HARDWARE GUIDE

iXon3

www.andor.com

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SECTION 1 - INTRODUCTION TO iXon3 HARDWARE

Thank you for choosing the **Andor iXon3**. You are now in possession of a revolutionary new **E**lectron **M**ultiplying **C**harge **C**oupled **D**evice (**EMCCD**), designed for the most challenging low-light imaging applications. This manual contains useful information and advice to ensure you get the optimum performance from your new system. If you have any questions regarding your iXon3 system, please feel free to contact Andor directly, or via your local representative or supplier. You can find contact details below.

1.1 - TECHNICAL SUPPORT

If you have any questions regarding the use of this equipment, please contact the representative***** from whom your system was purchased, or:

Europe

Andor Technology
7 Millennium Way
Springvale Business Park
Belfast
BT12 7AL
Northern Ireland
Tel. +44 (0) 28 9023 7126
Fax. +44 (0) 28 9031 0792
http://www.andor.com/contact_us/support_request

USA

Andor Technology
425 Sullivan Avenue
Suite # 3
South Windsor
CT 06074
USA
Tel. (860) 290-9211
Fax. (860) 290-9566
http://www.andor.com/contact_us/support_request

Asia-Pacific

Andor Technology (Japan)
7F Ichibancho Central Building
22-1 Ichiban-Cho
Chiyoda-Ku
Tokyo 102-0082
Japan
Tel. +81 3 3511 0659
Fax. +81 3 35110662
http://www.andor.com/contact_us/support_request

China

Andor Technology
Room 1116
Zhejiang Building
No. 26
An Zhen Xi Li
Section 3
Chaoyang District
Beijing 100029
China
Tel. +86-10-5129-4977
Fax. +86-10-6445-5401
http://www.andor.com/contact_us/support_request

*** The latest contact details for your local representative can be found on our website.**

1.2 - COMPONENTS

The Andor iXon3 system comprises the following main items:

- Detector head (hereinafter referred to as a **Camera** - see **figure 1** below)
- 2 off BNC - SMB cables
- User manuals on CD
- Software disk (SDK and/or Solis if ordered)
- Andor Programmer guide to Andor Basic (if ordered)
- Software Development Kit manual (if SDK ordered)
- Camera specific performance booklet or sheet
- ESD wrist strap. **NOTE: This must be worn at all times when handling the PCI card**
- PCI / PCIe card (CCI-22, CCI-23 or CCI-24, camera model dependent)
- PCI / PCIe controller card to camera cable
- Power supply block and correct power cable for the country where the camera is to be used

The following items are also available as optional accessories:

- Mounting Posts (see **page 21**)

1.2.1 - Camera

Figure 1: iXon3 camera

1.2.2 - Controller Cards

Figure 2: CCI-22

Figure 3: CCI-23

Figure 4: CCI-24

The Controller cards **(CCI-22, CCI-23 or CCI-24)** buffer data from the camera, before transfer to the computer memory, via the PCI bus. The CCI-22 & CCI-23 require a PCI 2.2 slot; the CCI-24 requires a **PCIe x1** slot.

All boards are well shielded against electrical interference.

Your iXon3 will be supplied with the controller card that is optimal for your camera model. Controller cards have a 26-pin interface for connection via a cable to the PC and an auxiliary connector. The pin-outs for the cards are shown on **page 79**.

1.2.3 - Controller Card Cables

The controller cards require the following connecting cables for correct operation:

Figure 5: PCI controller card to camera cable

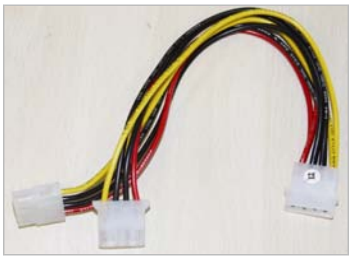


Figure 6: Internal “Molex” connector cable

- The controller card connector cable is used to connect the camera to the PCI controller card.
- The power cable is connected within the computer between any available “Molex” connector and the PCI controller card. **NOTE: The internal power cable is wired to leave a free connector for supplying power to other devices, should it be required.**

1.2.4 - Cooler Power Supply Block

The iXon3 system is designed to be powered from a PS-20 external **P**ower **S**upply **B**lock (PSB) which is used to supply power to the Thermoelectric cooler within the camera. The PSB requires an AC mains input between 90-264 V, 47-63 Hz and a maximum supply current of either 1A @ 115V or 0.5A @ 230V. The output of the PS-20 is 7.5V DC at 4.0A maximum. The PS-20 is fitted with a 3-pin IEC connector for the electrical supply input and the wall socket must be connected to the building's protective earth system. The connection to the iXon3 is made via a 2.1 mm jack connector.

