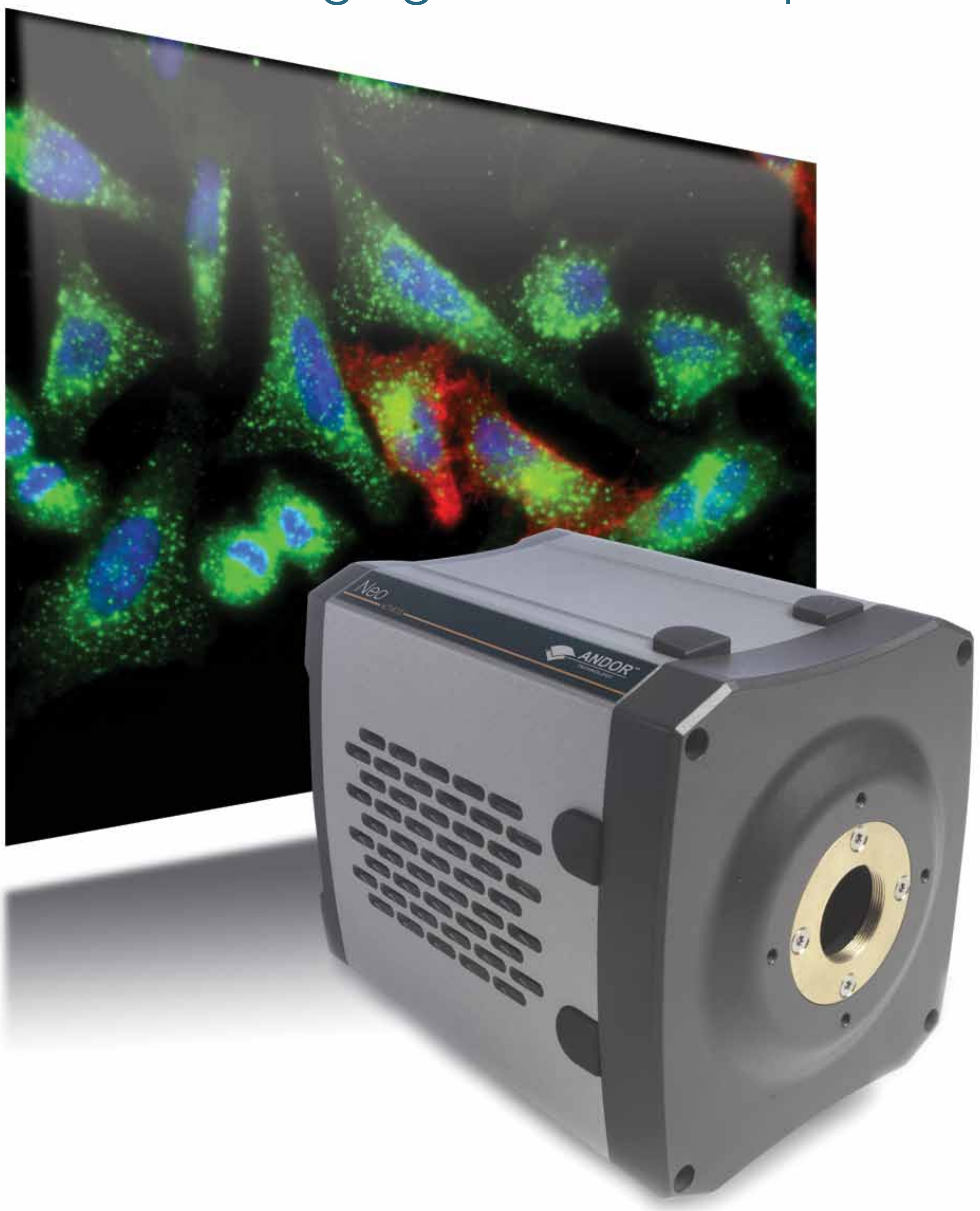
ANDOR™
TECHNOLOGY

Neo

sCMOS

Imaging without compromise



ANDOR™ TECHNOLOGY

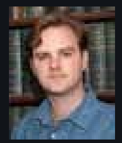
Scientific CMOS (sCMOS) technology overview

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 (sCMOS) 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)



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

"Neo cameras will literally allow one to see cells in a new light with ultrasensitive 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."

See page 28 for 'Comparing sCMOS with other detectors' technical note

sCMOS - No need to compromise

The 5.5 megapixel sensor offers a large field of view and high resolution, without compromising read noise, dynamic range or frame rate.

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

The low noise readout is complemented by 30,000:1 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 frames/sec.

Parameter	sCMOS (Neo)	Interline CCD	EMCCD
Sensor Format	5.5 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	1 e^- @ 30 frames/sec 1.4 e^- @ 100 frames/sec	4 - 10 e^-	< 1e^- (with EM gain)
Full Frame Rate (max.)	100 frames/sec @ full resolution	3 to 16 frames/sec	~ 30 frames/sec
Quantum Efficiency (max.)	57%	60%	90% 'back-illuminated' 65% 'virtual phase'
Dynamic Range	30,000:1 (@ 30 frames/sec)	~ 3,000:1 (@ 11 frames/sec)	8,500:1 (@ 30 frames/sec with low EM gain)
Multiplicative Noise	None	None	1.41x with EM gain (effectively halves the QE)

Neo

sCMOS

Andor's highly anticipated Neo sCMOS camera platform has been designed from the ground up, specifically to realize the performance potential of this exciting new sensor technology. Neo is unique in its ability to simultaneously offer ultra-low noise, extremely fast frame rates, wide dynamic range, high resolution and a large field of view.

Neo breaks new boundaries in offering an exceptionally low read noise of 1 e^- RMS without the need for signal amplification technology. 100 frames/sec can be reached with full frame readout, much faster with region of interest selection. These speeds can be uniquely coupled in Neo to a dynamic range of 30,000:1 with 16-bit digitization.

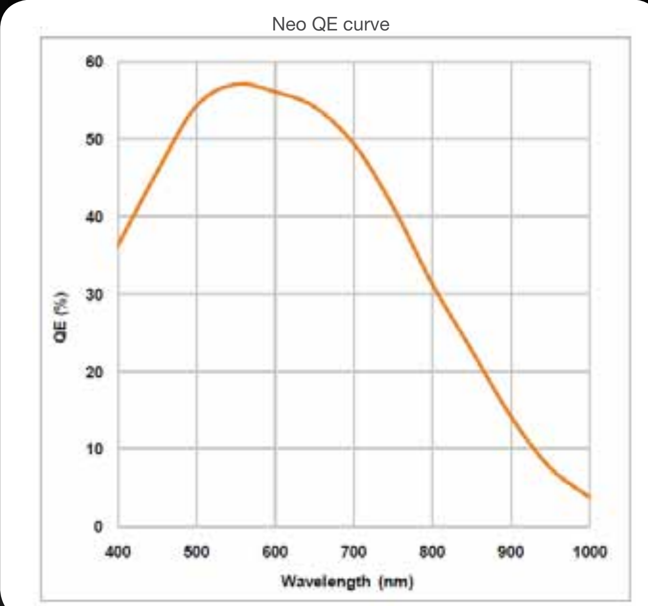
Neo offers an advanced, yet necessary, set of unique performance features and innovations, including extensive 'on-head' FPGA data processing capability, deep TE cooling to -40°C, 4 GB storage memory and a Data Flow Monitor. Andor's UltraVac™ vacuum process has been implemented to offer not only the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

- -40°C cooling
- Vacuum Longevity
- Data Flow Monitor
- Blemish Minimization
- 4 GB on-head Memory
- 5.5 Megapixel
- 100 frames/sec @ 16-bit
- 1 e^- noise
- 30,000:1 Dynamic Range
- Superior Image Quality
- Quantitative Stability
- Rapid Exposure Switching
- Superior FPGA Intelligence
- Single Input Window

Features	Benefits
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 and astronomy
Rapid frame rates	100 fps Full Frame; 208 fps @ 1.4 megapixel ROI; 1688 fps @ 144 x 128 ROI
TE cooling to -40°C	Minimization of dark current and pixel blemishes. Fan off mode
UltraVac™	Sustained vacuum integrity and unequalled cooling with 5 year warranty; complete sensor protection.
Dual-Gain Amplifiers	Extensive dynamic range of 30,000:1 @ 30 frames/sec
Extensive FPGA on-head data processing	Essential for best image quality and quantitative fidelity from sCMOS
4 GB on-head memory	Enables bursts of 100 frames/sec @ 16-bit and facilitates advanced data processing
16-bit digitization	For digitization of high dynamic range signals, even at 100 frames/sec.
Rolling and global shutter	Maximum flexibility across all applications
High Quantum Efficiency	Optimized for popular green/red emitting fluorophores
Data flow monitor	Innovatively manage acquisition capture rates vs data bandwidth limitations
iCam	Market leading exposure switching with minimal overheads
Dynamic Baseline Clamp	Essential for quantitative accuracy of dynamic measurements
Spurious Noise Filter	Realtime FPGA filter to identify and compensate for spurious high noise pixels
Single window design	Single input window with double AR coating ensures maximum photon throughput
Comprehensive trigger modes & 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 (e^-)	1 @ 200 MHz
	1.4 @ 560 MHz
Pixel Well Depth (e^-)	30,000
Max Readout Rate (MHz)	560 MHz
	(280 MHz x 2 outputs)
Frame Rates (frames per sec)	100 @ full frame
	1688 @ 144 x 128 ROI
QE max	57%



Performance and Innovations

Lowest Noise Floor

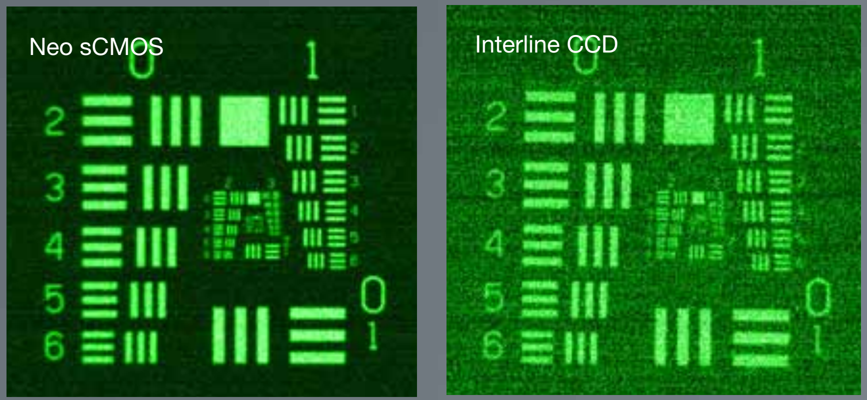
Andor's ultrasensitive Neo sCMOS camera has broken new ground in offering an unparalleled 1 electron RMS read noise floor, without amplification technology.

