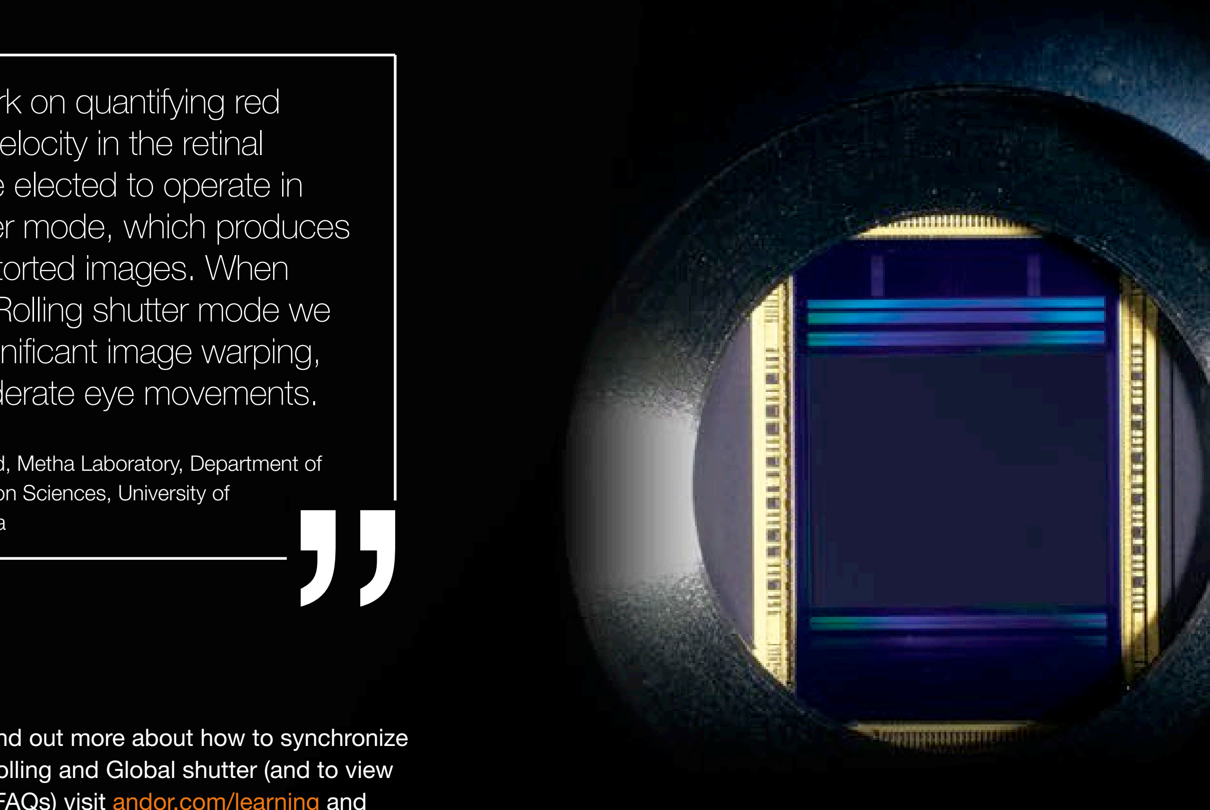
These images show the head of a moving sperm cell, imaged by the Neo sCMOS in both Global and Rolling shutter. Distortion of the shape of the sperm head is evident in Rolling shutter.

Global | Rolling



Correct | Distorted

> For our work on quantifying red blood cell velocity in the retinal capillaries we elected to operate in Global shutter mode, which produces minimally distorted images. When operating in Rolling shutter mode we observed significant image warping, even for moderate eye movements.
>
> Dr. Phillip Bedggood, Metha Laboratory, Department of Optometry and Vision Sciences, University of Melbourne, Australia



To find out more about how to synchronize to Rolling and Global shutter (and to view our FAQs) visit andor.com/learning and read the **'Synchronizing to Andor sCMOS Cameras'** technical note.

Performance and Innovations

Comprehensive trigger functionality

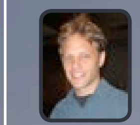
Neo and Zyla offer a selection of advanced trigger modes, designed to provide tight synchronization of the camera within a variety of experimental set-ups. Triggering is compatible with both Rolling and Global shutter modes.

- External TTL, Software and Internal trigger (including Simulated Global Exposure - Zyla 4.2)
- 'Time Lapse' and 'Continuous' (overlapped) kinetic series
- Fast exposure switching (iCam)

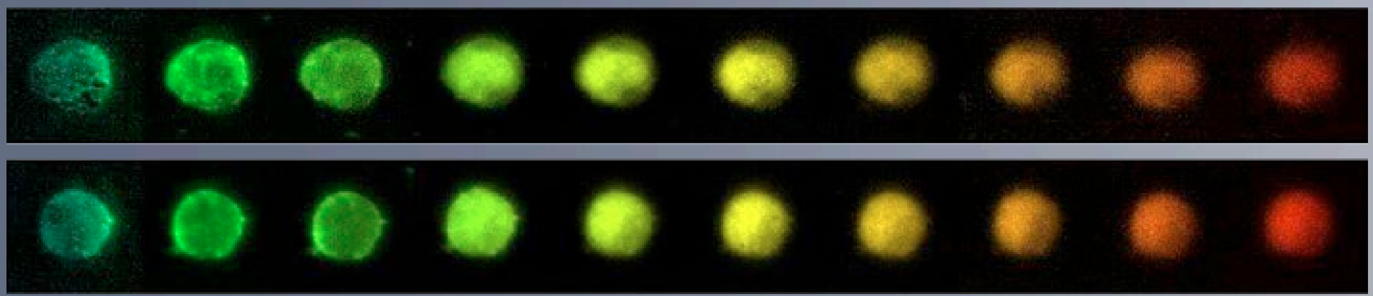
Trigger Mode	Description	Trigger Sources
Time Lapse	Each exposure started by a trigger event (e.g. TTL rising edge). Exposure duration is internally defined.	Internal, External Software
Continuous	Exposures run back to back with no time delay between them. Exposure time defined by time between consecutive trigger events.	Internal, External
External Exposure	Exposure time defined by TTL width (sometimes known as 'bulb mode').	External
External Start	TTL rising edge triggers start of internally defined kinetic series.	External trigger, followed by internal timer

Available Neo and Zyla trigger modes, applicable to both Rolling and Global shutter.

> "Andor's sCMOS camera is the ideal solution for imaging flow cytometry where both high speed and low read noise are critical."



Dr. Ethan Schonbrun, Rowland Institute, Harvard University, USA



Hyperspectral Imaging of Fluorescent Beads. Image courtesy of Dr. Ethan Schonbrun.

sCMOS Software Solutions

Andor Solis

Solis is a ready to run Windows package with rich functionality for data acquisition and image analysis/processing.

Andor Basic provides macro language control of data acquisition, processing, display and export.

Andor SDK

A software development kit that allows you to control the Andor range of cameras from your own application. Available as 32 and 64-bit libraries for Windows (XP, Vista and 7) and Linux. Compatible with C/C++, LabView and Matlab.

Andor iQ

A comprehensive multi-dimensional imaging software package. Offers tight synchronization of EMCCD with a comprehensive range of microscopy hardware, along with comprehensive rendering and analysis functionality. Modular architecture for best price/performance package on the market.

Bitplane Imaris

Imaris delivers all the necessary functionality for visualization, segmentation and interpretation of multidimensional datasets.

By combining speed, precision and intuitive ease-of-use, Imaris provides a complete set of features for handling multi-channel image sets of any size up to 50 gigabytes.

Third Party Software Compatibility

The range of third party software drivers for Andor's sCMOS camera platforms is expanding steadily. Please enquire for further details.

NIS-Elements | MetaMorph NX | µManager | LabVIEW | MATLAB
NORPIX | Windows XP | Windows Vista | Windows 7 | Linux

The Andor Imaging Range

Have you found what you are looking for? As an alternative to the sCMOS cameras, Andor offers an extensive portfolio of high performance low light imaging camera technologies.

iKon CCD

Deep cooled, low noise CCD

- -100°C cooling
- Back-illuminated > 90% QE
- 1 megapixel to 4 megapixel
- Enhanced NIR versions
- ‘PV Inspector’ model (Optimized for EL / PL in-line inspection)
- USB 2.0 true plug and play

Clara Interline CCD

High-performance Interline CCD

- Industry lowest Interline read noise (2.4 e-)
- -55°C fan cooled; -40°C vibration free mode
- 1.4 megapixel
- USB 2.0 true plug and play

Zyla sCMOS

Fast, sensitive, compact, light sCMOS

- 1 electron read noise @ 30 fps
- 5.5 and 4.2 megapixel sensors / 6.5 µm
- 0°C cooling at +35°C ambient
- 100 fps sustained (10-tap Camera Link)
- Cost effective USB 3.0 option
- 16-bit data range

Neo sCMOS

Vacuum cooled, lowest noise sCMOS

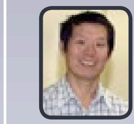
- 1 electron read noise @ 30 fps
- 5.5 megapixel / 6.5 µm
- -40°C vacuum cooling
- 30 fps sustained; 100 fps burst
- 4 GB on head memory
- 16-bit data range
- Fan off vibration free mode

iXon EMCCD

High performance EMCCD platform

- Single photon sensitive and back-illuminated
- Industry fastest frame rates
- -100°C cooling
- Flexible yet intuitive
- Quantify in electrons or photons

> “We tested the Andor sCMOS camera in conjunction with a popular cooled CCD camera, and compared with results from a similar test of a competitor’s scientific CMOS camera. Andor’s camera showed lowest dark noise, biggest field of view with very good sampling resolution (number of pixels), fastest frame rate, compatible signal to noise ratio and potentially largest dynamic range of detection. It is the most suitable camera on the market for our project.”



Dr. Yan Gu, Confocal Imaging and Analysis Lab National Institute for Medical Research, London, UK

Technical Notes

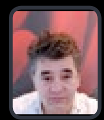
New technology and innovation heralds a lot of new questions!

The following section is dedicated to providing a greater depth of understanding of the performance and innovations associated with the Andor scientific CMOS camera platform. Deeper insight is provided into areas such as the unique dual amplifier architecture (for extended dynamic range), sCMOS read noise distribution, dark noise effects, vacuum sensor protection and Rolling vs. Global shutter readout modes.

We also present a comprehensive overview of how new sCMOS technology compares to existing 'gold standard' scientific imaging cameras such as Interline CCD and EMCCD technology.

- **LightScan PLUS** NEW
- **Rolling and Global Shutter**
- **Dual Amplifier Dynamic Range**
- **The Importance of TE Cooling to sCMOS Technology**
- **Comparing sCMOS With Other Scientific Detectors**
- **Andor sCMOS PC Recommendations and Data Flow Considerations**
- **Understanding Read Noise in sCMOS**
- **Interpolative Blemish Corrections on sCMOS** NEW
- **PIV Mode for Neo and Zyla** NEW

"We developed a quantitative phase scanner, capable of imaging an entire microscope slide in less than 50 minutes, with submicron resolution. Due to the high sensitivity of Andor sCMOS cameras we were able to reduce our exposure time from 100 ms to 15 ms. The fast frame rates and large field of view of Zyla 5.5 are instrumental in studying fast dynamics in cells and reducing the number of frames to be acquired."



Prof. Gabriel Popescu, Beckman Institute for Advanced Science and Technology, University of Illinois, USA

Technical Note

Application of LightScan PLUS to Light Sheet Microscopy

LightScan PLUS is a new feature which has been added to Zyla 4.2 to allow the end-user to have more control over the Rolling Shutter scanning mechanism. In standard rolling shutter cameras, the user typically has no control over parameters such as scan direction or scan speed. This new mode introduces significant flexibility, enabling the user to finely synchronize to a range of illumination scan options and to minimize dead time between scans. The primary applications for these new scanning modes are Scanned Light Sheet Microscopy and Line Scanning Confocal Microscopy.

The bulk of light sheet microscopy applications today investigate the development of living embryos and the samples under examination can be relatively large and thick. By virtue of this, it can be quite difficult to acquire highly resolved images due to the scattering of light and low contrast in such samples. One proposed alternative approach is to sweep a laser beam across the focal plane, resulting in a planar illumination equivalent to a sheet but with improved illumination efficiency and uniform intensity distribution. LightScan PLUS functionality allows the user to synchronize the scanning of their illumination beam to a defined scan row height on the sensor. Image quality is improved since the scan row height can act as a slit detector, rejecting scattered light, improving contrast and SNR and hence providing sharper and more resolved images.

With LightScan PLUS the user can scan their light source from the top to the bottom of the sensor or vice versa in one continuous sweep. An enhanced degree of precision control is available through FlexiScan, allowing independent control over the scan row height, scan speed and exposure time. Furthermore, LightScan PLUS provides multiple different readout directions allowing the user further flexibility. CycleMax functionality ensures minimum deadtime between scans by enabling the laser sweep and corresponding rolling shutter scan direction to alternate from top-bottom to bottom-top, thus avoiding the need to reset the laser to the same starting position for each subsequent image.

The figure below illustrates the Rolling Shutter mechanism, and is adapted from one of the first papers (1) which describes the approach

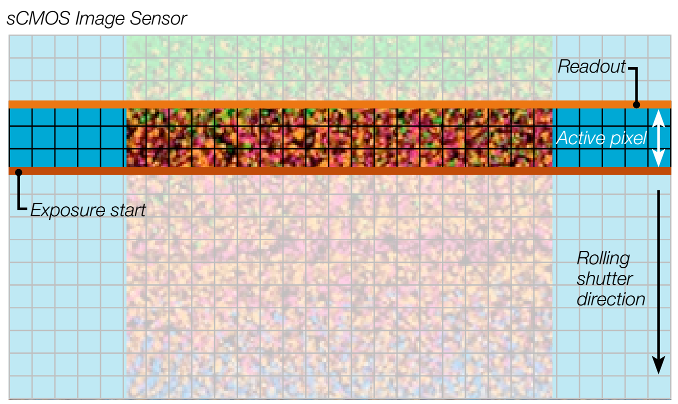


Figure 1: Rolling Shutter mode. The band of pixel rows in between is exposed to light simultaneously. This band moves from the top to the bottom of the sensor. Its width is defined by the single row exposure time, and can be adjusted from a minimum of one line up to the whole chip.

of scanned light sheet microscopy with confocal slit detection. As each row starts to expose sequentially down the sCMOS sensor, the sensor essentially acts as a rolling exposure window of pixels that are exposing simultaneously. By synchronizing the scanning light source with the rolling exposure window one can combine the rolling shutter exposure slit with the scanning light source.

LightScan PLUS

The standard mode of operation in sCMOS cameras is to read out from the centre of the sensor out to the edge with two halves of the sensor exposing simultaneously. LightScan PLUS provides the user with a range of different readout options. Firstly, the rolling shutter can now be scanned from the top to the bottom of the sensor or vice versa in one continuous sweep (Figure 2) and at the same time the user has the ability to control the slit width (number of rows to expose), exposure time and scan speed.