Power requirements	
Voltage	+7.5V ± 5%
Current	4A maximum at peak cooling
Ripple & Noise	1% maximum
Total Power	30 W maximum

NOTE:

1. Cooling is only available when the PSB is connected to the camera.

2. In the UK (or other countries using UK standard electrical systems), the mains input into the PS-20 must be fitted with a 240V 5A fuse.

1.2.5 - Software

Your iXon3 may have been supplied with Andor Solis or Andor iQ software, or with the Andor SDK. However it is also compatible with a range of 3rd party software options offering optimized acquisition control and analysis functionality. For further details of Andor software capabilities and software options, please go to the following page on our website: **http://www.andor.com/products/software/**

1.3 - SAFETY PRECAUTIONS & MAINTENANCE

1.3.1 - Care of the camera

WARNINGS:

1. **The camera is a precision scientific instrument containing fragile components. Always handle with the care necessary for such instruments.**
2. **There are no user serviceable parts inside the camera. If the head is opened the warranty will be void.**
3. **The camera should be mounted so that the mains supply can be easily disconnected. In case of emergency, the disconnecting device is the mains lead. This will either be the mains lead connected to the product, or in the case of a cabinet-based system the mains lead to the cabinet.**
4. **To prevent accidental internal damage to the camera, objects small enough to enter the slots on the sides of camera should be placed well away from these slots.**
5. **Ensure that a minimum clearance of approximately 100 mm (4”) is maintained in front of all ventilation slots and the fan inlet. Cooling performance cannot be guaranteed unless these criteria are observed.**
6. **Only use a dry, clean, lint free cloth to clean all painted surfaces. If necessary, use a water diluted detergent to lightly dampen the cloth - do not use Isopropyl alcohol, solvents or aerosols.**
7. **To clean the window, remove loose particulate matter with an air blower. If the component is still not clean, it may be drag wiped using folded lint free, clean, soft white tissue dampened with pure methanol.**
8. **If the equipment is used in a manner not stated by Andor, the protection provided by the equipment may be impaired.**

1.3.2 - Environmental conditions

- Indoor use only
- Altitudes up to 2000 m
- Operating temperature 0°C to 40°C
- Maximum relative humidity < 70% (non-condensing)
- Other voltage fluctuations as stated
- Over voltage category 1: This is designed to withstand the normal transient voltages expected at a mains socket
- Pollution degree 2: Only non-conductive pollution occurs. Occasionally, temporary conductivity caused by condensation is to be expected
- Electromagnetic compatibility: This is a Class A product. In a domestic environment this product may cause electromagnetic interference, in which case the user may be required to take adequate measures

1.3.3 - Regular checks

The state of the product should be checked regularly, especially the following:

- The integrity of the enclosure
- Any water hoses used
- The AC/DC External Power Supply
- The mains cable

NOTE: Do not use equipment that is damaged.

1.3.4 - Annual electrical safety checks

It is advisable to check the integrity of the insulation and protective earth of the product on an annual basis, e.g. U.K. PAT testing.

NOTE: Do not use equipment that is damaged

1.3.5 - Replacement parts

A PS-20 (see **page 20**), which is the only external power supply recommended for use with the iXon camera, has been supplied to you. If this unit fails or is damaged, please contact Andor for a replacement. Depending on the Terms and Conditions of your Warranty, you may be charged for this replacement.

1.3.6 - Fuse replacement

The camera itself does not have a fuse. However, if a U.K. (BS 1363) mains lead has been supplied, it contains a fuse, whose characteristics are as follow:

- **Rated Current:** 5 A
- **Rated Voltage:** 240 Vac.
- **Type:** BS 1362
- **Size:** 0.25 × 1 inch

1.3.7 - Overheating

Care should be taken to ensure that the camera does not overheat, as this can cause system failure. Overheating may occur if either of the following situations arises:

- The air vents on the sides of the detector head are accidentally blocked or there is insufficient or no water flow
- The ambient air temperature is higher than 30ºC

To protect the camera from overheating, a thermal switch has been attached to the heat sink. If the temperature of the heat sink rises above predefined limit, the power supply to the cooler will cut off and a buzzer will sound. The cut-out will automatically reset once the head has cooled. It is not recommended that you operate in conditions that would cause repeated cut-outs as the thermal switch has a limited number of operations.

