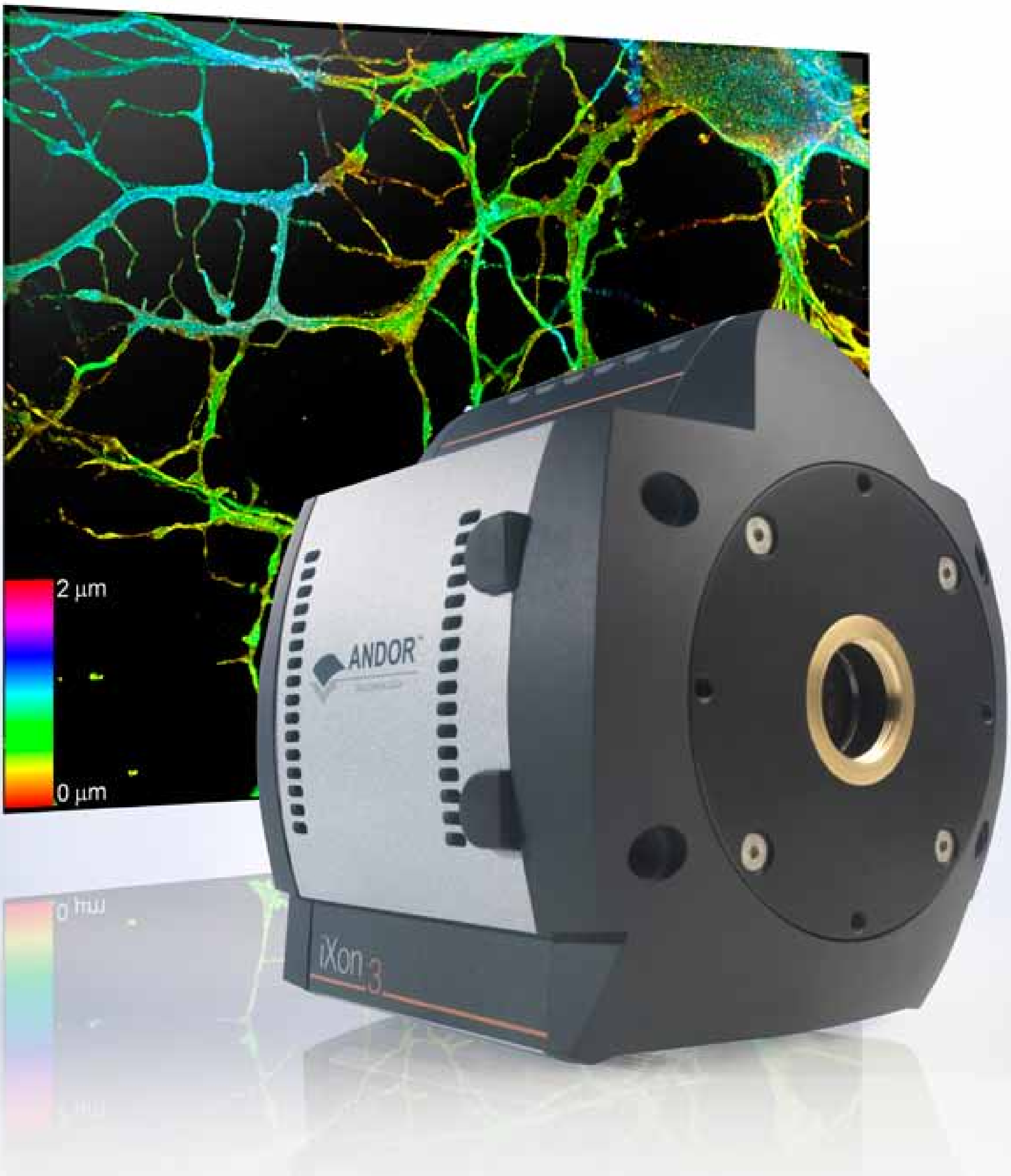
iXon 3

Driving the absolute best from EMCCD technology



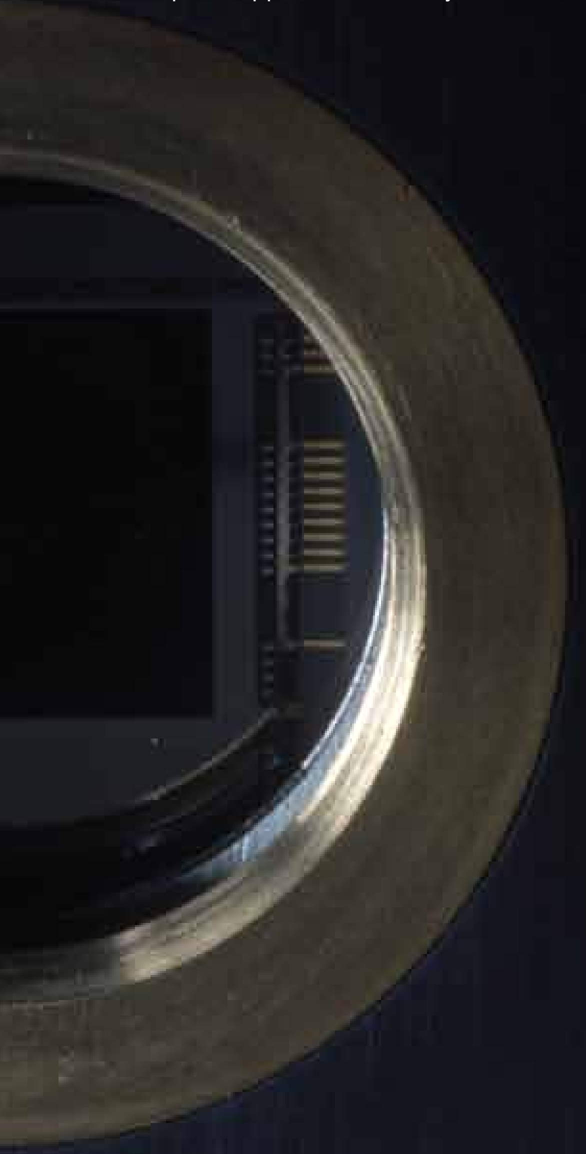
iXon3

The Industry's Highest Performance Scientific EMCCD Cameras

Andor Technology pioneered the world's first scientific EMCCD cameras, shipping the initial cameras back in 2000 and winning the Photonics Circle of Excellence award. Andor coined the term 'Electron Multiplying CCD (EMCCD)', which has now been adopted right across this burgeoning industry.

Since then, Andor have consistently set higher and higher EMCCD performance standards with our deep-cooled, vacuum-sealed iXon and iXon+ quantitative EMCCD camera range. For example, we introduced the first back-illuminated EMCCDs in January 2002, alongside our unique baseline clamp solution for enhanced quantitative performance. Andor's method for achieving industry-lowest Clock Induced Charge was introduced in early 2003 and our benchmark quantitative and linearized EM gain control (RealGain™) and patented EM gain recalibration technology (EMCAL™) was innovated in January 2006.

The new iXon3 sets the bar higher still, offering industry leading high-end EMCCD performance alongside a range of new customer requested functionality. The iXon3 presents an exceptional combination of low noise sensitivity, quantitative stability, fast speed, application flexibility and ease of use.



2000 **Andor shipped first EMCCD cameras**

- Pioneering EMCCD cameras
- Photonics Circle of Excellence Award

2001 **iXon**

- Flagship high-end EMCCD camera platform
- First to introduce back-illuminated sensors
- Deepest cooling and lowest noise
- Fastest frame rates

2006 **iXon+**

- New innovations and greater sensitivity
- Patented EMCAL™ and RealGain™
- World's first megapixel back-illuminated EMCCD (888 model)
- Maintaining market leader status

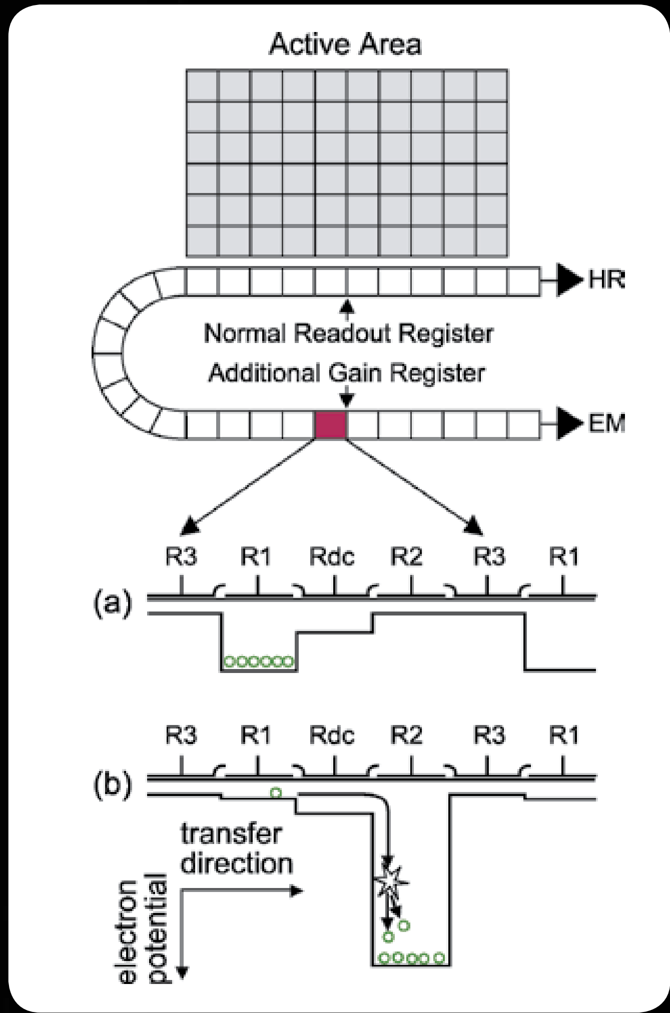
2010 **iXon3**

- New customer requested functionality
- Superior ease of optimization
- Quantify data in electrons or photons
- Enhancements in noise, speed and quantitative stability

The EMCCD advantage…

Current trends in photon measurement are placing unprecedented demands on detector technology to perform at significantly higher levels of sensitivity and speed. Electron Multiplying CCD (EMCCD) technology has been designed to respond to this growing need, unlocking new and innovative experimental prospects.

EMCCDs operate by amplification of weak signal events (down to single photons) to a signal level that is well clear of the read noise floor of the camera at any readout speed. Importantly, this 'on-chip' amplification process is realized without sacrificing the photon collection capability of the sensor, with back-illuminated sensors offering up to 95% Quantum Efficiency (QE).



'With the iXon3, Andor have delivered a dedicated, truly high-end ultra-sensitive scientific camera platform, designed specifically to extract the absolute best from Electron Multiplying CCD (EMCCD) technology across all critical performance specs and parameters.'

iXon3 - The Microscopist's Choice

In applications such as single molecule microscopy, super-resolution, live cell microscopy (including confocal), calcium signalling, transport/motile imaging and intracellular bioluminescence, where weak, rapidly changing fluorescent signals from cells must be dynamically imaged, Andor's iXon3 technology offers an ideal detection solution. Ultrasensitive detection capability in fluorescence microscopy facilitates use of lower excitation powers (thereby reducing photobleaching and phototoxicity) and lower dye concentrations.

Since its pioneering introduction in 2000, Andor's EMCCD technology has been widely and highly successfully employed by microscopists throughout the world, resulting in an outstanding level of representation in high-profile publications.

iXon3 - The Physicist's Choice

The unique high-performance specifications of the optimized iXon range have been serving the physical scientist and astronomer in scenarios that demand more than simply an EM sensor in a camera. Andor have worked with numerous scientists to deliver solutions that work for their particular application requirements, such as providing effective charge purging immediately prior to acquisition, specific coatings, coupling to fiber optic scintillators and also specific interface requirements.

As such the Andor iXon brand has been prevalent across a variety of demanding applications, such as photon counting, lucky astronomy, adaptive optics, Bose Einstein condensation (BEC) / ion trapping, single molecule detection / nanotechnology, neutron tomography, X-ray/Gamma tomography, plasma diagnostics, Raman detection, sono-and thermo-luminescence detection.

Why choose Andor's iXon3 high-performance EMCCD?

The principal reason for making use of Andor's iXon3 EMCCD technology is to ensure the absolute **highest sensitivity** from a **quantitative** scientific digital camera, particularly under **dynamic** measurement conditions (faster frame rates). Andor's proven UltraVacTM vacuum technology, carrying a **7 year warranty,** is critical to ensure both **-100ºC deep cooling** and complete **protection** of the sensor.

The iXon3 is designed to be the most **flexible** yet **easy to use** EMCCD on the market, optimisable for a wide variety of application requirements in a single click via the new OptAcquireTM feature. Furthermore, signal can be quantitatively calibrated in units of **electrons or photons**, either in real time or post-processing. Patented, pioneering technology offers **automated recalibration** of EM gain, alongside anti-ageing protection.

Crucially, the iXon brand carries an outstanding reputation within the industry for **quality** and **reliability**, brandishing an unparalleled track record of minimal field failures.

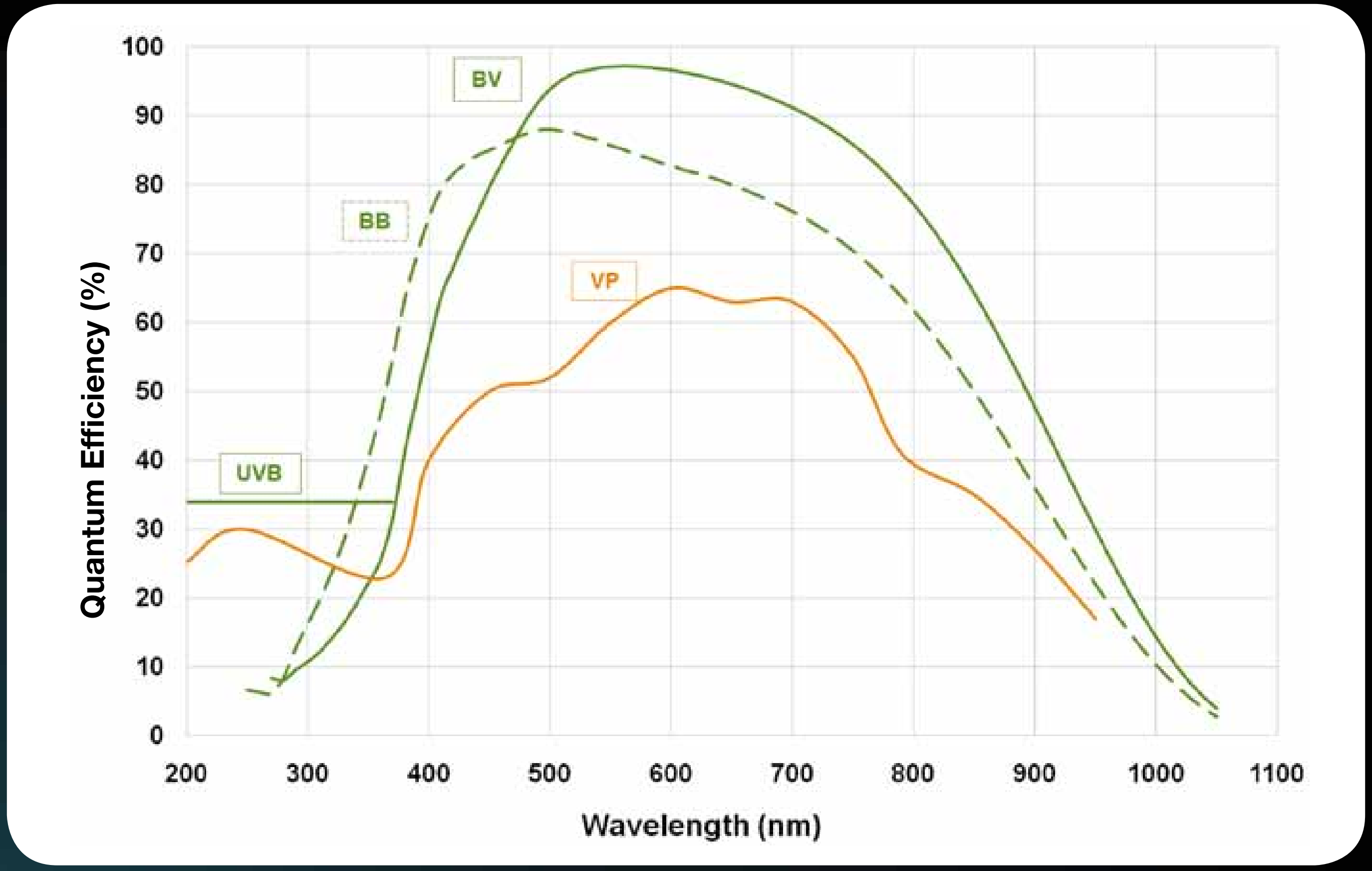
Andor's iXon3 EMCCD cameras deliver best performance in these core areas:

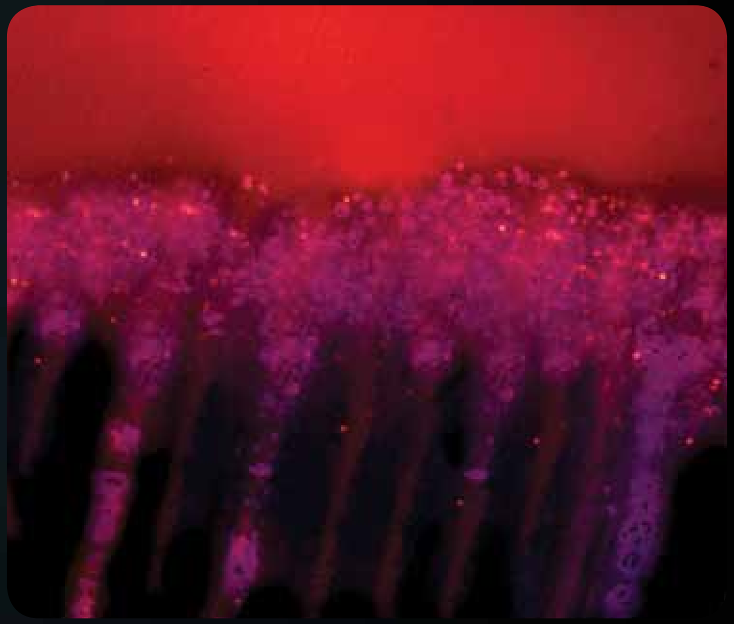
- **Highest Sensitivity EMCCD** – Deepest cooling & lowest clock induced charge parameters complement the high QE performance of back-illumination. Now with optional advanced filters to eradicate spurious noise.
- **Fastest Frame Rates** – Industry-fastest full frame rate; up to 60% faster than competitors under sub-array/binning conditions. Unique cropped sensor mode solution for additional frame rate boost.
- **Ultimate Longevity** – Essential vacuum protection of back-illuminated sensor meaning no QE degradation. Anti-Ageing technology and EMCALTM, a pioneering auto-recalibration of EM gain. Unparalleled long-term performance capability.
- **User Friendliness** – iXon3 remains the most versatile EMCCD camera available. Now, via OptAcquire, it can be optimized for different application requirements with a single click.
- **RealGainTM** - Select absolute EM gain direct from a linear and directly quantitative software gain scale. The EM gain you ask for is the EM gain you get.
- **Quantitative: Baseline (bias) Clamp & Stabilized EM gain** – all that should vary is your signal. Now with option to display signal in electrons or photons, real time or post convert.
- **Flexibility** – Ability to fine tune performance for a wide range of application scenarios. '2 in 1' EMCCD and low noise CCD amplifiers (model dependent). Ability to turn off EM gain completely for rapid imaging under brighter conditions.
- **Advanced Performance through Software** – iCam encompasses a set of unique innovations that empower iXon3 cameras to operate with market-leading exposure switching through imaging software packages.

iXon3 Range

The iXon3 portfolio encompasses a number of model variations, offering solutions for a wide range of application requirements. Whether your needs are guided more by resolution, speed, field of view, wavelength dependence or simply budget, the iXon3 family of market leading EMCCD cameras will provide a match.

iXon	Requirement	Pixel Format	QE options	Pixel Size	Frame Rate
897	Ultimate Sensitivity	512 x 512	BV, UVB, BB	16µm	35 fps
885	Resolution, Speed & Sensitivity	1004 x 1002	VP	8 µm	31 fps (60 fps with binning)
860	Lightening Speed & Sensitivity	128 x 128	BV, UVB	24 µm	513 fps
888	Field of View & Sensitivity	1024 x 1024	BV, UVB, BB	13µm	9 fps





Resin-dentine interface created using an etching-and-rinse bonding agent previously mixed with 0.1% rhodamine-b and subsequently applied in deep dentine to aid the visualization of resin tags, adhesive and interdiffusion layers. A reflective 10% silver nitrate solution was used to evaluate the microporosities within the hybrid layer of the resin-dentine interfaces. The silver grains located within the microporosities of the interdiffusion layer have diameters of <1 µm.

Image courtesy of Salvatore Sauro, King's College London Dental Institute

iXon3 897

Ultimate Sensitivity…

This highly popular back-illuminated 512 x 512 frame transfer model delivers unequalled thermoelectric cooling down to -100°C, industry-lowest clock induced charge noise, and operates at 35 frames/sec (full resolution). Andor's rapid vertical shift capability gives distinct speed advantages when operated with binning/sub-array, whilst minimizing vertical smear. EMCCD and conventional CCD readout modes provide heightened application flexibility.

The 897 model has proven to be an extremely popular choice within biophysics, presenting an excellent combination of superior sensitivity, superb quantitative stability and rapid frame rate performance. The ultra low noise has also rendered this model superb for single photon counting experiments, which offer a means to circumvent EMCCD multiplicative noise and further boosting S/N ratio.