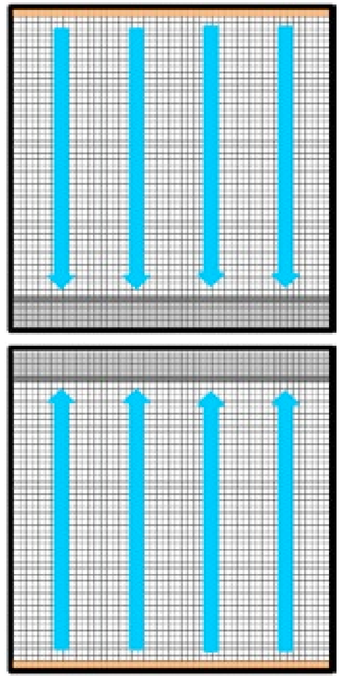


Figure 2: LightScan PLUS enables the rolling shutter to scan the sensor from top to bottom or vice versa in one continuous sweep. This is also known as "Single Port Readout"

It has been reported previously (1), that the length of the exposure time governed the slit width and therefore as the exposure time was increased the slit width was also increased. This ensured that for weakly fluorescent samples the exposure time could be extended to increase the fluorescent signal in the sample but the slit width would also be increased thereby reducing the contrast and confocality in the final image.

In order to have an increased signal and confocality concurrently, it would require that the exposure time and slit width be controlled independently. LightScan PLUS makes this possible. As well as this, the user has the ability to alter the speed with which the slit width scans the sensor. This becomes important when investigating highly dynamic samples where speed is crucial. In addition, the light source

Parameter	*216 MHz	*540 MHz
Scan speed range (Rows/ms)	2.98 – 41.67	7.43 - 104
Exposure range for slit width of 10 rows (ms)	0.240 – 3.36	0.096 – 1.344
Scan time for one full image (ms)	49 - 686	19.69 – 275.66

Table 1: Independent control of scan speed and exposure time in LightScan PLUS (*Due to a granularity of 290/118 (216/540 MHz) ns with the scan speeds the user can fine tune the readout speed with excellent precision)

which is synched to the sensor may not have the ability to scan at very high speeds and in this instance it would be useful to slow down the scan speed of the 'virtual slit'. Table 1 below denotes the flexibility of LightScan PLUS in terms of scan speed and exposure time.

Furthermore, LightScan PLUS offers the user multiple new scanning directions for the rolling shutter mechanism in which both sides of the sensor can be used. As well as the standard mode of operation whereby the sensor is scanned from the centre outwards in both

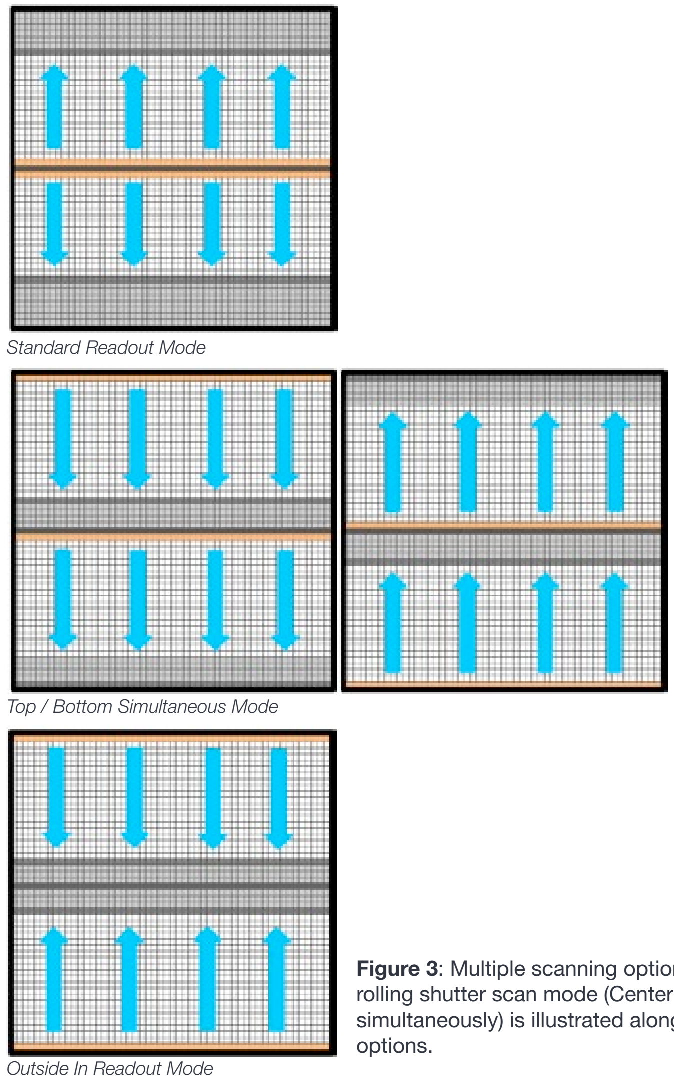


Figure 3: Multiple scanning options available. The standard rolling shutter scan mode (Center outwards in both directions simultaneously) is illustrated along with the three new scanning options.

directions simultaneously, the user now has the option to change the direction of the scan on either half of the sCMOS sensor. These new scanning options for sCMOS are ideal in multi-wavelength applications where two light sources are scanning across the image sensor with different wavelengths. These are illustrated below in Figure 3.

For all the various scanning options it would be advantageous to have the minimum deadtime between alternate frames and have the ability to capture multiple frames as fast as possible. LightScan PLUS offers a unique mechanism which enables this functionality. CycleMax functionality ensures minimum deadtime between scans by enabling the laser sweep and corresponding rolling shutter scan direction to alternate from top-bottom to bottom-top, thus avoiding the need to reset the laser to the same starting position for each subsequent image.

References

1. Optics Express, Vol. 20, Issue 19, pp. 21805-21814, 2012

ANDOR
an Oxford Instruments company

Technical Note

Rolling and Global Shutter Flexibility - Neo 5.5 and Zyla 5.5

The CIS2051 sCMOS sensor in both Neo 5.5 and Zyla 5.5 cameras was designed with a 5T (5 transistor) pixel architecture to offer choice of both Rolling and *true* Global shutter modes (also called Rolling and Global exposure modes). This provides superior application and synchronization flexibility and the ability, through Global shutter, to closely emulate the familiar ‘Snapshot’ exposure mechanism of Interline CCDs.

Rolling and Global shutter modes describe two distinct sequences through which the image may be read off a sCMOS sensor. In Rolling shutter, different lines of the array are exposed at different times as the read out ‘wave’ sweeps through the sensor. In Global shutter mode each pixel in the sensor begins and ends the exposure simultaneously, analogous to the exposure mechanism of an Interline CCD. However, absolute lowest noise and fastest non-synchronized frame rates are achieved from Rolling shutter mode.

Traditionally, most CMOS sensors offer either one or the other, but Neo 5.5 and Zyla 5.5 offer the choice of both Rolling and Global from the same sensor. The user benefits from the ability to select (via software selection) either readout mode from the same sensor, such that the most appropriate mode can be chosen dependent on specific application requirements.

Rolling shutter

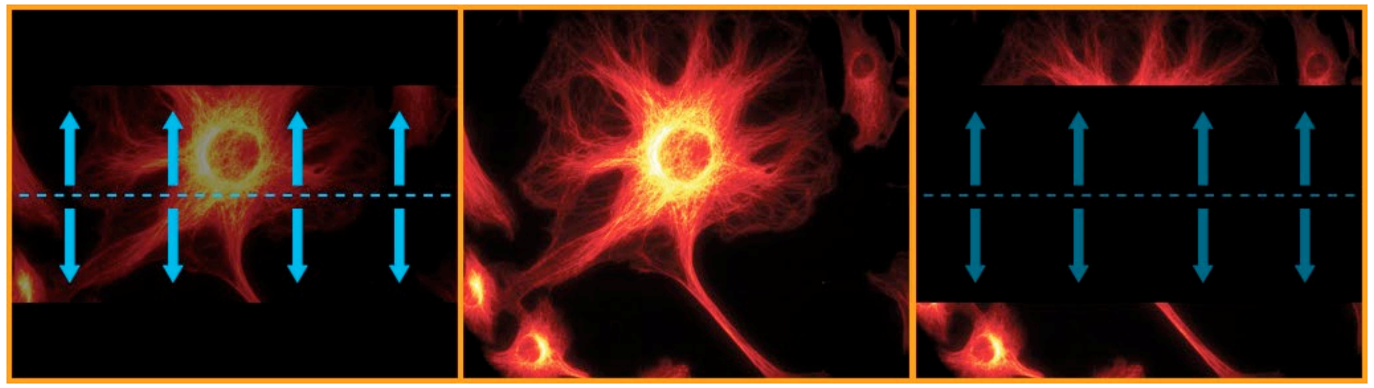
Rolling shutter mode essentially means that adjacent rows of the array are exposed at slightly different times as the readout ‘waves’ sweep through each half of the sensor. That is to say, each row will start and end its exposure slightly offset in time from its neighbor. At the maximum readout rate this offset between adjacent row exposures is 10 µs. The Rolling shutter readout mechanism is illustrated in Figure 1. From a point of view of readout, the sensor is split in half horizontally, and each column is read in parallel from the center outwards simultaneously, row after row. At the start of an exposure, the wave sweeps through each half of the sensor, switching each row in turn from a ‘keep clean state’, in which all charge is drained from the pixels in the anti-bloom structure, to an ‘exposing state’ in which light induced charge is collected in each pixel. At the end of the exposure, the readout wave again sweeps through the sensor, transferring the charge from each row into the readout node of each pixel. The important point is that each row will have been subject to exactly the same exposure time, but the row at the top or bottom of each sensor half would have started and ended its exposure 10 ms (1000 rows x 10 µs/row) after the rows at the center of the sensor.

Rolling shutter can be operated in a continuous ‘overlap’ mode when capturing a kinetic series of images, whereby after each row has been read out, it immediately enters its next exposure. This ensures a 100% duty cycle, meaning that no time is wasted between exposures and, perhaps more importantly, no photons are wasted. At the maximum frame rate for a given readout speed (e.g. 100 fps at 560 MHz) the sensor is continuously reading out in overlap mode, i.e. as soon as the readout fronts reach the top and bottom of the sensor, they immediately return to the center to readout the next exposure.

A potential downside of Rolling shutter is spatial distortion, resulting from the above described exposure mechanism. Distortion would be more apparent in cases where larger objects are moving at a rate that the image readout could not match. However, distortion is less likely when relatively small objects are moving at a rate that is being temporally oversampled by the frame rate.

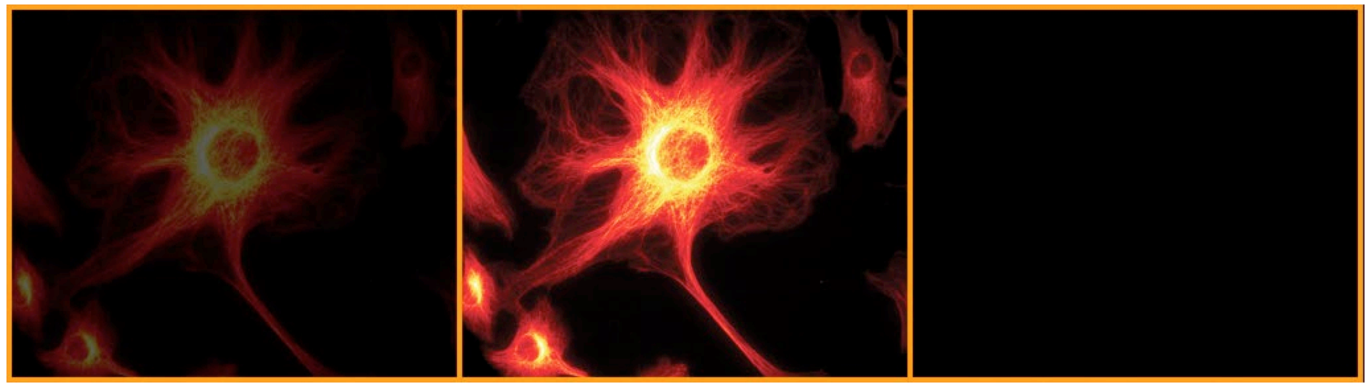
A further downside is that different regions of the exposed image will not be precisely correlated in time to other regions, which can be essential for some usages. A final, and very important, factor is that synchronizing (e.g. light source activation or peripheral device movement) to Rolling shutter readouts can be complex and also can result in slower cycle times and frame rates, relative to those achievable in Global shutter.

Rolling shutter exposure sequence (single frame)



Exposure start Exposure Readout

Global shutter sequence (single frame)



Exposure start Exposure Exposure End

Figure 1 - Simplified illustration showing sequence of events in Rolling and Global shutter modes. Note that while a single image acquisition is represented, each mode is also compatible with ‘overlap’ readout, whereby the next exposure begins simultaneously with image readout.

Global shutter – ‘Interline CCD mode’

Global shutter mode, which can also be thought of as a ‘Snapshot’ exposure mode, means that all pixels of the array are exposed simultaneously, thus enabling ‘freeze frame’ capture of fast moving or fast changing events. In this respect, Global shutter can be thought of as behaving like an Interline CCD sensor. Before the exposure begins, all pixels in the array will be held in a ‘keep clean state’, during which charge is drained into the anti-bloom structure of each pixel. At the start of the exposure, each pixel simultaneously begins to collect charge and is allowed to do so for the duration of the exposure time. At the end of exposure, each pixel transfers charge simultaneously to its readout node.

Global shutter can be configured to operate in a continuous ‘overlap’ mode (analogous to Interline CCD), whereby an exposure can proceed while the previous exposure is being readout out from the readout nodes of each pixel. In this mode, the sensor has a 100% duty cycle, again resulting in optimal time resolution and photon collection efficiency. During this entire cycle, there is no period of ‘transient’ readout as found in Rolling shutter.

Importantly, Global shutter mode is very simple to synchronize to and often yields faster frame rates than efforts to synchronize with Rolling shutter with the same exposure time. Global shutter can also be regarded as essential when exact time correlation is required between different regions of the sensor area.

However, the mechanism of Global shutter mode demands that a reference readout is performed ‘behind the scenes’, in addition to the actual readout of charge from each pixel. This additional digitized readout is required to eliminate reset noise from the Global shutter image. Due to this additional reference readout, Global shutter mode carries the trade-off of halving the maximum unsynchronized frame rate that would otherwise have been achieved in Rolling shutter mode.

	Rolling	Global
Snapshot exposure	No	Yes
Interline CCD similarity	No - very different ‘transient’ exposure sequence	Yes – extremely similar exposure sequence
Temporal correlation between different regions of image area	No - up to 10 ms (@ 560 MHz) difference between centre and top or bottom of image	Yes - all pixels represent exact same time of exposure
Synchronization capability	Complex to synchronize Requires strobe light source Longer cycle times.	Simple to synchronize Any light source Shorter cycle times
Fast double exposure capability	No	Yes
Maximum Frame Rate	Maximum available (non- synchronized)	Maximum frame rates are halved
Read Noise	Lowest possible (1 e^- to 1.3 e^-)	Slightly higher (2.3 e^- to 2.6 e^-)
Spatial Distortion	Possible if not temporally oversampling object dynamics or shuttering light source	None
Duty Cycle Efficiency	Reduced, e.g. if require to shutter illumination off during ‘transient’ readout phases	Typically much larger since no ‘transient’ readout phase to avoid

Table 1 - Comparing the pros and cons of Rolling vs Global shutter.