NOTE: When using water cooling, always use water that is above the dew point of the ambient environment otherwise condensation may occur (please see page 14).

1.3.8 - Working with electronics

The computer equipment that is to be used to operate the iXon3 should be fitted with appropriate surge/EMI/RFI protection on all power lines. Dedicated power lines or line isolation may be required for some extremely noisy sites. Appropriate static control procedures should be used during the installation of the system. Attention should be given to grounding. All cables should be fastened securely into place in order to provide a reliable connection and to prevent accidental disconnection.

The power supply to the computer system should be switched off when changing connections between the computer and the camera. The computer manufacturer's safety precautions should be followed when installing the PCI Controller Card into the computer.

The circuits used in the camera head and the PCI controller card are extremely sensitive to static electricity and radiated electromagnetic fields and therefore they should not be used (or stored close to) EMI/RFI generators, electrostatic field generators, electromagnetic or radioactive devices, or other similar sources of high energy fields. Types of equipment that can cause problems include Arc welders, Plasma sources, Pulsed-discharge optical sources, Radio frequency generators and X-ray instruments.

1.3.9 - Condensation

You may see condensation on the outside of the camera body if the temperature of the cooling water is too low or if the water flow is too great. The first signs of condensation will usually be visible around the connectors where the water tubes are attached. In such circumstances switch off the system and wipe the camera with a soft, dry cloth. It is likely there will already be condensation on the cooling block and cooling fins inside the camera. Please also carry out the following actions:

- Set the camera aside to dry for several hours before you attempt re-use
- Before re-use blow dry gas through the cooling slits on the side of the camera to remove any residual moisture
- Use warmer water or reduce the flow of water when you start using the device again

1.3.10 - Dew Point graph

The graph in **figure 7** below plots the relationship between **Relative Humidity** and **Dew Point** at varying ambient temperature. This can be used to calculate the minimum temperature the cooling water should be set to.

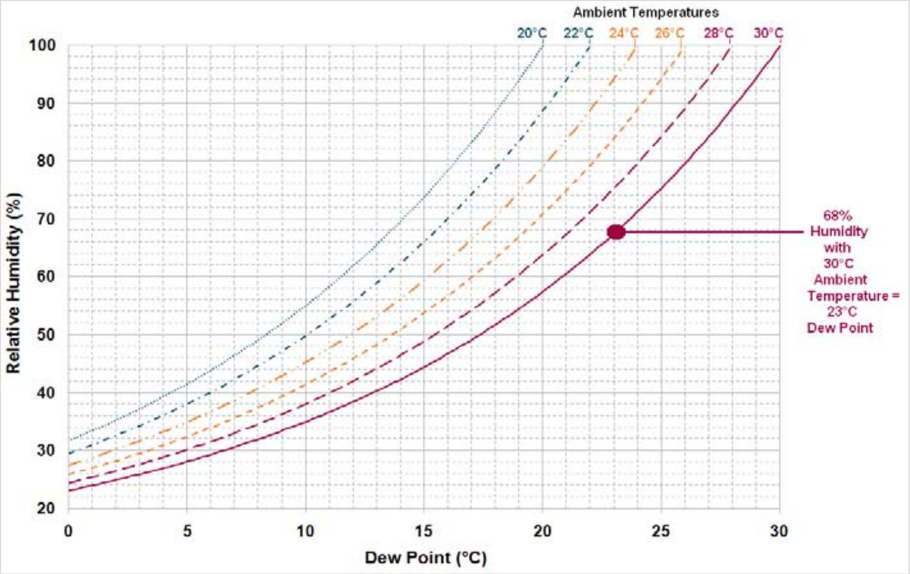


Figure 7: Dew point graph

For example, when using an iXon3 897, you will need 10ºC cooling water to guarantee performance down to -100ºC. In the relatively dry atmosphere of an air-conditioned lab, cooling water at 10ºC should not present any problems.

However, in humid conditions (such as exist in some parts of the world) condensation may occur, resulting in damage to the head. In such conditions you will have to use warmer water (20ºC or even higher if it is very humid). The minimum CCD temperature would then be limited to a higher value.

1.3.11 - EM Gain ageing

It has been observed that some EMCCD sensors, more notably in cameras that incorporate L3Vision sensors from E2V, are susceptible to EM gain fall-off over a period of time. It is important to note that this ageing effect applies to any EMCCD camera manufacturer that incorporates L3Vision sensors into their cameras. In the Andor iXon3 range, this refers to the 897, 888 & 860 models.