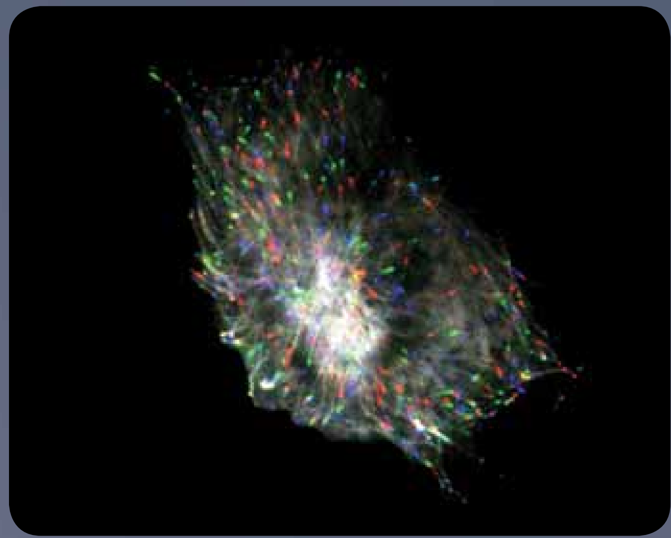
Key Specifications

Active Pixels	512 x 512
Pixel Size (W x H; μm)	16 x 16
Image Area (mm)	8.2 x 8.2
Active Area Pixel Well Depth (e^-)	160,000
Max Readout Rate (MHz)	10
Frame Rates (frames per sec)	35 (full frame) - 549
Read Noise (e^-)	49 @ 10 MHz < 1 with EM gain
QE max	> 90%

Features	Benefits
TE cooling to -100°C	Critical for elimination of Darkcurrent detection limit.
RealGain™	Absolute EMCCD gain selectable directly from a linear and quantitative scale.
OptAcquire	Optimize the highly flexible iXon3 for different application requirements at the click of a button.
Count Convert	Quantitatively capture and view data in electrons or incident photons. Applied either in real time or post-processing, Count Convert does this important conversion for you.
EMCAL™	Patented user-initiated self-recalibration of EM gain.
iCam	Exposure time fast switching provides market leading acquisition efficiency.
Minimal Clock-Induced Charge	Unique pixel clocking parameters, yielding minimized spurious noise floor.
UltraVac™	Critical for sustained vacuum integrity and to maintain unequalled cooling and QE performance, year after year. 7 year vacuum warranty.
Cropped Sensor Mode	Specialized acquisition mode for continuous imaging with fastest possible temporal resolution
Spurious Noise Filter	Intelligent algorithms to filter clock induced charge events from the background. Real time or post-processing.
Enhanced Photon Counting Modes	Intuitive single photon counting modes to overcome multiplicative noise. Real time or post-processing.
Superior Baseline Clamp and EM Stability	Essential for quantitative accuracy of dynamic measurements.
Selectable amplifier outputs – EMCCD and conventional	'2 in 1' flexibility. EMCCD for ultra-sensitivity at speed, conventional CCD for longer acquisitions.

Applications of the 897 include:

- Single molecule detection
- Super resolution (PALM, STORM)
- TIRF microscopy
- Spinning disk confocal microscopy
- Selective/single plane illumination microscopy (SPIM)
- Calcium flux
- Whole genome sequencing
- FRET / FRAP
- Microspectroscopy / Hyperspectral imaging
- Lucky astronomy
- Single Photon Counting



Composite triple color image of a microtubule protein (EB1-GFP) imaged with objective-type TIRFM (60x 1.45NA), using the iXon+ back-illuminated 897 model. The different colors reveal the dynamics of the microtubules over time: frame 1 = red; frame 10 = green; frame 20 = blue.

Courtesy of Dr Derek Toomre, CINEMA laboratory, Dept. Cell Biology, Yale University.

ANDOR™ TECHNOLOGY

iXon3 888

Field of View and Sensitivity…

Andor's megapixel back-illuminated EMCCD combines a large field of view, single photon detection capability and > 90% Quantum Efficiency. The 1024 x 1024 frame transfer format offers unequalled thermoelectric cooling down to -95°C, industry-lowest clock induced charge noise, and operates at ~ 9 frames/sec (full resolution). EMCCD and conventional CCD readout modes provide heightened application flexibility.

The enhanced field of view of the 888 model has rendered it effective in applications such as astronomy, tomography and in vivo luminescence. In live cell microscopy the 888 can be employed to capture more of the microscope's field of view, or in combination with a suitable image splitter to perform dual wavelength FRET whilst still visualizing a useful area of the specimen.

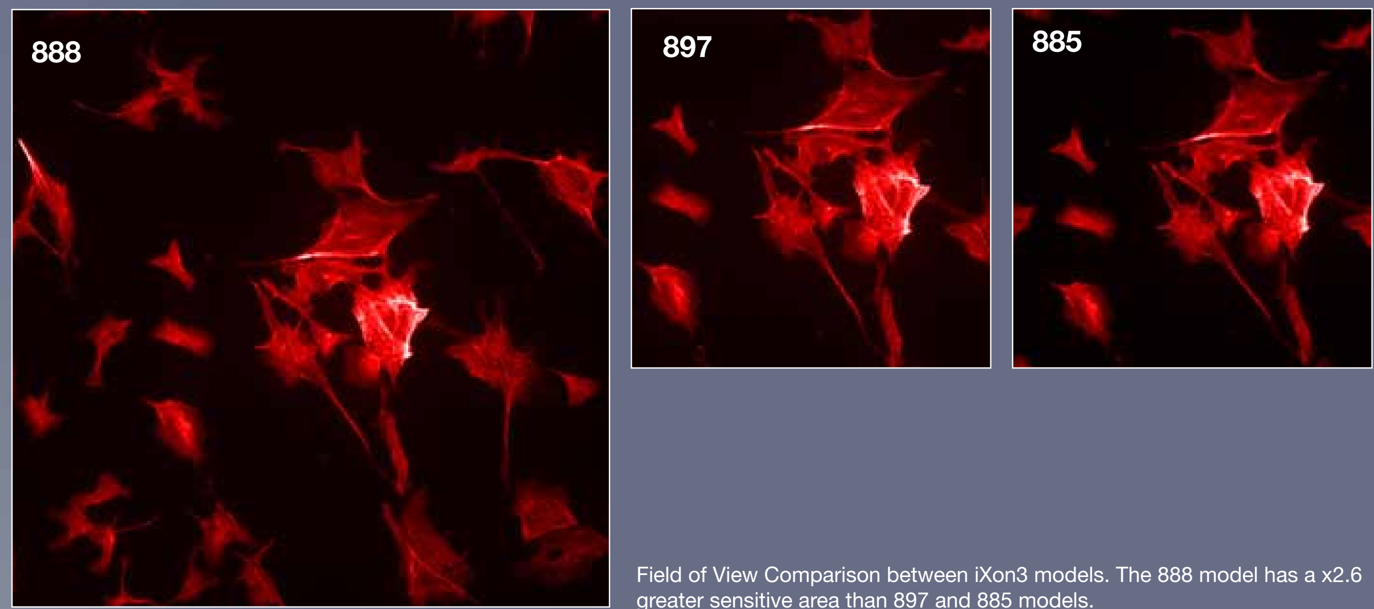
Key Specifications

Active Pixels	1024 x 1024
Pixel Size (W x H; μm)	13 x 13
Image Area (mm)	13.3 x 13.3
Active Area Pixel Well Depth (e^-)	80,000
Max Readout Rate (MHz)	10
Frame Rates (frames per sec)	8.9 (full frame) - 310
Read Noise (e^-)	47 @ 10 MHz < 1 with EM gain
QE max	> 90%

Applications of the 888 include:

- Single molecule detection
- Tomography
- In vivo luminescence
- TIRF microscopy
- Spinning disk confocal microscopy
- FRET
- Microspectroscopy / Hyperspectral imaging
- Astronomy
- Single Photon Counting

Features	Benefits
13.3 x 13.3 mm sensor	Largest field of view EMCCD available
TE cooling to -95°C	Critical for elimination of Darkcurrent detection limit.
RealGain™	Absolute EMCCD gain selectable directly from a linear and quantitative scale.
OptAcquire™	Optimize the highly flexible iXon3 for different application requirements at the click of a button.
Count Convert	Quantitatively capture and view data in electrons or incident photons. Applied either in real time or post-processing, Count Convert does this important conversion for you.
EMCAL™	Patented user-initiated self-recalibration of EM gain.
iCam	Exposure time fast switching provides market leading acquisition efficiency.
Minimal Clock-Induced Charge	Unique pixel clocking parameters, yielding minimized spurious noise floor.
UltraVac™	Critical for sustained vacuum integrity and to maintain unequalled cooling and QE performance, year after year.
Cropped sensor mode	Specialised acquisition mode for continuous imaging with fastest possible temporal resolution
Spurious Noise Filter	Intelligent algorithms to filter clock induced charge events from the background. Real time or post-processing.
Enhanced Photon Counting Modes	Intuitive Single Photon Counting modes to overcome multiplicative noise. Real time or post-processing.
Superior Baseline Clamp and EM stability	Essential for quantitative accuracy of dynamic measurements.
Selectable amplifier outputs – EMCCD and conventional	'2 in 1' flexibility. EMCCD for ultra-sensitivity at speed, conventional CCD for longer acquisitions.



Field of View Comparison between iXon3 models. The 888 model has a x2.6 greater sensitive area than 897 and 885 models.

ANDOR TECHNOLOGY

iXon3 860

Lightning Speed and Sensitivity…

Andor's iXon3 860 back-illuminated EMCCD is designed for very rapid imaging of low light events, combining > 500 frames/sec with single photon detection capability and > 90% Quantum Efficiency.

Thermoelectric cooling down to -100°C minimizes EM-amplified Darkcurrent, whereas industry fastest vertical shift speeds minimize both clock induced charge noise and vertical smear during frame transfer. The absolute EM gain multiplication can be varied linearly from unity up to a thousand times directly via RealGain™, a true quantitative EM gain scale.

Sub-millisecond biology is readily accessible through use of sub-array selection and pixel binning. The speed and sensitivity of the 860 also renders it ideal for adaptive optics.

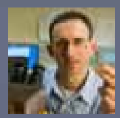
Key Specifications

Active Pixels	128 x 128
Pixel Size (W x H; µm)	24 x 24
Image Area (mm)	3.1 x 3.1
Active Area Pixel Well Depth (e⁻)	160,000
Max Readout Rate (MHz)	10
Frame Rates (frames per sec)	513 (full frame) up to several thousands
Read Noise (e⁻)	48 @ 10 MHz < 1 with EM gain
QE max	> 90%

Applications of the 860 include:

- Single Molecule Detection
- Calcium flux
- Voltage sensitive dyes
- Adaptive optics
- FRET
- Fluorescence correlation spectroscopy (FCS)

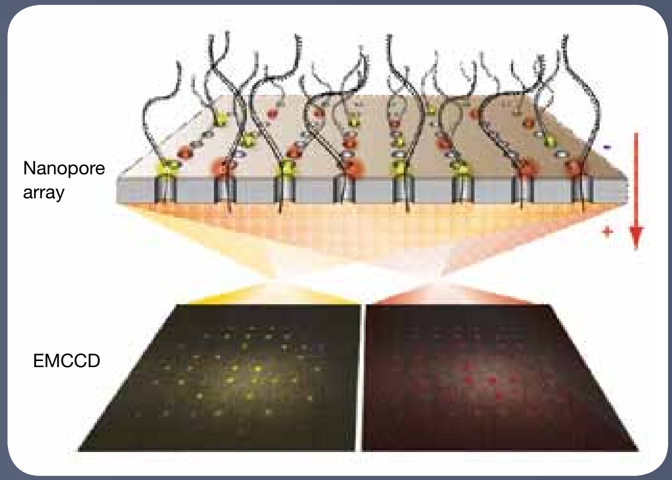
Features	Benefits
513 full frames/sec	Fast frame rates ideal for ion signaling microscopy and adaptive optics.
TE cooling to -100°C	Critical for elimination of Darkcurrent detection limit.
RealGain™	Absolute EMCCD gain selectable directly from a linear and quantitative scale.
OptAcquire™	Optimize the highly flexible iXon3 for different application requirements at the click of a button.
Count Convert	Quantitatively capture and view data in electrons or incident photons. Applied either in real time or post-processing, Count Convert does this important conversion for you.
iCam	Exposure time fast switching provides market leading acquisition efficiency.
UltraVac™	Critical for sustained vacuum integrity and to maintain unequalled cooling and QE performance, year after year.
Cropped sensor mode	Specialized acquisition mode for continuous imaging with fastest possible temporal resolution
Spurious Noise Filter	Intelligent algorithms to filter clock induced charge events from the background. Real time or post-processing.
Enhanced Photon Counting Modes	Intuitive single photon counting modes to overcome multiplicative noise. Real time or post-processing.
Superior Baseline Clamp and EM stability	Essential for quantitative accuracy of dynamic measurements.



Prof Amit Meller

Associate Professor of Biomedical Engineering and Physics, Boston University

"The use of the highly sensitive and ultra-fast back-illuminated iXon 860 EMCCD is central to our high-speed single molecule gene sequencing method, as we rely on fast multi-colour optical readout from many nanopores simultaneously."



Schematic diagram of the single molecule Optipore sequencing method, with an illustration of the 'enzyme-free' nanopore array through which DNA strands are electrophoretically drawn. iXon 860 cameras were used to capture two color sequencing data at 1000 frames/sec.

Courtesy of Prof. Amit Meller, Dept. of Biomedical Engineering & Physics, Boston University

ANDOR TECHNOLOGY

iXon3 885

Resolution, Speed and Sensitivity…

The 885 model benefits from the unique innovations and high-end performance specifications that characterized the iXon3 family as the industry's leading high-performance EMCCD range. The megapixel sensor format and 8 x 8 μm pixel size of the 885 presents an attractive combination of field of view and resolution, offering excellent Nyquist over-sampling for cell microscopy. A full resolution frame rate of 31 frames/sec is achievable; 60 frames/sec when 2 x 2 binned.

The absolute EM gain multiplication can be varied linearly from unity up to a thousand times directly via RealGain™, a true quantitative EM gain scale. Furthermore, the 885 is non-ageing and does not require routine EM gain recalibration. Extended red QE response is ideally matched to popular red-emitting fluorophores and for imaging of Bose Einstein Condensates using NIR probe laser.

The 885 has proven to be the camera of choice when it comes to affordably upgrading the performance of microscopy set-ups to open new application possibilities, redefining what you can expect from the 'workhorse camera' price category. Offering an impressive combination of fundamental high-end performance specifications and superb application versatility, the 885 sets a new benchmark for the camera performance that you can acquire within your budget.

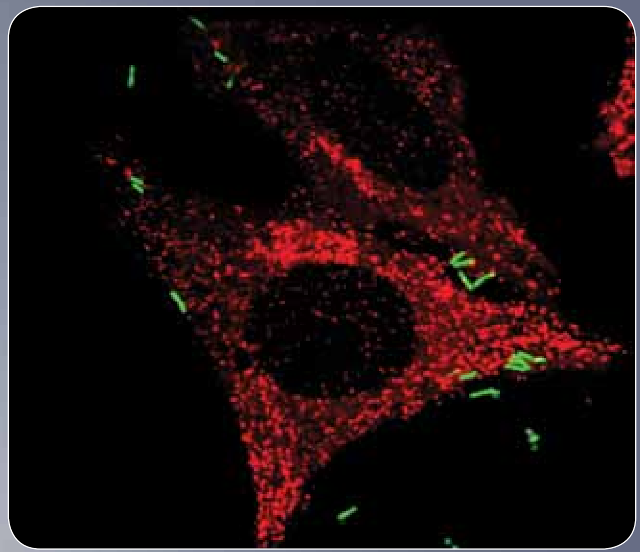
Key Specifications

Active Pixels	1004 x 1002
Pixel Size (W x H; μm)	8 x 8
Image Area (mm)	8 x 8
Active Area Pixel Well Depth (e^-, typical)	30,000
Max Readout Rate (MHz)	35
Frame Rate (frames per sec)	31.4 60.5 with 2 x 2 binning
Read Noise (e^-)	25 @ 35 MHz; < 1 with EM gain
QE max	65%

Applications of the 885 include:

- Super resolution (PALM, STORM)
- TIRF microscopy
- Spinning disk confocal microscopy
- Selective/single plane illumination microscopy (SPIM)
- Calcium flux
- FRET / FRAP
- Bose Einstein Condensation

Features	Benefits
35 MHz readout	31 frames/sec at full megapixel resolution; 60 frames/sec when 2 x 2 binned
8 x 8 μm pixel size (fully binnable)	Excellent balance of NyQuist resolution and photon collection.
RealGain™	Absolute EMCCD gain selectable directly from a linear and quantitative scale.
Negligible EM gain ageing	No requirement for gain recalibration.
OptAcquire™	Optimize the highly flexible iXon3 for different application requirements at the click of a button.
TE cooling to -95°C	Critical for elimination of Darkcurrent detection limit.
Cropped sensor mode	Specialized acquisition mode for continuous imaging with fastest possible temporal resolution
Spurious Noise Filter	Intelligent algorithms to filter clock induced charge events from the background. Real time or post-processing.
Extended red response	Significantly higher sensitivity to red-emitting dyes such as CY5, mCherry, dsRed and Alexa680. Bose Einstein Condensation in NIR.
iCam	Exposure time fast switching provides market leading acquisition efficiency.
UltraVac™	Critical for sustained vacuum integrity and to maintain unequalled cooling and QE performance, year after year.
Minimal Clock-Induced Charge	Unique pixel clocking parameters, yielding minimized spurious noise floor.
Enhanced Baseline Clamp and EM stability	Essential for quantitative accuracy of dynamic measurements.
Charge Purging	Anti-bloom structure drains unwanted charge/signal outside of the acquisition period



'With single photon sensitivity, great resolution, very fast frame rates, high 'virtual phase' QE, enhanced quantitative stability, exceptionally low Darkcurrent, UltraVac™ hermetic vacuum and non-ageing EM gain technology - all at an affordable price tag – the iXon3 885 deserves serious consideration as a worthy addition to your laboratory.'

GFP-tagged Listeria HeLa cells expressing the clathrin light chain tagged with fluorescent protein (Tomato). Full resolution, 100 ms exposure images, taken with the iXon+ 885K integrated into the Andor Revolution confocal spinning disk system.

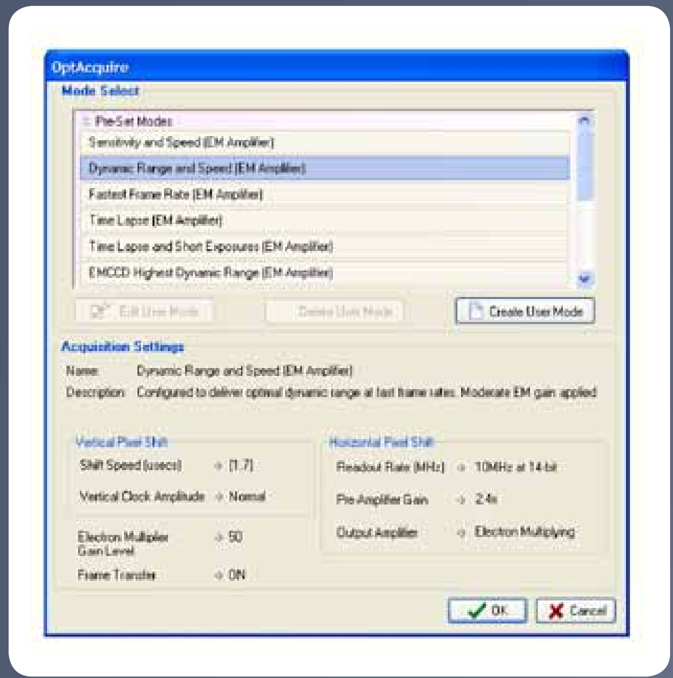
Courtesy of Dr. Esteban Veiga, Institute Pasteur, Paris.

iXon3 Performance and Innovations

OptAcquire – Flexibility need not be complicated…

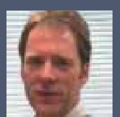
The control architecture of the iXon3 is extremely flexible, meaning the camera can be adapted and optimized for a wide variety of quantitative experimental requirements, ranging from single photon counting through to slower scan, 16-bit dynamic range measurements. However, we are starkly aware that optimizing EMCCD technology is far from trivial, with various set-up parameters influencing and trading off between different camera performance characteristics. We have developed OptAcquire, a unique interface allowing users to conveniently choose from a prvdetermined list of camera set-up configurations.

The user need only choose how they would like their camera to be optimized, e.g. for 'Sensitivity & Speed', 'Dynamic Range & Speed', 'Time Lapse'. Parameters such as EM gain value , vertical shift speed, vertical clock amplitude, pre-amp sensitivity and horizontal readout speed will then be optimized accordingly, 'behind the scenes'. Furthermore, the option exists to create additional user-defined configurations.



- Convenient 'one-click' set-up
- Opens the market leading flexibility of the iXon3 to less advanced users
- Optimize for range of experimental requirements
- Create additional user defined modes

See page 31 for 'OptAcquire' technical note



Prof Mark Nelson
Dept of Pharmacology
University of Vermont

Commenting on the use of the iXon 897 (as part of Andor Revolution XD spinning disk confocal system) for taking the first ever images of calcium "pulsars" from inositol 1,4,5-trisphosphate receptors (IP3Rs) in arterial walls.

"The signals are hard to pick out unless you know what you're looking for and have a good camera that is fast enough. The calcium events last for less than half a second. They also occur in a very small volume so you have to have both the spatial and temporal resolution to see them. The Andor system has exceptional sensitivity, critical to image very small signals that control the function of living cells."

Count Convert

iXon3 offers the capability to quantitatively capture and present data in units of electrons or photons, this important conversion is applied either in real time or as a post-conversion step.