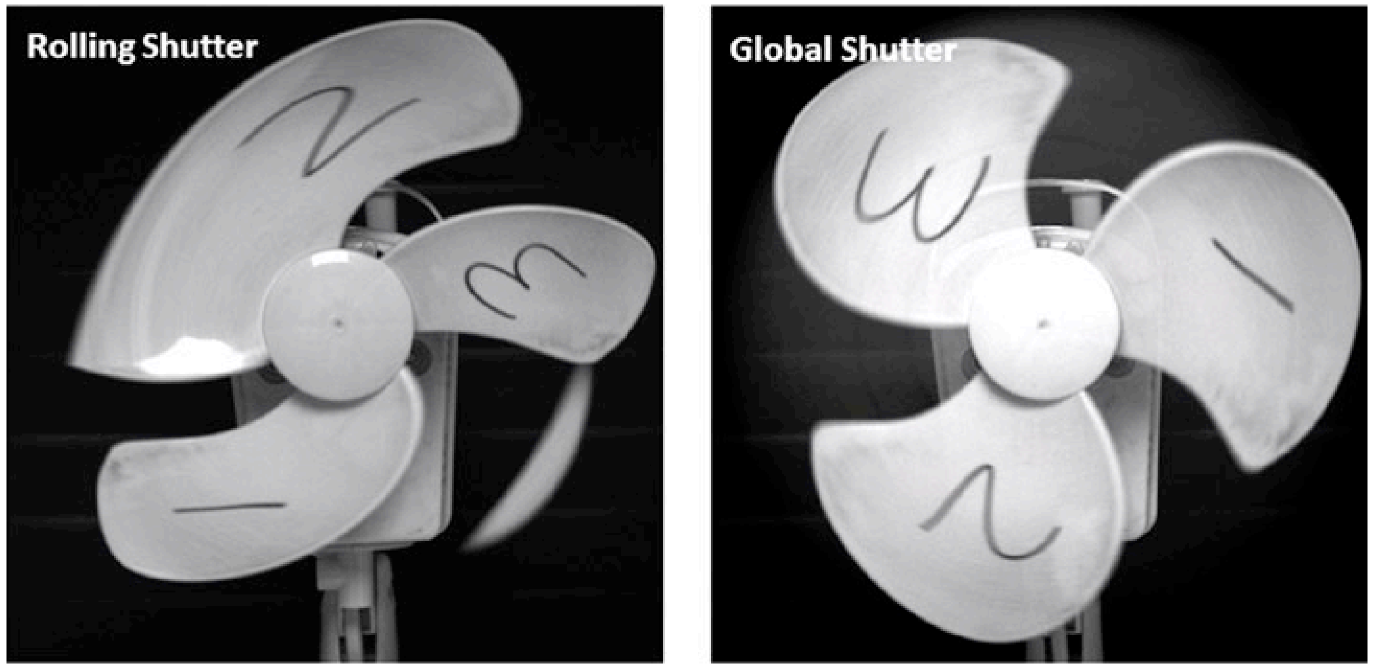


Figure 2 – Images of a moving fan, acquired with Neo sCMOS camera with Rolling and Global shutter exposure modes, same exposure time. The spatial distortion associated with the 'Rolling shutter effect' is apparent in the left image. Global shutter is a 'Snapshot' acquisition mode and avoids spatial distortion.

Rolling or Global?

Whether Rolling shutter or Global shutter is right for you will depend very much on the experiment. Global shutter has a 'non-transient' exposure mechanism that is entirely analogous to that of Interline CCDs, and for many will provide the reassurance of 'freeze frame' capturing of moving objects or transient events during a kinetic acquisition series with zero spatial distortion, as well as offering simpler and faster synchronization performance. For particular applications, for example where it is required that different regions of the image maintain temporal correlation or where it is required to accurately synchronize to relatively short lived events, Global shutter will be viewed as a necessity.

Figure 2 shows images of a moving fan, imaged with both Rolling and Global shutter exposure modes of the Neo sCMOS camera, identical exposure time. Significant spatial distortion (beyond motion blur) of the fan blades is apparent in the image captured with Rolling shutter. The reason for this is that the blades are moving fast relative to the time taken for the 'transient' exposure activation / readout fronts of Rolling shutter to transverse the blade width. This spatial distortion is often referred to as the 'Rolling shutter effect'.

However, Rolling shutter mode, with the enhanced non-synchronized maximum frame rate possibility and lower read noise, is still likely to suit many scientific applications, e.g. where one simply needs to track relatively small objects in 2D as a function of time. As long as the frame rate is such that the camera is temporally oversampling object dynamics within the image area, negligible spatial distortion will be observed in Rolling shutter mode. Such oversampling is good imaging practice, since it is generally undesirable to have an object travel a significant distance during a single exposure. However, it must always be borne in mind that, even if distortion is not manifest, an object at the top or bottom of the image will be captured up to 10 ms apart from an object at the center of the image: if this is a factor for your experiment, then Rolling shutter should not be used.

Short charge transfer time between two consecutive exposures with Global shutter

The Global shutter mode of Neo can be used to affect electronic gating, similar to that possible with Interline CCDs. Before the exposure, all pixels in the array will be held in a 'keep clean state', during which charge is drained into the anti-bloom structure of each pixel, thus acting as an 'electronic shutter'. The exposure 'switch on' is electronic and extremely fast (sub-µs). At the end of exposure, each pixel transfers charge simultaneously to its readout node, again acting as an electronic shutter close mechanism. The transfer time specification for this step is only 2 µs and has been optically measured to be less than 1 µs.

The short transfer time between two consecutive images in Global shutter mode lends the Neo to fast 'double exposure' applications, such as Particle Imaging Velocimetry (PIV).

Synchronizing to Rolling and Global shutter

Flexibility to offer both Rolling shutter and true Global shutter can be considered highly advantageous. Rolling shutter delivers absolute lowest read noise and is best used for very fast streaming of data (> 50 fps full frame) without synchronization to a light source or peripheral device. However, it carries the risk of spatial distortion, especially when imaging relatively large, fast moving objects. There is no risk of spatial distortion when using true Global shutter. To avoid spatial distortion in Rolling shutter a simulated Global exposure synchronization approach must be used, which requires a pulsed light source and also significantly reduces the duty cycle of photon collection (i.e. reduces photons collected per cycle). By contrast, a 100% duty cycle can be maintained in Global shutter.

When synchronizing to fast switching peripheral devices, true Global shutter mode is relatively simple and can result in faster

frame rates. While the read noise in Global shutter mode (~2.5 e-) is approximately double that of Rolling shutter (~1.2 e-), this can often be offset against the higher duty cycles (therefore increased photon collection per cycle) and higher synchronized frame rates possible in true Global shutter mode.

View the tech note entitled – 'Synchronizing to sCMOS cameras – Importance of both Rolling and true Global Exposure' at andor.com/learning.

'Simulated' Global Exposure in Zyla 4.2

Whilst the '4T' sensor of the Zyla 4.2 offers only the Rolling shutter exposure mechanism, the camera offers the possibility to 'simulate' the Global shutter effect, which can be employed to overcome risk of distortion when imaging rapidly moving objects.

To achieve this, the following conditions are required:

- Use of the 'Global Clear' function of the sensor – this is selectable through software and cannot be used in image overlap mode (note, the true Global shutter of the Zyla 5.5 can be operated with overlap for minimized photon loss).
- A pulsable light source – either laser or LED.
- TTL communication – the Zyla is capable of producing a specific 'FIRE' output that can be used to pulse light on during the part of the Rolling shutter exposure sequence during which nothing is transient. Alternatively, the camera can operate as the slave.

Note: At the full 100 fps frame rate (full frame), there is no 'non-transient' part of the exposure cycle, meaning this configuration is not usable.

'Gen I' vs 'Gen II' sCMOS?

It has been noted with interest that another prominent player in the sCMOS field has opted to apply the term 'Gen II' to a 4T (4 transistor) variant of the low noise pixel architecture. This particular sensor is utilized also in the Zyla 4.2 model. While a 4T design can be considered beneficial in affording an improved Quantum Efficiency response, it does so at the expense of Global shutter capability, thus limiting application flexibility and synchronization performance.

In the author's opinion, it is misleading to apply an aggressive 'Gen II' marketing label to such a sensor variant, when both 4T (Rolling shutter) and 5T (Global shutter) CMOS concepts have been around for some time and are extremely well documented. The two sensor types are no more than design variations, offering distinct pros and cons that must be considered from an application context.

Technical Note

Dual Amplifier Dynamic Range

The Dual Amplifier architecture of sCMOS sensors across all Neo and Zyla models uniquely circumvents the need to choose between low noise or high capacity, in that signal can be sampled simultaneously by both high gain and low gain amplifiers respectively. As such, the lowest noise of the sensor can be harnessed alongside the maximum well depth, affording the widest possible dynamic range.

Traditionally, scientific sensors including CCD, EMCCD, ICCD and CMOS demand that the user must select ‘upfront’ between high or low amplifier gain (i.e. sensitivity) settings, depending on whether they want to optimize for low noise or maximum well depth. Since the true dynamic range of a sensor is determined by the ratio of well depth divided by the noise floor detection limit, choosing either high or low gain settings will restrict dynamic range by limiting the effective well depth or noise floor, respectively.

For example, consider a large pixel CCD, with 16-bit Analogue to Digital Converter (ADC), offering a full well depth of 150,000 e^- and lowest read noise floor of 3 e^-. The gain sensitivity required to give lowest noise is 1 e^-/ADU (or ‘count’) and the gain sensitivity required to harness the full well depth is 2.3 e/ADU, but with a higher read noise of 5e^-. Therefore, it does not automatically follow that the available dynamic range of this sensor is given by 150,000/3 = 50,000:1. This is because the high sensitivity gain of 1e/ADU that is used to reach 3 e^- noise means that the 16-bit ADC will top out at 65,536 e^-, well short of the 150,000 e^- available from the pixel. Therefore, the actual dynamic range available in ‘low noise mode’ is 65,536/3 = 21,843:1. Conversely, the lower sensitivity gain setting means that the ADC will top out at ~150,000 e^-, but the higher read noise of 5 e^- will still limit the dynamic range to 150,000/5 = 30,000:1 in this ‘high well depth mode’.

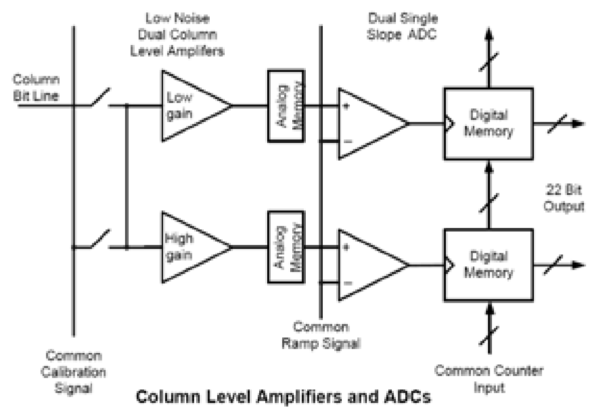


Figure 1 - Schematic layout of sCMOS Columns Level Amplifiers and Analogue to Digital Converters (ADCs).

Andor’s sCMOS sensors offer a unique dual amplifier architecture, meaning that signal from each pixel can be sampled simultaneously by both high and low gain amplifiers. The sensor also features a split readout scheme in which the top and bottom halves of the sensor are read out independently. Each column within each half of the sensor is equipped with dual column level amplifiers and dual analog-to-digital converters, represented as a block diagram in Figure 1. The dual column level amplifier/ADC pairs have independent gain settings, and the final image is reconstructed by combining pixel readings from both the high gain and low gain readout channels to achieve a wide intra-scene dynamic range, uniquely so considering the relatively small 6.5 µm pixel pitch.

The method of combining signal from two individual ADCs can be divided into four basic steps:

1) At the end of the analogue chain the “Signal” voltage is applied to two independent amplifiers: the high gain amplifier and the low gain amplifier. This results in two separate digital data streams from the sensor.
2) In the camera, the FPGA selects which data stream to use on a pixel per pixel, frame by frame basis using a threshold method.
3) The data is then corrected for DC offset and gain. Again, this is done on a pixel by pixel basis using the correction data associated with the data stream. The gain corrects for pixel to pixel relative QE, pixel node amplifier and the high and low amplifier relative gains.
4) The pixels are then combined into a single 16-bit image for transfer to the PC.

NOTE: Due to the splicing together of the low and high gains, the transition region between them is not seamless but has been optimized as far as possible.

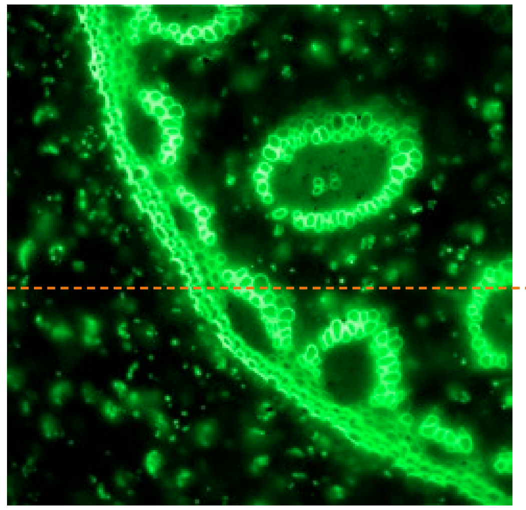
There are available individual settings and one dual amplifier 16-bit setting per shutter mode for both ‘slow’ and ‘fast’ readout rates, as shown in Table 2. The user maintains the choice of opting to stay with single gain channel data if dynamic range is not critical, resulting in smaller file sizes. This in turn offers faster frame rates when continuously spooling through the Camera Link interface and writing to hard disk.

Amplifier Gain	Electrons/count	Noise	Signal to Noise Ratio	Effective Well depth (limited by ADC)
High	Fewer	Lower	Higher	Lower
Low	More	Higher	Lower	Higher

Table 1 - The ‘traditional’ limiting choice: the mutually exclusive effect of high vs low gain amplifier choice on noise floor and effective well depth.

Amplifier Gain (Current Andor SDK / Solis description)	Mode	Sensitivity e-/ADU (typical)	Data Range	Effective pixel saturation limit / e^-	Spooling file size (5.5 megapixel)
12 bit (high well capacity)	GS/RS	7.5	12-bit	30,000	8.5 MB
12 bit (low noise)	GS	0.43	12-bit	3,690	8.5 MB
12 bit (low noise)	RS	0.3	12-bit	1,230	8.5 MB
16 bit (low noise and high well capacity)	GS	0.46	16-bit	30,000	11.3 MB
16 bit (low noise and high well capacity)	RS	0.46	16-bit	30,000	11.3 MB

Table 2 – Typical performance of supported gain settings of Andor sCMOS cameras.



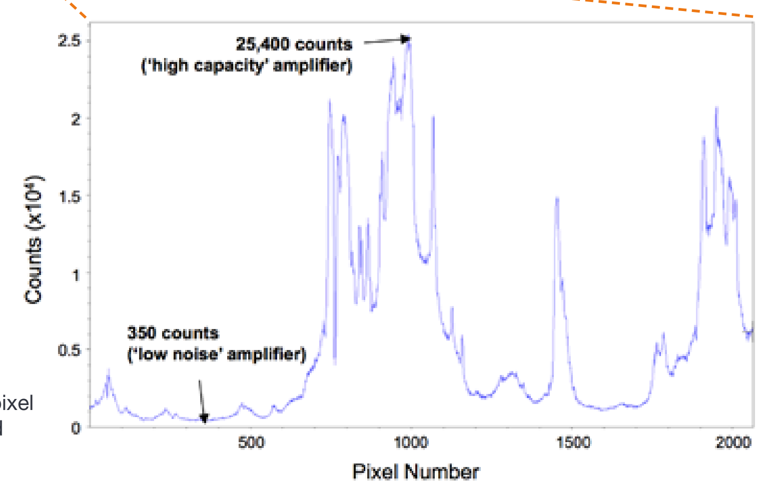


Figure 2 - High contrast image of fixed labelled cell. Intensity line profile through single row demonstrates pixel regions that were sampled by high gain (low noise) and low gain (high capacity) amplifiers.