EMCCD cameras incorporating Impactron (EMCCD) sensors from Texas Instruments have shown rates of EMCCD saturation-induced ageing that are orders of magnitude slower than those with E2V sensors, exposed to comparable light intensity and gain. **NOTE: 885 iXon3 models contain Impactron sensors from Texas Instruments, which do not exhibit EM gain ageing (or at most exhibits it at a negligible level).**

A technical note entitled: "**Longevity in EMCCD and ICCD**" , which further explains this phenomenon, can be downloaded from the following website: **http://www.andor.com/library/publications/?app=543**

If left unchecked, this ageing phenomenon has the potential to significantly compromise the long-term quantitative reliability of EMCCD cameras. Andor have recognized this ageing issue and have implemented 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 and if these highly sensitive sensors are used with due care and attention, ageing can be minimized and should not present any real problem to the user.

More details of this ageing effect and Andor's solutions can be found on **page 32,** but listed below are some guidelines to minimize the EM gain ageing process:

- **Do not** use EM gain values greater than necessary to overcome the read noise. A rule of thumb is that a gain of x4 or 5 the rms read noise (accessible from the spec sheet or performance sheet) is more than sufficient to render this noise source negligible. In practice, this can always be achieved with EM Gain of less than x300 (often much less). Pushing gain beyond this value would give little or no extra Signal to Noise benefit and would only reduce dynamic range
- Only select the extended EM gain scale of x1000 for **single photon counting** applications and always ensure that the signal falling onto the sensor is indeed within the regime of low numbers of photons per pixel.
- Turn **down** the gain when the camera is not acquiring
- Try **not** to **over-saturate** the EMCCD sensor

1.3.12 - Minimizing particulate contamination

It is important that particulate contamination of the exterior of the camera window is kept to a minimum, such that images are kept free of ‘shadowing’ particles directly in the focal path. The iXon3 range comes equipped with an internal C-mount shutter. Whilst not being required for frame transfer operation (which is a shutter-free readout mode) it is good practice to close the shutter when the camera is not in acquisition use for a reasonable period. It is also advisable to use the software to close the shutter when exposing the camera to the ‘open environment’ (i.e. removed from a microscope C-mount or focusing lens) whilst power is still flowing to the camera.

When the camera power is turned off, the C-mount shutter closes automatically and the camera can be moved freely. We recommend that the C-mount opening is covered when the camera is not in use.

If there is evidence of particulate contamination on the front window it is possible to clean the window by blowing dry air gently over the window surface. However, the shutter has to be kept open for this procedure, which means that the camera has to be powered up. Therefore since light can access the EMCCD sensor during this time, we recommend that EM Gain is turned off, (readily selectable through the software).

SECTION 2 - INSTALLATION

2.1 - INSTALLING THE HARDWARE

2.1.1 - PC requirements

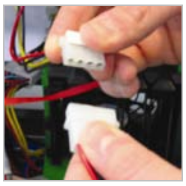
The system requires a PCI/PCIe compatible computer (**PCI 2.2** for CCI-22 & CCI-23 cards or **PCIe** for the CCI-23 controller cards shown on **page 9**) and the minimum recommended PC specifications are as follows:

- 3.0 GHz single core or 2.6 GHz multi core processor
- 2 GB RAM
- 100 MB free hard disc to install software (at least 1GB recommended for data spooling)
- PCI 2.2, PCI-X 1.0 or PCIe slot
- 10,000 rpm SATA hard drive preferred for extended kinetic series - SATA RAID 0 recommended for iXon 885 (e.g. Seagate Barracuda, Western Digital Caviar RE or VelociRaptor etc.)
- Windows (XP, Vista and 7) or Linux

In all cases, the operating system should be on a separate hard drive and the hardware controller should be on a separate PCI bus.

1. Firmly press the connector into the chosen expansion slot, e.g.:

2. For maximum cooling, when the supplied PCI card has an Auxiliary Power connector (“flylead”), this can be connected to a suitable point on the power supply of the PC, e.g.:



NOTE: Should any problems be experienced with this connection, please contact your nearest technical representative.

3. Making sure that the card’s mounting bracket is flush with any other mounting brackets or filler brackets to either side of it, secure the Controller Card in place.
4. Replace the cover of the computer and secure it with the mounting screws if applicable.
5. Reconnect any accessories you were using previously.