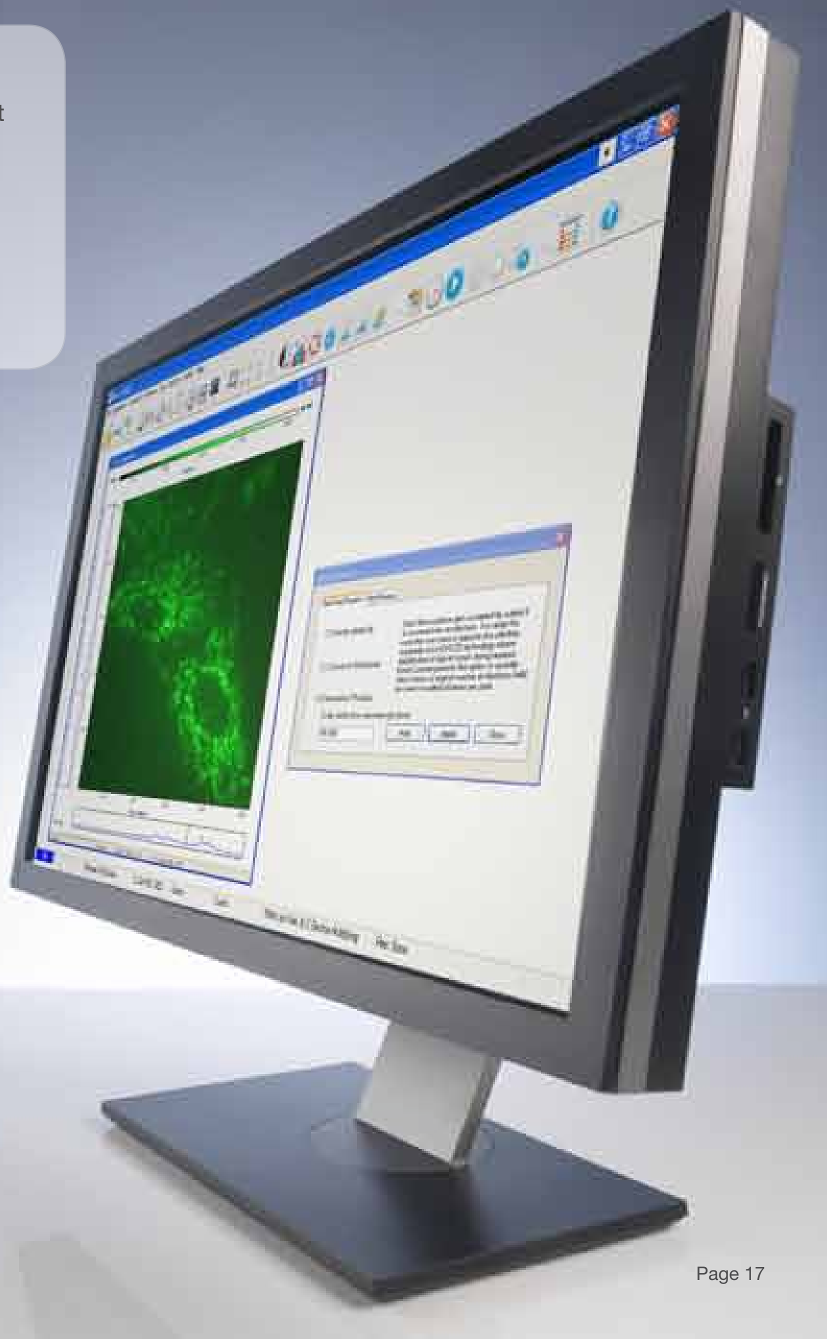
The standard way to present quantitative data in scientific detectors has been in units of 'counts', relating to the digitized steps of the Analogue to Digital Converter (ADC) used in the camera. Each Analogue to Digital Unit (ADU) relates to a precise number of 'photo-electrons' that were generated originally from photons striking and being captured by the detector pixel.

In the iXon3, this conversion factor is very accurately recorded within the camera. Knowing this value, alongside the EM gain (RealGain^{TM}) and baseline (bias) offset, facilitates back calculation from the signal in ADU counts per pixel to the signal in electrons per pixel. Furthermore, knowledge of the Quantum Efficiency (QE) and light throughput properties of the camera at each wavelength enables this process to be taken a step further, allowing the signal to be estimated in photons incident at each pixel, provided the spectral spread of the signal is not too broad.

The Count Convert functionality of the iXon3 provides the flexibility to acquire data in either electrons or incident photons, with negligible slow down in display rate. Furthermore, the option exists to record the original data in counts and perform this important conversion to either electrons or photons as a post-conversion step, while retaining the original data.

- Quantify data in electrons or incident photons
- Convenient estimate of sample signal intensity at the detector
- Real time or post-convert
- Reference between different samples, users and set-ups
- Meaningful signal relating to PALM/STORM localization accuracy

See page 30 for 'Count Convert' technical note

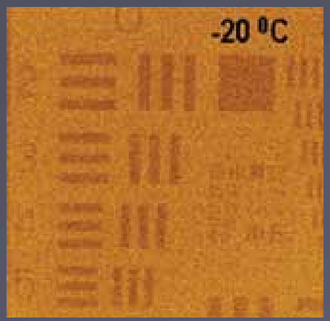


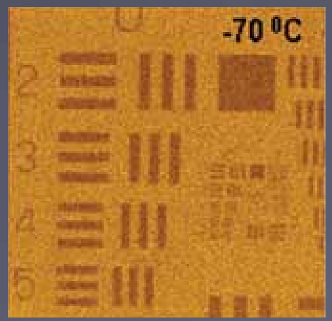
iXon3 Performance and Innovations

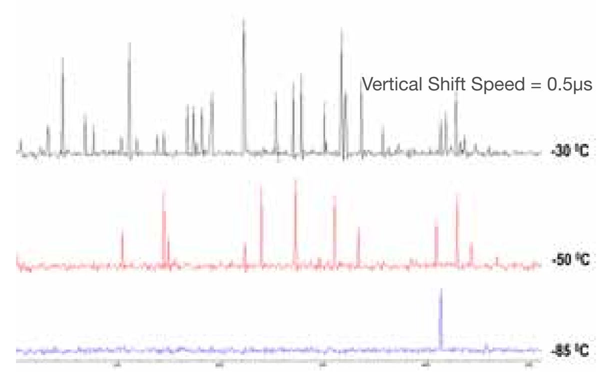
Deep Thermoelectric Cooling

Single thermal electrons are amplified by the EMCCD gain mechanism. Deep vacuum TE cooling is critical to optimize the sensitivity performance of EMCCD sensors, otherwise the raw sensitivity will be compromised, even under conditions of short exposures

- Cooling down to -100°C
- Lowest EM-amplified Darkcurrent
- Fewer pixel blemishes (hot pixels)
- Low power consumption vacuum cooling

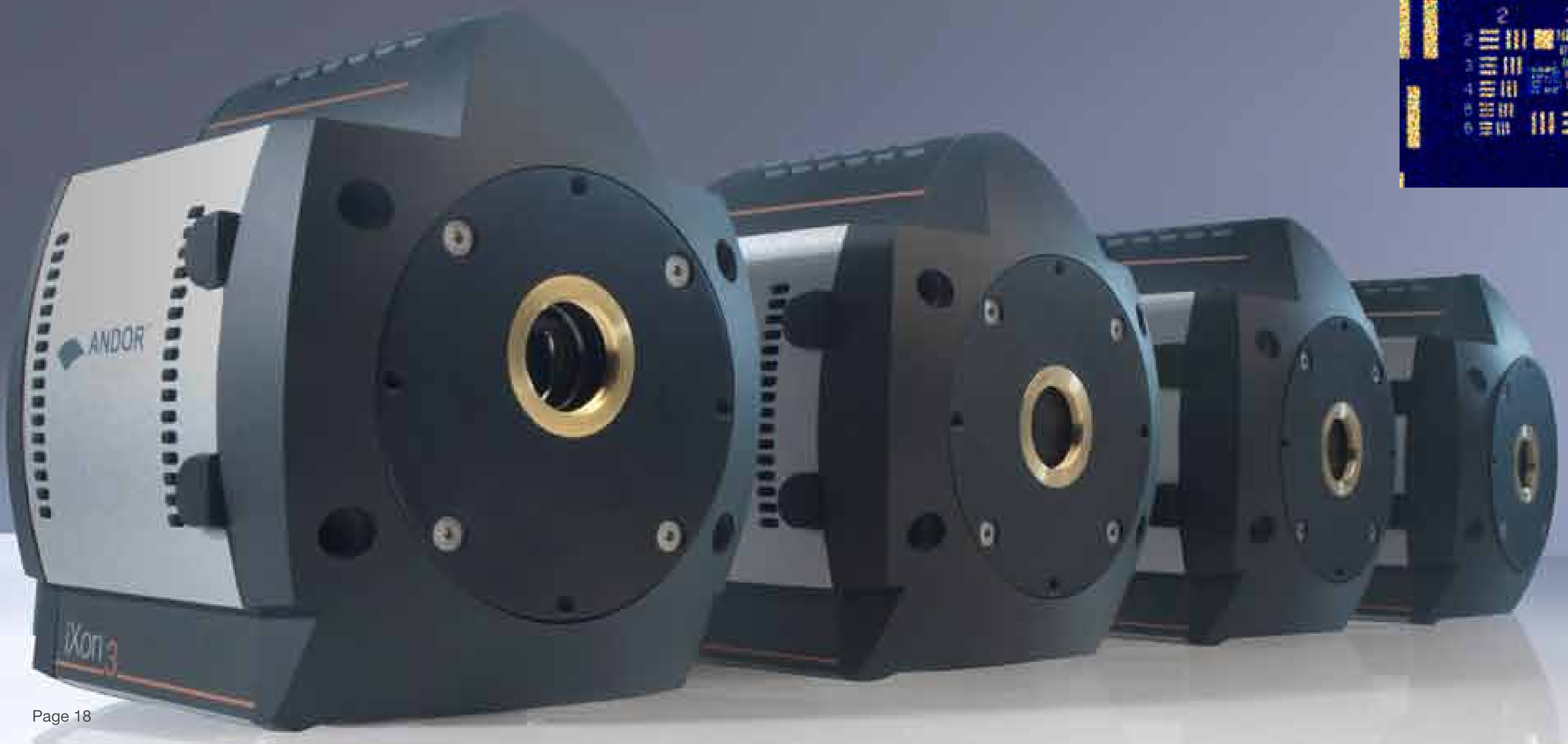






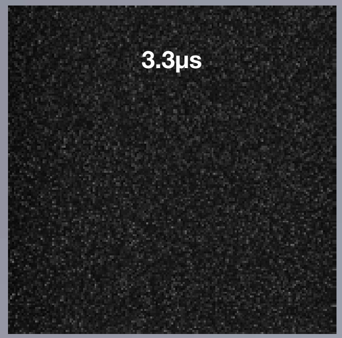
Line intensity profiles across a row of 512 pixels of the 897 model, taken from DARK images at three different cooling temperatures, 29 ms exposure times. The cleanest noise floor is clearly seen under conditions of deep cooling, even for such short exposure times.

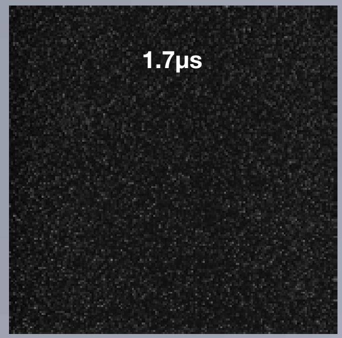
885 images from an extremely weak LED illuminating a resolution chart in a light-tight environment, taken in each case with x1000 EM gain, 15s exposure time at 2 different cooling temperatures. -20°C shows significantly poorer contrast (and hence resolution) due to elevated levels of EM-amplified Darkcurrent in the 'dark' regions. Note that with EM gain off, this signal level would be completely absorbed in the read noise floor.

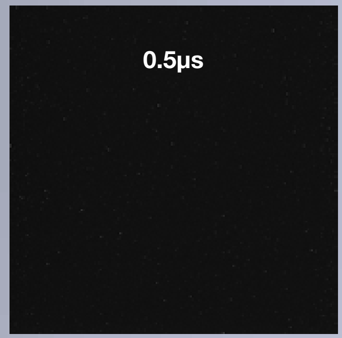


Minimized Clock-Induced Charge

After having minimized Darkcurrent through deep cooling, the remaining detection limit in back-illuminated EMCCDs is given by the number of Clock-Induced Charge noise events. Andor's industry-exclusive combination of high resolution clocking parameters and sub-microsecond clock speeds are fundamental to minimizing CIC, enabling truly 'high-end' EMCCD sensitivity to be claimed.





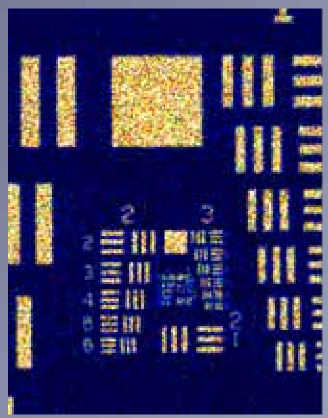


DARK IMAGES taken with the iXon 897 at x1000 gain at different vertical shift speeds, 29 ms exposure time. Cooling temperature was -85°C to ensure minimal Darkcurrent contribution.

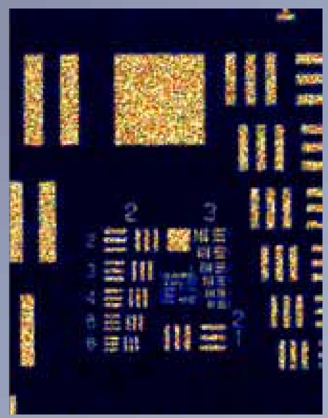
Spurious Noise Filter

It can still be desirable to optionally filter the remaining spurious noise (Clock-Induced Charge or photons) to give as 'black' a background as possible, eradicating any remaining such 'salt and pepper' noise. It is important to utilize noise selection and filter algorithms that are intelligent enough to accomplish this task without impacting the integrity of the signal itself. This is realized through the new Spurious Noise Filter (SNF) functionality of iXon3, which offers the user a choice of advanced algorithms to try. SNF can be applied either in real time or as a post-processing step.

Before SNF



After SNF



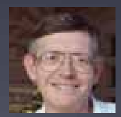
Before and after application of the iXon3 Spurious Noise Filter

iXon3 Performance and Innovations

Rapid Frame Rates

Maximum frame rate performance in EMCCDs is a function of two parameters; (1) Pixel Readout Speed (horizontal); (2) Vertical Clock speed. The former dictates how rapidly charge is pushed horizontally through the EM gain register and the remaining readout electronics, while the latter dictates the speed at which charge is vertically shifted down through both the exposed sensor area and masked frame transfer area of the chip. iXon3 industry fastest vertical shift speeds result in faster frame rates and reduced smearing, significantly faster under commonly employed conditions of sub-array/binning.

- Fastest Full Frame Rates
- Up to 60% faster than competing EMCCDs under conditions of sub-array/binning
- Minimized smearing



Prof W.E. Moerner
Dept of Chemistry
Stanford University

"As the localization precision of our super-resolution technique improves at a rate of one over the square root of the number of photons detected, it was essential to use a camera that allowed us to detect every possible photon from each single molecule. However, the speed of imaging is also important. Since we need to acquire multiple images for each reconstruction, it is always best to record the images as fast as possible."

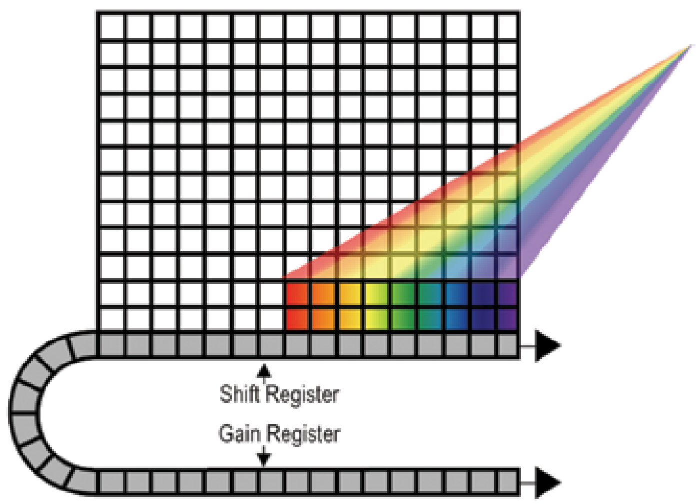
See page 36 for 'Maximising frame rate' technical note

iXon3 with OptoMask enabling faster frame rates in crop sensor aquisition mode

Pushing Frame Rates with Cropped Sensor Mode

Through this acquisition mode, significant increases in frame rates are accomplished by 'fooling' the sensor into thinking it smaller than it actually is. In standard sub-array/ ROI readout mode each frame still carries the time overhead to readout all pixels to the left and right of the selected area and to vertically shift all pixels above and below the selected area. The charge from these pixels is then dumped before an image is sent from camera to PC. In cropped sensor mode, the number of pixel readout steps outside of that required to read out the requested sub-array is significantly reduced, resulting in markedly higher frame rates.

However, this mode requires that light is not allowed to fall onto the area of the sensor outside of the defined active sub-area. In optical microscopy, this can be realized in conjunction with the new **OptoMask** accessory, which inserts easily between the microscope output and the camera. Using the OptoMask, a sub-array can be readily defined through positioning of the masking blades, and a cropped area matched to this in software.



Cropped Sensor Mode. The active imaging area of the sensor is defined in a way that only a small section of the entire chip is used for imaging. The remaining area has to be optically masked to prevent light leakage and charge spill-over that would compromise the signal from the imaging area. By cropping the sensor, one achieves faster frame rates because the temporal resolution will be dictated only by the time it requires to read out the small section of the sensor.

	Array Size						
Binning	**256 x 256**	**128 x 128**	**64 x 64**	**32 x 32**	**512 x 100**	**512 x 32**	**512 x 1**
1 x 1	69	395	988	2577	176	538	7980
2 x 2	136	743	1764	5400	342	1025	-
4 x 4	260	1327	2902	6068	649	1877	-
8 x 8	483	2184	4285	7375	1268	3209	-

Frame rates achievable by the iXon3 897 in cropped sensor mode

	Array Size					
Binning	**502 x 501**	**251 x 250**	**125 x 125**	**75 x 75**	**32 x 32**	**1004 x 1**
1 x 1	62	231	465	763	1704	13812
2 x 2	118	426	859	1401	2976	-
4 x 4	213	735	1474	2404	4746	-
8 x 8	361	1144	2341	3637	6757	-

Frame rates achievable by the iXon3 885 in cropped sensor mode

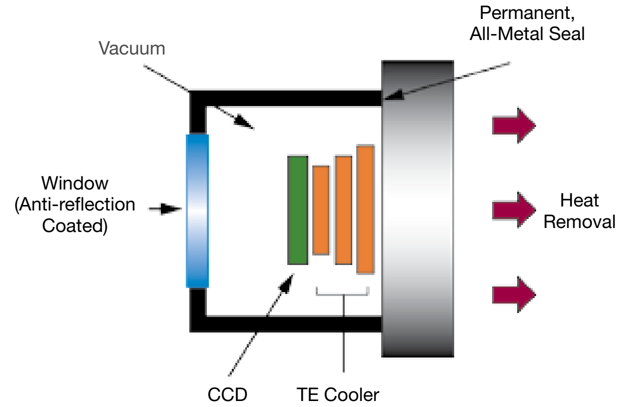
iXon3 Performance and Innovations

UltraVac™ Permanent Vacuum Head

It is important that a back-illuminated sensor is housed in a hermetically sealed permanent vacuum head with minimized outgassing, otherwise both cooling performance and the sensor QE will steadily degrade. It is this compelling reason that drove Andor to develop UltraVac.

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. UltraVac also enables use of only one input window, improving photon-throughput by 8%.

- No QE degradation
- Sustained deep TE cooling
- No maintainence/re-pumping
- One input window
- No condensation



Schematic of a Permanent Vacuum Head

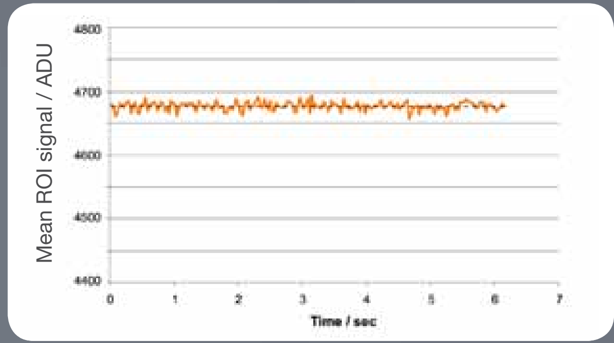
7 Year Vacuum Warranty -

Unlike other vacuum EMCCDs on the market, the iXon family has now been shipping with a vacuum enclosed sensor for almost 10 years, with statistical data that substantiates our extremely robust vacuum claims. With the iXon3, Andor are proud to offer an extended 7 year warranty on the vacuum enclosure as standard.

Superior Quantitative Stability

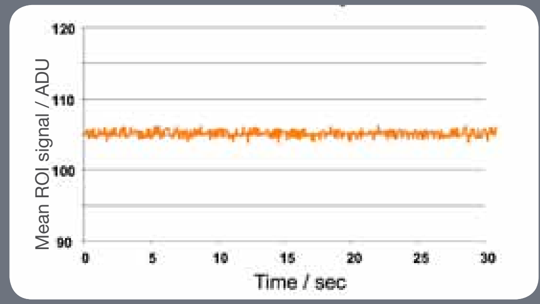
The iXon3 is well regulated in terms of both Baseline (bias offset) rigidity and superior EM gain stability, lending for enhanced quantitative reliability throughout and between measurements.

EM gain Stability

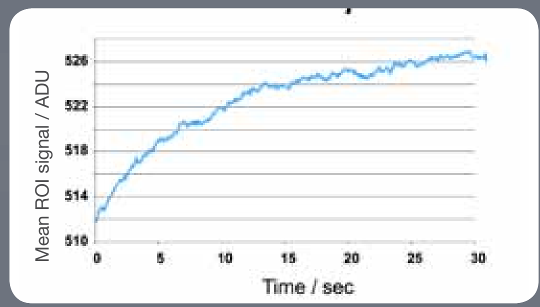


EM gain Stability in the iXon3 897: 200 frame kinetic series; frame transfer (overlapped) acquisition; 30 ms exposure time; x300 EM gain

Baseline Clamp ON



Baseline Clamp OFF



iXon3 Baseline Clamp (bias stability) in operation

RealGain™, Anti-Ageing and EMCAL™

In early 2006, Andor once again raised the bar by introducing some significant new technology innovations. These particular pioneering steps, were to set new high standards in quantitative EMCCD usage and general EMCCD longevity expectations, which others in the industry are now adopting.