What is truly extraordinary is that this performance level is achievable at 30 frames/sec, representing 200 MHz pixel readout speed. Furthermore, even at full readout speed, the read noise floor is negligibly compromised, maintaining 1.4 e^- RMS at 100 frames/sec. 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 the Neo sCMOS suitable for a wide variety of challenging low light imaging applications.

Readout noise (e^-) [2]	
200 MHz	1
400MHz	1.2
560 MHz	1.4

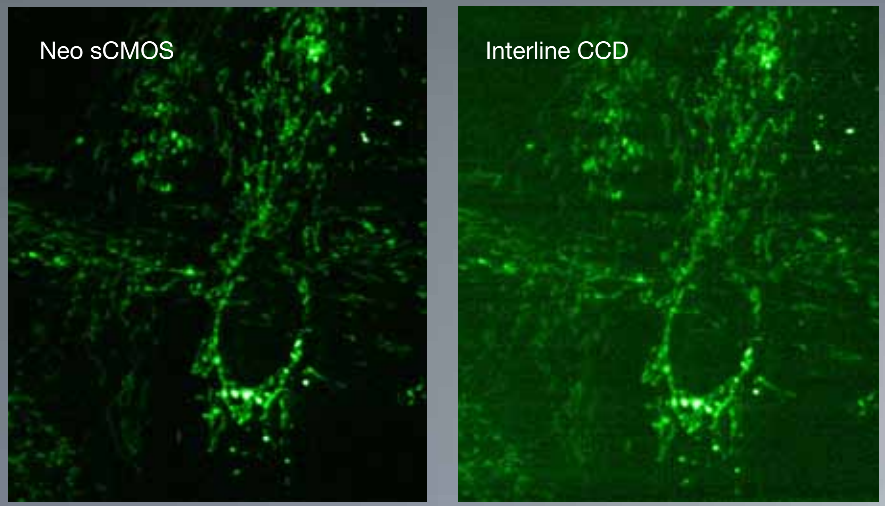
See page 26 for
'Understanding Read Noise'
technical note

(a)



Comparative low light images taken with Neo sCMOS (1.2 electrons read noise @ 400 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 comes equipped with an optional in-built FPGA filter that operates in realtime to reduce the frequency of occurrence of high noise pixels that would otherwise would appear as spurious 'salt and pepper' noise spikes in the image background

Extended Dynamic Range

The Andor Neo is designed to make use of the innovative dual 'column-level' amplifier design of the sensor.

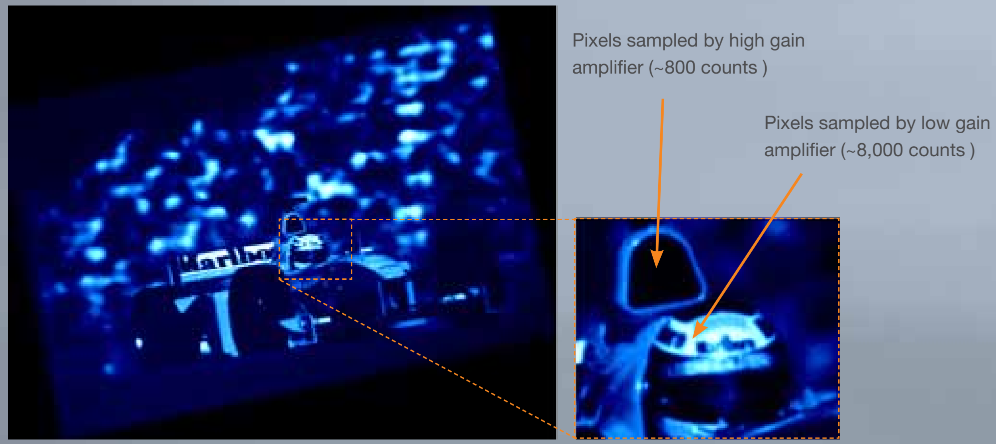
Traditionally, sensors require that the user must select upfront between high or low amplifier gain (i.e. sensitivity) settings, depending on whether they want to optimize for low noise or maximum well depth. The dual amplifier architecture of the sCMOS sensor in Neo circumvents this need, in that signal 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 30,000:1.

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.

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



See page 20 for
'Dual Amplifier Dynamic Range'
technical note

2 Neo cameras mounted on the Andor TuCam Dual Camera Image Splitter

Performance and Innovations

Rapid Frame Rates

The parallel readout nature of the 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. Neo is uniquely designed to harness this speed potential.

	Cameralink Base		Burst to 4GB Internal Memory	
Array Size	Rolling Shutter	Global Shutter	Rolling Shutter	Global Shutter
2560 x 2160 (full frame)	30	15	100	50
2064 x 2048	32	16	106	54
1392 x 1040	63	31	208	105
528 x 512	125	63	403	211
144 x 128	500	250	1688	844

Maximum frame rates achievable from the Neo sCMOS camera.

4 Gigabyte on-head memory

Neo is the only scientific CMOS camera on the market with on-head memory. This renders it unique in its ability to acquire bursts of data at the full 100 frames/sec with 16-bit digitization.

The Neo comes with dual CameraLink connection ports, for future upgrade to 'Turbo' (10-tap) Cameralink capability, which carries sufficient capacity to transfer images continuously from the camera at full speed. Very high end PC solutions are recommended to handle the high data rates associated with such fast speed operation.

iCam

Neo benefits 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.

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

"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."

Data Flow Monitor

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

For sustained kinetic series measurements, yielding a data set that exceeds the 4 GB on-head storage capacity of Neo, it is possible to be rate limited by:

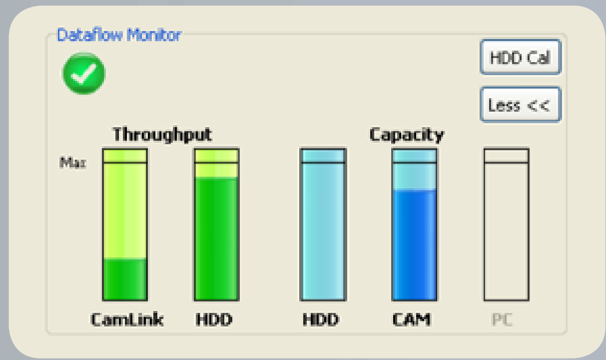
(a) bandwidth of the Camerlink interface connecting the camera to the PC

(b) hard drive write speed

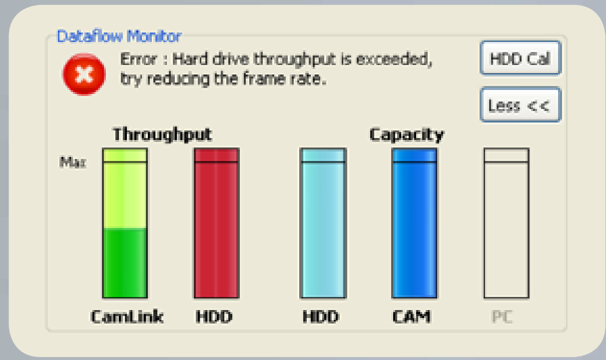
In such circumstances the true frame rate threshold also depends on many set-up factors, including exposure time, ROI size, binning, pixel readout rate and choice of single or dual amplifier data.

The Data Flow Monitor 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.

The Data Flow Monitor can be regarded as an essential tool for day-to-day usage of sCMOS technology.



Eg.1 Requested kinetic series within capability of Cameralink data transfer bandwidth and Hard Disk Drive write speed.



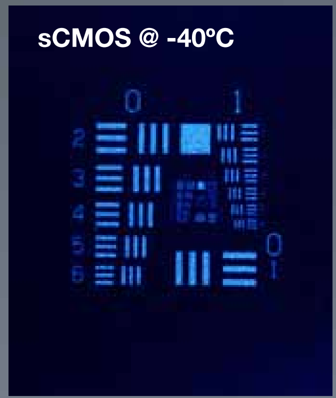
Eg.2 Hard Disk Drive will not write data fast enough for the requested kinetic series. Advised to first reduce data rate.

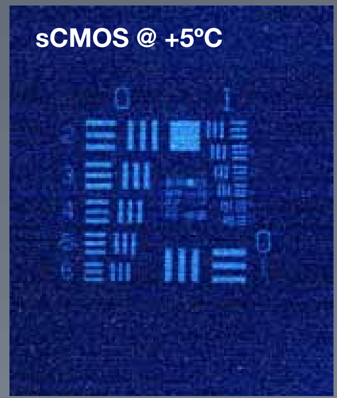
Performance and Innovations

Deep Thermoelectric Cooling

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

Cooling Temperature	Darkcurrent
-30°C (fan cooling)	0.07 e⁻/pixel/sec
-40°C (10°C liquid)	0.03 e⁻/pixel/sec





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 (x2) readout giving 1.2 electron read noise.