Technical Note

The Importance of TE Cooling to sCMOS Technology

Since the read noise of scientific CMOS technology is extremely low, very careful attention must be given to the contribution of thermal noise, which if left unchecked carries potential to sacrifice the low noise floor advantage of the technology. Deep thermoelectric cooling coupled with intelligent electronic integration of the sensor provides the key to maintaining a minimized detection limit through suppression of dark current, whilst simultaneously reducing the occurrence of hot pixel blemishes.

Part 1 - Effect on Noise Floor

The ultra-low value of 1 electron rms read noise available from sCMOS cameras is entirely unprecedented, and dramatically outperforms even the best CCD to date. Read noise is an important contributor to the noise floor detection limit of a camera, but the noise associated with thermal signal, dark current, should never be overlooked. In CMOS cameras especially, even modest exposure times can result in a significant increase in dark noise. Furthermore, since scientific CMOS cameras have a much lower read noise baseline, then the percentage increase in dark current can be proportionally larger.

Andor's sCMOS cameras have each been designed to implement both effective sensor cooling, and minimisation of dark current through intelligent electronic clocking of the sensor (Dark Noise Suppression Technology). In fact, the Andor Neo sCMOS platform is unique in the market in that it is the only commercially available CMOS camera with vacuum technology, offering the level of deep thermoelectric cooling necessary to absolutely minimize the detrimental influence of dark noise. Figure 1 shows theoretical plots of noise floor versus exposure time, for three different camera types utilizing the 5.5 megapixel '5T' sensor type, including a competitor's camera. The parameters used in determining the overall noise floor are based on a typical read noise 'baseline' of ~1 electrons, combined with the measured typical dark current of the sensor at each of the temperatures, the values for 0°C and -30°C from the Andor Zyla 5.5 and Neo 5.5 sCMOS cameras respectively. The dark current value used for +5ºC has been taken from the spec sheet of a competitive camera using the same sensor. Combined noise is calculated in quadrature, i.e. using the 'square root of the sum of the squares method'.

Even within the exposure range up to 2 seconds, the low noise floor can be notably sacrificed by a factor of x2 in the case of the +5ºC competitor camera. Cooling to -30°C maintains the 1 electron noise floor over this short exposure range. At an exposure time of 10 seconds, the noise floor associated with the +5°C competitor's camera is significantly compromised to a value greater than 4.5 electrons, i.e. x4.5 greater than the read noise, whereas the noise is maintained to values less than 1.5 electrons with deeper cooling.

For very low light measurements, such as in chemiluminescence detection, it can sometimes be desirable to apply exposure times up to or greater than 10 minutes. At 600 seconds, unless deep cooling is applied, the thermal contribution to the noise floor would become excessively large, shown in graph (c) as reaching 35 electrons for the +5°C camera. The noise floor associated with the -30ºC cooled Neo is held at a more modest 2.4 electrons over this extensive exposure period.

Figure 2 shows dark images of a 2 second exposure, taken with Neo sCMOS versus that of a competitor's sCMOS (same sensor type) at +5°C. The same relative intensity scaling (in terms of absolute electrons) is used to display each. The detrimental effect of elevated bulk dark current is evident, manifest also in the comparative single row intensity profiles derived from each image.

Part 2 - Effect on Hot Pixel Blemishes

CMOS sensors are particularly susceptible to hot pixel blemishes. These are spurious noise pixels that have significantly higher dark current than the average. Through deep TE cooling of the sensor, it is possible to dramatically minimize the occurrence of such hot pixels within the sensor, meaning that these pixels can still be used for useful quantitative imaging. Table 1 shows that the typical number of pixels with higher than average dark current can be dramatically limited in practice through cooling of the sensor, meaning that they are not required to be treated by interpolation filters. Such interpolation over pixel blemishes can be detrimental in some applications that depend on total quantitative integrity over a limited set of pixels, for example in localization based super-resolution microscopy (such as PALM and STORM techniques).

Cooling Temperature	# hot pixels with > 2e-/pix/sec
+5°C	28,500
-30°C	1,800

Table 1 – Typical number of hot pixels (i.e. higher than average dark current pixels) of the 5.5MP sCMOS sensor that show dark current greater than 2 e-/pix/sec at cooling temperatures of +5°C and -30°C.

Figure 3 (a) shows a 3D intensity plot of the same region of a 5.5 megapixel sCMOS sensor at a number of different cooling temperatures, each recorded with only 1 second exposure time in Rolling shutter mode. It is clear that cooling to -30°C and beyond is highly effective in reducing the occurrence of hot pixel spikes, thus offering both an aesthetically cleaner image and a greater proportion of useable and meaningful pixels. This in turn means that significantly fewer pixels need be treated using a nearest neighbor median replacement algorithm. Even using very short exposure conditions of 30 ms, there are still significant numbers of hot pixels present at higher cooling temperatures, as illustrated in figure 3 (b).

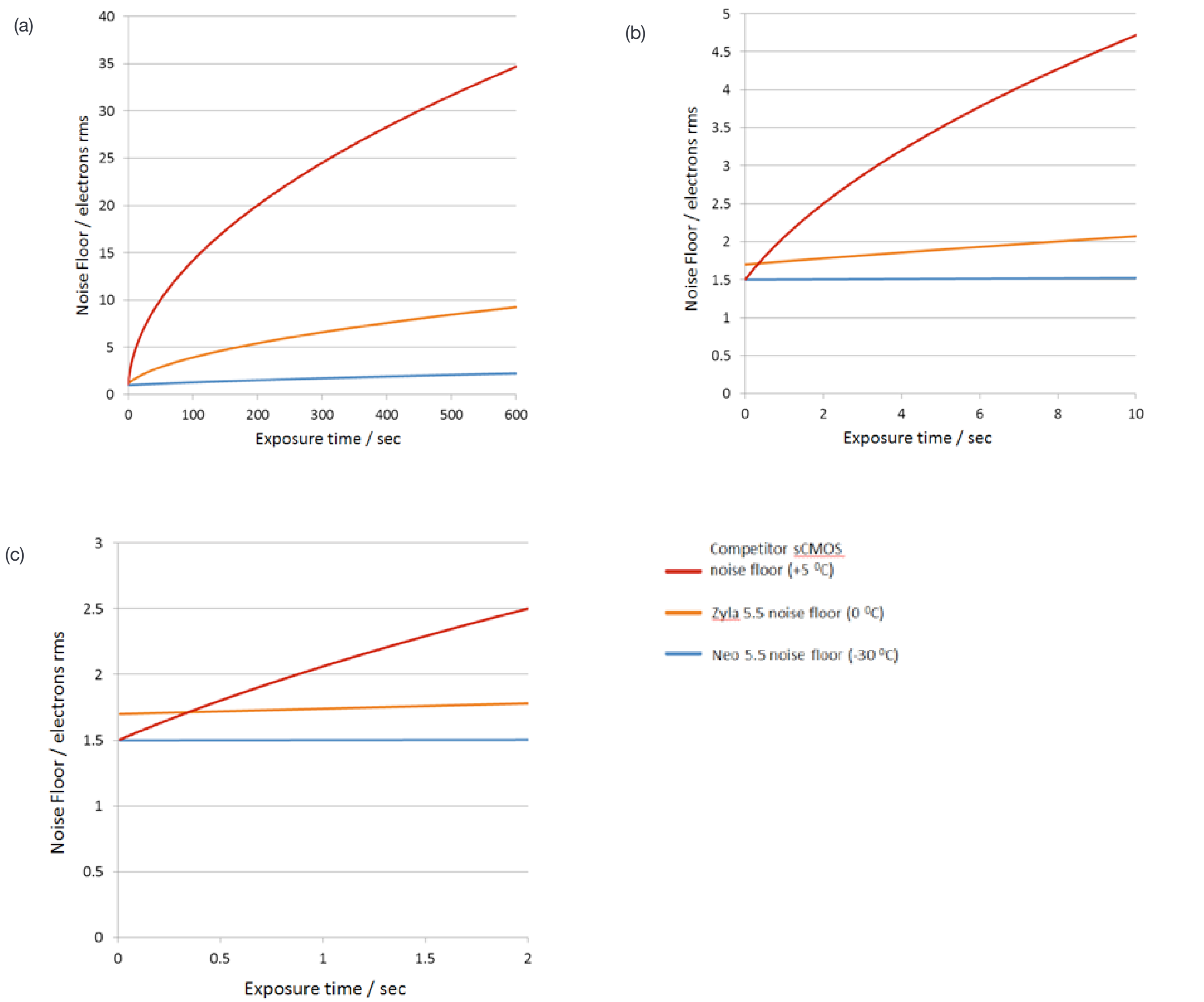


Figure 1 - Plots of sCMOS noise floor (read noise and dark noise combined in quadrature) versus exposure time, for three difference cameras all using the same 5.5 megapixel '5T' sensor type, cooled to their standard air cooled temperatures; a competitor's sCMOS camera cooled to +5ºC, the Zyla 5.5 cooled to 0ºC and the Neo 5.5. cooled to -30ºC. Plots are displayed over three ranges of exposure time: 0 - 2 sec, 0 - 10 sec and 0 -600 sec.

Part 3 – Minimization of Vibration

Many optical configurations are sensitive to vibrations from the camera fan, such as patch clamp or combined optical/AFM set-ups. The deep cooling advantage of Neo means that the internal fan can be turned off by instead opting to flow water through the conveniently located connections. Andor's Neo offers:

- Two fan speeds
- Ability to turn off fan completely

'Liquid cooling' through the camera allows minimization of vibration while still stabilizing at -40°C. Alternatively, if complete vibration free operation is required without water cooling, the Neo fan can be turned off for a limited period of time, during which the camera is passively cooled. Table 2 shows typical fan-off durations that apply when the Neo camera is operated in a + 25°C ambient environment.

Note however that the Zyla 4.2 is now shipping with a new low noise fan, that has reduced vibration by a factor of 3x. This makes the Zyla also suitable for many vibration sensitive experiments.

Furthermore, both Zyla 4.2 and Zyla 5.5 are now available as water cooled options. In this configuration, vibration will be minimized and cooling to -10ºC is achievable.

Sensor Readout Speed	Selected Sensor Temperature	Duration Before Fan Is Forced On
560 MHz	0°C	60 minutes
560 MHz	5°C	79 minutes
560 MHz	15°C	93 minutes
560 MHz	-15°C	9 minutes
560 MHz	-30°C	5 minutes
200 MHz	-15°C	18 minutes
200 MHz	-30°C	12 minutes

Table 2 - Examples of what fan-off durations are achievable across a range of cooling temperatures and readout speeds when operating Neo sCMOS in an ambient environment of 25°C.

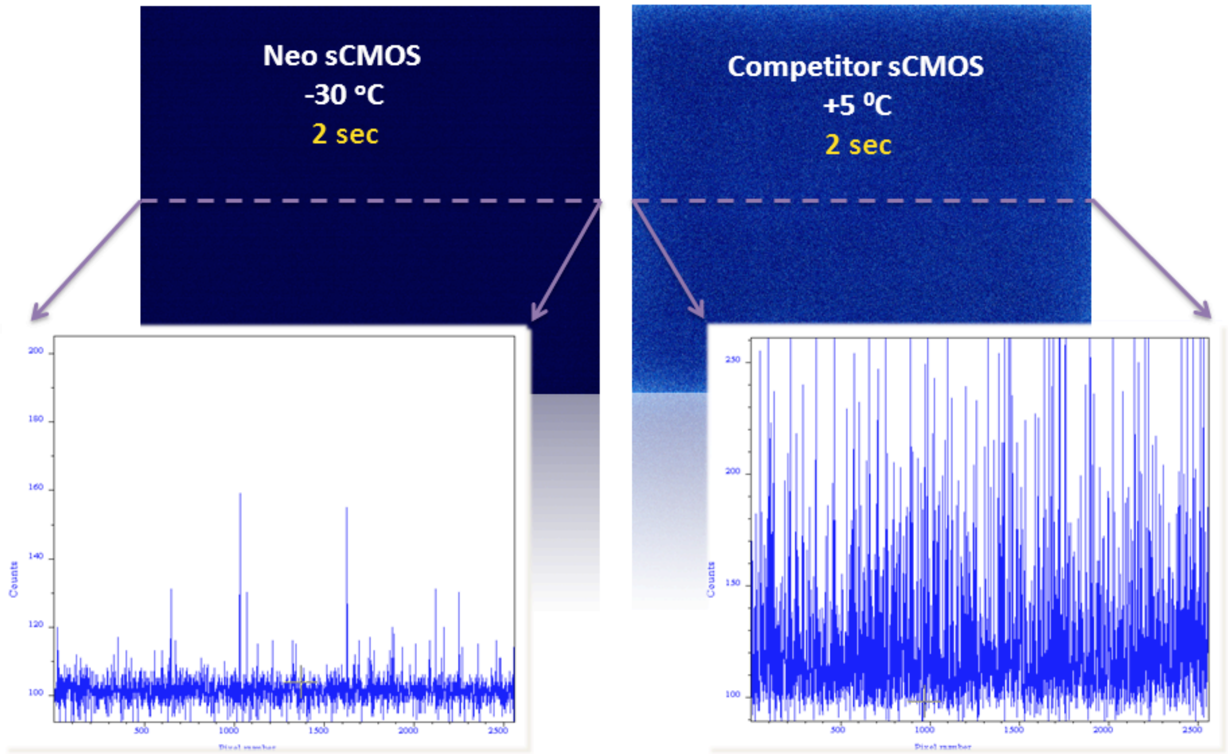


Figure 2 - Thermal noise can sacrifice the sCMOS low detection limit. Low light images recorded with a Neo sCMOS at -30°C versus a competing sCMOS using the same sensor, operated at +5°C. Shown with same relative intensity scaling; 2 sec exposure time; 560MHz readout speed. Comparative line intensity profiles from a single row is also shown for each case.