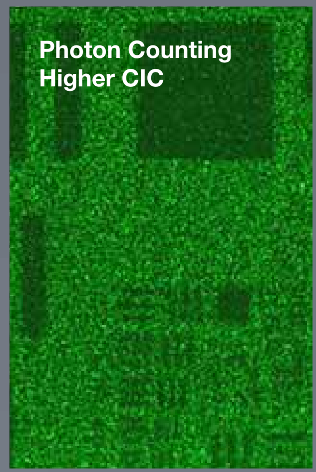
- **RealGain™** – Select absolute EM gain direct from a linear and directly quantitative software scale, x1 to x1000. The EM gain you ask for is the EM gain you get.
- **Anti-Ageing** – Internally configured to significantly inhibit saturation-induced decay of EM gain.
- **EMCAL™** - Innovative user-initiated self-recalibration of EM gain, utilizing a patented method of automated EM gain assessment and Andor's unique Linear and Real Quantitative gain implementation.
- **Temperature Compensated** - Calibration holds across all cooling temperatures. No need to recalibrate on each use in multi-user laboratories and facilities.

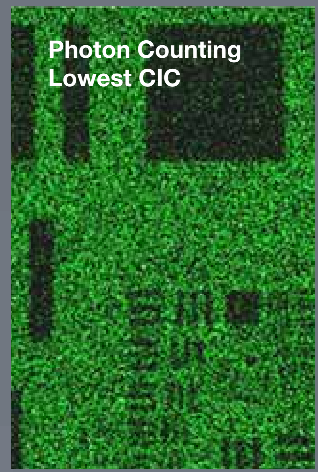
Enhanced Photon Counting

To successfully photon count with EMCCDs, there has to be a significantly higher probability of seeing a 'photon spike' than seeing a Darkcurrent/CIC 'noise spike'.
The iXon3 897 combines deepest thermoelectric cooling and low CIC performance, yielding market leading photon counting performance and higher contrast images.

Real-time and post process photon counting…

The advanced photon counting modes of the iXon3 allow for both real time and post-process photon counting.
The latter offers the flexibility to 'trial and error' photon count a pre-recorded kinetic series, trading-off temporal resolution vs SNR.





iXon3 Software Solutions

Andor Solis

Solis is a ready to run Windows package with rich functionality for data acquisition and image analysis/processing. Available on 32-bit and 64-bit versions of Windows (XP, Vista and 7).

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

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.

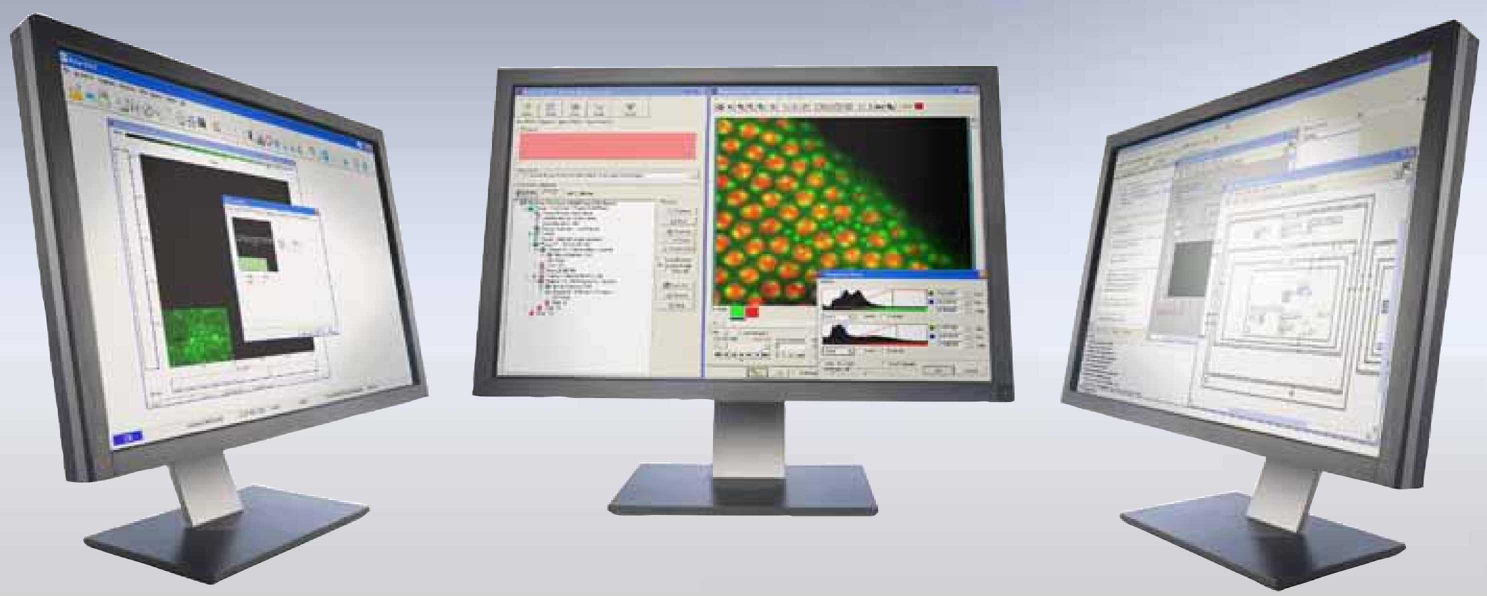
Andor SDK

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.

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.

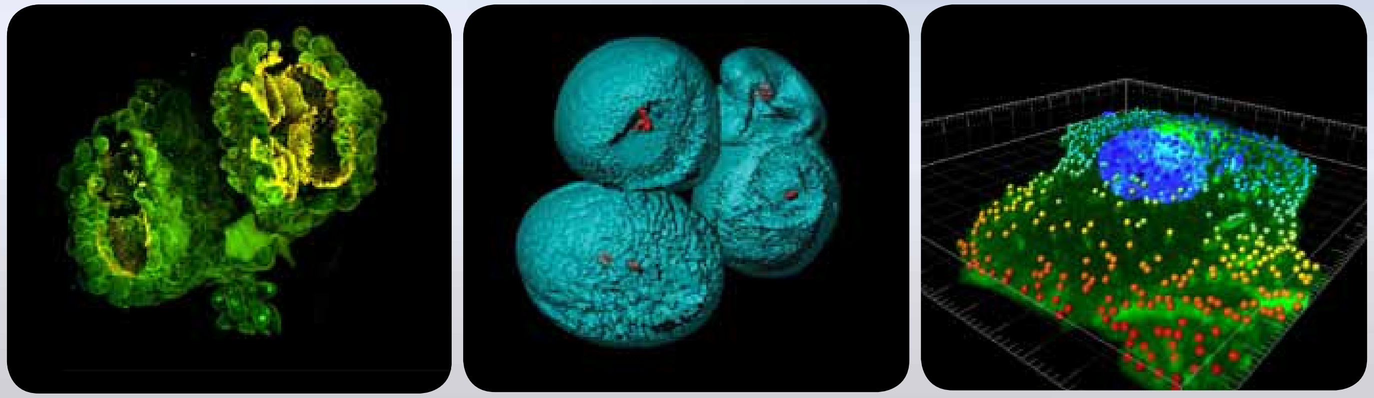


Third party software compatibility

Drivers are available so that the iXon3 range can be operated through a large variety of third party imaging packages, including:

- Metamorph (Molecular Devices Corporation)
- NIS Elements (Nikon)
- LAS (Leica)
- Xcellence (Olympus)
- Image Pro (Media Cybernetics)
- MicroManager (University of California, SF)
- Till Photonics Live Acquisition (Till Photonics)
- Imaging Workbench (Indec)
- WinFluor (University of Strathclyde)
- Maxim DL (Diffraction Limited)
- LabView (National Instruments)
- Matlab (MathWorks)

See Andor web site for detail: http://www.andor.com/software/software_support/



3D images rendered by Imaris

ANDOR™ TECHNOLOGY

The Andor Imaging Range

Have You Found What You Are Looking For? As an alternative to the iXon3, Andor offers an extensive portfolio of performance low light imaging camera technologies.

LucaEM - 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, 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

iXon3 - high performance EMCCD platform

- Single photon sensitive
- -100°C cooling
- Fastest frame rates
- Flexible yet intuitive
- Quantify in Electrons or Photons

Clara - high-performance interline CCD platform

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

Check out Andor's NEW sCMOS technology.

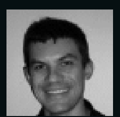
Simultaneously offering, ultra-sensitivity, high speed, high-resolution, large field of view & high dynamic range.

Technical Notes

Andor's high-performance EMCCD cameras have been the technology favourite of the vast majority of EMCCD-enabled laboratories across the globe.

The following section is dedicated to providing a greater depth of understanding of the performance innovations underlying the iXon3 family of high-end EMCCD cameras, outlining the core technical reasons why Andor are still very much considered the EMCCD industry leaders, notably so in the key areas of sensitivity, speed, stability, longevity, quality and accessibility.

- **Count Convert - Quantifying data in Electrons and Photons**
- **OptAcquire™ – Flexibility need not be complicated**
- **Deep Vacuum TE Cooling and Darkcurrent Elimination**
- **Minimizing Clock Induced Charge - finesse charge clocking**
- **UltraVac™ permanent vacuum head and performance longevity**
- **Maximizing frame rate performance in EMCCDs**
- **Quantitative Stability in EMCCDs**
- **RealGain™, Anti-Ageing and EMCAL™**
- **Photon Counting in EMCCDs**
- **Fast Kinetics Mode**
- **Dynamic Range & EMCCDs – Uncovering the Facts**
- **Making Sense of Sensitivity**
- **iXon3 Trigger Modes**



Dr Roberto Zoncu
School of Medicine
Yale University

Commenting on the use of the iXon 897 model in imaging fluorescently labelled endosomes in living cells using TIRF illumination.

"Because the vesicles are very small, the light sensitivity of the camera has to be as high as possible. This camera is the most sensitive available in our experience. It's better than any competitor around."

Technical Note

Count Convert - Quantifying Data in Electrons and Photons

One of the distinctive features of the iXon3 is the capability to quantitatively capture and present data in units of electrons or photons, the conversion applied either in real time or as a post-conversion step.

Photons that are incident on pixels of an array detector are captured and converted to electrons. During a given exposure time, the signal in electrons that is collected in each pixel is proportional to the signal intensity. In EMCCDs, the signal in electrons is further multiplied in the EM gain register. The average multiplication factor is selected in software from the RealGain™ scale.

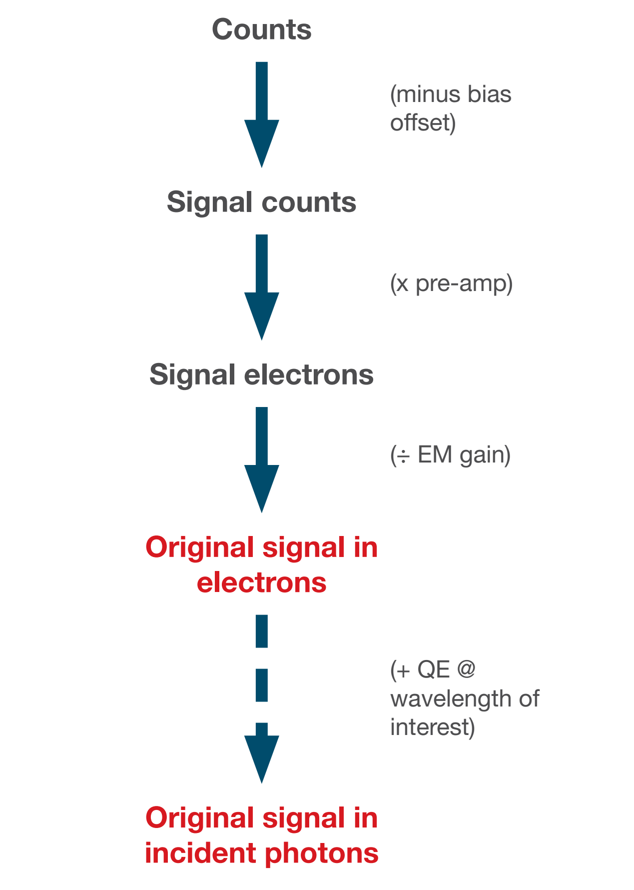
It can be desirable to directly quantify signal intensity either in terms of electrons per pixel or in terms of incident photons per pixel. However, during the readout process, array detectors must first convert the signal in electrons (the multiplied signal in the case of EMCCDs) into a voltage, which is then digitized by an Analogue to Digital Converter (ADC). Each Analogue to Digital Unit (ADU) is presented as a 'count' in the signal intensity scale, each count corresponding to an exact number of electrons. Furthermore, the signal value in counts will sit on top of an electronic bias offset value. In the iXon3 this 'baseline' can be clamped at 100 counts.

Therefore, in order to back calculate to the original signal in electrons, the electron to ADU conversion factor must be very accurately stored by the camera (which varies depending on the pre-amplifier gain selection chosen through software). Calculation of the signal as absolute electrons also requires knowledge of the bias offset and the EM gain. The calculation path is shown in Fig 1 (right).

Furthermore, knowledge of the Quantum Efficiency (QE) at each wavelength and light throughput properties of the camera window enables this process to be taken a step further, allowing the signal to be estimated in photons incident at each pixel. For this step, the user must input the signal wavelength. In fluorescence microscopy for example, this would correspond to the central wavelength defined by a narrow band emission filter matched to the fluorophore of interest. If the spectral coverage of the signal on the detector is too broad, such that the QE curve varies significantly throughout this range, then the accuracy of the incident photon estimation would be compromised.

The Count Convert functionality of the iXon3 provides the flexibility to acquire data in either electrons or incident photons, with negligible slow down in display rate. Furthermore, the option exists to record the original data in counts and perform this important conversion to either electrons or photons as a post-conversion step, while retaining the original data.

Fig 1



- Quantify data in electrons or incident photons
- Convenient estimate of sample signal intensity at the detector
- Real time or post-convert
- Reference between different samples, users and set-ups
- Meaningful signal relating to PALM/STORM localization accuracy

Technical Note

OptAcquire – Flexibility need not be complicated

OptAcquire is a unique control interface, whereby a user can conveniently choose from a pre-determined list of set-up configurations, each designed to optimize the camera for different experimental acquisition types, thus removing complexity from the extremely adaptable control architecture of the iXon3.

The control architecture of the iXon3 is extremely tunable, meaning the camera can be adapted and optimized for a wide variety of quantitative experimental requirements. The range from fast single photon counting through to slower scan, 16-bit dynamic range measurements. However, successfully optimizing EMCCD technology is not a trivial exercise, with various set-up parameters directly influencing different camera performance characteristics. OptAcquire has been designed as a unique interface whereby a user can choose from a pre-determined list of nine camera set-up configurations. A variety of set-up parameters are balanced behind the scenes through the OptAcquire menu. Furthermore, advanced users may wish to create their own additional OptAcquire modes to aid future set-up convenience.

iXon3 control parameters include:

- **EM gain** – this parameter has a direct bearing on both sensitivity and dynamic range.
- **Vertical clock speed** – flexibility in this parameter is critical to optimizing the camera for lowest noise, fastest speed, minimal frame transfer smear or maximum pixel well depth.
- **Vertical Clock Amplitude** – can be employed to help 'over-clock' the sensor to achieve faster frame rates and can also be used to reduce charge leakage into the image area when there is saturated signal in the frame transfer storage area (e.g. when combining very short exposure with a slow readout speed).
- **Horizontal readout speed** – ranging between faster frame rates and best dynamic range.
- **Pre-amplifier gain** – trading off reduced digitization noise versus accessing full pixel well depth.
- **EM / Conventional amplifier** – to choose between ultrasensitive EMCCD operation or traditional high dynamic range CCD operation, the latter recommended for relatively 'brighter' signals or when it is possible to apply long exposures to overcome read noise floor.
- **Frame Transfer (overlap)** – overlap readout is used to achieve 100% duty cycle, ideal for fastest frame rate measurements without switching exposure time between frames. This mode should be deselected for time-lapse experiments.

Pre-defined OptAcquire modes include:

Sensitivity & Speed (EM Amplifier)	Optimized for capturing weak signal at fast frame rates, with single photon sensitivity. Suited to the majority of EMCCD applications.
Dynamic Range & Speed (EM Amplifier)	Configured to deliver optimal dynamic range at fast frame rates. Moderate EM gain applied.
Fastest Frame Rate (EM Amplifier)	For when it's all about speed. Optimized for absolute fastest frame rates of the camera. Especially effective when combined with sub-array/binning selections.
Time Lapse (EM Amplifier)	Configured to capture low light images with time intervals between exposures. Overlap ('frame transfer') readout is deactivated.
Time Lapse & Short Exposures (EM Amplifier)	Configured to minimize vertical smear when using exposure times < 3 ms.
EMCCD Highest Dynamic Range (EM Amplifier)	Combines EMCCD low light detection with the absolute highest dynamic range that the camera can deliver. Since this requires slower readout, frame rate is sacrificed.
CCD Highest Dynamic Range (Conventional Amplifier)	Optimized for slow scan CCD detection with highest available dynamic range. Recommended for brighter signals or when it is possible to apply long exposures to overcome noise floor.
Photon Counting	Configuration recommended for photon counting with individual exposures < 10 sec.
Photon Counting with Long Exposures (> 10 sec)	Configuration recommended for photon counting with individual exposures > 10 sec.

Technical Note

Deep Vacuum TE Cooling and Darkcurrent Elimination

On harnessing EMCCD technology, Darkcurrent is an absolutely critical parameter to minimize, more so than in a standard sensitive CCD. The reason for this is that thermally generated electrons are amplified by EMCCD just as photon-generated electrons (signal) are amplified.

For optimal sensitivity in EMCCDs, thermoelectric cooling of the sensor must be deep enough that this noise source is virtually eliminated. It is important to recognize that this point applies very much to short exposure operation also; the sensor readout process alone results in significant Darkcurrent production if left untreated.

The above point is demonstrated by the following simple test:

- Figure 1(A) shows two dark images taken with a 512 x 512 back-illuminated sensor (from E2V) at two different cooling temperatures; –80ºC and –30ºC.
- All are in frame transfer mode at the maximum frame rate of 34 full frames per second, i.e. ~ 29 ms exposure.
- EM gain is set at X1000; at such an EM gain setting the vast majority of Darkcurrent and CIC events will be exposed.

The speckled 'salt and pepper' noise pattern that is quite obvious in the -30ºC condition is due almost entirely to amplified Darkcurrent electrons. Note that each of these images has ALREADY been optimized for minimal CIC, so only the effect of cooling is being demonstrated. If CIC had not been minimized, the –30ºC situation would have appeared bleaker still, with an extremely dense 'EM-amplified' noise floor.

As an alternative view Figure 1(B) shows a line intensity plot from a single row from each image; the readout noise is visible as the fuzzy baseline of each trace with the Darkcurrent 'spikes' sticking out of it; this is just what we expect. These spikes, or background events, are what set the remaining detection limit of the camera, not the readout noise.

It is instantly clear that cooling is beneficial. The performance at –80ºC is by far the best and there is no way that this level of low noise detection limit can be achieved at –30ºC, under any circumstances. It is important to note that even with a short exposure time, the -30ºC background events are predominantly from Darkcurrent. As such ,we are clearly better off with much deeper cooling, regardless of exposure time.

Single thermal electrons are amplified by the EMCCD gain mechanism. Deep vacuum TE cooling is critical to optimize the sensitivity performance of back-illuminated EMCCD sensors, otherwise the raw sensitivity will be compromised, even under conditions of short exposures.

Deep TE cooling can also make a tangible difference to signal to noise ratio for longer exposure conditions. Figure 2 shows extremely low light images, recorded at -70ºC and -95ºC with the iXon3 888 on a light tight imaging chamber using weak LED illumination through pinholes. The light levels used are typical of an experiment involving imaging of weak luminescence signal. The photon flux is so low, that a two minute exposure is required in order to visualize the pinhole signals. It is clear that from the significantly improved SNR, and therefore contrast, at -95ºC cooling, that such extremely low cooling temperatures are recommended for longer exposure acquisitions.

1 MHz EM mode readout limits background of long exposures

When back-illuminated EMCCD sensors from E2V are read out at faster horizontal speeds, such as 10 MHz, the EM-amplified background becomes notably higher over longer exposures. A slower 1 MHz readout mode is required to minimize background under these conditions.

This is not so apparent for shorter exposure times (e.g. < 1 sec) but when you go towards multiple secs to mins it is certainly apparent. Some example dark noise images are show in Fig 1 and Fig 2 for two different cooling temperatures, taken with the iXon3 888 camera at x1000 EM gain.

This basic trend can indeed be considered an oddity, because you would intuitively imagine that the readout speed should not influence the noise background that is built up during the exposure, but nevertheless it is fact.

This means that we recommend using a readout speed of 1 MHz for longer exposure times. This is not really disadvantageous since you have already compromised the frame rate with the longer exposure time. For shorter exposure times/faster frame rate measurements, it is still best to opt for 10 MHz readout speed because Darkcurrent (being time dependent) is lower and CIC is the prominent contributor. Since CIC is elevated by use of slower readout parameters, then are better using 10 MHz. Also, you tend to need the faster readout speeds to achieve faster frame rates.

Recommended Pixel Readout Speed	Cooling Temperature	
	-70ºC	-100ºC
10 MHz	< 1 sec	< 10 sec
1 MHz	> 1 sec	> 10 sec

A slow readout 1 MHz mode is required to minimize EM-amplified background under longer exposure acquisition conditions.