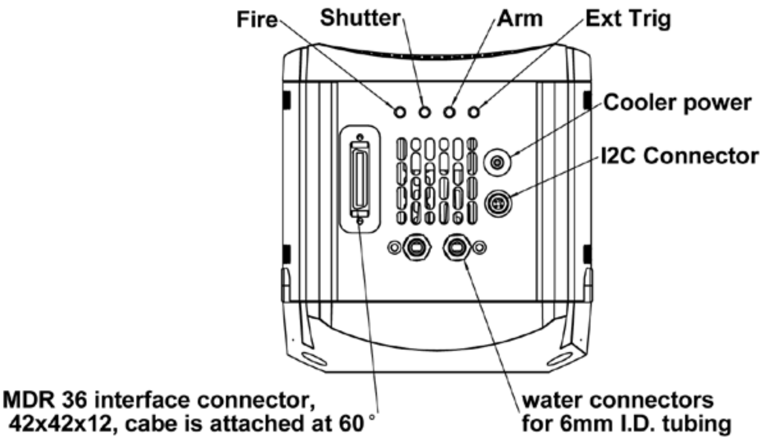


Figure 8: iXon3 connectors

There are six connections points on the iXon3 as shown in **figure 8** above. There are four industry-standard **SMB** (**S**ub **M**iniature **B**) connectors, details as follows:

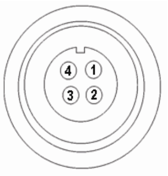
- **Fire** (please refer to **pages 51 - 63**)
- **Shutter** (see **page 64**)
- **Arm** (please refer to **pages 51 - 63**)
- **Ext. Trig (External Trigger Input)** (please refer to **pages 51 - 63**)

These are used to send/receive Trigger and Fire signals. The SMB outputs (Fire & Shutter) are CMOS compatible & series terminated at source (i.e. in the camera head) for a 50Ω cable.

NOTES:

1. **The termination at the customer end should be high impedance (>1KΩ) as an incorrect impedance match could cause errors with timing and triggering.**
2. **The External Trigger Input SMB is TTL level & CMOS compatible and has 470Ω impedance.**
3. **Signal diagrams of these connections can be found on page 79.**

There is an I^{2}C connection point and the pin-outs for this are shown in Figure 9 below:



PIN	FUNCTION
1	I^{2}C DATA
2	I^{2}C
3	+ 5V
4	GROUND

Figure 9: I^{2}C connection (facing in) with pin-outs

The connection for the 26 pin interface between the camera and the PCI controller card is made via an MDR 36 connector shown in **figure 8** above.

The Cooler Power connection is for the **P**ower **S**upply **B**lock (**PSB**) described on **page 11**.

2.3 - WATER PIPE CONNECTORS

Two connectors are fitted to the camera in order to allow water cooling pipes to be connected, e.g.:

These can be connected to a water cooler or recirculator to improve cooling.

2.4 - CONNECTING THE SYSTEM

Connect the elements of your system as follows:

1. Wherever possible, plug your PC into the mains outlet to ensure grounding, but keep the power switched off.
2. Connect the Camera to the **Controller Card** using the **Cable** provided. It is important that this cable is securely fastened to provide a good grounding between the camera and Controller Card.
3. Your system has been supplied with a **PSB** for cooling. The **PSB** connects to the camera via a 2.1mm Jack plug and to the mains electricity supply with a standard plug for your location.
4. There is only one socket on the camera that the **PSB** can be connected to and this is labeled **Cooler Power** (please see **figure 8** on **page 20)**.
5. For best performance the **PSB** should be plugged into the same power source as the computer.

2.5 - MOUNTING POSTS

- Mounting posts can be fitted on three sides of the camera. These can be used to mount the camera if the C-Mount is not used, and/or to mount accessories. **NOTE:.A bag containing two Ø1/2" x 80mm long x 1/4-20 UNC posts is included with all kits**
- There are 3 pairs of holes for the mounting posts, each with 2.0" spacing.