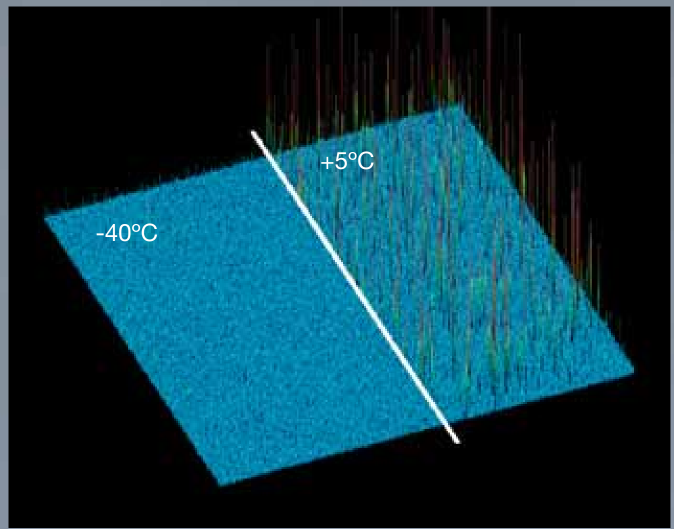
Deep TE cooling is critical for a number of reasons:

Minimization of darkcurrent

sCMOS cannot be considered a truly flexible, workhorse camera unless darkcurrent 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 darkcurrent 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 usable pixels



Hot pixel blemishes are significantly reduced at deeper cooling temperatures - shown above for 1 sec exposure

Minimization of vibration

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

Andor's Neo offers:

(a) Two fan speeds

b) Ability to turn off fan completely. Flowing liquid through the camera allows minimization of vibration while still stabilizing at -40°C

See page 23 for 'Importance of TE Cooling' technical note

Thermostatic Precision

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

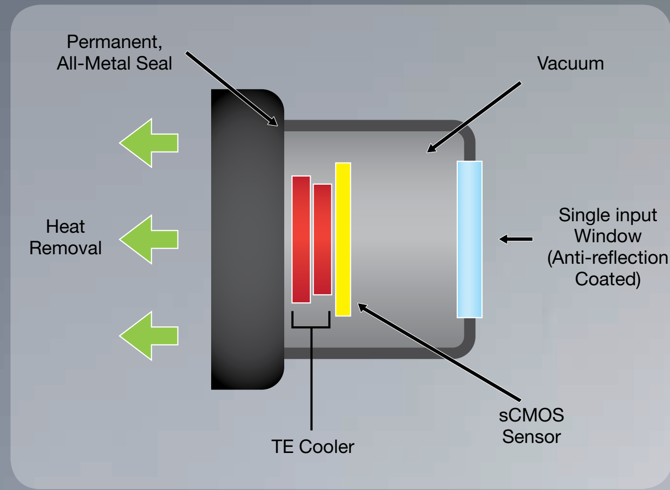
UltraVac™ – Permanent Vacuum Head

The Andor Neo is the only vacuum housed CMOS sensor available on the market. In terms of quality, performance and longevity, the importance of vacuum technology cannot be understated.

A sensor housed in a hermetically sealed permanent vacuum head with minimized out-gassing, means that neither cooling performance or sensor QE will steadily degrade over time. This design exclusively allows use of a single input window (AR coated on each surface), maximizing photon throughput to the sensor.

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.

- No QE degradation
- Sustained deep TE cooling
- Single input window (AR coated)
- No maintainence/re-pumping
- No condensation



Schematic of a Permanent Vacuum Head

5 Year Vacuum Warranty

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

See page 27 for 'Vacuum head performance and longevity' technical note

Performance and Innovations

Advanced FPGA on-head processing

The Andor Neo is 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. The superior dynamic processing capability of Neo 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 of Neo 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

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

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

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

The 6.5 µm pixels present in Neo 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 microscopy with x60 and x100 objectives. In low light measurements, CMOS sensors that have significantly smaller pixels are often operated with 2 x 2 binning in order to improve photon collection area per pixel, but this has the adverse effect of doubling the read noise.

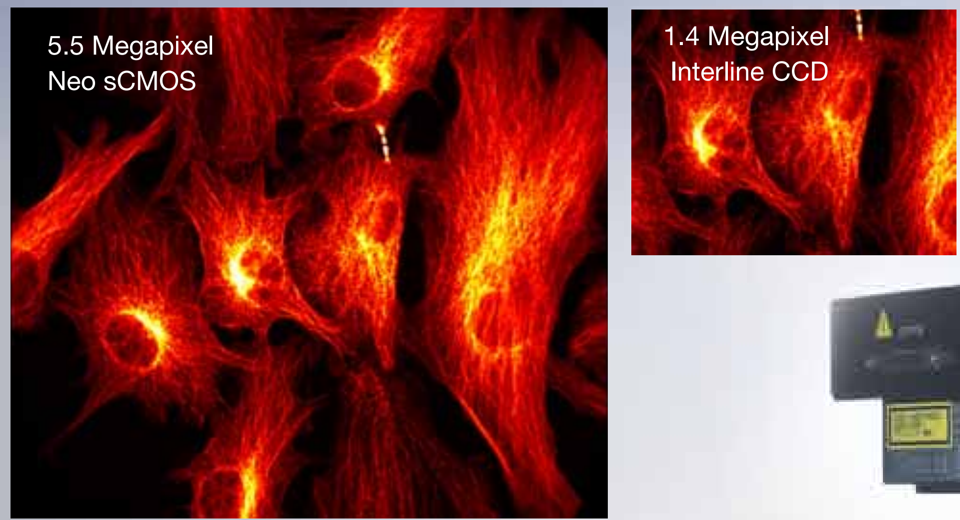
- Ideal balance of resolution, photon collection and well depth
- Superb 30,000 electron well depth
- No pixel binning required = no doubling of read noise
- No demagnification optics = no wasteful photon loss

Large Field of View

The 5.5 megapixel sensor in Neo offers an extended field of view with 22 mm diagonal, markedly exceeding the FOV available from alternative scientific Interline, EMCCD and CMOS detectors.

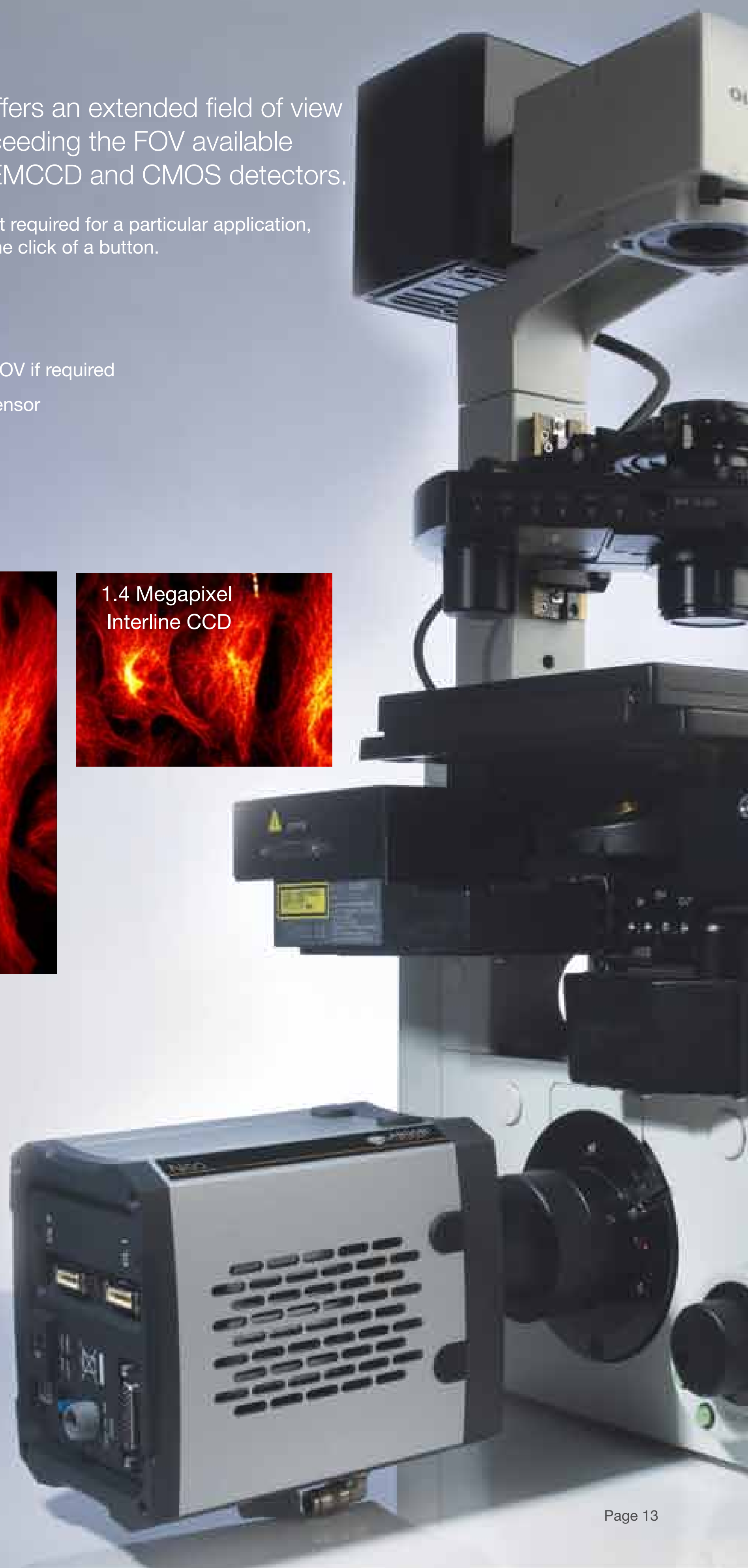
Flexibility is key however, and if a large FOV is not required for a particular application, Neo offers a range of pre-selected ROI sizes at the click of a button.

- 21.8 mm diagonal
- Closely matched to modern microscopes
- Pre-selected ROIs to quickly opt for smaller FOV if required
- x3.5 larger than popular 512 x 512 EMCCD sensor
- x3.9 larger than popular 1.4 MP Interline CCD sensor
- x6.4 larger than competing ‘scientific CMOS’ sensor



Field of View Comparison

Neo sCMOS vs popular 1.4 megapixel Interline CCD



Performance and Innovations

Rolling and Global Shutter modes

Neo offers the distinct capability to offer both Rolling Shutter and Global Shutter readout modes from the same sensor, such that the most appropriate mode can be selected dependent on application requirements.

Rolling shutter essentially means that different lines of the array are exposed at different times as the read out 'waves' sweep through the sensor. The fastest frame rates are available from this mode.