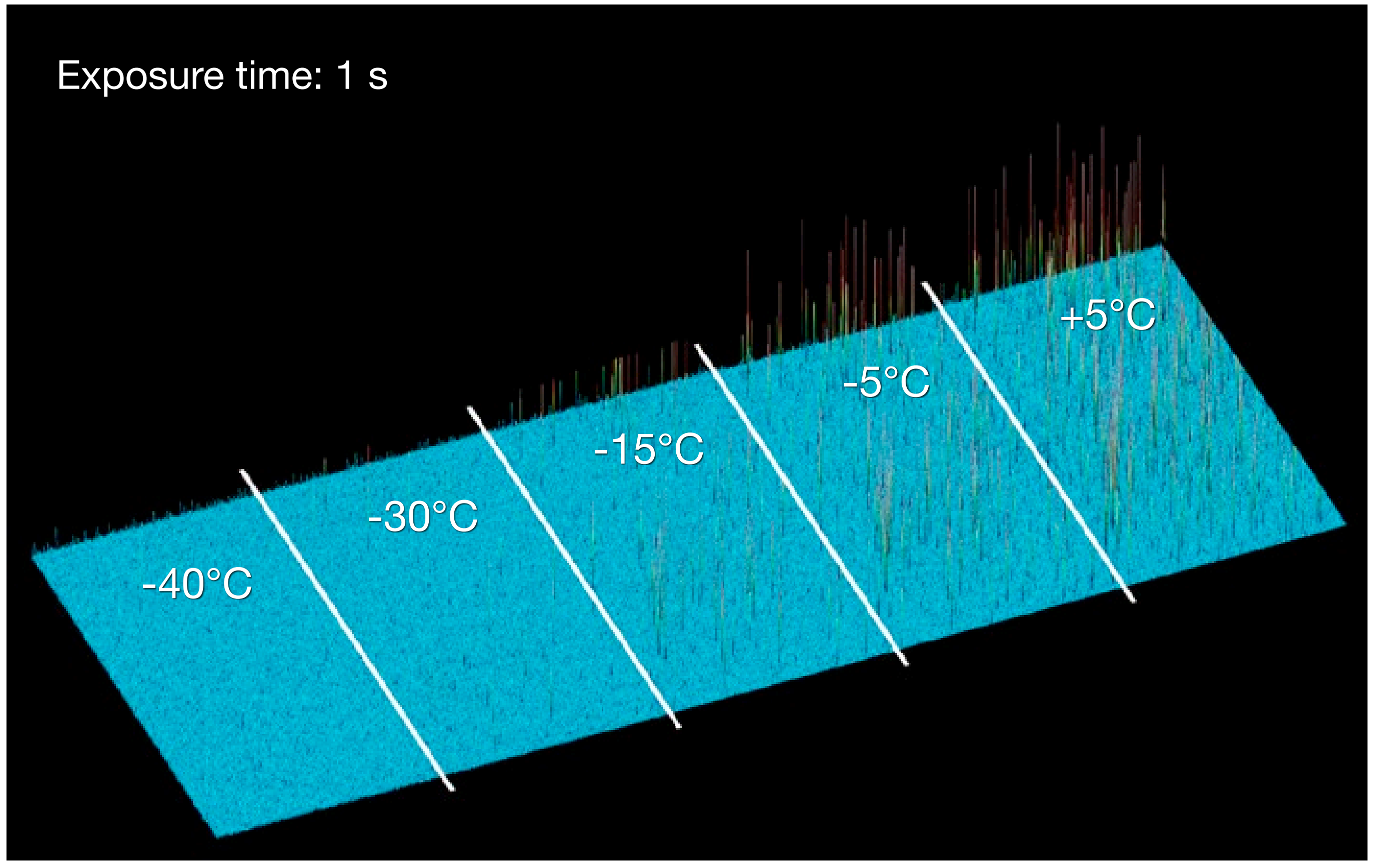


Figure 3 (a) – Blemishes at different temperatures for 1 sec exposure.

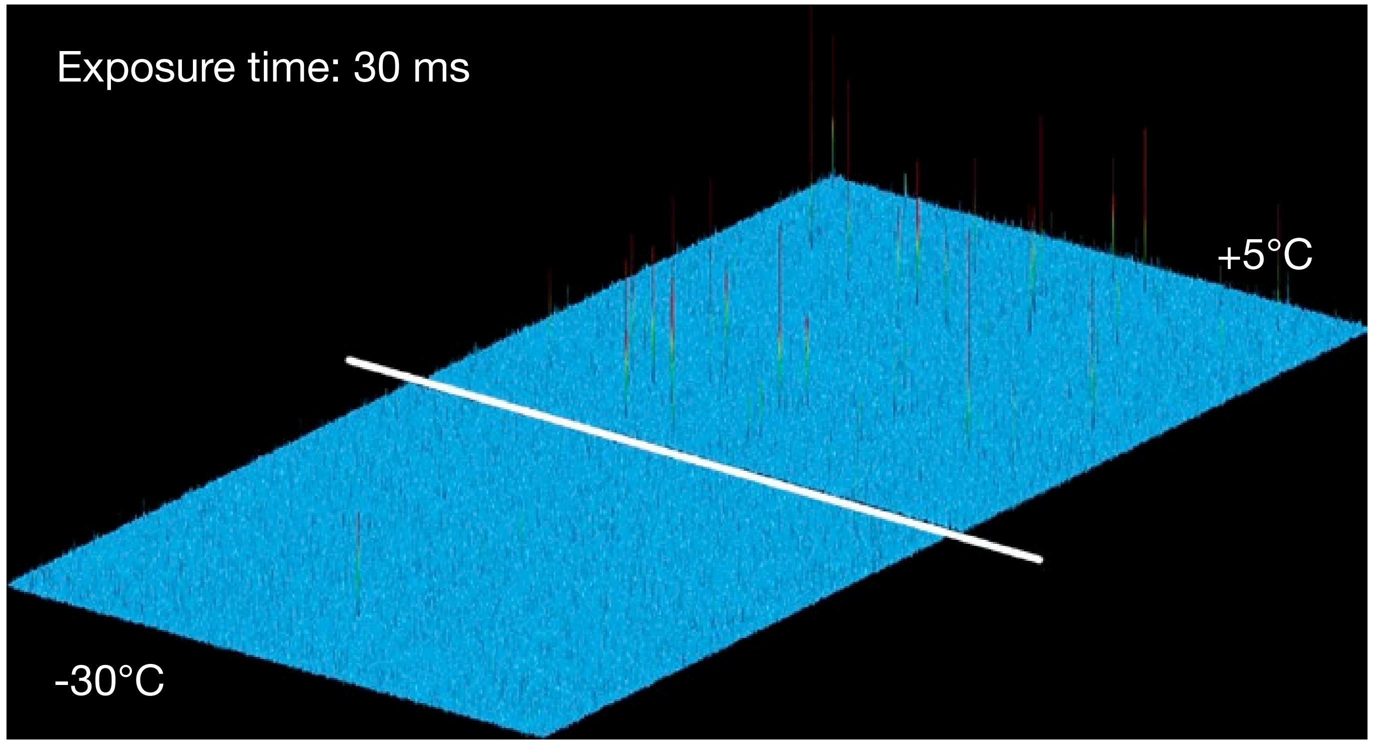


Figure 3 (b) – Blemishes at -30°C vs +5°C for 30 ms exposure.

Technical Note

Comparing sCMOS with Other Scientific Detectors

sCMOS technology is unique in its ability to overcome many of the mutual exclusivities that have marred other scientific detector technologies, resulting in an imaging detector that simultaneously optimizes a range of important performance parameters.

Part 1 - Current scientific imagers: Interline CCD and EMCCD

Many scientific imaging applications demand multi-megapixel focal plane sensors that can operate with very high sensitivity and wide dynamic range. Furthermore, it is often desirable that these sensors are capable of delivering rapid frame rates in order to capture dynamic events with high temporal resolution. Often there is a strong element of mutual exclusivity in these demands. For example, it is feasible for CCDs to achieve less than 3 electrons rms readout noise, but due to the serial readout nature of conventional CCDs, this performance comes at the expense of frame rate. This is especially true when the sensor has several megapixels of resolution. Conversely, when CCDs are pushed to faster frame rates, resolution and field of view are sacrificed (i.e. fewer pixels per frame to read out) or read noise and dynamic range suffer.

By way of illustration, consider one of the most popular, high-performance front-illuminated scientific CCD technologies on the market today – the Interline CCD. These devices are capable of reading out at 20 megapixel/s per output port with a respectable read noise of only 5 to 6 electrons rms. At this readout speed a single port 1.4 megapixel sensor can achieve 11 fps. Use of microlenses ensures that most of the incident photons are directed away from the Interline metal shield and onto the active silicon area for each pixel, resulting in peak QE greater than 60%. High performance combined with low cost has made the Interline CCD a very popular choice for applications such as fluorescence cell microscopy, luminescence detection and machine vision. However, even 5 to 6 e^- noise is too high for many low light scientific applications. For example, when imaging the dynamics of living cells, there is a need to limit the amount of fluorescence excitation light, such that both cell phototoxicity and photobleaching of the fluorescent dyes is minimized. The use of lower power excitation results in a proportionally lower fluorescent emission signal from the cell. Also dynamic imaging yields shorter exposure times per frame, thus fewer photons per frame. Ultra low light conditions mean that the read noise floor can often become the dominant detection limit, seriously compromising the overall signal-to-noise ratio (SNR) and hence the ability to contrast fine structural features within the cell. As such, the inability to maintain low noise at faster readout speeds limits the overall flexibility of the Interline CCD camera.

The Electron Multiplying CCD (EMCCD) was introduced into the market by Andor in 2000 and represents a significant leap forward in addressing the mutual exclusivity of speed and noise as discussed above. EMCCD cameras employ an on-chip amplification mechanism called 'Impact Ionization' that multiplies the photoelectrons that are generated in the silicon. As such, the signal from a single photon event can be amplified above the read noise floor, even at fast, multi-MHz readout speeds. Importantly, this renders the EMCCD capable of single photon sensitivity at fast frame rates (e.g. 56 fps with a 512 x 512 array). This attribute has rapidly gained recognition for EMCCD technology in demanding low light measurements, such as single molecule detection.

However, despite the sensitivity under extremely low light conditions, there are a few remaining drawbacks of EMCCD technology. The amplification mechanism required to reduce the effective read noise to < 1e^- also induces an additional noise source called multiplicative noise. This effectively increases the shot noise of the signal by a factor of 1.41, which is manifested as an increase in the pixel to pixel and frame to frame variability of low light signals. The net effect of multiplicative noise is that the acquired image has a diminished signal-to-noise ratio, to an extent that the QE of the sensor can be thought to have been effectively reduced by a factor of two. For example, a QE-enhanced back-illuminated EMCCD with 90% QE has effectively 45% QE when the effects of multiplicative noise are considered. Dynamic range limitations of EMCCDs must also be considered. It is possible to achieve respectably high dynamic range with a large pixel (13 to 16 µm pixel size) EMCCD, but only at slow readout speeds. As such, higher dynamic range can only be reached at slower frame rates (or with reduced array size) with modest EM gain settings. Application of higher EM gain settings results in the dynamic range being depleted further. Sensor cost of EMCCD technology is an additional consideration, along with the practical restriction on resolution and field of view that accompanies sensor cost. Presently, the largest commercially available EMCCD sensor is a back-illuminated 1024 x 1024 pixel device with 13 µm pixel pitch, representing a 13.3 x 13.3 mm sensor area. This already carries a significant cost premium, making further expansion to multi-megapixel devices a costly proposition.

Array Size (H x V)	Rolling shutter mode (fps)	Global (Snapshot) shutter mode (fps)
2560 x 2160 (full frame)	100	49
2064 x 2048 (4 megapixel)	105	52
1392 x 1040 (1.4 megapixel)	206	101
512 x 512	419	201
128 x 128	1,639	716

Table 1 - Frame rate vs sub-window size; Rolling and Global shutter readout modes. N.B. Same sub-window frame rates apply when using full horizontal width with the vertical heights indicated (see body text for further detail).

Part 2 - sCMOS: Circumventing the trade-offs

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 suitable to high fidelity, quantitative scientific measurement. sCMOS can be considered unique in its ability to simultaneously deliver on many key performance parameters, overcoming the 'mutual exclusivity' that was earlier discussed in relation to current scientific imaging technology standards, and eradicating the performance drawbacks that have traditionally been associated with conventional CMOS imagers.

The 4.2 and 5.5 megapixel sensors offer a large field of view and high resolution, without compromising read noise or frame rate. The read noise in itself is exceptional, even when compared to the highest performance CCDs. Not even slow-scan CCDs are capable of this level of read noise performance. High-resolution, slow-scan CCDs are typically characterized by seconds per frame rather than frames per second. The fact that the sCMOS device can achieve 1 electron median read noise while reading out up to 5.5 megapixels at 30 fps renders it truly extraordinary in the market. Furthermore, the sensor is capable of achieving 100 full fps with a read noise of between 1.1 to 1.45 electrons rms (camera dependent). By way of comparison, the lowest noise Interline CCD reading out only 1.4 megapixels at ~16 fps would do so with ~10 electrons read noise.

Greater speed is available through selection of 'region of interest' sub-windows, such that the field of view can be traded off to achieve extreme temporal resolution. Table 1 shows frame rates that can be expected from a series of sub-window sizes, in both Rolling shutter and Global shutter modes of operation (the distinction between these two modes is explained later in this paper). Note that each of the sub-windows can be expanded to full width in the horizontal direction and still maintain the same indicated frame rate. For example, both 1390 x 1024 and 2560 x 1024 sub-window sizes each offer 220 fps in Rolling shutter mode. This is important information for some applications that can take advantage of an elongated (letter box shape) region of interest.

The low noise readout is complemented by a high dynamic range of 33,000:1. Usually, for CCDs or EMCCDs to reach their highest dynamic range values, there needs to be a significant compromise in readout speed, yet sCMOS can achieve this value while delivering 30 fps. Furthermore, the architecture of sCMOS allows for high dynamic range by offering a large well depth, despite the small pixel size. By way of comparison, a 1.4 megapixel Interline with similarly small pixels achieves only ~1,800:1 dynamic range at 16 fps.

Part 3 - Comparing sCMOS to other leading scientific imaging technologies

A short comparative overview of sCMOS is provided in Table 2. For the purposes of this exercise, we limited the comparison to Interline CCD and EMCCD technologies, given their popularity across the range of scientific imaging applications. Interline CCDs are typified by a choice of 1.4 or 4 megapixel sensors. The most popular EMCCD sensors are 0.25 or 1 megapixel, typically offering up to 30 fps.

It is apparent that across most parameters, sCMOS presents a distinct performance advantage, notably in terms of noise, speed, dynamic range and field of view/resolution. Importantly, these advantages are met largely without compromise. Whilst the read noise of sCMOS is very low, EMCCD technology still maintains the distinct advantage of being able to multiply the input signal above the read noise floor, thus rendering it negligible (<1 e^-). The majority of EMCCD cameras purchased at this time are also back-illuminated, having ~90% QE max, which also feeds into the sensitivity comparison. For this reason, EMCCD technology will still hold firm in extreme low-light applications that require this level of raw sensitivity, and are willing to sacrifice on the enhanced resolution, field of view, dynamic range and frame rate that sCMOS can offer.

Figures 1 to 4 show the results of head to head sensitivity comparisons,

Parameter	Zyla sCMOS	Interline CCD	EMCCD
Sensor Format	4.2 megapixel	1.4 to 4 megapixel	0.25 to 1 megapixel
Pixel Size	6.5 µm	6.45 to 7.4 µm	8 to 16 µm
Read Noise	0.9 median (1.4 rms) e^- @ 30 fps 1.1 median (1.7 rms) e^- @ 100 fps	4 -10 e^-	<< 1e^- (with EM gain)
Full Frame Rate (max.)	Sustained: >30 fps full frame	3 to 16 fps	~30 fps
Quantum Efficiency (QE)	72%	60%	90% 'back-illuminated' 65 % 'virtual phase'
Dynamic Range	33,000:1 (@ 30 fps)	~3,000:1 (@ 11 fps)	8,500:1 (@ 30 fps with low EM gain)
Multiplicative Noise	none	none	1.41x with EM gain (effectively halves the QE)

Table 2 - Comparison summary of typical performance specifications specifications of Interline CCD and EMCCD technologies compared to sCMOS technology.

pitching a prototype 5.5 megapixel sCMOS camera against a 1.4 megapixel Interline CCD device, and also against 1 megapixel back-illuminated EMCCD. The sCMOS was set up to image at 560 MHz, this readout speed is capable of achieving 100 full fps, with only 1.3 electrons read noise. The Interline CCD camera, an Andor Clara, was read out at 20 MHz, achieving 11 fps with 5 electrons read noise (representing extreme optimization of this sensor at this speed). The EMCCD camera, an Andor iXon 888, was read out at 10 MHz with x300 EM gain amplification, achieving 9 fps with 0.15 electrons effective read noise. Low light imaging conditions were created using (a) a light tight imaging rig, fitted with a diffuse, intensity-variable 622 nm LED light source and mask overlay (consisting either an array of holes or a USAF resolution chart); (b) both confocal spinning disk and conventional widefield fluorescence microscopes, imaging fixed bovine epithelial cells labelled with BODIPY FL (emission max. ~510 nm).