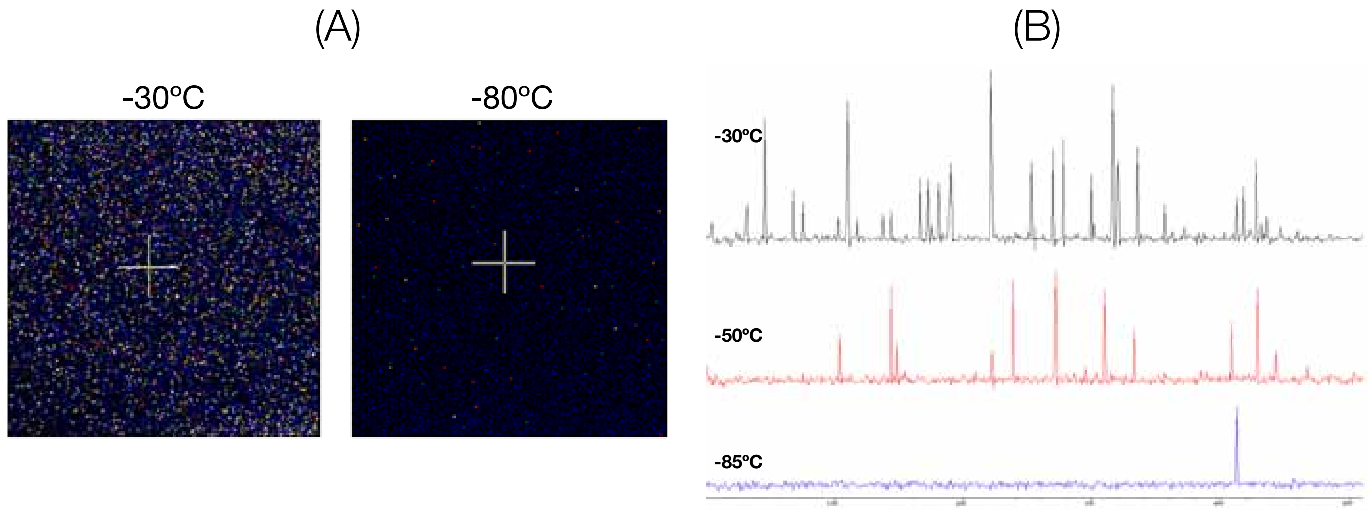


Figure 1: (A) shows DARK IMAGES taken at x1000 gain at different cooling temperatures, 29 ms exposure time. Vertical shift speed was 0.5 µs/row to ensure minimal CIC. (B) shows typical line intensity profiles across a row of 512 pixels, taken from such dark images at three different cooling temperatures. The cleanest noise floor is clearly seen under conditions of deep cooling, even for such short exposure times.

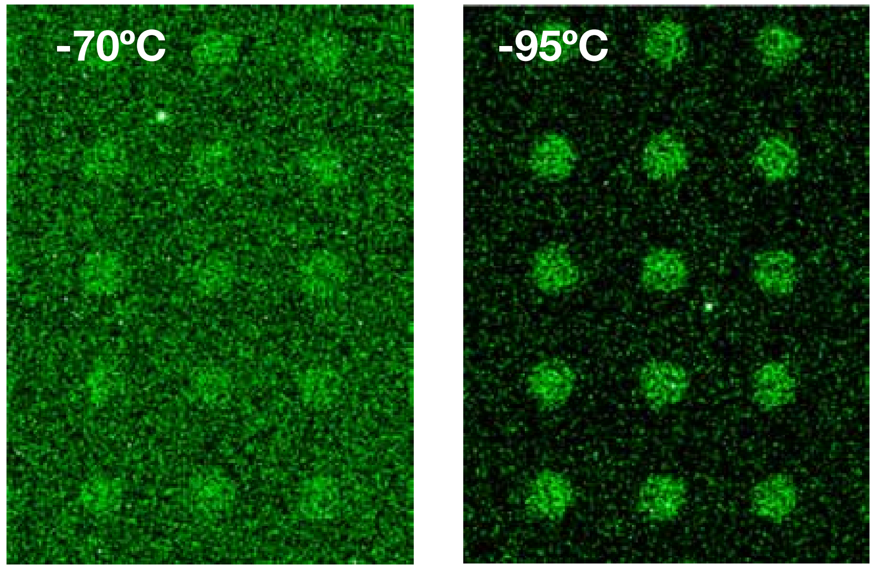


Figure 2: Images of extremely weak LED signal (signal intensity typical of weak luminescence experiments) acquired with iXon3 888 at cooling temperatures -70ºC and -95ºC (water cooling to achieve latter), 120 sec exposure times, sub-region show. The need to push to such deeper cooling temperatures can be readily observed under such extreme low light conditions.

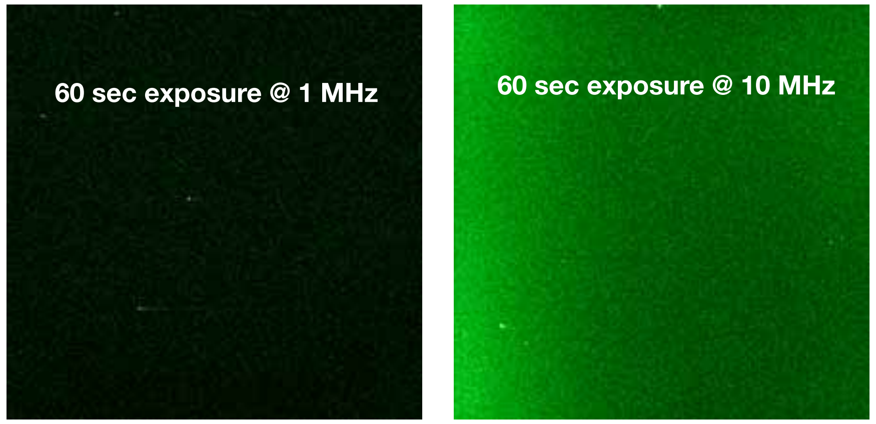


Figure 3: Dark noise background at 1 MHz and 10 MHz readout speeds;
60 sec exposure and -70ºC cooling.

Technical Note

Minimizing Clock Induced Charge - Finesse Charge Clocking

Clock Induced Charge (CIC) can be considered the remaining detection limit in EMCCD and must be minimized. Careful and rapid clocking are crucial contributors to achieving this.

The remaining limiting factor for EMCCD sensitivity, provided Darkcurrent has already been minimized through effective TE cooling, is a spurious noise source called Clock Induced Charge (CIC). This form of electron generation can occur even under normal clocking in any CCD, but when properly optimized the rate of occurrence is very small; i.e. CIC occurrence can be minimized down to the order of 1 in 200 pixels. For an EMCCD at high EM gain, such individual electrons can be seen as sharp spikes in the image and any CIC will become visible.

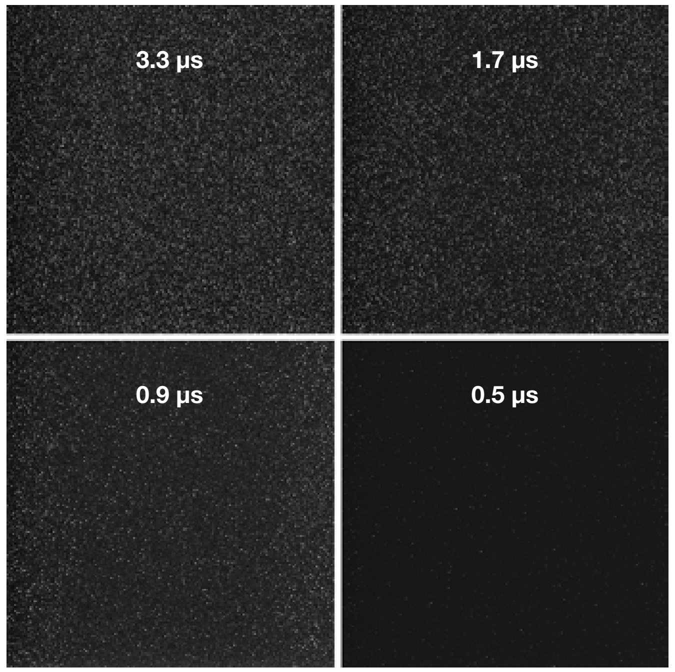
For several years, Andor have had very fine nanosecond resolution over EMCCD clockings and are well aware of the important parameters for reducing CIC, such as fine temporal control over the clock edges. Furthermore, there is a well-established direct link between pushing vertical clocks faster and achieving lower CIC. This clock speed dependence is amply illustrated in the following basic test:

Figure 1 shows a series of dark images and corresponding intensity profiles (across a random row from each image), using 30 ms exposures and EM gain of x1000. The cooling temperature in each case was set at -85ºC to ensure virtual elimination of Darkcurrent contribution, so any amplified noise spikes are derived primarily from the remaining CIC.

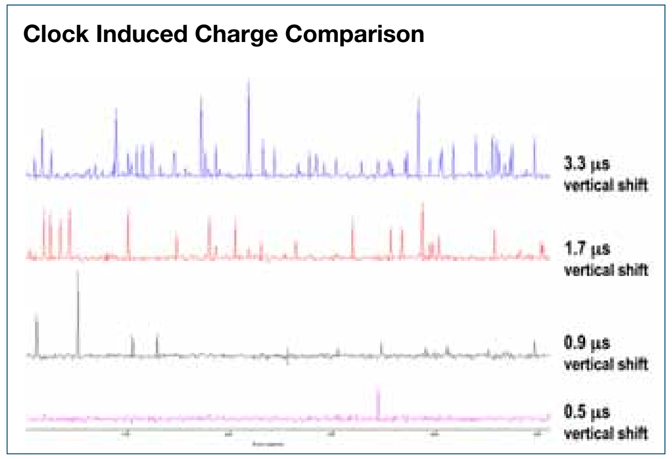
You can see that the vertical clock speeds have a significant impact on further reducing CIC, with the speed of 0.5 µs/shift offering the lowest CIC and therefore highest EMCCD sensitivity. This iXon3 clock speed performance is faster than other EMCCDs in the industry.

After having minimized Darkcurrent through < -80ºC cooling, the remaining detection limit in back-illuminated EMCCDs is given by the number of Clock-Induced Charge noise events.

Andor's industry-exclusive combination of high resolution clocking parameters and sub-microsecond clock speeds are fundamental to minimizing CIC, enabling truly 'high-end' EMCCD sensitivity to be claimed.



(A)



(B)

Figure 1 – (A) shows DARK IMAGES taken at x1000 gain at different vertical shift speeds, 29 ms exposure time. Cooling temperature was -85ºC to ensure minimal Darkcurrent contribution.
(B) shows typical line intensity profiles across a row of 512 pixels, taken from such dark images at three different vertical shift speeds. The cleanest noise floor is clearly seen under conditions of faster vertical shifts, an exclusive Andor capability.

Technical Note

UltraVac™ Permanent Vacuum Head and Performance Longevity

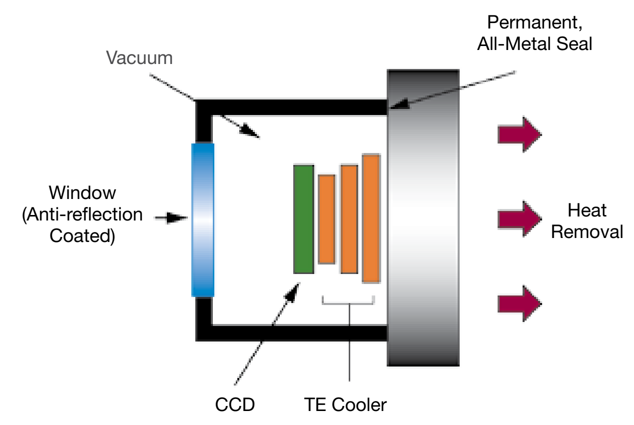
Andor's UltraVac™ vacuum process was designed not only to facilitate deep TE cooling, but also to provide absolute protection of the exposed sensor.

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 outgassed contaminants, the Quantum Efficiency of a back-illuminated EMCCD will decline proportionally. Furthermore, the sensor can fail if excessive condensation forms.

It was these compelling reasons that drove Andor to develop permanent vacuum technology more than 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.

A back-illuminated EMCCD sensor must be housed in a hermetically sealed vacuum head with minimized outgassing, otherwise both cooling performance and the sensor QE itself will degrade.



Schematic of a Permanent Vacuum Head

Figure 1

Benefits of Permanent Vacuum Head:

- Sustained vacuum performance over many years operation – proprietary process to minimize outgassing. 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 more than 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 outgassing 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.

Technical Note

Maximizing Frame Rate Performance in EMCCDs

The iXon3 is capable of market leading frame rate performance, achieved from ‘over-clocking’ the vertical shifts during readout. Furthermore, fastest possible continuous sub-region frame rates can be attained using the new ‘cropped sensor’ mode.

Part 1. Fastest vertical shifts for fastest speeds

Maximum frame rate performance in EMCCDs is a function of two parameters; (1) Pixel Readout Speed (horizontal); (2) Vertical Clock Speed. The former dictates how rapidly charge is pushed horizontally through the EM gain register and the remaining readout electronics, while the latter dictates the speed at which charge is vertically shifted down through both the exposed sensor area and masked frame transfer area of the chip. Significant advantages are gained through optimizing the camera electronics to enable vertical shifts to be speeded up. Andor’s iXon3 vertical shift speeds are the industry fastest.

Table 1 shows frame rates available from the iXon3 897 frame rates versus those of two other competing EMCCD brands with the same sensor. It is clear that while iXon3 still delivers the fastest full frame rate (512 x 512; no binning), the beneficial effect of ‘overclocking’ the iXon3 vertical shift speeds are most apparent under conditions of sub-array and/or binning, e.g. ~60% faster with 128 x 128 sub-array and 2 x 2 binning. This is particularly significant as many EMCCD applications require routine use of sub-arrays to examine a smaller active region of the sample under faster speeds (also used to reduce the file size of a large kinetic series of images).

	iXon3 897	Competing EMCCD 1	Competing EMCCD 2
512 x 512 / 1 x 1 binning	34.5	31.9	33.7
256 x 256 / 2 x 2 binning	132	107	124.4
128 x 128 / 2 x 2 binning	248	172	224
128 x 128 / 4 x 4 binning	439	254	376

Table 1 - Frame rate comparisons under various combinations of sub-array / binning: iXon3 897 vs. principal competing EMCCDs utilizing the same 512 x 512 back-illuminated sensor.

iXon3 industry-fastest vertical shift speeds result in faster frame rates and reduced smearing, markedly so under commonly employed conditions of sub-array/binning. ‘Overclocking’ the vertical shifts also reduces ‘clock induced charge’ spurious noise.

Part 2. Pushing Frame Rates with Cropped Sensor Mode

The iXon3 offers Cropped Sensor Mode, which carries the following advantages:

- Specialized readout mode for achieving very fast frame rates (sub-millisecond exposures) from ‘standard’ cameras.
- Continuous rapid spooling of images/spectra to hard disk.
- User selectable cropped sensor size – highly intuitive software definition.
- Ideal for super-resolution microscopy, ion signalling, voltage sensitive dyes and adaptive optics.
- The iXon3 is now available with the complementary OptoMask accessory, which can be used to shield the region of the sensor outside of the cropped area.

If an experiment demands fast temporal resolution, but cannot be constrained by the maximum storage size of the sensor (as is the case for ‘Fast Kinetics Mode’ of readout), then it is possible to readout the iXon3 in ‘Cropped Sensor Mode’. In this mode, the user defines a ‘sub-array’ size from within the full image sensor area, such that it encompasses the region of the image where change is rapidly occurring (e.g. a ‘calcium spark’ within a cell). The sensor subsequently “imagines” that it is of this smaller defined array size, achieved through software executing special readout patterns, and reads out at a proportionally faster frame rate. The smaller the defined array size, the faster the frame rate achievable. Table 2 shows frame rates that are achievable with the iXon3 897 when in Cropped Sensor Mode.

In order to use Cropped Sensor Mode, one has to ensure that no light is falling on the light sensitive area outside of the defined region. Any light collected outside the cropped area could corrupt the images that were acquired in this mode. For microscopy set-ups, this is now aided with an accessory called OptoMask, available from Andor.

Cropped Sensor Mode has the end result of achieving a much faster frame rate than that obtainable in a conventional ‘sub-array’ / ROI readout (during which we would still have to vertically shift the unwanted rows). The frame rate increase is achieved by not reading out (i.e. discarding) the unwanted pixels.

Cropped Sensor Mode is ideally suited to a number of challenging applications across many diverse fields of research. In terms of technology matches, cropped sensor is well suited to rapid applications of EMCCD cameras. The fundamental advantage and a distinct feature of EMCCD technology is its ability to virtually eliminate the camera readout noise detection limit at any readout speed. This allows EMCCD detectors to be successfully used for applications where raw sensitivity and exposure time requirements ultimately prevent the use of conventional CCD systems.

In biological imaging Cropped Sensor Mode can be successfully used to enhance performance and throughput in super-resolution ‘nanoscopic’ applications including STORM and PALMIRA. Imaging frame rates exceeding 1000 / s can be achieved with a sufficiently small crop area. A series of measurements done on the Andor iXon3 885 have demonstrated that Cropped Sensor Mode in conjunction with binning has pushed the speed beyond 4000 frames per second. Table 3 shows a comparison of frame rates achievable by the iXon3 885 camera in standard sub-array readout versus the same size of region selected in Cropped Sensor Mode. It is apparent that frame rates of between x2 and x4 faster are readily achievable in Cropped Sensor Mode.

Cropped Sensor Mode can also be employed to achieve extremely fast temporal resolution in ion signalling measurements, such as observing calcium sparks. Samples labelled with voltage sensitive dyes also benefit from extremely fast imaging, with thousands of frames per second not being uncommon.

EMCCD-based adaptive optics, for which smaller format EMCCD sensors are often used, can benefit from cropped sensor readout. Small area EMCCDs can already operate at >500 fps and can be flexibly optimized in cropped mode to exceed 2000 fps. Use of cropped sensor mode opens new possibilities for very fast adaptive optics imaging enabling the users to reach into several thousands of frames per second.

There is also potential to use cropped EMCCDs for multi-spectral fluorescence confocal scanning, as an alternative to the arrays of PMTs that have traditionally been used in this approach. The greater than 90% Quantum Efficiency of the back-illuminated sensor, single photon sensitivity, array architecture and rapid pixel readout speed can be exploited to markedly improve this approach. The laser dwell-time should be set to coincide with the time to expose and read-out a short row of approximately 32 pixels - sufficient spectral channels to yield effective un-mixing of several known emitting dyes, resulting in a data cube of 512 x 512 x 32 (spectral) taking less than 1 second to generate. There is a clear sensitivity advantage of EMCCD pixels over the usually employed PMT-technology, which is circa 5-fold in the blue-green and up to tenfold in the red.

	Array Size						
Binning	256 x 256	128 x 128	64 x 64	32 x 32	512 x 100	512 x 32	512 x 1
1 x 1	69	395	988	2577	176	538	7980
2 x 2	136	743	1764	5400	342	1025	-
4 x 4	260	1327	2902	6068	649	1877	-
8 x 8	483	2184	4285	7375	1268	3209	-

Table 2 - Frame Rates achieved by the iXon3 897 in cropped sensor mode

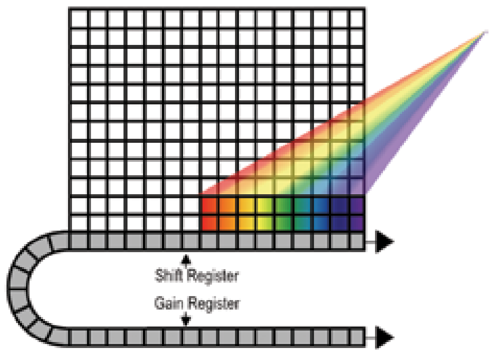


Figure 1. Cropped Sensor Mode. The active imaging area of the sensor is defined in a way that only a small section of the entire chip is used for imaging. The remaining area has to be optically masked to prevent light leakage and charge spill-over that would compromise the signal from the imaging area. By cropping the sensor one achieves faster frame rates because the temporal resolution will be dictated by the time it requires to read out small section of the sensor.

	‘Standard’ sub-array					
Binning	**502 x 501**	**251 x 250**	**125 x 125**	**75 x 75**	**32 x 32**	**1004 x 1**
1 x 1	62	118	218	340	588	1,370
2 x 2	116	215	376	543	826	-
4 x 4	208	366	581	775	1,031	-

	‘Cropped Mode’ sub-array					
Binning	**502 x 501**	**251 x 250**	**125 x 125**	**75 x 75**	**32 x 32**	**1004 x 1**
1 x 1	62	231	465	763	1,704	13,812
2 x 2	118	426	859	1,401	2,976	-
4 x 4	213	735	1,474	2,404	4,746	-

Table 3 - Comparing frame rates achievable by the iXon3 885 under both ‘standard’ sub-array and ‘Cropped Sensor’ modes of readout.

Technical Note

Quantitative Stability in EMCCDs

EMCCDs are susceptible to various sources of data instability. Each of these sources have been addressed in iXon3 to ensure reliable quantitative performance throughout a kinetic acquisition and also repeatability between measurements.

Baseline Clamp
The baseline (or bias level) is an electronic offset added to the output signal from the EMCCD sensor to ensure that the displayed signal level is always a positive number of counts. No actual noise is associated with this positive counts value and thus it is important to recognise that it does not affect sensitivity. However one must remember to subtract the baseline offset value from the signal intensity when performing signal to noise calculations.

Traditionally, when acquiring data, small changes in heat generation of the driving electronics within the detector head may cause some drift in the baseline level. This is often particularly observable during long kinetic series.

Since 2002, Andor have been addressing this undesirable effect in our high-end EMCCD cameras.

Any drift in the baseline level can be corrected by using our innovative Baseline Clamp option. Baseline Clamp corrects each individual image for any baseline drift by subtracting an average bias signal from each image pixel and then adding a fixed value to ensure that the displayed signal level is always a positive number of counts. As such, the baseline remains at a rock-steady value during a fast kinetic series, as shown in Figure 1.

Note: The baseline bias level is also susceptible to variation at different EM gain settings. Again the iXon3 baseline clamp corrects for this, ensuring the bias level is clamped no matter what EM gain setting is selected.

Electron Multiplication (EM) Stability
It is a well recognized fact that EM multiplication factor is temperature dependent. That is why iXon3 pioneering RealGain™ – a linear and quantitative EM gain calibration - is temperature compensated, i.e. the same precise correlation between software EM gain selection and actual EM gain holds at whatever cooling temperature is selected. This will be addressed in more detail later.

However, another by-product of this temperature dependence is that we must also pay close attention to optimized temperature stability regulation. The results of such attention to detail are best understood through observation of the stability via measurement of a stable light source, as shown in Figure 2.

Here we used the back-illuminated iXon3 897 to measure signal from a stable LED source overlayed with a mask of arrayed holes, imaged in conditions of zero ambient background light (use of a light-tight darkbox).

Kinetic series were recorded over 500 frames, 60 ms exposure times, frame transfer mode, Baseline Clamp activated (such that absolute bias stability is in place also). A moderately intense signal, such that instability would not be lost in the signal shot noise, was recorded with 'EM gain off", then 'EM gain on'.

Region of Interest kinetic plots were derived from each data set type and two such representative plots are shown. Significantly, there is no additional relative signal variation observable over the duration of the kinetic series of the 'EM gain on' data.