Rolling Shutter exposure and readout (single scan)

Exposure start | Exposure | Readout

Global shutter, which can also be thought of as a 'snapshot' exposure mode, means that all pixels of the array are exposed simultaneously.

Global Shutter exposure and readout (single scan)

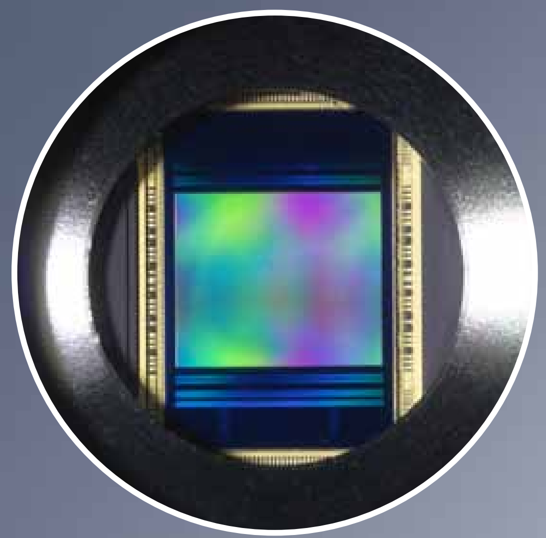
Exposure start | Exposure | Exposure End

See page 24 for 'Rolling and Global Shutter' technical note

Comprehensive trigger functionality

Neo offers 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
- Rolling and Global shutter trigger modes
- 'Time Lapse' and 'Continuous' (overlapped) kinetic series
- Rapid 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 trigger modes applicable to both Rolling and Global shutter

Neo 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++, C#, Delphi, VB6, VB.NET, 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.

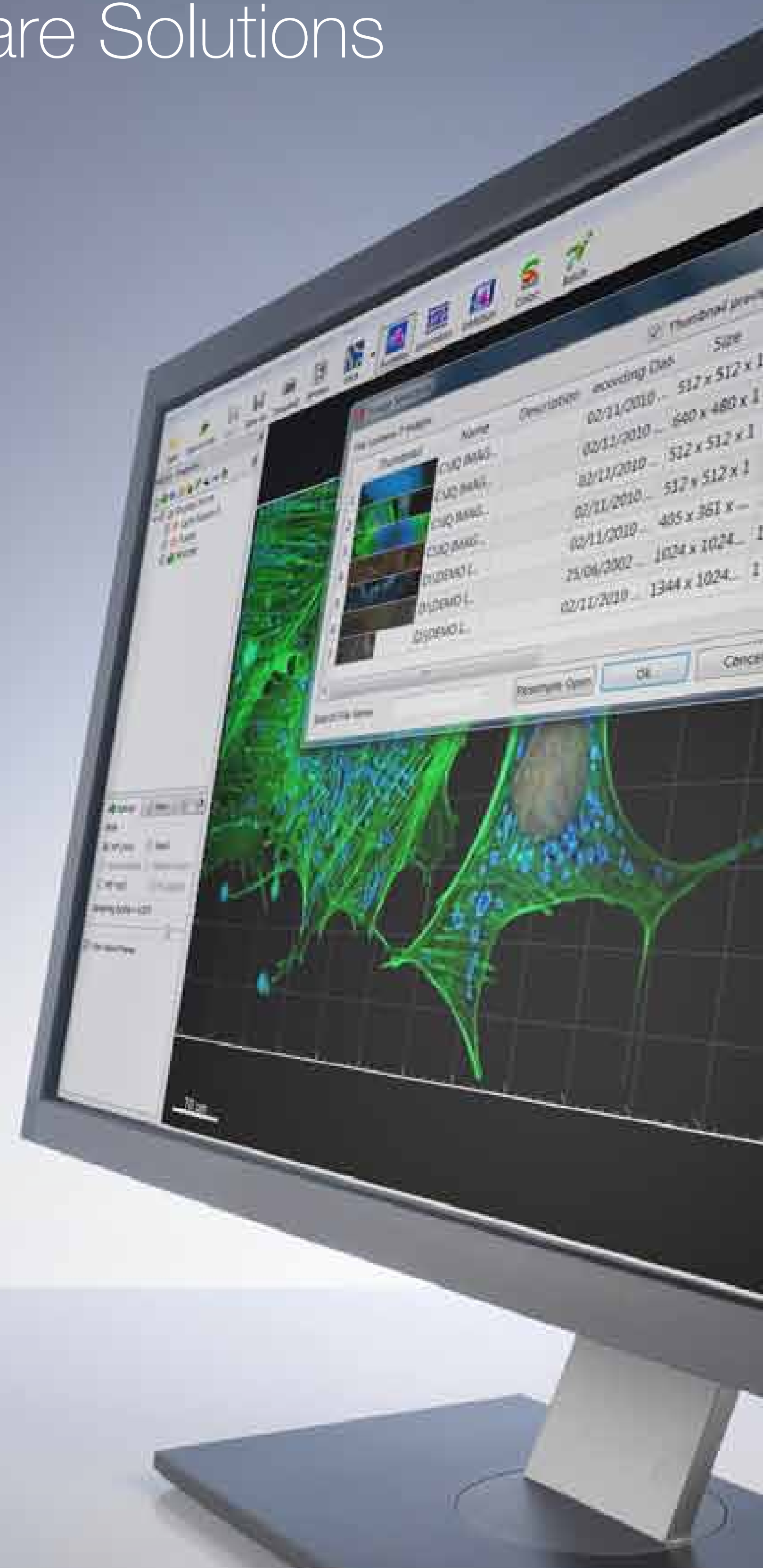
Bitplane Imaris

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 this new camera platform is expanding steadily. Please enquire for further details



The Andor Imaging Range

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

Luca^{EM} - price/performance EMCCD platform

- Single photon sensitive
- Compact
- Luca R – megapixel format; 12.4 fps
- Luca S – VGA format; 37 fps
- USB 2.0 true plug and play

iKon - deep cooled, back-illuminated, low noise CCD

- -100°C cooling
- Back-illuminated > 90% QE
- 1 megapixel to 4 megapixel
- Enhanced NIR versions
- 'PV Inspector' optimized for EL/PL inspection
- USB 2.0 true plug and play

Neo – high performance sCMOS

- 1 electron read noise
- -40°C cooling
- 5.5 Megapixel / 6.5 µm pixels
- 100 frames/sec
- 16-bit digitization

iXon3 - high performance EMCCD platform

- Single photon sensitive
- Back-illuminated > 90% QE
- -100°C cooling
- Fastest EMCCD frame rates
- Flexible yet intuitive
- Quantify in Electrons or Photons

Clara - high-performance Interline CCD platform

- 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

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 Neo 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.

- **Dual Amplifier Dynamic Range**
- **Comparing sCMOS with other scientific detectors**
- **UltraVac™ permanent vacuum head and performance longevity**
- **Rolling and Global Shutter**
- **Understanding Read Noise in sCMOS**
- **The Importance of Deep TE Cooling to CMOS Technology**

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

‘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. Yan Gu
Confocal Imaging and Analysis Lab
National Institute for Medical Research

‘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’

Technical Note

Dual Amplifier Dynamic Range

The Dual Amplifier architecture of sCMOS sensor CIS 2051 in Neo 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 optimise 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, then 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'.

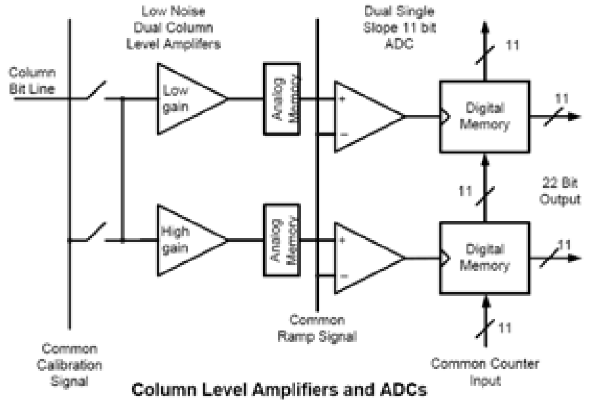


Figure 1: Schematic layout of sCMOS Columns Level Amplifiers and Analogue to Digital Converters (ADCs)

sCMOS sensor CIS 2051 offers 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 11-bit 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 (Gain 4) and the low gain amplifier (Gain 1). This results in two separate digital data streams from the sensor.
2) In the camera FPGA, Neo 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.

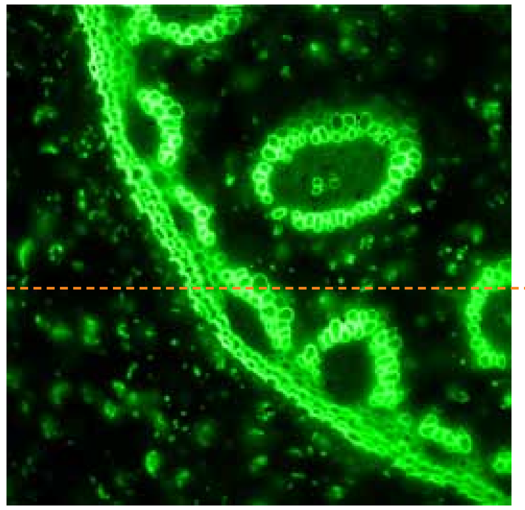
The sensor has four available individual 11-bit gain settings and one dual amplifier 16-bit setting, as shown in table 2. The user maintains the choice of opting to stay with 11-bit single gain channel data if dynamic range is not critical, resulting in smaller file sizes. This in turn offers faster frame rates when continuously spooling through the Cameralink 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 (software setting)	Sensitivity e^-/ADU (typical)	Digitization	Effective pixel saturation limit / e^-	Spooling file size
Gain 1	22	11-bit	30,000	8.5 Mb
Gain 2	9.4	11-bit	19,250	8.5 Mb
Gain 3	1.8	11-bit	3,690	8.5 Mb
Gain 4	0.6	11-bit	1,230	8.5 Mb
DUAL (1 and 4)	22 and 0.6	16-bit	30,000	11.3 Mb