The LED rig proved excellent for comparing sensitivity under extreme low light conditions, using two low light intensity settings; 10 photons/6.5 µm and 32 photons/6.5 µm. The SNR superiority of sCMOS over even well-optimized Interline CCD technology can clearly be observed, manifest as better contrast of signal against a less noisy read noise background, resulting also in better resolution of features. However, comparison of the two technologies against back-illuminated EMCCD (Figure 2) at the weakest LED setting, showed that the < 1 electron noise floor and higher QE of the EMCCD resulted in notably superior contrast of the weak signal from the noise floor.

Figures 3 and 4 show clear differences in low light signal contrast between sCMOS and Interline cameras, employed on both spinning disk and widefield fluorescence microscopy set-ups. Again the contrast difference arises from the read noise difference between the two technologies.

To further supplement the relative sensitivity performance of these imaging technologies, theoretical SNR plots that are representative of these three technologies are given in Figures 5 and 6. For this comparative exercise, specifications were used that reflect the most sensitive Interline CCD and back-illuminated EMCCD sensors on the market today.

Figure 5 shows how the SNR of sCMOS compares to that of Interline CCD across a range of photon fluxes (i.e. incident light intensities). The pixel size differences between the two sensor types is negligible, thus there is no need to further correct for differing areas of light collection per pixel. Note that rms noise figures were used for the sCMOS plot (as opposed to median noise). This has been determined to yield a theoretical plot that is much closer to that which is measured experimentally. The sensitivity differences between the two technology types are reflected in the marked variance between the respective SNR curves at low to moderate photon fluxes. At higher photon fluxes, there is no 'cross-over' point between sCMOS and Interline CCD curves. Similar QE and pixel size ensures that the Interline CCD will never surpass the SNR performance of sCMOS. In fact, due to the significantly lower read noise, the sCMOS exhibits markedly better signal-to-noise than the Interline CCD until several hundred photons/pixel, at which point the two curves merge as the read noise of both sensors becomes negligible compared to the shot noise.

Figure 6 shows SNR plots that compare sCMOS and Interline CCD sensors with that of back-illuminated EMCCD sensors. The plot assumes that all three sensors have the same pixel size, which could effectively be the case if the ~6.5 µm pixels of both the sCMOS and Interline CCD sensors were to be operated with 2 x 2 pixel binning, to equal a 13 µm EMCCD pixel (representative of a popular back-illuminated EMCCD sensor on the market). As such, the photon flux is presented in terms of photons per 13 µm pixel (or 2 x 2 binned super-pixel), relating to an actual pixel area of 169 µm^2. Note that the input QE for each sensor type was taken as the average QE between the range 500 nm and 750nm. There are two notable cross-over points of interest, relating to where the EMCCD S/N curve crosses both the sCMOS and Interline CCD curves, which occur at photon flux values of ~126 photons/pixel and ~330 photons/pixel, respectively. At photon fluxes lower than these cross-over points the EMCCD delivers better S/N ratio, and worse S/N ratio at higher photon fluxes. The reason that a back-illuminated EMCCD with negligible read noise does not exhibit higher S/N right throughout the photon flux scale, is due to the multiplicative noise of the EMCCD plot (which effectively increases the shot noise).

Figures 7 and 8 show widefield fluorescence microscope images, taken using x60 and x100 magnifications respectively, comparing 5.5 megapixel sCMOS to 1.4 megapixel Interline CCD technology. Each clearly reveal the markedly larger field of view capability of the 5.5 megapixel sCMOS sensor. Since each sensor type has ~6.5 µm pixel pitch, allowing for adequate NyQuist oversampling at the diffraction limit, it is unsurprising that each show virtually identical resolution of fine intracellular structure under brighter conditions, as shown in Figure 8. At low photon fluxes however, typified in Figures 3 and 4, the higher read noise of the Interline device results in greater sacrifice in resolution and contrast. This is a decisive point for live cell measurements, which often necessitate the use of low illumination energies.

Conclusion

After several decades of technology maturation, we have now reached a 'leap forward' point, where we can confidently claim that the next significant wave of advancement in high-performance scientific imaging capability has come from the CMOS imaging sensor technology stable. Scientific CMOS (sCMOS) technology stands to gain widespread recognition across a broad gamut of demanding imaging applications, due to its distinctive ability to simultaneously deliver extremely low noise, fast frame rates, wide dynamic range, high quantum efficiency, high resolution and a large field of view. Comparisons with other current 'gold standard' scientific image detector technologies show that the sCMOS technology out-performs even the high-performing Interline CCD camera in most key specifications.

For extremely low light applications that require absolute raw sensitivity at respectably fast frame rates, a high performance back-illuminated EMCCD camera (present in the Andor iXon range) maintains an application advantage.

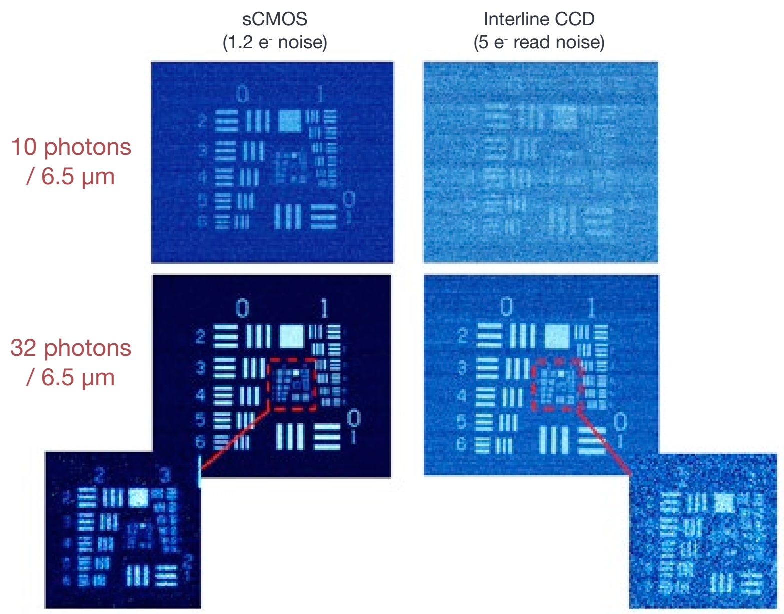


Figure 1 - Comparative low light images of a USAF resolution chart, showing Andor sCMOS (1.3 electrons read noise @ 560 MHz) vs Interline CCD (5 electrons read noise @ 20 MHz), under the two lowest LED settings.

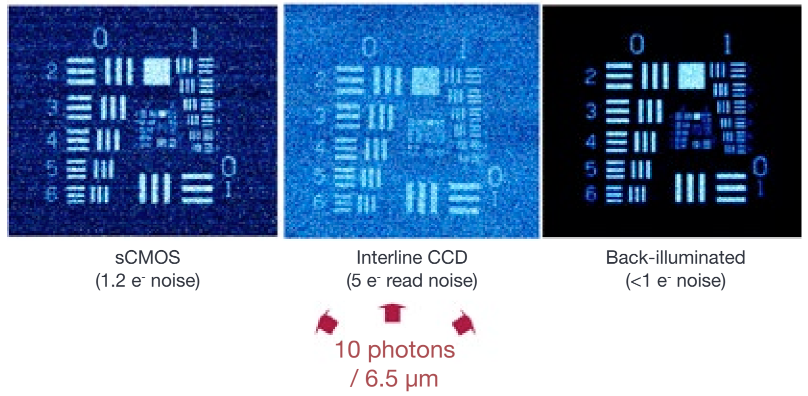


Figure 2 - Comparative low light images taken with Andor sCMOS (1.3 electrons read noise @ 560 MHz) vs Interline CCD (5 electrons read noise @ 20 MHz) vs back-illuminated EMCCD (< 1 e- read noise), under extremely low light conditions (10 photons / 6.5 µm setting). sCMOS and Interline CCD were 2 x 2 binned in order to have the same effective pixel pitch (and light collection area per pixel) as the 13 µm pixel of the EMCCD sensor.

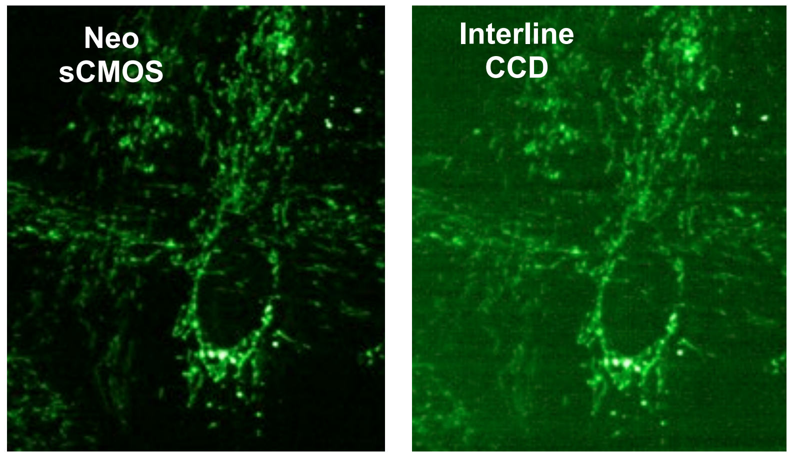


Figure 3 - Comparative low light images taken with Andor sCMOS (1.3 electrons read noise @ 560 MHz) vs Interline CCD (5 electrons read noise @ 20 MHz) of fluorescently labelled fixed cell using a CSU-X spinning disk confocal microscope (x60 oil objective), each 100 ms exposure, same laser power, displayed with same relative intensity scaling. Note, the field of view is limited by the aperture size of the CSU-X, which is matched to the 1.4 megapixel Interline sensor.

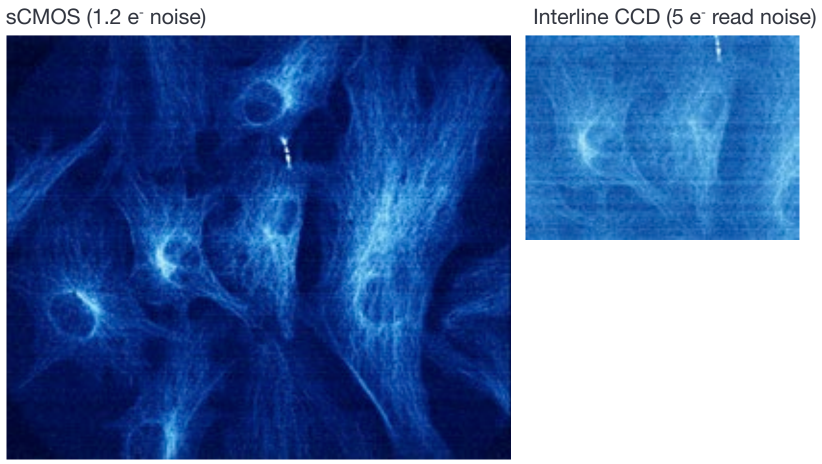


Figure 4 - Comparative low light fluorescence microscopy images taken with Andor sCMOS (1.3 e^- @ 560 MHz) vs Interline CCD (5 e^- @ 20 MHz) under low light conditions, typical of those employed in dynamic live cell imaging. ND filters on a widefield fluorescence microscope were used to reduce light levels relative to the read noise floor.

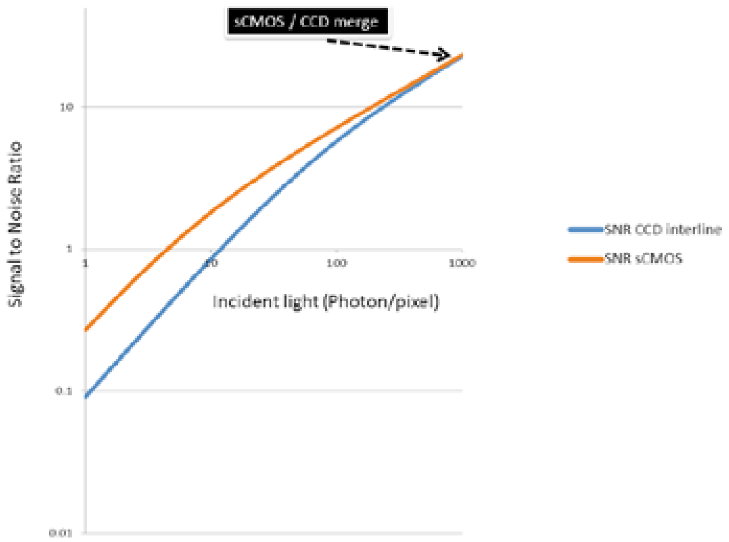


Figure 5 - Theoretical Signal to Noise plot comparisons for sCMOS vs Interline CCD sensors. Photon flux (i.e. input light intensity) is given in terms of photons per 6.5 µm pixel of each sensor type.

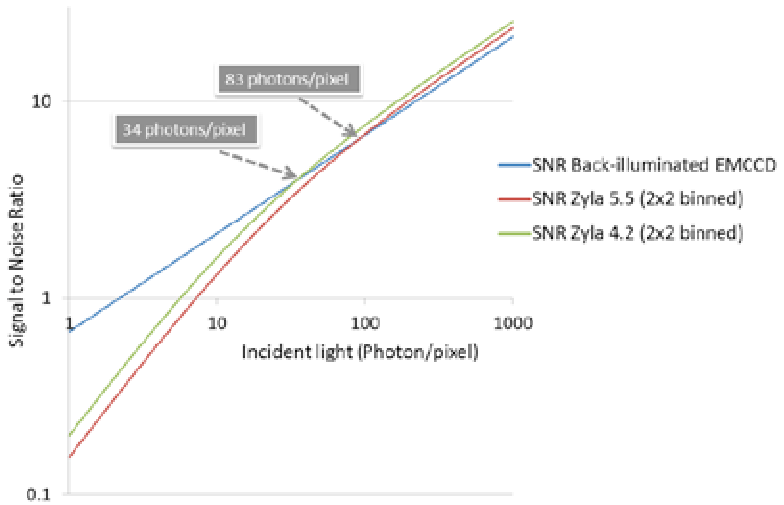


Figure 6 - Plot of Signal to Noise Ratio versus Incident Photon Intensity, comparing back-illuminated EMCCD iXon 888 (13 µm pixel size) to 2x2 binned Zyla sCMOS cameras (13 µm pixel size after binning). An average QE value for each sensor between 500-750 nm was used.

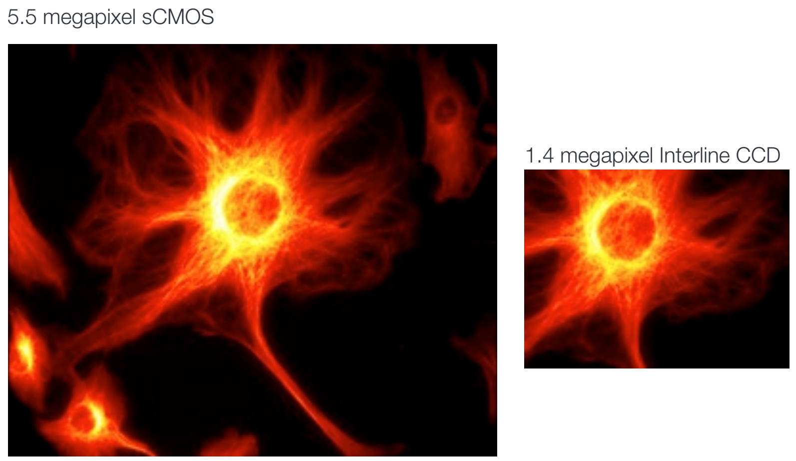


Figure 7 - Field of view comparison of two technologies; x60 magnification, 1.25 NA, 5.5 megapixel Andor sCMOS vs 1.4 megapixel Interline CCD (each have ~6.5 µm pixel pitch). sCMOS is capable of offering this larger field of view @ 100 frame/s with 1.3 e^- read noise.