Be careful of light source instability
When performing stability measurements, care must be taken to assess the stability of the light source. As an example, the same back-illuminated 512 x 512 camera that was used for the EM stability measurement in Figure 2 was subsequently transferred to a research grade widefield epifluorescence microscope and fixed cells, immuno-stained with fluorescence dyes (Invitrogen Molecular Probes), were imaged over a similar kinetic series, as shown in Figure 3.

In these experiments the variation of the signal intensity was significant, whether EM gain was on or off; the amplitude of variation was much higher than the shot noise on the signal. This indicates that the light source of the microscope itself can often be subject to much greater stability variations than could be derived from any EM gain instability observations. One needs to very carefully check for all sources of undesirable signal fluctuation, such as stability of illumination (background illumination fluctuations can also contribute) when conducting quantitative time course experiments.

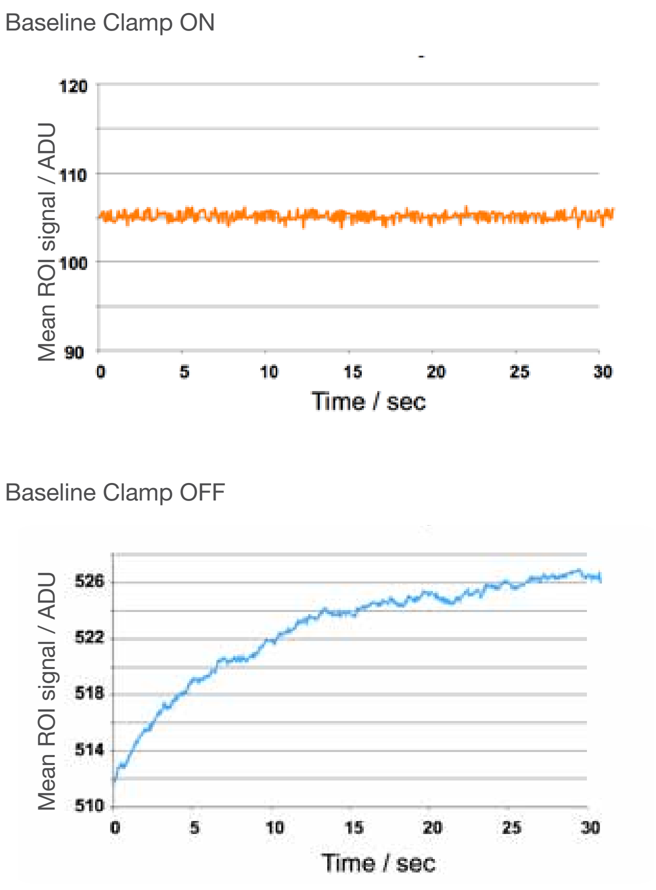


Figure 1 - iXon3 Baseline Clamp (bias stability) in operation. A standard feature in iXons since 2002.

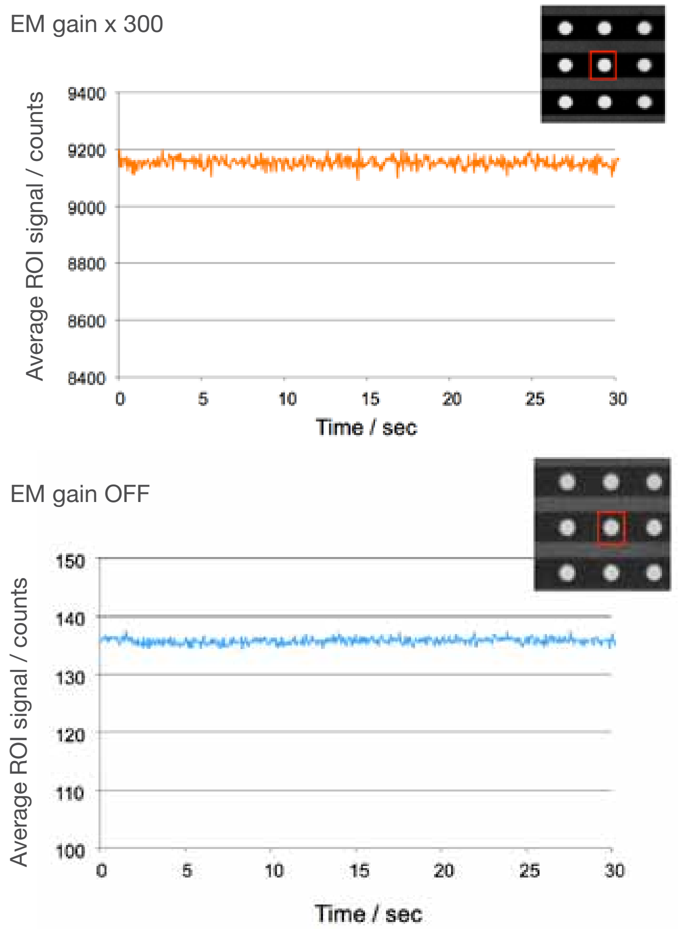


Figure 2 – EM gain stability in the back-illuminated iXon3 897 (512 x 512 pixels) – LED measurement.

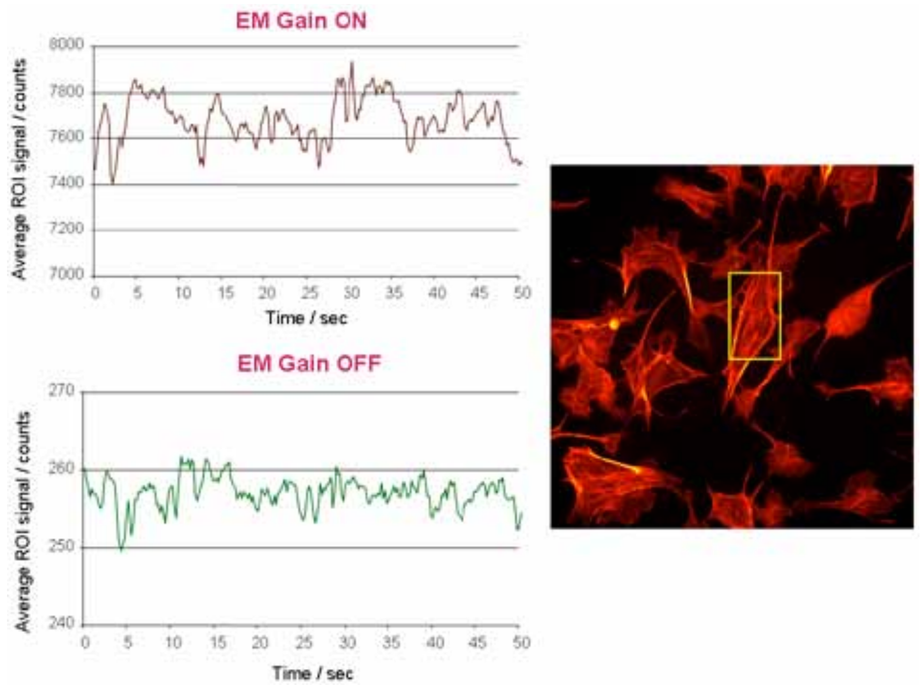


Figure 3 – Instability of the light source on a standard research grade epifluorescence microscope.

Technical Note

RealGain™, Anti-Ageing, EMCAL™ and Longevity

In 2006 Andor once again raised the bar by introducing some significant new technology innovations. These particular pioneering steps were to set new high standards in quantitative EMCCD usage and general EMCCD longevity expectations, which others in the industry are only now beginning to adopt.

Part 1: RealGain™, Anti-Ageing and EMCAL™

RealGain™

Linear - In response to considerable demand from our customers, Andor have set about a detailed analysis of the EM voltage dependence, and have successfully converted the non-linear relationship between EM gain and the EM software setting into a linear one.

Real - Importantly, the true EM gain (i.e. the absolute signal multiplication factor) is selected directly from the software linear gain scale, as shown in Figure 1. No more guesswork with arbitrary gain units across a non-linear scale - the gain you ask for is the gain you get. Select the best gain to overcome noise and maximize dynamic range.

Temperature Compensated

Although EM gain is temperature dependent, Andor's real/linear gain calibration extends to any EMCCD cooling temperature. Selecting x300 gain software setting at -50°C, or at –100°C gives the same x300 true EM gain. Importantly, this means that there is no need to recalibrate EM gain on each use in multi-user laboratories and facilities.

Anti-Ageing of EM gain

It has been observed that EMCCD sensors, more notably in cameras that incorporate L3Vision sensors from E2V, are susceptible to EM gain fall-off over a period of time. This phenomenon has been documented by E2V and can be viewed on their web site. All back-illuminated EMCCD sensors are of this brand and therefore all are susceptible to EM gain ageing.

It is important to note that the ageing effect applies to any EMCCD camera, by any manufacturer, that incorporates these L3Vision sensors. In Andor's iXon3 range, this corresponds to 897, 888 and 860 models. If left unchecked, this ageing phenomenon has the potential to significantly compromise the long-term quantitative reliability of EMCCD cameras. Fortunately, if these highly sensitive sensors are integrated into intelligently designed camera electronics, ageing can be minimized and should not present any real problem to the user.

Andor have recognized the ageing issue and have been busy implementing innovative measures to stabilize the EM gain on these sensors, ensuring that this ground-breaking ultra-sensitive technology can deliver a prolonged quantitative service to the user. iXon3 cameras have been internally configured to ensure that the rate of EM gain fall off is significantly reduced under standard operation. Part of the measures taken has been to invoke real EM gain limits, coupled with signal intensity feedback warnings (after EM amplification) to ensure that the user is more restricted in his/her ability to apply excessive EM gain and/or signal. The EM gain scales offered are more than sufficient to render the read noise floor as negligible for a given signal intensity and readout speed. These tight user restrictions significantly reduce the rate of EM gain fall off.

EMCAL™

Andor have developed a unique and patented method of user-initiated EM gain self-recalibration. Even after exercising due care during usage and availing of the above anti-ageing restrictions, the EM gain may deplete over an extended period of time. The EMCAL™ self-recalibration process is very easily initiated by the user. At the touch of a button, a routine is triggered that measures EM gain and uses the iXon3 in-built temperature compensated RealGain™ scales to reset the EM gain calibration (if required), to deliver the true values requested on the software scale – i.e. resetting the factory calibration. EMCAL™ stands to markedly prolong operational lifetime and quantitative reliability of the technology, and circumvents the need to return the camera to the factory for recalibration.

Part 2: Longevity -

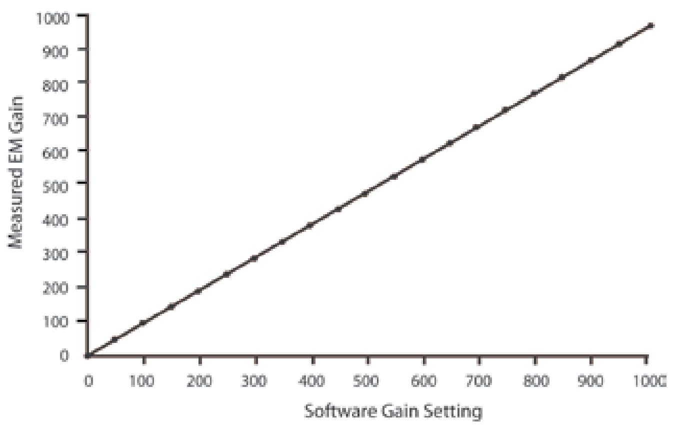


Figure 1 – RealGain™ calibration in the iXon3 – the same linear relationship holds across all cooling temperatures.

How extensively can Andor's back-illuminated EMCCDs be used before they can no longer be recalibrated (EMCAL™) to factory EM gain settings?

One common concern associated with EM gain ageing phenomenon and the associated EMCAL™ recalibration fix is that of longevity. The clock voltage setting, which must be adjusted as part of the recalibration routine, will eventually reach a maximum threshold value, after which further rescaling is not possible and EM gain will then fall off irreparably upon further extensive use of the camera. The question is, when is this likely to happen under typical use?

Andor ran extensive testing on the iXon3 897 camera in order to project the operational lifetime of the 'gain register' (where signal amplification occurs on-chip) in back-illuminated EMCCDs, the conditions of testing are described below:

- Overall duration of test: ~14 months
- Camera usage during this period: Continuous 24 hours per day, 7 days per week
- Frame rate: 30 frames / sec
- EM gain setting: x1000
- Photons / pixel / frame: 90
- Number of pixels illuminated: ~ 200,000 (~ 75% of array)
- EMCAL: Applied once per day.

Results

The clock voltage required to maintain the EM gain calibration was measured once daily using Andor's EMCAL™ routine and the plot shown was generated by extrapolating from the equation derived from the gain ageing trend. This shows that under the test conditions employed, the EMCCD calibration would only be expected to reach ~ 65% of the available clock voltage scale after 7 years of continuous operation.

Demanding test conditions

The combination of parameters employed in this test represents quite aggressive acquisition conditions. ~1.1 billion images were recorded during this period, with ~ 200,000 illuminated pixels per image, corresponding to ~ 220,000 billion pixels being amplified through the gain register with x1000 EM amplification per pixel.

Most users would not subject the camera to 24/7 continuous acquisition at 30 frames/sec. We also strongly recommend that, except for photon counting, the EM gain setting is limited to no more than x300 for the vast majority of applications, but x1000 was chosen here as a more rigorous test condition. Finally, it is quite rare that 75% of all pixels in the array will be subjected to uniform signal of this magnitude, as was imposed on the sensor here. In reality the light emitting species of typical user samples will project onto a much smaller fraction of pixels from frame to frame.

EMCAL™ does NOT accelerate EM gain ageing

It is important to recognize that the rate of ageing is not accelerated by routine application of Andor's EMCAL™ routine. The rate of ageing is determined by the illumination and EM gain conditions that the sensor is subject to through operation, irrespective of routine recalibration using EMCAL™. If the 'previous' mechanism of EM gain recalibration were to be used, whereby the camera is shipped back to factory less frequently for manual readjustment of the clock voltage, the progress along the ageing curve would not be any different from that shown here (adjusting for the additional time that the camera would be out of action).

Conclusion

For the vast majority of low-light applications and taking due care and attention to stay within recommended operating conditions, applying EMCAL™ as required, the gain ageing phenomenon is not considered to ever impose a restriction on the quantitative reliability of your Andor iXon3 camera.

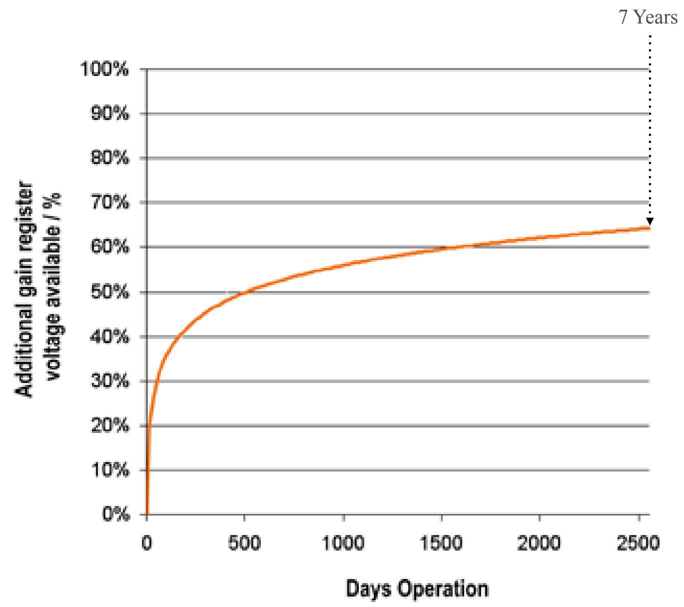


Figure 2 – Ageing profile of an Andor backlit EMCCD. Test conditions: 24/7 operation; 30 frames/sec; x90,000 electrons per pixel through gain register; ~ 200,000 pixels illuminated

ANDOR™ TECHNOLOGY

Technical Note

Photon Counting in EMCCDs

Photon Counting in EMCCDs is a way to overcome the multiplicative noise associated with the amplification process, thereby increasing the signal to noise ratio by a factor of root 2 (and doubling the effective Quantum Efficiency of the EMCCD). Only EMCCDs with a low noise floor can perform photon counting. The approach can be further enhanced through innovative ways to post process kinetic data.

The industry-leading Darkcurrent and Clock Induced Charge (CIC) specification of Andor's back-illuminated iXon3 897 model renders it uniquely suited to imaging by Photon Counting.

Photon Counting can only be successfully carried out with very weak signals because, as the name suggests, it involves counting only single photons per pixel. If more than one photon falls on a pixel during the exposure, an EMCCD (or an ICCD for that matter) cannot distinguish the resulting signal spike from that of a single photon event, and thus the dynamic range of a single frame exposure is restricted to one photon.

To successfully photon count with EMCCDs, there has to be a significantly higher probability of seeing a 'photon spike' than seeing a Darkcurrent/CIC 'noise spike'. The iXon3 897 has the lowest Darkcurrent/CIC performance on the market, yielding both lower detection limits and higher contrast images.

Under such ultra-low light conditions, 'photon counting mode' imaging carries the key benefit that it is a means to circumvent the Multiplicative Noise, also know as 'Noise Factor'. Multiplicative noise is a by-product of the Electron Multiplication process and affects both EMCCDs and ICCDs. In fact, it has been measured to be significantly higher in ICCDs. The noise factor of EMCCDs is well theorized and measured; to account for it you increase the shot noise of the signal by a factor of square root 2 (~x1.41). This gives the new 'effective shot noise' that has been corrected for multiplicative noise. The effect of this additional noise source on the overall Signal to Noise ratio can be readily viewed in the S/N plots in the technical note entitled 'EMCCD signal to noise plots'.

Photon Counting Mode does not measure the exact intensity of a single photon spike, it merely registers its presence above a threshold value. It does this for a succession of exposures and combines the individual 'binary' images to create the final image. As such, this mode of operation is not affected by the multiplication noise (which otherwise describes the distribution of multiplication values around the mean multiplication factor chosen). The end result is that low light images acquired through this mode of acquisition are improved by a factor of ~x1.41 Signal to Noise, compared to a single integrated image with the same overall exposure time.

To successfully photon count with EMCCDs, there has to be a significantly higher probability of seeing a 'photon spike' than seeing a Darkcurrent/CIC 'noise spike'. The lower the contribution of this 'spurious' noise source to a single exposure within the accumulated series, the lower the detection limit of photon counting and the cleaner the overall image will be, as demonstrated in Figure 1.

The iXon3 897 has the most effective combined cooling/CIC minimization on the market, lower than other competing EMCCDs utilizing the same 512 x 512 sensor. As such, the detection limit for Photon Counting is markedly lower. The iXon3 intuitively offers Photon Counting modes, either as a real time acquisition or as a post-processing step. OptAcquire can be used to first optimize the camera for Photon Counting acquisition.

Photon Counting by Post-Processing

As a post-processing analysis, the user holds the flexibility to 'trial and error' photon count a pre-recorded kinetic series, trading-off temporal resolution vs SNR by choosing how many images should contribute to each photon counted accumulated image. For example, a series of 1000 images could be broken down into groups of 20 photon counted images, yielding 50 time points. If it transpires that better SNR is required, the original dataset could be re-treated using groups of 50 photon counted images, yielding 20 time points.

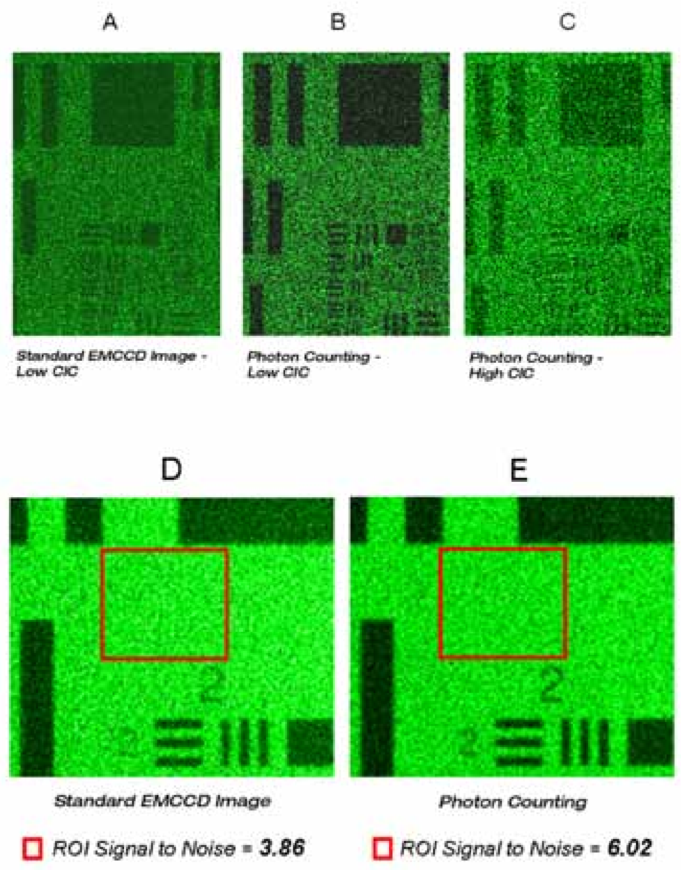


Figure 1 – 'Photon Counting' vs. 'Standard EM-on' Imaging for very weak signals:
Images A, B and C were recorded under identical illumination conditions, identical exposure times and each with EM gain set at x1000. The benefit of Photon Counting under conditions of low Clock Induced Charge (CIC) are evident. Images D and E are derived from a larger number of accumulated images, to yield a greater measurable signal to noise ratio. An identically positioned Region of Interest on each image was used to determine S/N of 3.86 and 6.02 for standard and photon counted images respectively. This factor improvement is in accord with the theory of Photon Counting circumventing the influence of multiplicative noise (noise factor) in EMCCD signals.