Table 2- Individual amplifier gain settings of the sCMOS CIS 2051 sensor



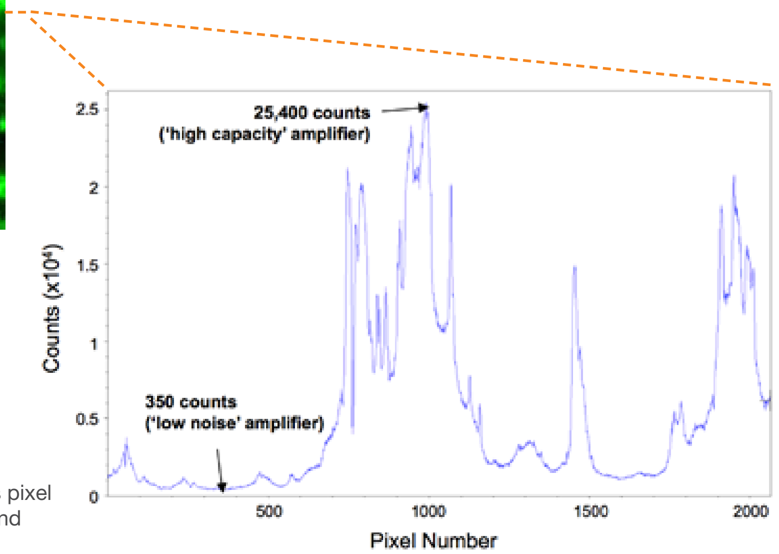


Figure 2- High contrast image of fixed labelled cell. Intensity line profile through single row demonstrates pixel regions that were sampled by high gain (low noise) and low gain (high capacity) amplifiers.

Technical Note

The Importance of Deep 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 provides the key to maintaining a minimized detection limit through suppression of darkcurrent, 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, darkcurrent, 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.

The Andor Neo sCMOS platform is unique in the market in that it is the only scientific CMOS camera to offer the level of deep thermoelectric cooling necessary to minimize the detrimental influence of dark noise. Figure 1 shows theoretical plots of noise floor versus exposure time, at three different cooling temperatures, +5ºC, -30ºC and -40ºC. 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 darkcurrent of the CIS 2051 sCMOS sensor at each of the temperatures. 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 1 sec, the low noise floor can be notably sacrificed by ~ 75% at the higher temperature of +5ºC. Cooling to either -30ºC maintains the 1 electron noise floor over this short exposure range. At an exposure time of 10 sec, the noise floor associated with +5ºC is significantly compromised to a value approaching 5 electrons, i.e. x5 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 sec, unless deep cooling is applied, the thermal contribution to the noise floor would become excessively large, shown in graph (c) as reaching 35 electrons. Holding the cooling temperature at -40ºC would result in the noise floor being held at a more modest 5 electrons over this extensive exposure period.

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 darkcurrent 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.

Figure 2 shows a 3D intensity plot of the same 500 x 1000 pixel region of an sCMOS CIS2051 sensor at a number of different cooling temperatures, each recorded with only 1 sec exposure time in rolling shutter mode. It is clear that cooling to -30ºC 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 pixels.

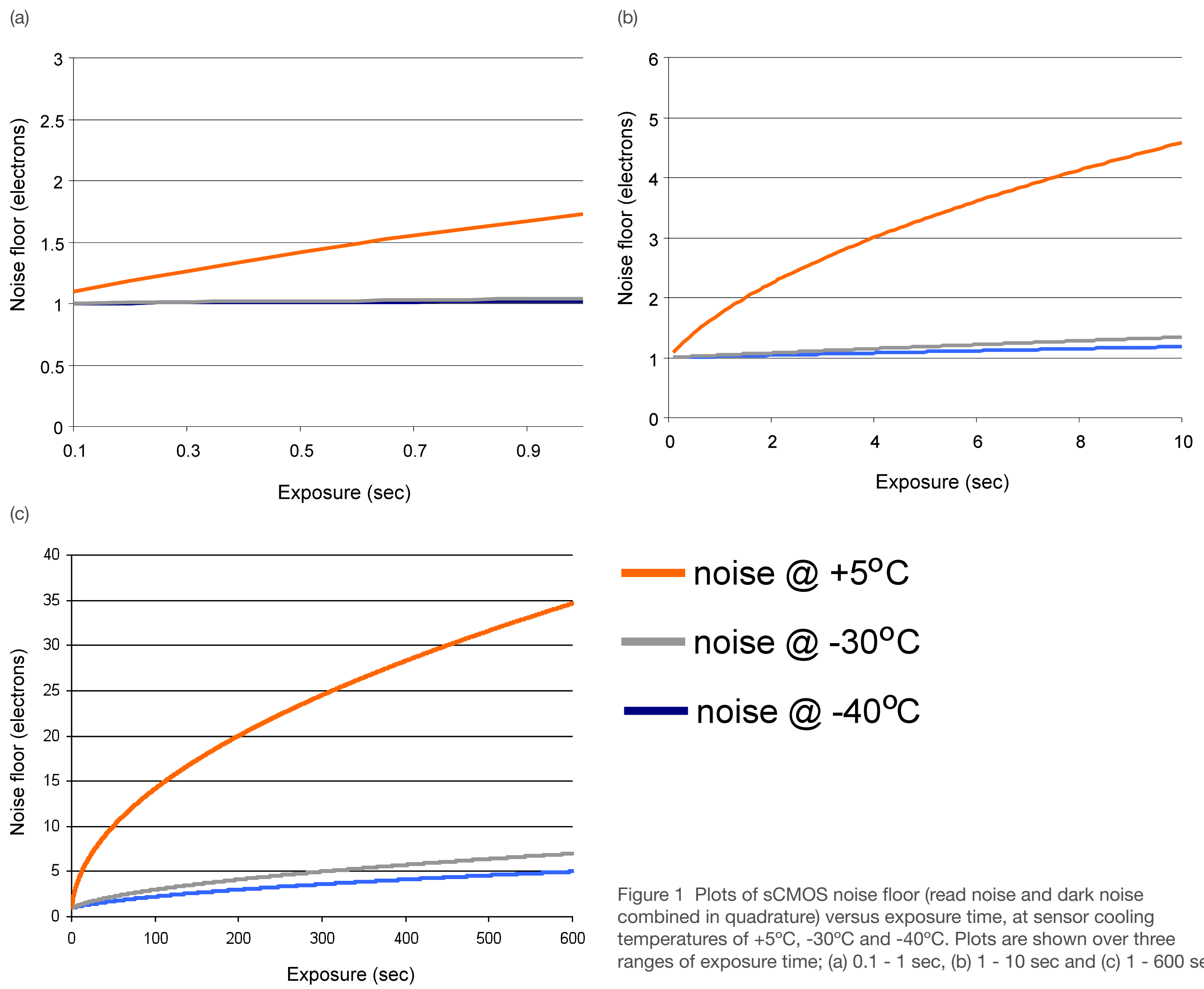


Figure 1 Plots of sCMOS noise floor (read noise and dark noise combined in quadrature) versus exposure time, at sensor cooling temperatures of +5ºC, -30ºC and -40ºC. Plots are shown over three ranges of exposure time; (a) 0.1 - 1 sec, (b) 1 - 10 sec and (c) 1 - 600 sec

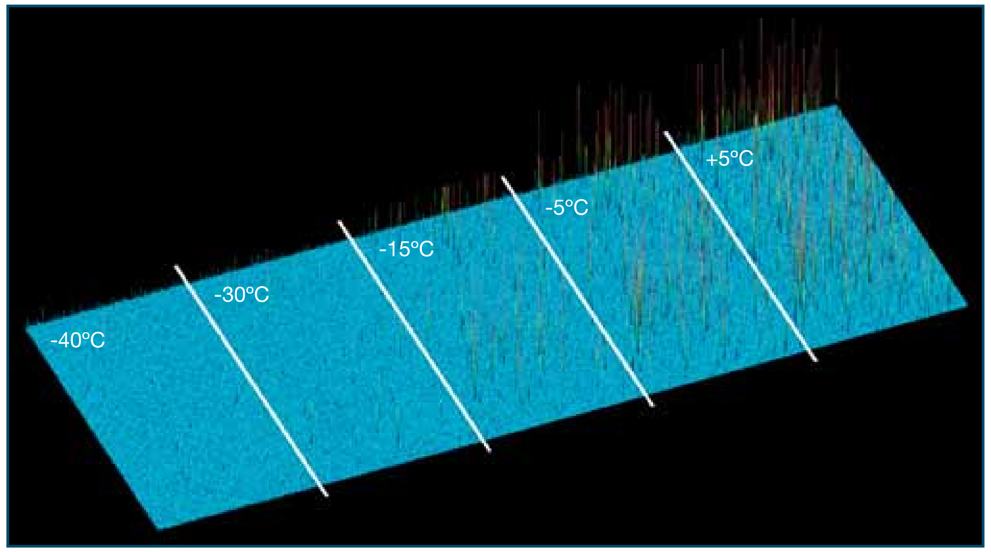


Figure 2 - 3D surface intensity plots derived from a 500 (w) x 1000 (h) region of interest at a series of cooling temperatures, showing the effect of sensor cooling in reducing hot pixel blemishes

Technical Note

Rolling and Global Shutter

The new CIS 2051 sCMOS sensor offers a choice of both Rolling and Global shutter, providing superior application flexibility.

Rolling and Global shutter modes describe two distinct sequences through which the image may be read off a CMOS sensor. In rolling shutter, charge is transferred from each row in sequence during readout, whereas in global shutter mode each pixel in the sensor effectively ends the exposure simultaneously. However, lowest noise and fastest frame rates are achieved from rolling shutter mode. Traditionally, most CMOS sensors offer either one or the other, but very rarely does the user have the choice of both from the same sensor. With sCMOS technology the user benefits from the capability to select between 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 of 560 MHz, this offset between adjacent row exposures is 10 µs. The rolling shutter readout mechanism is illustrated in Figure 1. From the point of view of readout, the sensor is split in half horizontally, and each column is read in parallel from the centre outwards, 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, 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 the extremes of the sensor halves would have started and ended its exposure 10 ms (1000 rows x 10 µs/row) after the rows at the centre of the sensor.