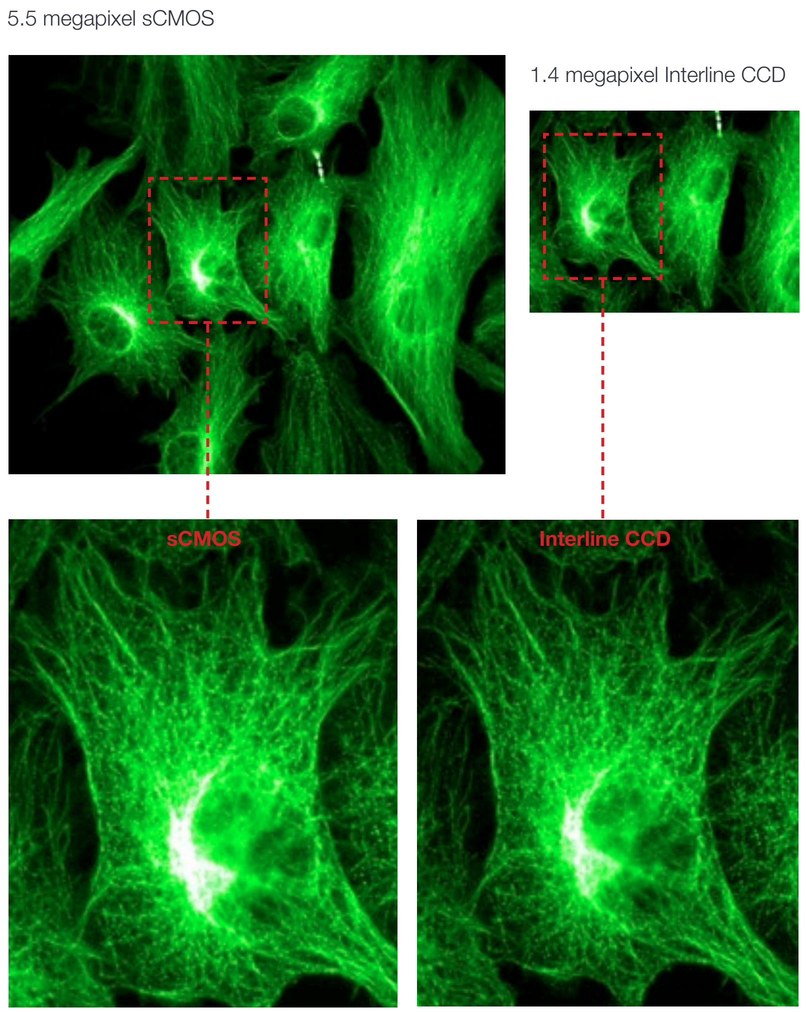


Figure 8 - Field of view and resolution comparison of two technologies; x100 magnification, 1.45 NA, 5.5 megapixel Andor sCMOS vs 1.4 megapixel Interline CCD (each have ~6.5 µm pixel pitch).

Technical Note

Andor sCMOS PC Recommendations and Data Flow Considerations

Andor's sCMOS camera solutions are capable of data rates that are markedly faster than other scientific camera technologies on the market. It is important to ensure that both the camera to PC interface, and the speed and capacity of the PC memory being used support the data output from the camera. The pixel readout speed that yields the fastest frame rate of 100 fps (full frame) relates to a data rate of ~850 MB/sec (single amplifier mode).

1.0 Optimizing data spooling: Identifying the 'bottlenecks'

Some applications require fast kinetic series acquisitions that are sustainable for a relatively long duration. In such cases, the data must be spooled continuously to either a suitable fast PC hard drive solution or PC RAM, each with sufficient storage capacity.

The data transfer rates achievable over more extended kinetic series are limited either by:

- The data bandwidth of the Camera Link/USB 3.0 interface between camera and PC or
- The write speed of the hard drive (if spooling to hard drive is selected)

The maximum sustained speeds are ultimately limited by the interface bandwidth. In addition, time is taken for a 'read request' to be sent by the software to retrieve the next image block.

1.1 Single Camera Link Interface ('3-tap')

The single Camera Link interface ('3-tap') has a bandwidth limitation of ~250MB/sec, which translates to ~30 frames per second (fps) of 5.5 MP image size, single/dual amplifier mode.

1.2 Dual Camera Link Interface ('10-tap')

The dual Camera Link interface ('10-tap') has a bandwidth limitation of 850 MB/sec, translating to the full 100 fps in single amplifier mode. Thus, to achieve maximum available sustained speeds, a PC configuration should be capable of writing/spooling data at faster than this rate (see Section 4.0).

The maximum length of a kinetic series is determined by the capacity of PC RAM or hard drive that is assigned for spooling. The issue of determining achievable speeds is further compounded by the fact that data rates are also adjusted by user selected variables such as exposure time, pixel readout speed, ROI size, hardware binning or single/dual amplifier dynamic range modes.

1.3 USB 3.0 interface

The USB 3.0 interface has a bandwidth limitation of 335 MB/sec, which translates to 40 fps of 5.5 MP image size and 53 fps of 4.2 MP image size, single amplifier mode.

2.0 Andor Dataflow Monitor

Andor developed the Dataflow monitor for the Neo 5.5 sCMOS and Zyla 5.5 and 4.2 sCMOS cameras to identify any system bottlenecks for a requested kinetic series. The Dataflow monitor is accessible through the set-up dialogue of the Andor Solis acquisition software. This provides 'up-front' estimation as to whether the kinetic series conditions that have been requested by the user are able to support the data transfer and write bandwidths available from Camera Link/USB 3.0 interface and PC hard drive respectively.

The Dataflow monitor also estimates if the available storage capacity of camera, PC RAM or hard drive is sufficient for the requested acquisition. This informs the user of any potential data speed or capacity issues in advance of beginning an acquisition. A scenario in which the Dataflow monitor has accepted and flagged a kinetic series request, respectively is shown in Figure 1.

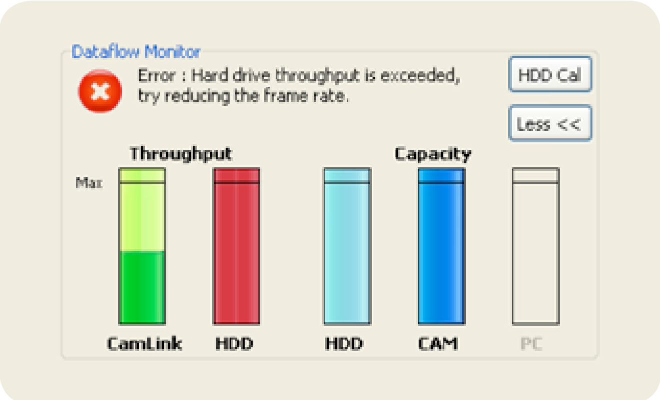


Figure 1: The Dataflow monitor has raised a warning against the requested kinetic series. In this case the data rate exceeds that of the hard drive write speed. Options to rectify this include: (a) reduce frame rate / lengthen exposure time (b) maintain frame rate, but reduce ROI size (otherwise the frame rate increases and the data rate remains the same) (c) use hardware binning (d) use single amplifier mode (d) reduce kinetic series length to be within the 4 GB on-head camera memory of the Neo 5.5 sCMOS (e) spool to PC RAM (if greater than 4 GB in the case of the Neo 5.5 sCMOS).

3.0 Exploiting the on-head memory buffer of the Neo 5.5 sCMOS

The Neo 5.5 sCMOS camera has a 4GB on-head memory buffer. This buffer can be used for a 'data burst' up to the maximum frame rate of the camera until it is full. Therefore a much higher frame rate can be attained during this time than can be supported over a more prolonged time. Once the on-head memory is full the frame rate will be limited by the Camera Link interface bandwidth, the hard drive write speed (if hard disk spooling is selected) and the other 'processing' overheads associated with the acquisition software.

In practice, one might expect a capacity of between 450 to 500 frames when operating at 100 fps, 5.5 megapixel (full resolution) in single amplifier mode (12-bit) i.e. the Neo on-head memory buffer can hold 4 to 5 seconds worth of kinetic series data under maximum frame rate conditions of the camera.

Using smaller ROI sizes to achieve the highest frame rates

It is also possible to use smaller ROI sizes and the Neo 5.5 on-head memory buffer to achieve exceptionally high frame rates.
For example:

- Maintain frame rate and extend kinetic series length beyond 5 seconds, or
- Achieve faster frame rates but stay within the 4 to 5 sec threshold

The Neo 5.5 sCMOS memory buffer can therefore be used to achieve high acquisition rates from even modestly specified PC configurations. For example, a hard drive may only be capable of writing data at 25 fps, yet a speed of 40 fps is required for a particular experiment. In this instance, it is still possible to acquire at 40 fps, the memory buffer filling at a rate of 15 fps. Under these circumstances, the kinetic acquisition could be run for up to ~30 seconds before the buffer becomes filled.

4.0 PC Recommendations - Speed tests

4.1 Zyla '10-tap' kinetic series tests

PC solutions for Zyla 5.5 and Zyla 4.2 sCMOS that were tested by Andor over extensive kinetic series lengths are shown in Table 1. The dual Camera Link '10-tap' configuration of the Zyla represents the option that is chosen for the fastest possible frame rate performance.

		System A (RAID SSD) and System B* (RAM)					
	Platform	Dell T7910 (A) and Dell T5810 (B)					
	Processor	Intel Xeon CPU E5-2640 v3 @ 2.6 GHz, eight core (A) Intel Xeon CPU E5-1620 v3 @ 3.5 GHz quad core (B)					
	Memory	8GB (A) and 64 GB (B)					
	SSD	1TB – 4x SSD in RAID0 >850MB/s – Samsung SM841N					
		Zyla 5.5 10-Tap			Zyla 4.2 10-Tap		
Array size / ROI	Data Range	Frame Rate		Series length (System A)	Frame Rate		Series length (System A)
		200 MHz	560 MHz		216 MHz	540 MHz	
2560 x 2160	RS 12-bit	36	100	120,000	-	-	120,000
	RS 16-bit	36	75		-	-	
	GS 12-bit	18	50		-	-	
	GS 16-bit	18	50		-	-	
2048 x 2048	RS 12-bit	38	105	160,000	40	100	160,000
	RS 16-bit	38	98		40	100	
	GS 12-bit	19	52		-	-	
	GS 16-bit	19	52		-	-	
1920 x 1080	RS 12-bit	72	198	340,000	76	189	340,000
	RS 16-bit	72	198		76	189	
	GS 12-bit	35	97		-	-	
	GS 16-bit	35	97		-	-	
512 x 512	RS 12-bit	152	419	2,600,000	161	398	2,600,000
	RS 16-bit	152	419		161	398	
	GS 12-bit	73	201		-	-	
	GS 16-bit	73	201		-	-	
128 x 128	RS 12-bit	596	1,639	40,000,000	631	1,559	40,000,000
	RS 16-bit	596	1,639		631	1,559	
	GS 12-bit	260	721		-	-	
	GS 16-bit	260	721		-	-	

Table 1 - Frame rates achieved by Zyla 5.5 and the Zyla 4.2 sCMOS '10-tap' on the Dell T7910 with 4x SSD (1 TB) in RAID 0 configuration (System A) and the Dell T5810 with 64 GB RAM (System B) and tested over extended kinetic series to fill the capacity of the SSD (1 TB) and the RAM (64 GB)

*System B with 64 GB RAM can acquire 6000 full frame (2560 x 2160 pixels) 12-bit images before the full capacity is utilized. For smaller ROI's this limit is significantly reduced.

Figure 2: The Zyla is available in both 4.2 megapixel 'Zyla 4.2' and 5.5 megapixel 'Zyla 5.5' sensor formats. Both formats are available with either USB 3.0 or 10-tap interface. The 'Neo 5.5' features a 5.5 megapixel sensor, USB 3.0 interface and 4GB on-head memory buffer. Neo 5.5, Zyla 5.5 and Zyla 4.2 all produce data rates of up to ~850 MB/s at frame rates of 100 fps.

Figure 3: Dell T7910 PC (System A) - 4 x 250 GB Solid State Drives (SSD) configured in RAID 0 are utilized for data spooling. An additional fifth hard drive is assigned to the operating system (Windows 7, 64-bit).

The test utilized Andor Solis acquisition software, internally triggered, Rolling Shutter and Global Shutter mode and 200 MHz/560MHz pixel readout speed, in both 12-bit (single amplifier) and 16-bit (dual amplifier) data range configurations.

The results indicate the sustainable frame rates, limited only by the capacity of the hard drive or PC RAM. The PC configuration is based on the T7910 with 2.6 GHz Eight Core and the T5810 with 3.5 GHz Quad Core.

The speed tests used extensive kinetic series lengths that filled the 1 TB capacity of the SSD

- 2560 x 2160 – 120,000
- 2064 x 2048 – 160,000 frames
- 1980 x 1080 – 340,000 frames
- 512 x 512 – 2,600,000 frames
- 128 x 128 – 40,000,000 frames

- System A utilizes 4 x 250 GB Solid State Drives (SSD) configured in RAID 0 for data spooling. An additional fifth hard drive is assigned to the operating system (Windows 7, 64-bit). (See Figure 3).

- System B utilizes 64 GB RAM (4 x 16 GB) for direct data spooling. Solis acquisition software is a 64-bit application and thus allows the user to utilise the full RAM capacity. It is important to note that spooling to RAM does not allow for permanent storage and the user would need to send the data to a hard drive with equivalent capacity following data acquisition in order to save the kinetic series.

In consideration of these results:

- System A has more capacity compared to the RAM solution in System B.

- Speeds attained from System B (RAM) can be considered to approximate the maximum sustained frame rate possible for each ROI size.

- Performance achieved for System A was limited more by the write capacity of the hard drive configuration.

From an SDK integration and driver development standpoint, it is important to note:

- Maximum sustained frame rates relate to how fast data can be retrieved from the camera.

- Additional processing overheads, including saving data to the hard drive, could impact these figures.

4.2 – Neo 5.5 and Zyla 5.5 USB 3.0 kinetic series tests

Table 2 outlines the PC solution for Neo 5.5, Zyla 5.5 and Zyla 4.2 USB 3.0 sCMOS that were in-house tested by Andor over extensive kinetic series lengths. The test utilized Solis acquisition software, internally triggered, Rolling Shutter and Global Shutter mode and 200 MHz / 560 MHz pixel readout speed, in both 12-bit (single amplifier) and 16-bit (dual amplifier) data range configurations.

The PC configuration is based on the Dell T5810 with 3.5 GHz Quad Core. This system utilizes 64 GB RAM (8 x 8 GB) for direct data spooling. Solis acquisition software is a 64-bit application and thus allows the user to utilise the full RAM capacity. It is important to note that spooling to RAM does not allow for permanent storgae and the user would need to send the data to a hard drive with equivalent capacity following data acquisition in order to save the kinetic series.