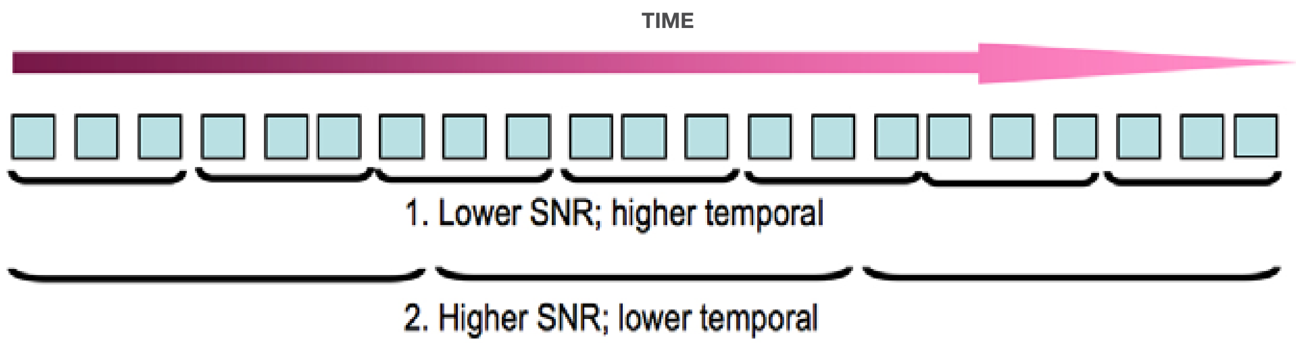


Figure 2 – Schematic illustration of how photon counting can be applied to a kinetic series as a post processing step, affording increased flexibility in 'trial and error' trading SNR vs temporal resolution.

Technical Note

Fast Kinetics Mode

Fast Kinetics Acquisition Mode can be used to acquire bursts of data with sub-microsecond time resolution. The iXon3 is configured to make available not only the rows of the image area, but also rows under the frame transfer mask for storage of acquired data prior to readout. The 'overclocked' vertical shift speeds of the iXon3 renders it ideal for extremely fast temporal resolution in Fast Kinetics Mode.

Fast Kinetics is a special read out mode of iXon3 that uses the actual sensor as a temporary storage medium and allows an extremely fast sequence of images to be captured. The capture sequence is illustrated here:

Step 1: both the Image and Storage areas of the sensor are fully cleaned out (the Keep Clean Cycle)

Step 2: the Keep Clean Cycle stops and the acquisition begins. The image builds up on the illuminated section of the sensor.

Step 3: the sensor remains in this state until the exposure time has elapsed, at which point the complete sensor is clocked vertically by the number of rows specified by the user.

Steps 4 & 5: the process is continued until the number of images stored equals the series length set by the user. The iXon3 is set up to utilize the entire area of the frame transfer mask for additional signal storage.

Step 6: at this point the sequence moves into the readout phase by first vertically shifting the first image to the bottom row of the sensor. The sensor is then read out in the standard method.

Points to consider for Fast Kinetics Mode:

- Light MUST only be allowed to fall on the specified sub-area. Light falling anywhere else will contaminate the data.
- The maximum number of images in the sequence is set by the position of the sub-area, the height of the sub-area and the number of rows in the CCD (Image and Storage area)
- There are no Keep Cleans during the acquisition sequence.
- The industry fastest vertical shift speeds of the iXon3 enables fastest time resolution with minimal vertical smearing.
- A range of internal trigger and external trigger options are available for Fast Kinetics Readout.

The Fast Kinetics Mode capability of the iXon3 renders it uniquely suited to microsecond-order kinetic measurements, facilitated by rapid vertical shifts and extensive on-chip storage areas.

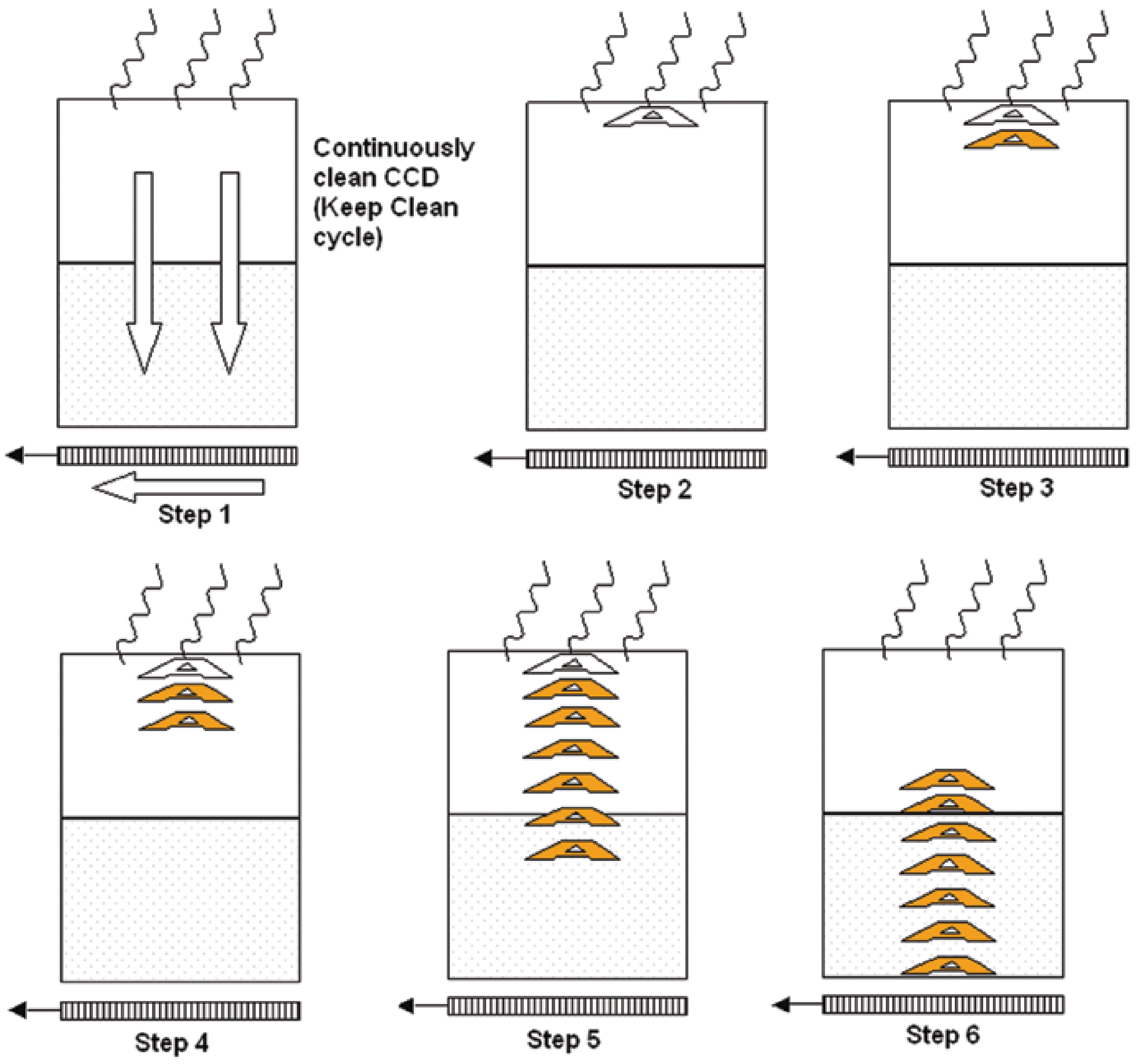


Figure 1 – Illustration showing Fast Kinetics Mode capture sequence of the iXon3

Technical Note

Dynamic Range & EMCCDs – Uncovering the Facts

Calculating dynamic range in EMCCDs has often been a source of confusion, due to the additional requirement to factor in EM gain and the extended well capacity of the gain register. High dynamic range can be accessed in EMCCDs with careful fine tuning of EM gain.

Dynamic Range (DR) is given by:
Calculating Dynamic Range in an EMCCD camera is a slightly more complicated story than for conventional CCDs. This is because of the favourable effect of EM gain on the detection limit vs. the limiting effect of EM gain on the full well capacity. The easiest way to address this is to first take each parameter separately:

Detection Limit and EM gain:
The main function of EMCCD is to eliminate the read noise detection limit and enable detection of weak photon signals that would otherwise be lost within this noise floor. With EM gain, the detection limit is given by the 'Effective Read Noise', i.e. the read noise divided by the gain multiplication, down to one electron. Why never less than one? This stems from the definition of detection limit, which is essentially "the signal equal to the lowest noise level". Since you can't get a signal less than one photon, then the detection limit should never be taken as less than one electron.
For example, the iXon3 897 has a read noise of ~50 electrons @10 MHz with EM gain off. At EM gain x2, the new detection limit can be considered to be 25 electrons effective read noise, at x5 it will be 10 electrons, at x50 it will be 1 electron. At x100, the Effective Read Noise will be 0.4 electrons, but as far as the Detection Limit is concerned, this must still be taken as 1 electron.

Full Well Capacity and EM gain:
One might imagine that applying EM gain will decrease the full well pixel capacity proportionally. This is indeed the case, but a buffer has been built into EMCCD cameras to enable at least some EM gain to be applied while maintaining the original well capacity. This buffer is in the form of a higher capacity in the gain register pixels, where the multiplication actually takes place. So, the true capacity is given by the capacity of the pixels of the sensor, but as you apply EM gain this holds only up until the point where the larger capacity of the gain register pixels also become saturated by applied EM gain. After that point, you have to correct the 'effective' full well of the sensor to be equal to the full well of the gain register divided by the gain.

Dynamic Range and EM gain:
These above factors combined mean that as EM gain is increased, Dynamic Range will increase with gain to a maximum, level off and then reach a point at which it begins to deplete again with further gain. This can seem complicated, but fortunately these DR vs EM gain relationships can be readily plotted out and visualized in graphical form, as exemplified in Figure 1.

iXon3 back-illuminated EMCCDs can be read out at either 10, 5, 3 or 1 MHz speeds. This offers extended flexibility to balance Dynamic Range vs frame rates. Furthermore OptAcquire can be used to select optimal Dynamic Range settings at fastest and slowest speeds.

There are a number of interesting points to note from these plots:
1. The rationale behind offering readout speeds slower than 10 MHz through the EM-amplifier is so that frame rate can be traded off against dynamic range. You can see that the highest dynamic range through an EM amplifier comes from the slowest 1 MHz readout speed.
2. At any readout speed through the EM-amplifier, the best combination of Dynamic Range and sensitivity can be obtained at an EM gain setting equal to the readout noise at that speed. At this point the DR is at maximum and the effective readout noise is 1 electron (i.e. just on the verge of single photon sensitivity).
3. At x1000 EM gain the dynamic range is only 400:1. Excessively high EM gain can also accelerate EM gain ageing in back-illuminated EMCCDs (see section 7). EM gains of x300 or less are more than sufficient to optimize sensitivity, while ensuring dynamic range is not excessively compromised. The only occasions when Andor recommends extending EM gain to x1000 is for single photon counting experiments.
4. The highest dynamic range is through the conventional amplifier at 1 MHz.
5. It is clear that the actual sensor Dynamic Range only exceeds 14-bits at 1 MHz, through either EM or a conventional amplifier. Therefore, it is at 1 MHz that we require an option to match this higher dynamic range output with a scientific grade, noise free 16-bit A/D digitization. The iXon3 is uniquely designed to do just that, making use of a real scientific grade A/D that is optimized for 1 MHz readout.

Note: There is a direct relationship between readout noise and maximum dynamic range at a given readout speed. Lower readout noise affords higher dynamic range. The readout noise specification used in calculating dynamic range must be with EM gain turned off, as quoted in all iXon3 spec sheets.

We note, however, that another prominent EMCCD provider chooses to quote their lowest read noise value, not for EM gain-off, but only for EM gain x4, x6 or x10 (model dependent). In this case, to arrive at the real read noise spec you would have to multiply the quoted figure by x4, x6 or x10.

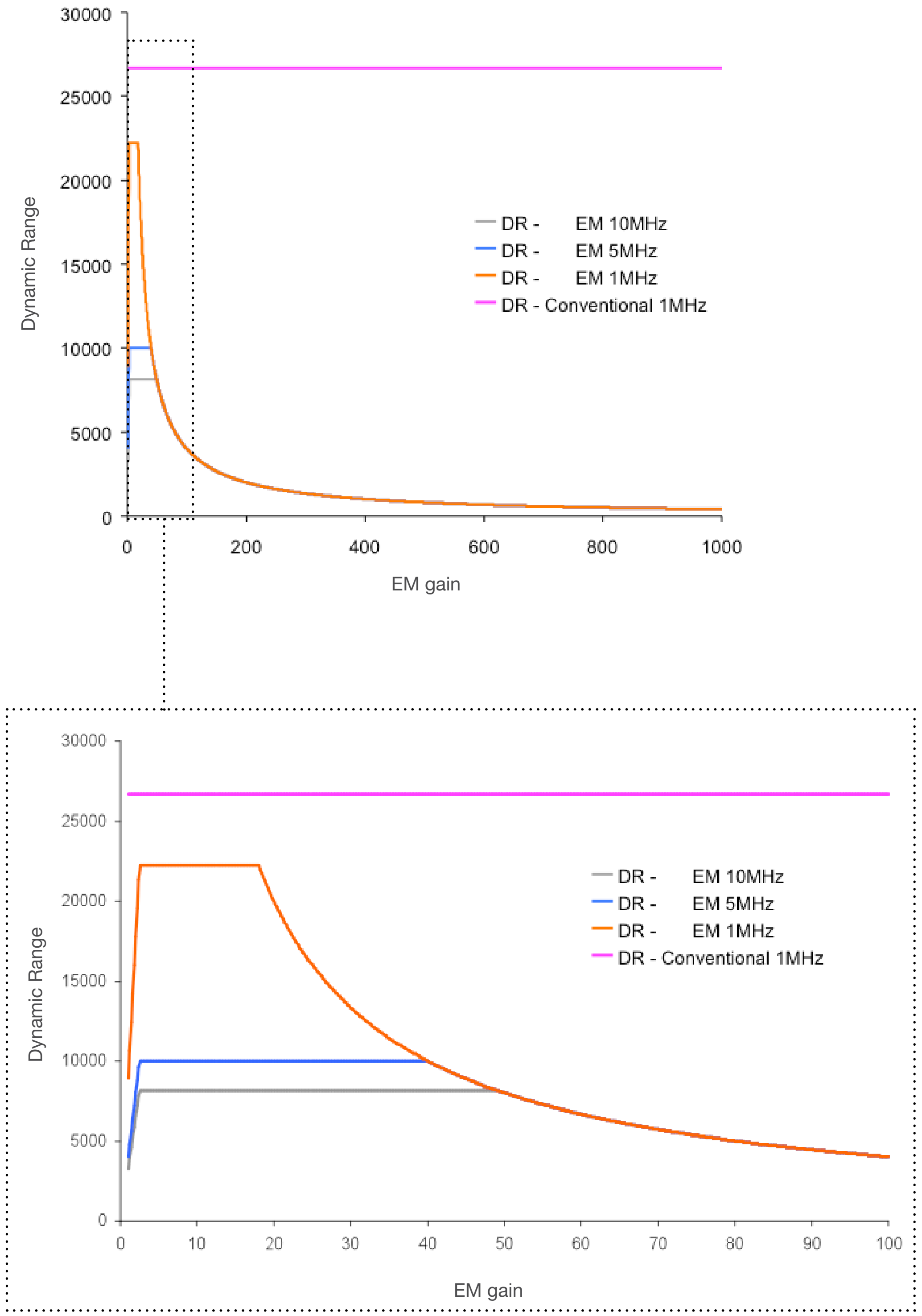


Figure 1 – Dynamic Range vs EMCCD gain for iXon3 897. Shown for EM amplifier @ 10, 5 and 1 MHz readout speed and for Conventional amplifier at 1 MHz readout speed.
Well capacities used in DR calculation are characteristic of the CCD97 512 x 512 back-illuminated L3 sensor from E2V. Dynamic range only exceeds 14-bits max @ 1 MHz, through either amplifier.

Technical Note

Making Sense of Sensitivity

It is often questioned whether or not to use EMCCD gain or whether to use EM or conventional CCD amplifiers (model dependent). The answer usually depends both on required frame rate and on light levels. Plots of Signal to Noise ratio vs Signal Intensity can be instructive in making such decisions. Here we introduce the concept of Signal to Noise in EMCCDs and discuss such plots.

Part 1 - Understanding Noise Sources in EMCCDs

Read Noise

Read Noise in many instances can be considered the true CCD detection limit, particularly the case in fast frame rate experiments because, (a) short exposures combined with low Darkcurrent make the Darkcurrent contribution negligible and (b) faster pixel readout rates, such as 5 MHZ and higher, result in significantly higher readout noise. The fundamental advantage of EMCCD technology is that gains are sufficient to effectively eliminate readout noise, therefore eliminating the detection limit.

Multiplicative Noise

This noise source is only present in signal amplifying technologies and is a measure of the uncertainty inherent to the signal multiplying process.

For example, during each transfer of electrons from element to element along the gain register of the EMCCD, there exists only a small probability that the process of impact ionization will produce an extra electron during that step. This happens to be a small probability but when executed over more than 590 steps, very large potential overall EM gains result. However, the downside to this process results from the probabilities. Due to this, there is a statistical variation in the overall number of electrons generated from an initial charge packet by the gain register. This uncertainty is quantified by a parameter called 'Noise Factor' and detailed theoretical and measured analysis has placed this Noise Factor at a value of , or 1.41 for EMCCD technology. This is an additional form of noise that must be taken into account when calculating Signal/Noise for these detectors. Note that this noise source is significantly greater from the MCP of ICCDs than from the gain register of the EMCCD. ICCDs have noise factors typically ranging from 1.5 to >2.

However, one way to better understand the effects of this noise source is in terms of an addition to the shot noise of the system. Extra multiplicative noise has the same form as shot noise in that each noise type results in an increase in the variation of number of electrons that are read out of a CCD (under constant uniform illumination).

Indeed multiplicative noise can be thought to contribute directly to the overall shot noise, in that one should multiply the Shot Noise by the Noise Factor when calculating overall noise.

Simply put, multiplicative noise does not in any way reduce the average signal intensity or reduce the number of photons that are detected, it simply increases the degree of variation of the signal around the mean value, in addition to the variation that already exists from the shot noise (variation from pixel to pixel or from frame to frame).

Dark Current

Due to the effective cooling inherent to Andor's cameras, dark current is minimized, and may often be considered practically negligible. The extent of contribution is dependent on exposure time, since the dark current is quoted in electrons/pixel/sec. It is particularly important to eliminate Darkcurrent with EMCCD technology as even single thermally generated electrons in the silicon will be amplified in the gain register just as a single photoelectron, and will appear in the final signal as a single noise spike. Fortunately, for fast frame rate experiments combined with iXon3 very low Darkcurrent, this noise source may be ignored.

Spurious Noise

Spurious Noise appears in the form of Clock Induced Charge (CIC) in EMCCD technology. CIC is independent of exposure time and generally single electron events generated during charge shift (EBI is the form of spurious noise in ICCDs and is exposure dependent). CIC is generated in every CCD but is normally buried in the readout noise. In the EMCCD however, these single electrons are amplified by the gain register just as a single photoelectron would be. In the EMCCD, CIC can in some ways be considered the true limit of detection, in that at the single photon detection level, a single photon spike will be indistinguishable from a CIC spike. Andor has specialized electronics however, that enable this source of noise to be minimized. In practical terms, ultra-weak signals of the single photon nature would be distinguishable from CIC spikes in that one could generally expect to see 'groupings' of photon spikes from adjacent pixels, even from diffraction limited single molecule emissions.

Simplified consideration of EMCCD noise

From the above list it is apparent that in most uses of Andor's iXon3, since read noise and Darkcurrent can be virtually eliminated (i.e. the noise sources that would define the detection limit have been rendered effectively negligible), the principal sources of noise that must be considered are shot noise, noise factor (multiplicative noise) and spurious noise.

It is easy to combine shot noise and multiplicative noise in an overall noise equation, using:

Overall Noise = Shot Noise x 1.41

Shot Noise can be determined if the average signal is measured in electrons - by measuring in electrons, the calculation is independent of the sensor's QE – i.e. the photons have already been converted to photoelectrons so the QE corrected signal is being measured. If the average signal in photons is already known (e.g. estimated from other measurements with PMTs), the shot noise can be corrected for sensor QE at that wavelength:

i.e.

$$\textbf{Overall Noise} = \left(\sqrt{\frac{QE}{100} \times PhotonSignal}\right) \times 1.41$$

therefore

$$\textbf{Overall Signal/Noise} = \frac{\frac{QE}{100} \times PhotonSignal}{\left(\sqrt{\frac{QE}{100} \times PhotonSignal}\right) \times 1.41}$$

Since spurious noise is very different in nature to shot noise, it is best to consider spurious noise separately. Each EMCCD will have a measured figure for the levels of CIC spikes to be expected during a readout. This will present a figure for the average number of random spurious single electron spikes that will appear within the image. If the measured signal is at the very low photon level (one or two electrons per pixel), this noise source will be more significant. If the signal is slightly more intense than this, it may become less of an issue, and may even be filtered out. Note that often, the minimal amount of spurious noise remaining from the iXon3 is minor compared to the level of background photons in the image.

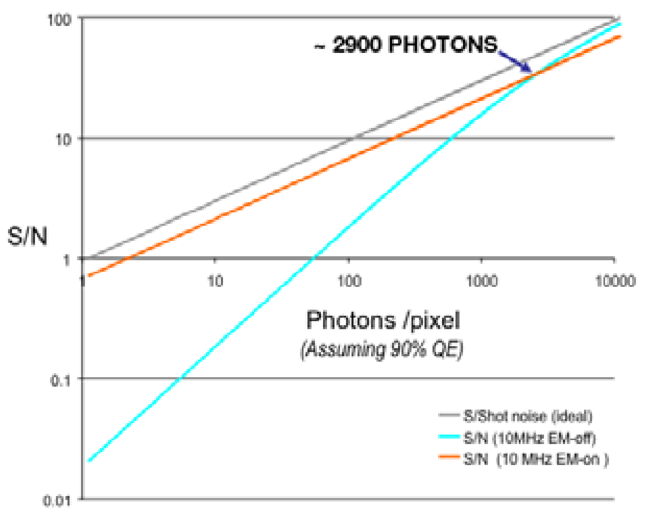


Figure 1 – EM gain-ON vs EM-gain-OFF signal to noise plots for back-illuminated iXon3 EMCCDs at 10 MHz readout speed – applies to 897, 860 and 888 models.