Rolling shutter can be operated in a 'continuous' mode when capturing a kinetic series of images, whereby after each row has been read out it immediately enters it's 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, i.e. as soon as the readout fronts reach the top and bottom of the sensor, they immediately return to the centre to readout the next exposure.

The potential downside of rolling shutter, which is spatial distortion resulting from the above described exposure mechanism, has historically been more apparent in devices such as CMOS camcorders, where the entire image field could be moved (for example by the user rapidly panning the camera) at a rate that the image readout could not match; thus, objects could appear at an angle compared to their actual orientation. In reality, despite the time-offset readout pattern, rolling shutter mode will be used for the majority of scientific applications, especially where the exposure time is equal to or greater than the sensor readout time, discussed later.

Global Shutter

Global shutter mode, which can also be thought of as a 'snapshot' exposure mode, means that all pixels of the array are exposed simultaneously. In most respects, 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 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. Importantly, 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.

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. Due to this additional reference readout, global shutter mode carries the trade-off of halving the maximum frame rate that would otherwise have been achieved in rolling shutter mode. In addition, global shutter also increases the RMS read noise by a factor of 1.41 over rolling shutter readout.

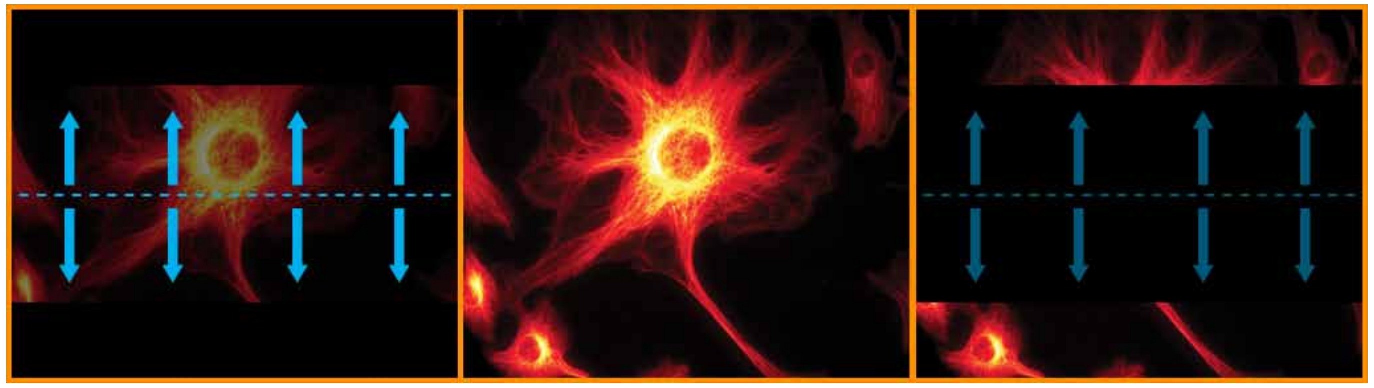
	Rolling	Global
Frame Rate	Maximum available	Maximum frame rates are halved
Read Noise	Lowest	Increased by x1.41
Spatial Distortion	Possible if not temporally oversampling object dynamics	None

Table 1 – Comparing the pros and cons of rolling vs global shutter

Rolling or Global?

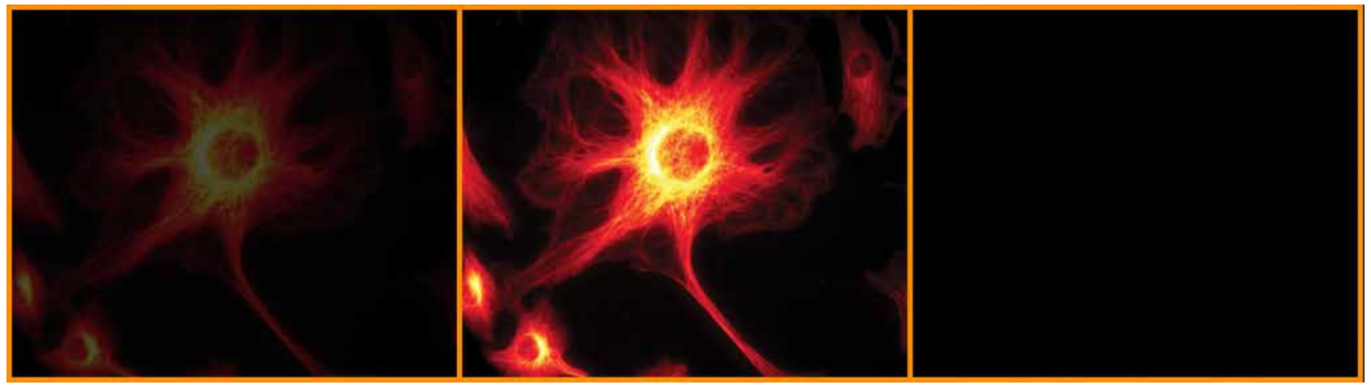
Whether rolling shutter or global shutter is right for you will depend very much on the experiment. Rolling shutter mode, with the enhanced frame rates and lower noise, is likely to suit the majority of scientific applications. As long as the frame rate is such that the camera is temporally oversampling object dynamics within the image area, negligible spatial distortion will be observed. Such oversampling is good imaging practice, since it is undesirable to have an object travel a significant distance during a single exposure. That is to say, if you experience distortion of an object in rolling shutter, you were likely to see smearing of the object in a CCD camera operated with the same exposure time and frame rate. This same principal holds true for global shutter mode or any other method of controlling exposure time. For some particular applications however, for example where it is required to accurately synchronise to relatively short lived events, global shutter will be viewed as a necessity.

Rolling Shutter exposure and readout



Exposure start | Exposure | Readout

Global Shutter exposure and readout



Exposure start | Exposure | Exposure End

Figure 1 – Simplified illustration showing sequence of events in global shutter mode. N.B. does not apply to all triggering options of global shutter.

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 CIS 2051 sCMOS sensor, 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.

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 Neo sCMOS camera at the fastest readout speed of 560 MHz (or 280 MHz x 2). It is standard to describe noise in CMOS technology by citing 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. While there will be a small percentage of pixels with noise greater than 2 or 3 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 read noise typically ranging between 6 and 10 electrons RMS (depending on camera manufacture).

Insight into the sCMOS architecture

The sensor 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 (ADC); see technical note of Dual Column Amplifiers 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 5 transistors ('5T' design), enabling the novel 'global shutter' mode and also facilitating correlated double sampling (CDS), to further reduce noise, and a lateral anti-blooming drain. The sensor is integrated with a microlens array that serves to focus much of the 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).

The sensor is configured to offer low dark current and extremely low read noise with true CDS. Non-linearity is less than 1% and is further correctable to < 0.2%. 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.

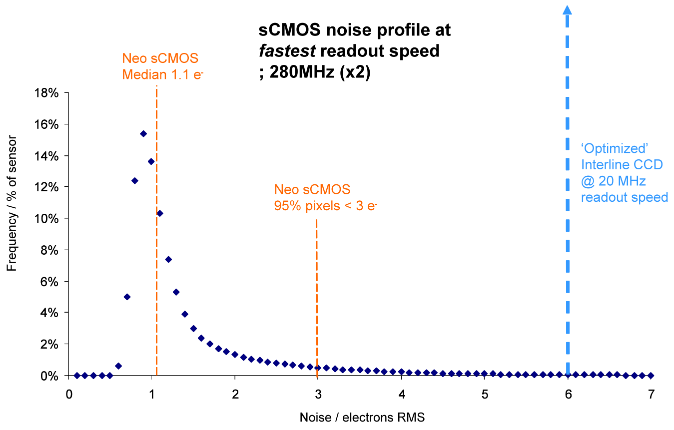


Figure 1 – Representative histogram showing read noise distribution at fastest readout speed of 280 MHz (x2). The median value of 1.1 e- means 50% pixels have less than 1 e- and 50% have greater than 1 e-. The line at 6 e- represents a typical read noise value from a well optimized Interline CCD – all pixels in a CCD share the same noise value.

Spurious Noise Filter

Andor's Neo sCMOS camera comes 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 5 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 neighbouring pixels.

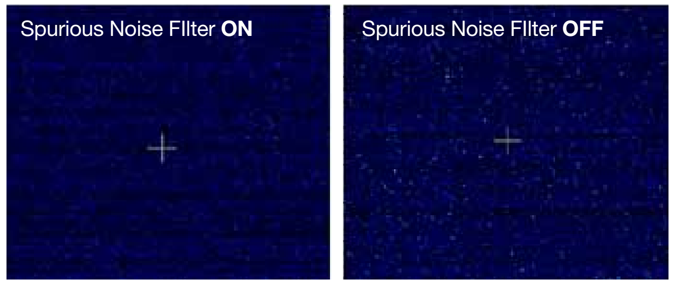


Figure 2 – Demonstration of Spurious Noise Filter on a dark image, 20 ms exposure time, 200 MHz (x2) readout speed (~ 1.2 e- readnoise)

Technical Note

UltraVac™ permanent vacuum head: performance and longevity

Andor's UltraVac™ vacuum process was designed not only to facilitate deep TE cooling, but also to provide absolute protection of the exposed sensor and to allow use of only a single input window, maximising photon throughput to the sensor.

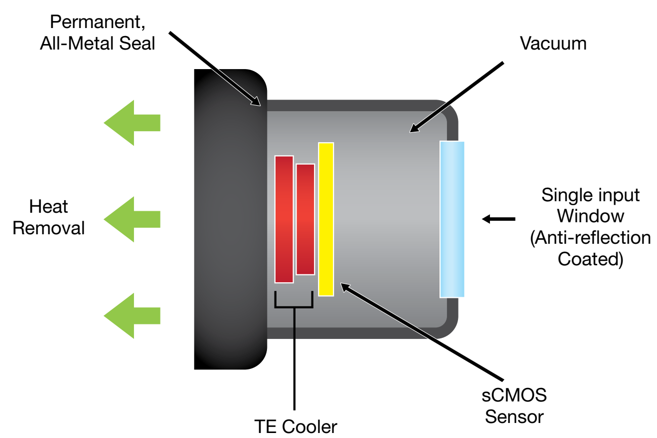
Unless protected, cooled sensors will condense moisture, hydrocarbons and other gas contaminants. Such contaminants are particularly damaging towards the detecting surface of back-illuminated sensors. Exposed to such out-gassed contaminants, the Quantum Efficiency of a sensor will decline proportionally. Furthermore, the sensor can fail if excessive condensation forms.