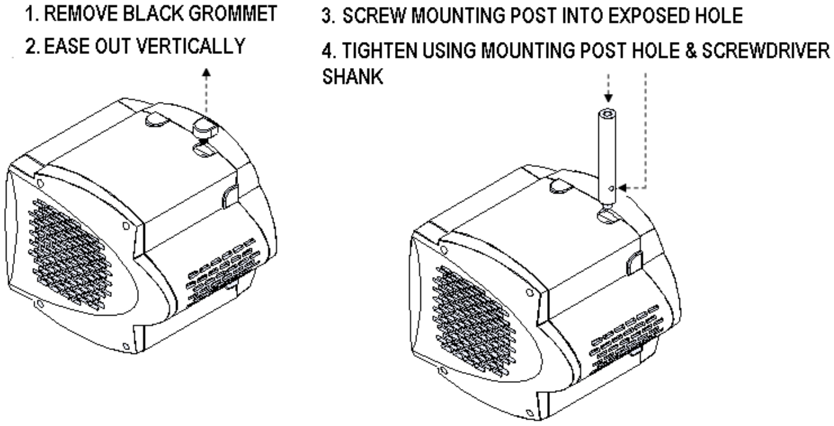
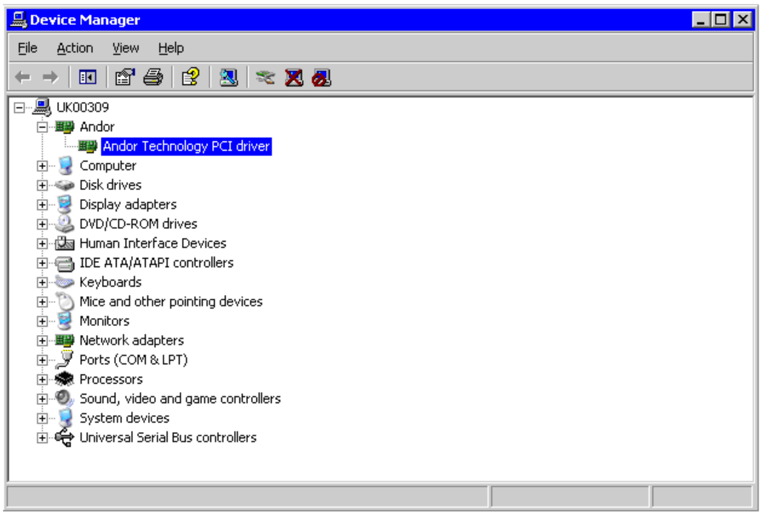


Figure 10: Mounting post installation

2.6 - INSTALLING PCI/PCIe DRIVER - WINDOWS O/S (2000/XP/VISTA)

During the start up sequence the operating system will detect the Andor PCI / PCie controller card and a dialogue box will prompt you for the location of the device driver.

- Insert the **CD** containing the driver file. With Andor Solis or iQ software, this is located on the main application CD. 3rd party software packages may supply a separate driver installation CD containing the Andor drivers. Navigate to the **Setup Information File (atmcd.inf**).
- Select the device driver file and click **OK.**
- Restart the PC. This completes the device driver installation.
- The **Andor Technology PCI driver** should now be shown in the **Device Manager**, e.g.:



2.6.1 - Installing Linux driver

The Andor PCI / PCIe controller card device driver is compiled from source and installed automatically during installation of the Andor Linux SDK using the **install_andor** script.

2.6.2 - Software installation

For detailed instructions on how to install the program software, please refer to the software manual supplied with your camera.

SECTION 3 - FEATURES & FUNCTIONALITY

3.1 - EMCCD OPERATION

3.1.1 - Structure of an EMCCD

Advances in sensor technology have led to the development of a new generation of ultra-sensitive, low light **E**lectron **M**ultiplying **C**harged **C**oupled **D**evices **(EMCCD**s). At the heart of your iXon3 camera is the latest **EMCCD**, a revolutionary technology, capable of single photon detection. An **EMCCD** is a silicon-based semiconductor chip bearing a two-dimensional matrix of photo-sensors or **pixels**. This matrix is usually referred to as the image area. The pixels are often described as being arranged in rows and columns, the rows running horizontally and the columns vertically. The **EMCCD** in the camera is identical in structure to a conventional **C**harged **C**oupled **D**evice (**CCD)** but with the **shift register** extended to include an additional section, the **Multiplication** or **Gain** Register as shown in **figure 11** below:

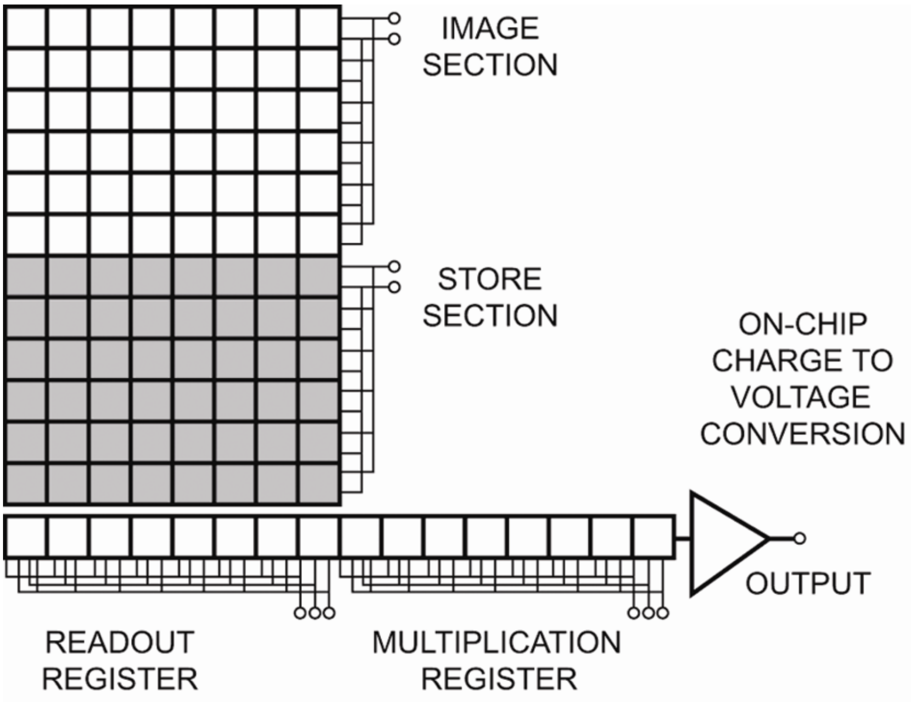


Figure 11: EMCCD structure

During an acquisition using a conventional **F**rame **T**ransfer **CCD** (**FT CCD**), the image area is exposed to light and an image is captured. This image in the form of an electronic charge is then automatically shifted downwards behind the masked region of the chip before being read out. To read out the sensor, charge is moved vertically into the readout register, and then horizontally from the readout register into the output node of the amplifier. As stated on the previous page, the readout register is extended to include the **multiplication** (gain) register. The amplification occurs in this register through the scheme highlighted below in **figure 12** below. When moving charge through a register there is a very tiny but finite probability that the charges being transferred can create additional charge by a process known as “impact ionization”. Impact ionization occurs when a charge has sufficient energy to create another electron-hole pair and hence a free electron charge in the conduction band can create another charge. Hence, amplification occurs. To make this process viable, EMCCD’s tailor the process in two ways, as follows:

1. Firstly, the probability of any one charge creating a secondary electron is increased by giving the initial electron charge more energy. This is typically done by replacing one of the electrodes (phases) of this readout section with two electrodes. The first is held at a fixed potential and the second is operated as normal, except that much higher voltages are employed than are necessary for charge transfer alone. The large electric field generated between the fixed voltage electrode and the clocked electrode is sufficiently high for the electrons to cause “impact ionization” as they transfer. The impact ionization causes the generation of new electrons, i.e. multiplication or gain.
2. Secondly, the EMCCD is designed with hundreds of cells or pixels in which impact ionization can occur and although the probability of amplification or multiplication in any one pixel is small (only around x1.01 to x1.015 times) over the entire length of the EM register the probability is very high and substantial gains of up to thousands can be achieved.

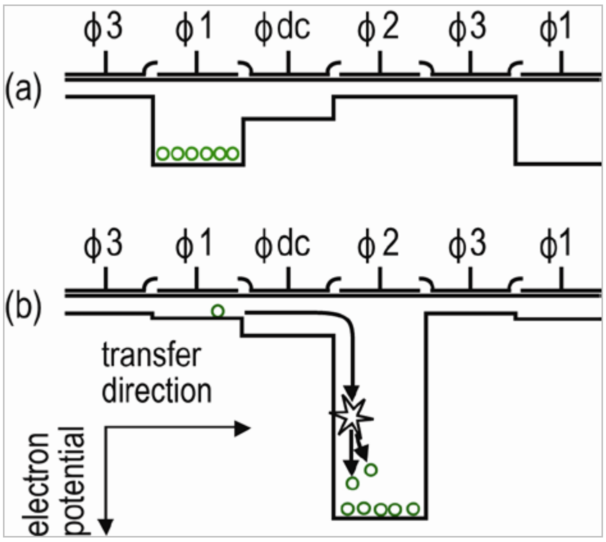


Figure 12: Gain register operation

3.1.2 - EM Gain & Read Noise

As explained on the previous pages, EMCCD sensors allow the detected signal to be amplified on the actual sensor itself before being readout through the output amplifier and digitized by the **A**nalog to **D**igital (**A/D**) converter. The reason that this on-chip-multiplication process gives such a spectacular improvement in low light detection is that it negates the effect of any electronic noise that may be generated by the read out electronics.