Part 2: Signal to Noise Plots

EM gain ON vs EM gain OFF (faster speeds)

Figure 1 shows Signal to Noise plots derived from the specs of the back-illuminated iXon3 EMCCDs, read out at 10 MHz (for fastest frame rates). A photon wavelength at which the QE of the sensor is 90% is assumed. Such plots are very useful to gauge the signal intensity at which it becomes appropriate to use EM gain to increase S/N. It is clear that at 10 MHz readout, one needs to encounter relatively intense signals of > 2900 photons / pixel before it becomes advantageous to operate with EM gain off. Note that the 'ideal' curve represents a pure Signal to Shot Noise and is shown for reference – if the detector had no sources of noise, this is what the curve would appear like. Even with EM gain turned on we encounter uniformly lower signal to noise than the ideal curve. This is due to the influence of EM Multiplicative Noise, which has the effect of increasing the shot noise by a factor of square root 2 (~1.41). Note, Multiplicative Noise (Noise Factor) is generally higher for ICCDs.

EM vs Conventional Amplifier (slower speeds)

Figure 2 shows Signal to Noise plots derived from the specs of the back-illuminated iXon3 EMCCDs at 1 MHz (slower frame rate operation), read out either with EM gain ON or alternatively through the Conventional amplifier (i.e. standard CCD operation). Again, a photon wavelength at which the QE of the sensor is 90% is assumed. Specifically this figure applies to 897 and 888 models for which this choice of amplifier is available.

Under slower speed operations, when one has the choice to read out as a 'conventional CCD', it can often be advantageous to do so in terms of achieving better signal to noise. Indeed the plots show that the cross-over point is at ~ 42 photons / pixel, below which it is advised still to readout through the EM amplifier with gain applied.

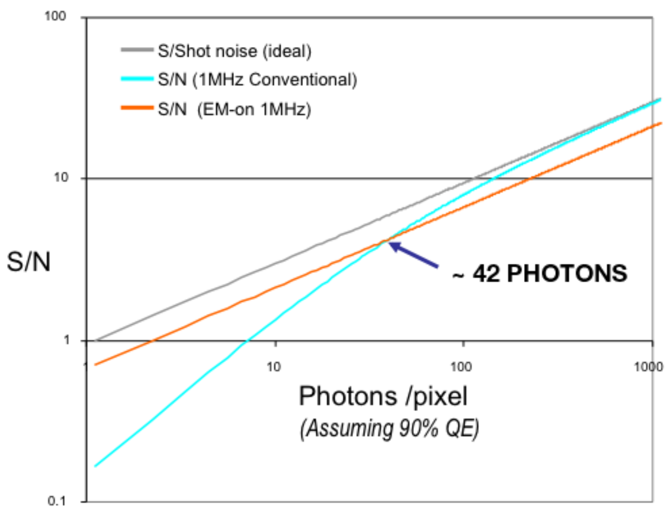


Figure 2 – EM gain-ON vs Conventional Amplifier signal to noise plots for back-illuminated iXon3 EMCCDs at 1 MHz readout speed – applies to 897 and 888 models.

Technical Note

iXon3 Trigger Modes

Andor's EMCCDs offer a comprehensive range of internal, software and external trigger modes. Furthermore, software and internal trigger modes avail of cutting-edge firmware and SDK enhancements, delivering enhanced speed performance during complex software acquisition protocols (iCam). On-head storage of multiple exposure times facilitates rapid exposure time switching upon receiving a trigger.

Part 1 - External Trigger Modes

The iXon3 back-illuminated range of cameras have several different external trigger modes:

External Trigger in Frame Transfer Mode (Simultaneous Exposure and Readout)
In this mode, the camera sits in its 'External Keep Clean' cycle, which can be interrupted by the external trigger with a jitter of only a couple of microseconds (exact dependent on camera model). Upon receiving a trigger, the camera stops all vertical clocking and waits for the programmed user delay period before starting the read phase. During the readout phase the Image area is transferred rapidly to the Storage area. The Storage area is then read out in the normal way.

Once the read out is complete the camera continues to wait for the next external trigger event. While the camera is waiting for the trigger event the shift register is continually clocked but the Image and Storage areas are not. On the next trigger the camera again waits for the programmed delay before starting the read out phase. The camera continues in this cycle until the number of images requested have been captured. Since the Image area is not cleaned between trigger events, the exposure time is defined by the time between trigger events. This sequence is illustrated in Figure 1.

External Exposure combined with Frame Transfer Mode (available only on iXon3 885 cameras)
This mode is not available for other iXon3 models, since it requires an 885 Global Clear function, whereby the anti-bloom structure of each pixel is used to drain the pixel of charge outside of the exposure period. This mode is distinct from the trigger modes discussed previously in that the exposure period is fully controlled by the width of the external trigger pulse. The exposure period starts on the positive edge and concludes on the negative edge. As illustrated in the timing diagrams below (Figure 2) the positive edge can occur either after the previous image has been completely read out or while it is still being read. The ability to overlap the readout with the exposure period allows for very high frame rates. In order to ensure that light falling on the image area before the start of the exposure does not contribute to the measured signal, the CCD is placed in a special keep clean mode. This keep clean mode uses the feature Global Clear, which is only available on a limited range of CCD sensors and hence not available on all iXon3 cameras. Although the start of the exposure can overlap with the read out phase of the previous image, the end of the exposure cannot. This is because the end of the exposure is marked by shifting the image area into the storage area. It is not possible to use the same feature as is used to prevent light that fell before the exposure starts from contributing to the measured signal as this would cause the already accumulated charge to be cleared.

NOTE: If the falling edge occurs during the read out phase it will be ignored and the next falling edge will terminate the exposure.

'External Trigger' in Non-Frame Transfer Mode
In this mode, the camera is once again sitting in 'External Keep Clean'. As can be seen from Figure 3, the External Keep Clean Cycle runs continuously until the external trigger event is detected, at which point the exposure phase starts. Once the exposure time has elapsed the charge built up in the Image area is quickly transferred into the Storage area. From the Storage area the charge is read out as normal. At the completion of the read out the camera restarts the external keep clean cycle and will perform a minimum number of cleans before accepting the subsequent trigger event.

'Fast External Trigger' in Non-Frame Transfer Mode
This mode is for the most part identical to External Trigger Mode and differs in only one respect: after an acquisition and readout, the camera will not wait for a sufficient number of keep clean cycles to have completed before allowing a trigger event to start the next acquisition. As a result Fast External Trigger allows a higher frame rate than standard external trigger mode. Fast External Trigger is most useful in those cases where there is very little light falling on the sensor outside of the exposure times and the user is looking for fast frame rates.

'External Exposure' in Non-Frame Transfer Mode
Figure 4 shows a timing scheme for External Exposure mode, another external trigger option for non-frame transfer readout. This mode is similar to the external trigger mode discussed above, in that the type of keep cleans are identical and the exposure is started by the positive edge of the trigger pulse. Where these two trigger modes differ is in how the exposure time is controlled. With standard External Trigger, the user (via software) controls the exposure time. With External Level Trigger mode, the level of the trigger pulse (i.e. the time spent in the 'on' state of the TTL signal) controls the exposure time. The exposure period starts on the positive edge of the trigger pulse and stops on the negative edge. The exposure is physically ended by shifting the Image area into the Storage area. The Storage area is then read out in the normal manner. On completion of the readout, the external keep clean cycle is started again.

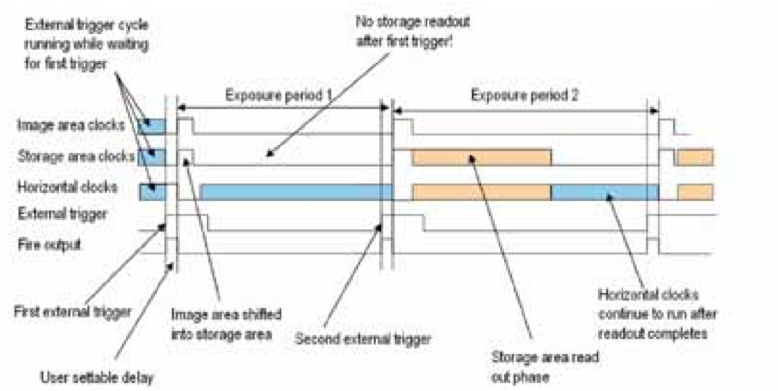


Figure 1: External Trigger in Frame Transfer mode

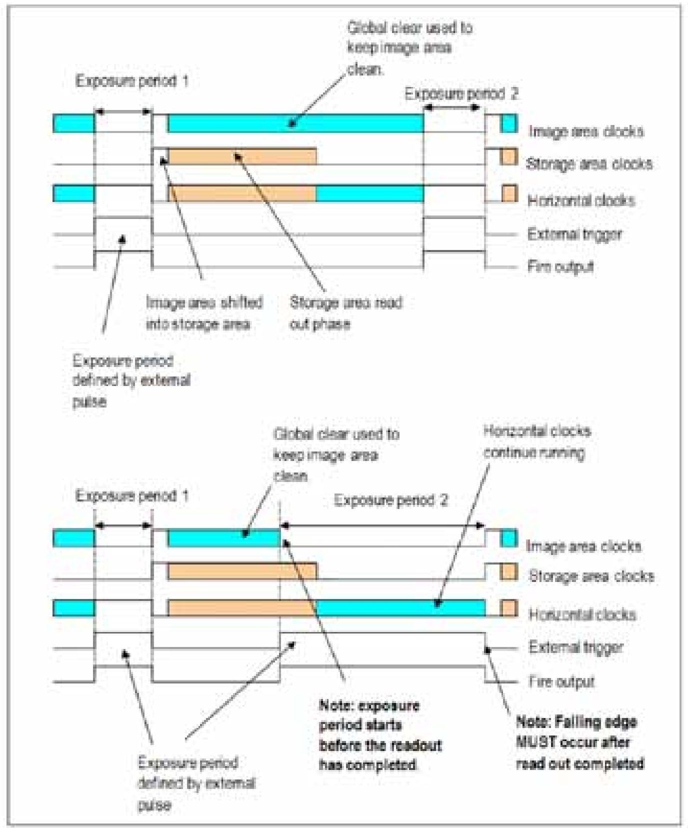


Figure 2: External Exposure Trigger in Frame Transfer mode (885 model only)

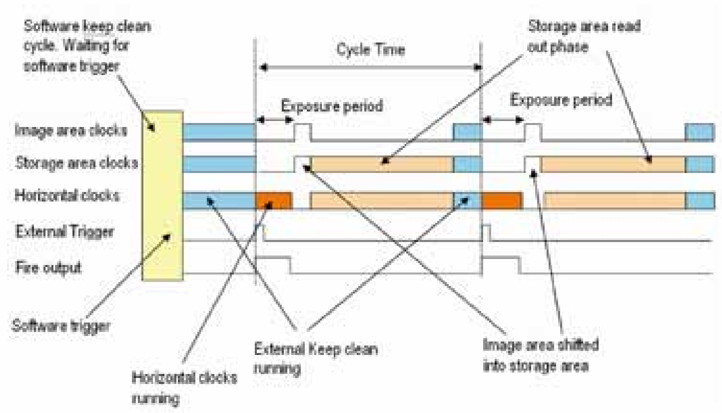


Figure 3: External Trigger in non-Frame Transfer mode

Technical Note

iXon3 Trigger Modes continued

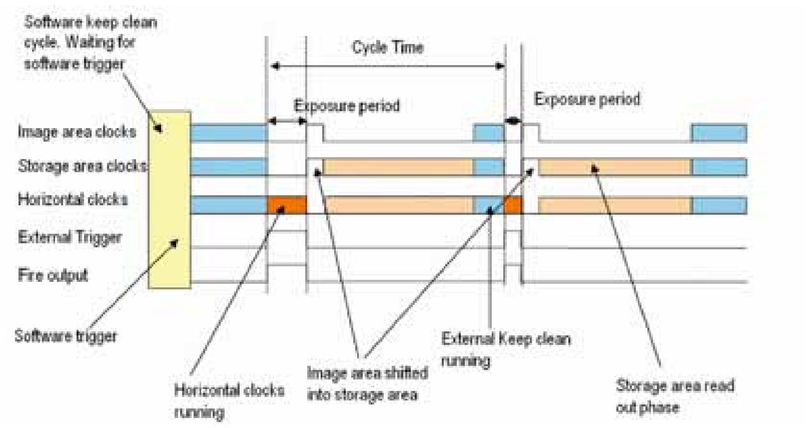


Figure 4: External Exposure in non-Frame Transfer mode

Notes...

Part 2 – iCam Fast Exposure Switching

iCam encompasses a set of unique innovations that empower Andor iXon3 cameras to operate with complete acquisition efficiency through Andor iQ multi-dimensional microscopy suite and other 3rd party imaging software packages.

	Frame Rate (fps)
iXon3 897 with iCam	23
Other EMCCD	12

Table 1 - Comparison of exposure switching speed in iXon vs competing EMCCD camera. Free-run imaging sequence involving dual channel acquisition protocol using rapid toggle between 1 ms and 2 ms for each channel. Both cameras have the same 512 x 512 back-illuminated sensor.

Imaging cameras in a heightened state of readiness…

Andor's iCam technology is a combined firmware and software innovation, a highly efficient and performance-optimized solution that is now integrated across all new Andor imaging cameras and Andor's iQ and SDK software platforms. iCam functionality has been integrated into a number of popular 3rd party software drivers, including MDC Metamorph and Nikon NIS Elements. iCam offers heightened EMCCD performance during tightly synchronized and complex multi-dimensional microscopy experiments.

Using state of the art bi-directional communication between camera and PC, iCam is particularly effective for multi-channel acquisitions during which different exposure times are rapidly toggled between channels, whether software triggered or hardware (externally) triggered, with absolute minimal overheads.

iCam delivers:

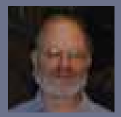
- Enhanced software triggering during acquisition – highly efficient upload of acquisition parameters from software to camera with minimized overheads.
- Ring Mode – ultimate in exposure switching during multi-channel protocols. Software pre-loads up to 16 acquisition channels onto the camera.
- Enhanced external trigger mode – optimized speed performance across all of Andor's comprehensive external trigger options.
- Asynchronous frame transfer mode (AFTM) – enhanced speed and synchronization in overlapped/frame transfer acquisition mode.
- Bi-directional communication – between PC and camera
- Further enhanced baseline stability – takes Andor's market-leading quantitative baseline stability to a whole new level.
- 3rd party software compatibility – most popular imaging suites can take advantage of iCam.

Enhanced data exchange between camera and PC

iCam allows for faster frame rates in software by using dedicated timing patterns that shorten unnecessary overhead times. These time lags prevent other EMCCD and interline cameras on the market from achieving fast frame rate during complex experimental protocols.

Furthermore, iCam's 'Ring Mode' offers the capacity to use up to 16 different timing patterns uploaded into the camera head, thus external triggers can result in virtually instantaneous switching between channels, facilitating unparalleled synchronization with other peripheral equipment such as filter wheel, laser-AOTF or z-stage.

Notes...



Prof John Sedat
Professor of Biochemistry & Biophysics, University of California, San Francisco

"Using a parallel array of Andor's iXon 897 EMCCD cameras as detectors, we have been able to develop our 3D-structured illumination instrument with enhanced sensitivity and speed, critical to realize the implementation of the technique for high resolution imaging of challenging live specimens within a reasonable measurement period."

Dr Tobias Walther
Max Planck Institute of Biochemistry, Munich

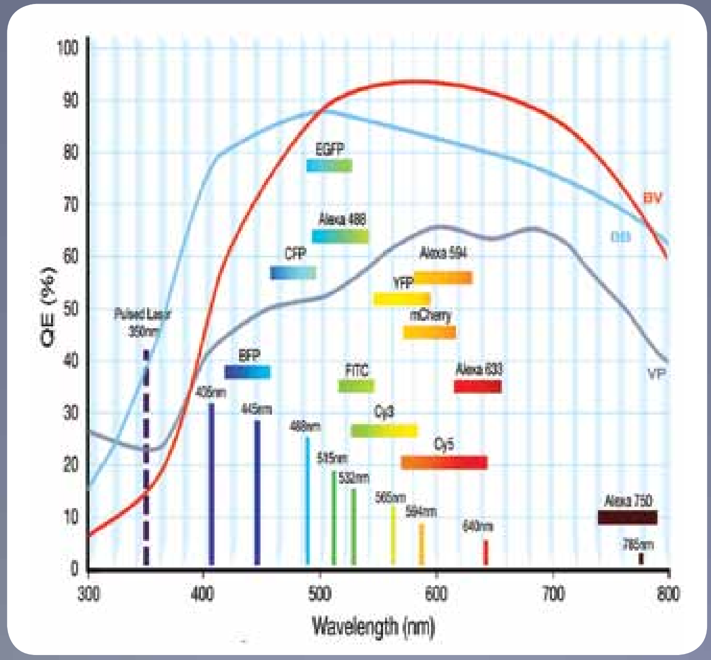
Commenting on the use of the iXon 897 (as part of Andor Revolution XD spinning disk confocal system) to directly image the localisation of Target of Rapamycin kinases within a cell.

"My lab does a lot of microscopy and for all of our applications these new cameras have been a breakthrough. For ten years, scientists have tried to localize these kinases indirectly. But, with this new generation of cameras, we can see single molecules in living cells – something which was impossible before."

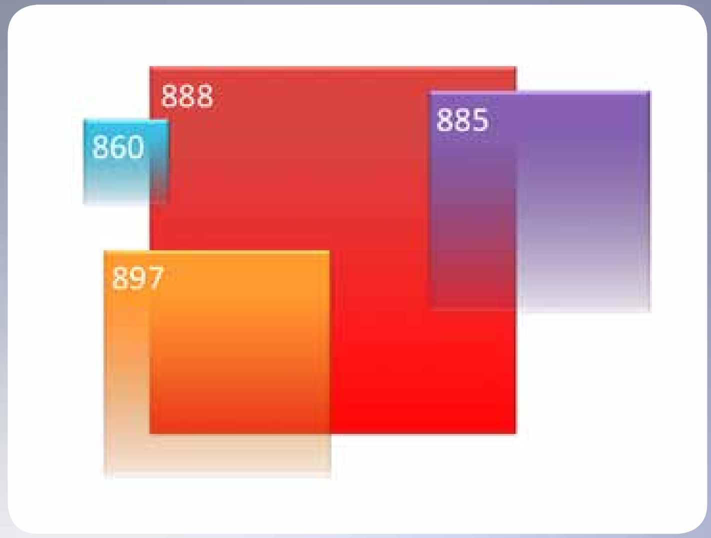
Front cover image:

A large scale 3D STORM image of a cultured hippocampal neuron colored in z.

Courtesy of Melike Lakadamyali, Institute of Photonic Sciences, ICFO, Barcelona, Spain and Hazen Babcock from Harvard University, Cambridge, MA



QE curves of iXon3 models vs emission of common fluorophores



The relative sensor size of the iXon3 models

ANDOR™
TECHNOLOGY

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.

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 lifecycle of the products.

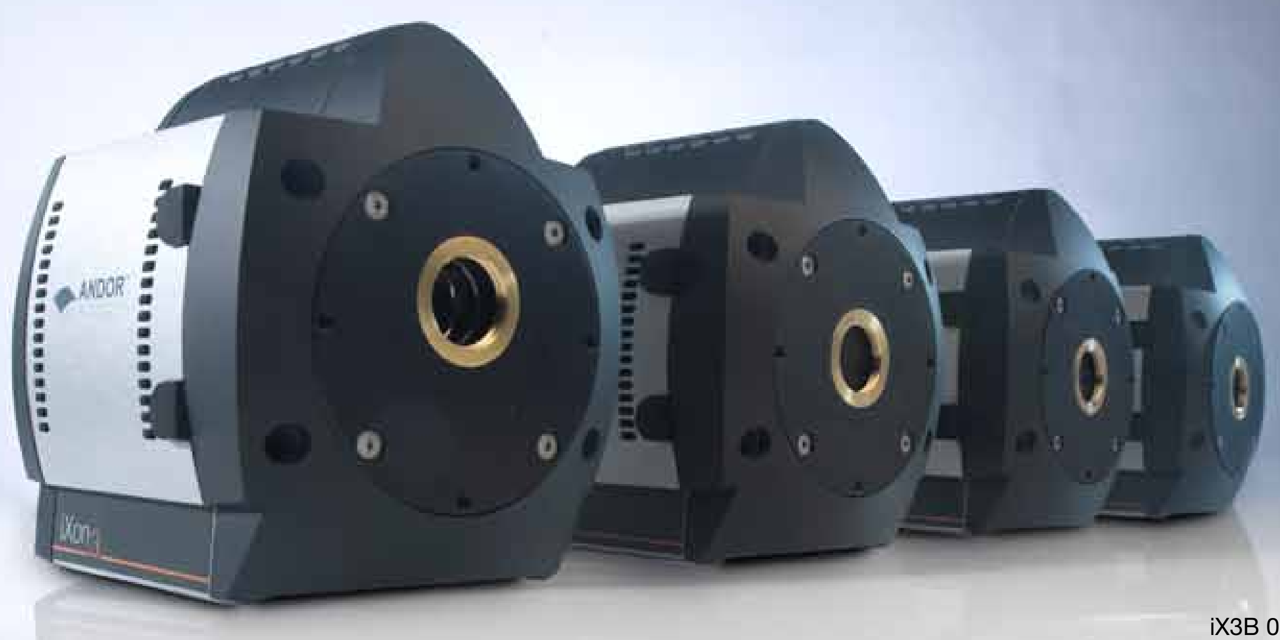
ISO14001: 2004 ISO9001: 2008 CE Windows XP Windows Vista Windows 7 Linux

Head Office
7 Millennium Way
Springvale Business Park
Belfast BT12 7AL
Northern Ireland
Tel: +44 (28) 9023 7126
Fax: +44 (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 NE Sarugakucho Building
2-7-6 Sarugaku-Cho
Chiyoda-Ku
Tokyo 101-0064
Japan
Tel: +81 (3) 3518 6488
Fax: +81 (3) 3518 6489

China
Room 502 Yu Yang Zhi Ye Building
A 2 Xiao Guan Bei Li
An Wai, Chaoyang District
Beijing 100029 China
Tel: +86 (10) 5129 4977
Fax: +86 (10) 6445 5401



iX3B 03/11