A high performance scientific sensor must be housed in a hermetically sealed vacuum head with minimized out-gassing, otherwise both cooling performance and the sensor QE itself will degrade.

It was these compelling reasons that drove Andor to develop permanent vacuum technology > 15 years ago. Andor have indeed perfected a proprietary Permanent Vacuum Head, essential not only to optimize cooling performance, but also to ensure that the sensor is protected and that this performance is retained year after year. Only Andor have shipped thousands of vacuum systems, enabling us to unequivocally substantiate our longevity claims with real reliability data.

Benefits of Permanent Vacuum Head:

- Sustained vacuum performance over many years operation – proprietary process to minimize out-gassing. Peak QE and cooling will not degrade.
- Benefit from a thoroughly proven solution. More than 15 years of shipping vacuum systems to the field and a negligible failure rate - MTBF (mean time between failure) figure of > 100 years. No one else can make or substantiate this claim with real data.
- Performance improves because the temperature of the chip can be reduced significantly. Better cooling (down to –100ºC with an enhanced thermoelectric peltier design) translates into substantially lower darkcurrent and fewer blemishes.
- Elimination of condensation and out-gassing means that the system can use only a single entrance window, with double antireflection coating – you can believe the QE curve!
- Prevent convection heat transport from the front window which would otherwise lead to condensation on the outside window.



Schematic of a Permanent Vacuum Head

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 frames/sec. 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. 34 frames/sec 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 (frames/s)	Global Shutter mode (frames/s)
2560 x 2160 (full frame)	100	50
2064 x 2048 (4 megapixel)	106	54
1392 x 1040 (1.4 megapixel)	208	105
528 x 512	403	211
144 x 128	1688	844

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.

Performance highlights of sCMOS CIS 2051sensor:

- Sensor format: 5.5 megapixels (2560(h) x 2160(v))
- Read noise: 1 e^- RMS @ 30 frames/s;
  1.4 e^- RMS @ 100 frames/sec
- Maximum frame rate: 100 frames/s
- Pixel size: 6.5 µm
- Dynamic range: 30,000:1 (@ 30 frames/s)
- QE max.: 57%
- Read out modes: Rolling and Global shutter (user selectable)

The 5.5 megapixel sensor offers 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 RMS read noise while reading out 5.5 megapixels at 30 frames/sec renders it truly extraordinary in the market. Furthermore, the sensor is capable of achieving 100 full frames/sec with a read noise 1.4 electrons RMS.

By way of comparison, the lowest noise Interline CCD reading out only 1.4 megapixels at ~ 16 frames/sec 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 frames/sec 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 30,000:1. 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 frames/sec. 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 frames/sec.

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 megapixel or 4 megapixel sensors. The most popular EMCCD sensors are 0.25 or 1 megapixel, typically offering up to 30 frames/sec.

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

Parameter	Neo sCMOS	Interline CCD	EMCCD
Sensor Format	5.5 megapixel	1.4 to 4 megapixel	0.25 to 1 megapixel
Pixel Size	6.5 µm	6.45 to 7.4µm	8 to 16 µm
Read Noise	1 e^- @ 30 frames/sec 1.4 e^- @ 100 frames/s	4 -10 e^-	< 1e^- (with EM gain)
Full Frame Rate (max.)	100 frames/sec @ full resolution	3 to 16 frames/s	~ 30 frames/s
Quantum Efficiency (QE)	57%	60%	90% 'back-illuminated' 65 % 'virtual phase'
Dynamic Range	30,000:1 (@ 30 frames/s)	~ 3,000:1 (@ 11 frames/s)	8,500:1 (@ 30 frames/sec with low EM gain)
Multiplicative Noise	none	none	1.41x with EM gain (effectively halves the QE)

Table 2: Comparison summary of typically specifications of Interline CCD and EMCCD technologies compared to sCMOS technology.

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 of 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, 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 400 MHz, this readout speed capable of achieving 70 full frames/s, with only 1.2 electrons read noise. The Interline CCD camera, an Andor 'Clara', was read out at 20 MHz, achieving 11 frames/sec 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 frames/sec 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. ~ 510nm).

The LED rig proved excellent for comparing sensitivity under extreme low light conditions, using two LED intensity settings, labeled 'LED1' and 'LED2'. The photon flux intensities at each setting, given as photons per 6.5 µm pixel, are approximately as follows: LED 1 ~ 10 photons/pix; LED 2 ~ 32 photons/pix. 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 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. The sensitivity differences between the two technology types is 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 13µm pixel (or 2 x 2 binned super-pixel), relating to an actual pixel area of $169\mu m^2$. 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 ~ 22 photons/pixel and ~ 225 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 CIS 2051 sCMOS sensor, optimized in the Andor Neo camera, out-performs even 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 iXon3 range) maintains an application advantage.

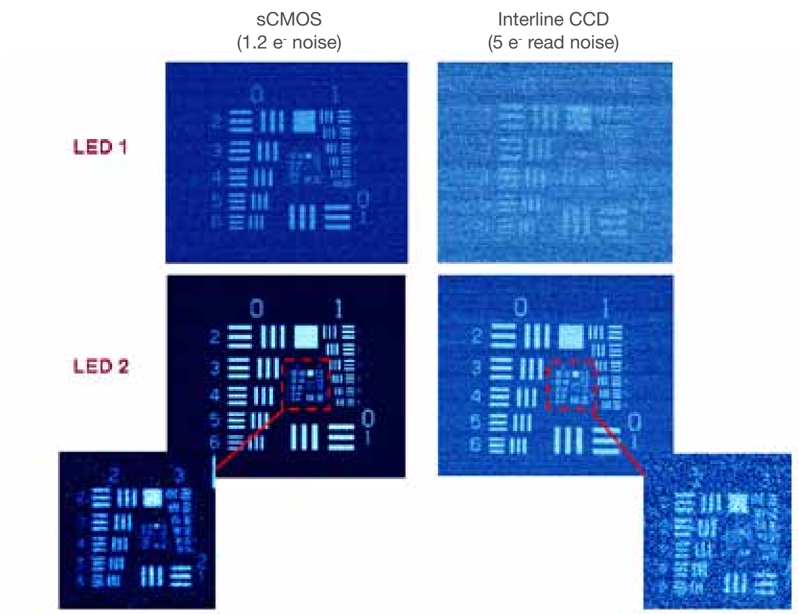


Figure 1: Comparative low light images of a USAF resolution chart, showing Andor sCMOS (1.2 electrons read noise @ 400 MHz) vs Interline CCD (5 electrons read noise @ 20 MHz), under the two lowest LED settings.

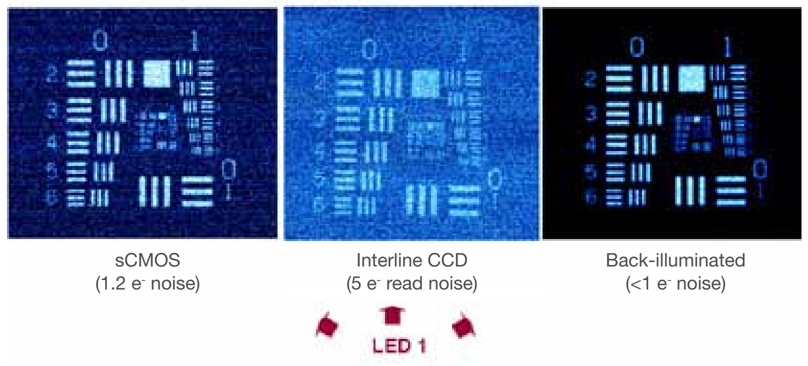


Figure 2: Comparative low light images taken with Andor sCMOS (1.2 electrons read noise @ 400 MHz) vs Interline CCD (5 electrons read noise @ 20 MHz) vs back-illuminated EMCCD (< 1e- read noise), under extremely low light conditions ('LED 1' setting). sCMOS and Interline CCD were 2 x 2 binned in order to have the same effective pixel pitch (and light collection area per pixel) as the 13 µm pixel of the EMCCD sensor.

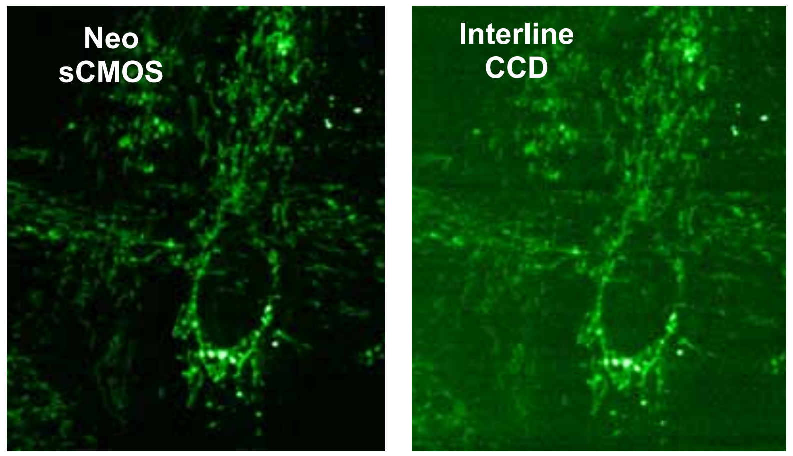
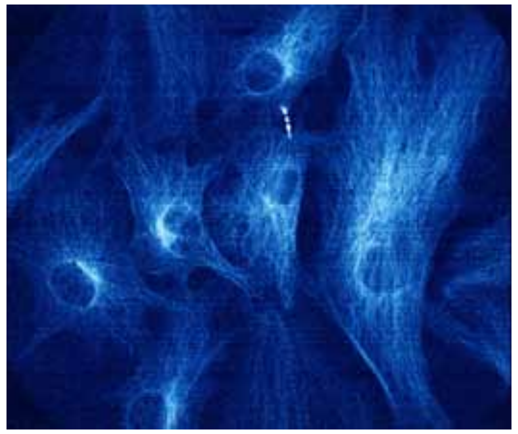


Figure 3 -Comparative low light images taken with Andor sCMOS (1.2 electrons read noise @ 400 MHz) vs Interline CCD (5 electrons read noise @ 20 MHz) of fluorescently labelled fixed cell using a CSU-X spinning disk confocal microscope (x60 oil objective), each 100 ms exposure, same laser power, displayed 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.

sCMOS (1.2 e⁻ noise)



Interline CCD (5 e⁻ read noise)

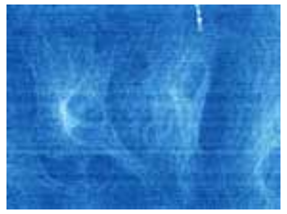


Figure 4 - Comparative low light fluorescence microscopy images taken with Andor sCMOS (1.2 e⁻ @ 400 MHz) vs Interline CCD (5 e⁻ @ 20 MHz) under low light conditions, typical of those employed in dynamic live cell imaging. ND filters on a widefield fluorescence microscope were used to reduce light levels relative to the read noise floor.