	Dell T5810 (RAM System)
Platform	Dell T5810
Processor	Intel Xeon CPU E5-1620 v3 @ 3.5GHz quad core
Memory	64 GB

Table 2 - PC solution for Neo 5.5 and Zyla USB3 sCMOS. This PC configuration with 64 GB RAM can acquire 6000 full frame (2560 x 2160 for Neo 5.5 and Zyla 5.5 and 2048 x 2048 for Zyla 4.2) 12-bit images before the full capacity is utilised. For smaller ROI's this limit is significantly reduced.

Summary

- System A makes use of the RAID 0 SSD configuration for direct spooling.

- System B utilizes 64 GB of PC RAM for direct spooling.

- Solis acquisition software is a 64-bit application and thus allows the user to utilise the full RAM capacity.

- It is important to note that spooling to RAM does not allow for permanent storage and the user would need to send the data to a hard drive with equivalent capacity following data acquisition in order to save the kinetic series.

Appendix

- In order to drive fastest data spooling rates, especially from 10-tap Zyla sCMOS, it is highly recommended that acquisition PCs are dedicated for this purpose and that other functions or applications are not being performed in the background, particularly during data acquisition. Tests represented in this technical note were under such stringent conditions. Both SDK 3 and Solis are compatible with a 64-bit OS. A 64-bit OS is recommended for most efficient spooling, where possible.

- Note however that iQ software requires a 32-bit OS.

- 64-bit 3rd party software packages must use the 64-bit SDK3 and operate on 64-bit OS.

- 64-bit software can spool directly to PC RAM that is greater than 4 GB.

- RAMDISK is the ONLY way to make use of > 4 GB PC RAM in a 32-bit application.

Technical Note

Understanding Read Noise in sCMOS

New sCMOS technology boasts an ultra-low read noise floor that significantly exceeds that which has been available from even the best CCDs, and at several orders of magnitude faster pixel readout speeds. For those more accustomed to dealing with CCDs, it is useful to gain an understanding of the nature of read noise distribution in CMOS imaging sensors.

Read Noise

CCD architecture is such that the charge from each pixel is transferred through a common readout structure, at least in single output port CCDs, where charge is converted to voltage and amplified prior to digitization in the Analogue to Digital Converter (ADC) of the camera. This results in each pixel being subject to the same readout noise. However, CMOS technology differs in that each individual pixel possesses its own readout structure for converting charge to voltage. In the sCMOS sensors, each column possesses dual amplifiers and ADCs at both top and bottom (facilitating the split sensor readout). During readout, voltage information from each pixel is directly communicated to the appropriate amplifier/ADC, a row of pixels at a time; see tech note on Rolling and Global shutter modes, page 30.

As a consequence of each pixel having its own individual readout structure, the overall readout noise in CMOS sensors is described as a distribution, as exemplified in Figure 1, which is a representative noise histogram from a camera at the fastest readout speed of 540 MHz (or 270 MHz x 2). Since the read noise of sCMOS is a distribution, the question then becomes how should the noise be cited in specification sheets? A standard method for CMOS has been to cite the median value of the distribution. In the data presented, the median value is 1.1 electron rms. This means that 50% of pixels have a noise less than 1.1 electrons, and 50% have noise greater than 1.1 electrons. However, it is also of value to consider the rms noise of the distribution. In the example given, the rms value is 1.6 e^-. When comparing Signal to Noise Ratio of sCMOS (with noise filters turned off) to that of other detector technologies, it is prudent to utilize rms values, as this takes more into account the noise distribution profile of sCMOS. Indeed, efforts to generate a set of 'measured vs theory' plots of Signal to Noise Ratio vs Incident Photon Intensity a much closer match when rms noise value is used in the theory curves. In the Andor sCMOS cameras specification sheets both median and rms noise values are provided. While there will be a small percentage of pixels with noise greater than two or three electrons, observable as the low level tail towards the higher noise side of the histogram, it must be remembered that a CCD Interline camera reading out at 20 MHz would have 100% of its pixels reading out with a narrow distribution of higher read noise, typically ranging between six and ten electrons rms (depending on camera manufacture).

Insight into the sCMOS architecture

The sensors feature a split readout scheme in which the top and bottom halves of the sensors are read out independently. Each column within each half of the sensor is equipped with dual column level amplifiers and dual analog-to-digital converters (ADC); see technical note of Dual Column amplifiers, page 34 for more detail. This 'split' sensor format was designed to help minimize read noise while maintaining extremely fast frame rates. Each pinned-photodiode pixel has either five transistors ('5T' design, used in Neo 5.5 and Zyla 5.5) or four transistors ('4T design, used in Zyla 4.2), and a lateral anti-blooming drain. The sensor is integrated with a microlens array that serves to focus much of the

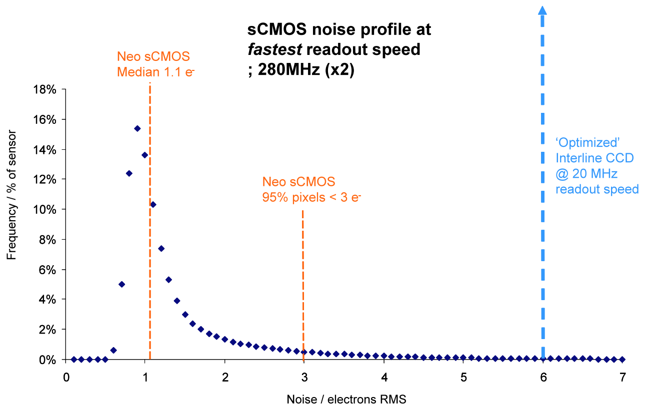


Figure 1 - Representative histogram showing read noise distribution at fastest readout speed of 560 MHz. The median value of 1.1 e^- means 50% pixels have less than 1 e^- and 50% have greater than 1 e^-. The line at 6 e^- represents a typical read noise value from a well optimized Interline CCD – all pixels in a CCD share the same noise value.

incident light per pixel away from the transistors and onto the exposed silicon, enhancing the QE (analogous to use of microlenses in Interline CCDs to focus light away from the column masks).

Sensors are configured to offer low dark current and extremely low read noise with true CDS. Non-linearity is less than 1%. The sensor also has anti-blooming of >10,000:1, meaning that the pixels can be significantly oversaturated without charge spilling into neighboring pixels. It is also possible to use the anti-blooming capability to hold all or parts of the sensor in a state of 'reset', even while light is falling on these pixels.

Spurious Noise Filter

Andor's sCMOS cameras come equipped with an optional in-built FPGA filter to reduce the frequency of occurrence of high noise pixels. This real time filter corrects for pixels that are above five electrons rms and would otherwise appear as spurious 'salt and pepper' noise spikes in the image. The appearance of such noisy pixels is analogous to the situation of Clock Induced Charge (CIC) noise spikes in EMCCD cameras, in that it is due to the fact that we have significantly reduced the noise in the bulk of the sensor, such that the remaining small percentage of spuriously high noise pixels can become an aesthetic issue. The filter employed dynamically identifies such high noise pixels and replaces them with the mean value of the neighboring pixels.

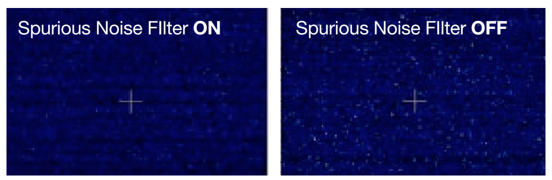


Figure 2 - Demonstration of Spurious Noise Filter on a dark image, 20 ms exposure time, 200 MHz (x2) readout speed (~1.2 e^- read noise).

Technical Note

Interpolative Blemish Corrections on sCMOS

Andor's Neo and Zyla cameras are equipped with considerable FPGA processing power which is utilized to filter the small percentage of blemishes from the image sensor. This filter identifies and compensates for three types of blemishes which are corrected for during the FPGA processing step:

1. 'Hot Pixels' - These are pixels with darkcurrent significantly above the average. To identify, Andor take a long exposure image and create a map of dark current across the image. Pixels that have a dark current value above a set threshold are labelled as 'Hot Pixels'.

2. 'Noisy Pixels' - These are pixels with read noise significantly above the average. To identify, Andor create a map of read noise for every pixel in the sensor. Pixels that have a read noise value above a set threshold are labelled as 'Noisy Pixels'.

3. 'Variable Responsivity Pixels' - Andor take a uniformly illuminated image at half well depth. This image is flat fielded to correct for any illumination non-uniformity. Pixels that have responsivity less than 75%, or greater than 125% of the global average are labelled as 'Variable Responsivity Pixels'.

The above blemish types are processed in the FPGA of the camera in real time, using an Interpolative Filter. This filter works by calculating the mean of the surrounding 8 pixel values and replacing this central blemish pixel with this mean value.

However, such interpolation over pixel blemishes can be considered detrimental in some specific applications that depend on total quantitative integrity over a limited set of pixels, for example in localization based super-resolution microscopy (such as PALM and STORM techniques) and astronomy. In these applications it can be beneficial for the user to be able to switch off interpolative corrections, which Andor have made possible. In this technical note, the instructions for turning this correction switch on/off are described for both Andor Solis acquisition software and Andor SDK3.

Turning Blemish Corrections ON/OFF

From the latest general release of Andor SDK3 (minimum version 3.7.30004) and Solis (minimum version 4.24.30004) this blemish correction can be switched on and off by the user.

SDK3

In SDK3 the Boolean feature is named StaticBlemishCorrection. Details of SDK3 feature control can be found in the Andor SDK3 manual.

Feature	Type	Description	Availability
Static Blemish Correction	Boolean	Enables or Disables Static Blemish Correction	Neo, Zyla

Solis

In Solis the user can switch this feature on and off using the following command in Andor basic:

booleanfeatureenable("StaticBlemishCorrection", 0)
This Turns off Static Blemish Corrections

booleanfeatureenable("StaticBlemishCorrection", 1)
This Turns on Static Blemish Corrections

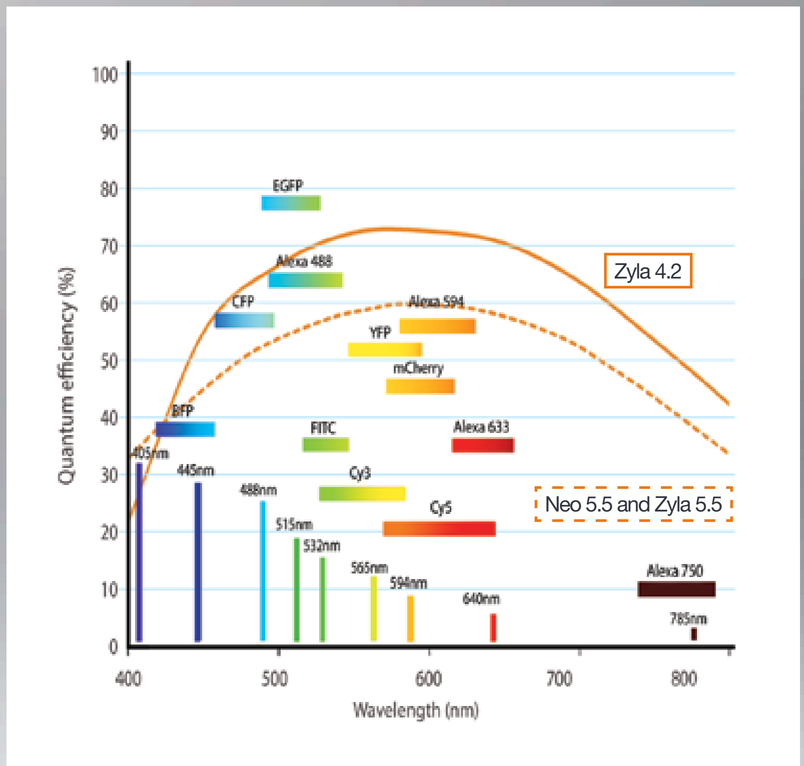
Access to Hot Pixel Maps

Some applications can be particularly susceptible to hot pixel blemishes. Thermoelectric cooling of the sensor (e.g. to -30ºC in the Neo), is instrumental in dramatically minimizing the occurrence of such hot pixels within the sensor, meaning that these pixels can still be used for useful quantitative imaging. However, knowledge of the location of the remaining hot pixel population can still be useful to some users, such that they can account for them in their processing stages.

Andor can work with the end user to generate a bespoke 'hot pixel map' of their sCMOS sensor. This map will be generated based on the experimental conditions outlined by the end user, such as exposure conditions and intensity threshold.

"Our tests with Andor's new sCMOS camera have been highly encouraging. The combination of very low noise sensitivity at rapid frame rates, coupled with high pixel resolution, will enable us to reach previously unattainable throughput from our massively parallel, nanopore-based, single molecule sequencing approach."

Prof Amit Meller, Associate Professor of Biomedical Engineering and Physics, Boston University, USA

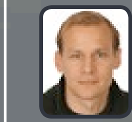


sCMOS Quantum Efficiency (QE) curves, incorporating laser excitation lines and emission ranges of common fluorophore labels.

Main front cover image:

Embryonic muscle cells where actin starts to form contractile fibers (cyan). Microtubules are shown in yellow. Large unstained inclusions in the cytoplasm are yolk deposits.
Image courtesy of Dr. Ulrike Engel, Nikon Imaging Center, Heidelberg, Germany

"Without pushing it to the limit we managed to take 131 planes of the drosophila embryo in just 4 seconds (5.5 megapixels mode), which is practically instantaneous compared to the morphogenetic processes and out-perform by far everything we have tried before. The camera is made for SPIM microscopy!"



Dr. Lars Hufnagel, Developmental Biology Unit, EMBL Heidelberg, Germany.

ANDOR
an Oxford Instruments company

Customer Support

Andor products are regularly used in critical applications and we can provide a variety of customer support services to maximize the return on your investment and ensure that your product continues to operate at its optimum performance.

Andor has customer support teams located across North America, Asia and Europe, allowing us to provide local technical assistance and advice. Requests for support can be made at any time by contacting our technical support team at andor.com/support.

Andor offers a variety of support under the following format:

- On-site product specialists can assist you with the installation and commissioning of your chosen product
- Training services can be provided on-site or remotely via the Internet
- A testing service to confirm the integrity and optimize the performance of existing equipment in the field is also available on request.

A range of extended warranty packages are available for Andor products giving you the flexibility to choose one appropriate for your needs. These warranties allow you to obtain additional levels of service and include both on-site and remote support options, and may be purchased on a multi-year basis allowing users to fix their support costs over the operating life cycle of the products.

Windows XP Windows Vista Windows 7 Windows 8 Linux

Project part financed by the European Regional Development Fund under the European Sustainable Competitiveness Programme for Northern Ireland.

Head Office
7 Millennium Way
Springvale Business Park
Belfast BT12 7AL
Northern Ireland
Tel: +44 (0)28 9023 7126
Fax: +44 (0)28 9031 0792

North America
425 Sullivan Avenue
Suite 3
South Windsor, CT 06074
USA
Tel: +1 860-290-9211
Fax: +1 860-290-9566

Japan
4F TK Sarugakucho Building
2-7-6 Sarugaku-Cho
Chiyoda-Ku
Tokyo 101-0064
Japan
Tel: +81 (0)3-3518-6488
Fax: +81 (0)3-3518-6489

China
Room 1213, Building B
Luo Ke Time Square
No. 103 Huizhongli
Chaoyang District
Beijing 100101
China
Tel: +86 (0)10-5129-4977
Fax: +86 (0)10-6445-5401

Find us on

OXFORD INSTRUMENTS
The Business of Science®