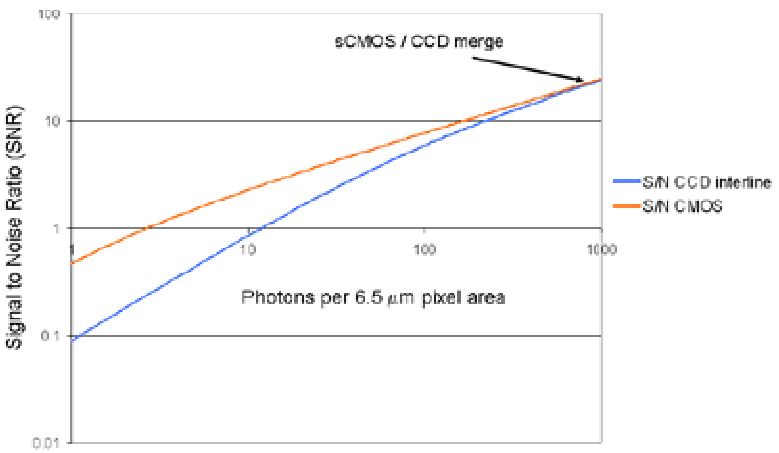


Figure 5 - Theoretical Signal to Noise plot comparisons for sCMOS vs Interline CCD sensors. Photon flux (i.e. input light intensity) is given in terms of photons per 6.5 µm pixel of each sensor type.

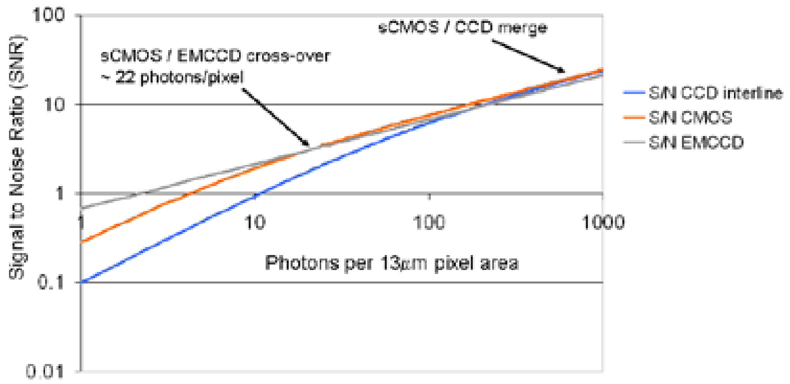
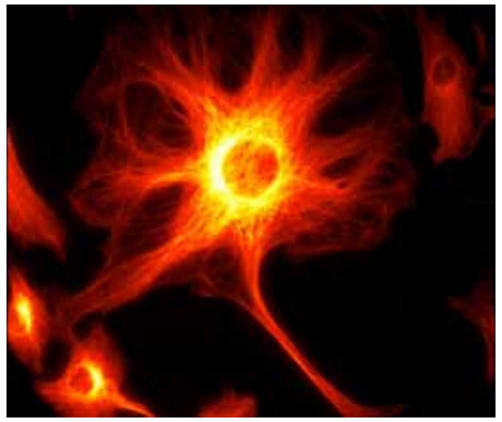


Figure 6 - Theoretical Signal to Noise plot comparisons for sCMOS vs Interline CCD vs back-illuminated EMCCD sensors. For purposes of a objective comparison, it is assumed that the ~6.5 µm pixels of the sCMOS and Interline CCD sensors are 2 x 2 binned in order to equal a 13 µm pixel of a back-illuminated EMCCD.

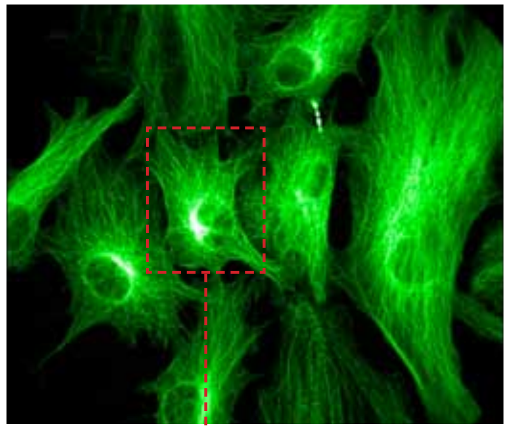
5.5 Megapixel sCMOS



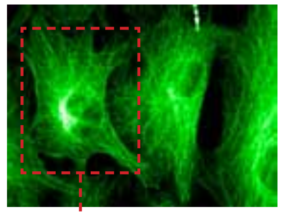
1.4 Megapixel Interline CCD

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 1.4 e⁻ read noise.

5.5 Megapixel sCMOS



1.4 Megapixel Interline CCD



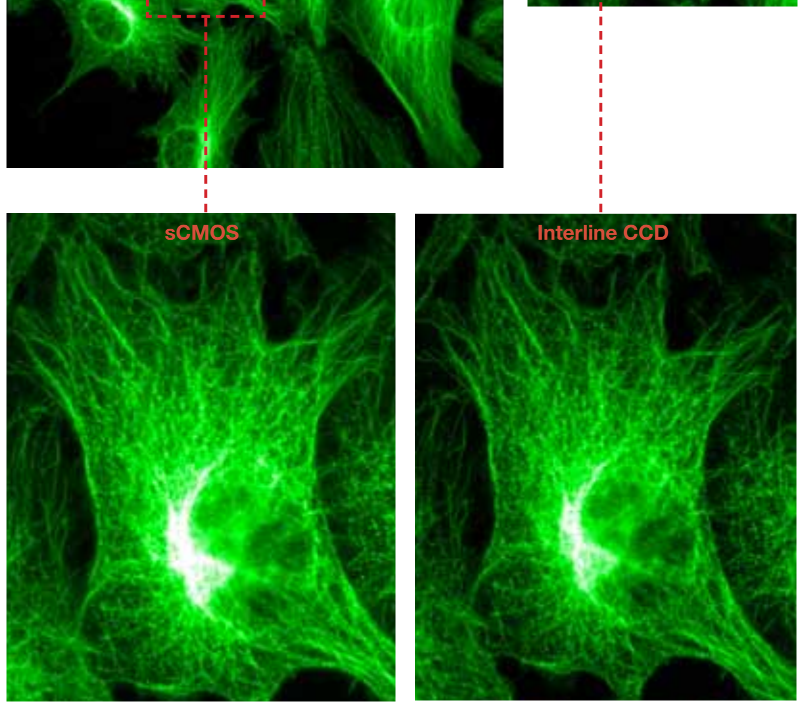
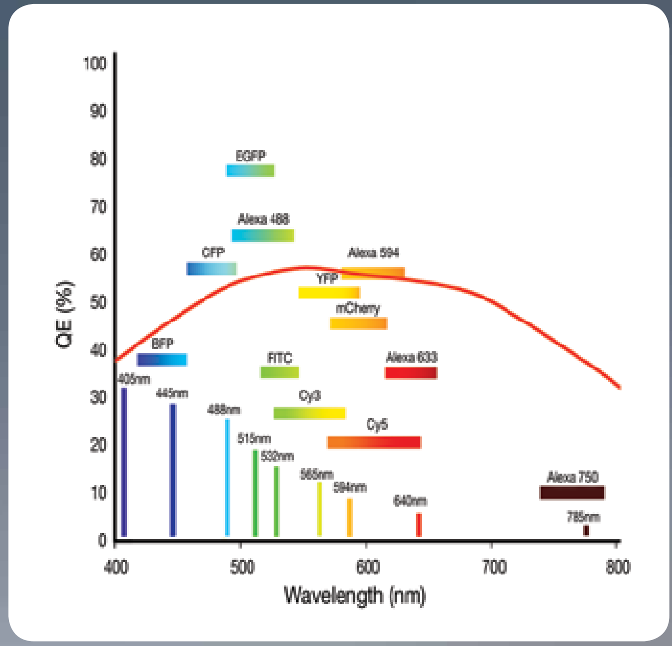


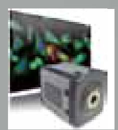
Figure 8: Field of view and resolution comparison of two technologies; x100 magnification; 1.45 NA; 5.5 megapixel Andor sCMOS vs 1.4 megapixel Interline CCD (each have ~ 6.5 µm pixel pitch).

Notes...



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

"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."



Front cover image:

This image shows TNFalpha-mCherry expressed at the surface of HeLa cells that were transiently transfected. The green label shows the localization of the endosomal protein EEA1 stained using a monoclonal antibody followed by an anti-mouse secondary antibody labelled with Alexa488.

Courtesy of Dr. Frank Perez, Institut Curie, Paris, France

Andor Customer Support

Andor products are regularly used in critical applications and we can provide a variety of customer support services to maximise 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 www.andor.com/customersupport.

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