All CCD cameras have an associated minimum electronic noise floor which is often termed the **Read Noise** of the system. Read noise is produced during the readout process mostly by the output amplifier but also has contributions from the digitization electronics. This sets the minimum signal level that can be detected by the camera, as any signal level below the read noise level will be indistinguishable from the read noise itself.

Read noise has therefore been the major limiting factor for low light level detection in CCDs for many years until the introduction of EMCCD cameras by Andor Technology in 2000. By applying EM gain, a weak signal that would otherwise be indistinguishable from the read noise can be amplified above the read noise level and thus be read out as a useful signal. This amplification of the signal before being read out effectively reduces the read noise level of the camera and even at relatively modest EM gain settings the effective read noise can be reduced to less than 1 electron r.m.s.

One other point to note is that since read noise increases with increased readout rate the application of EM gain really comes into its own at higher readout rates as any increase in the read noise can be overcome simply by increasing the EM gain. For example, an iXon3 897 typically has a read noise of 50 electrons rms when reading out at 10MHz. This can easily be reduced to < 1 electron by applying > x50 EM gain.

3.1.3 - EM Gain ON vs EM Gain OFF

Figure 13 below shows **S**ignal to **N**oise (**S/N**) plots derived from the specifications of the back-illuminated iXon3 EMCCDs, read out at 10MHz for a photon wavelength at which the **Q**uantum **E**fficiency (**QE**) of the sensor is assumed to be 90%. Such plots are very useful to gauge at what signal intensity it becomes appropriate to use EM Gain to increase S/N.

It is clear that at 10MHz 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 ratio and is shown for reference – if the camera 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 Multiplicative Noise, which has the effect of increasing the shot noise by a factor of $\sqrt{2}$ or ~1.41.

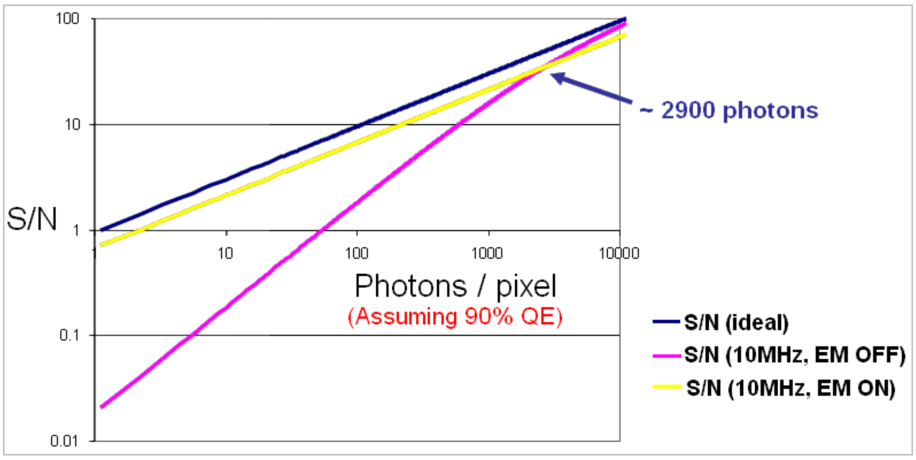


Figure 13: EM Gain ON vs. EM-Gain OFF signal to noise plots for back-illuminated iXon3 EMCCDs at 10MHz readout speed (applies to 897, 860 and 888 models).

Figure 14 below shows S/N plots derived from the specifications of the back-illuminated iXon3 EMCCDs at 1MHz (slower frame rate operation), read out either with EM Gain ON or alternatively through the conventional amplifier (i.e. standard CCD operation). Again, this plot assumes a photon wavelength at which the QE of the sensor is 90%. Specifically this figure applies to 897 and 888 models where the user has the choice of either EMCCD or conventional amplifiers.

At these **slower speed operations** when one has the choice to read out as a “conventional” CCD it can often be advantageous to do so in order to achieve better signal to noise. Indeed the plots show that the cross-over point is at ~42 photons/pixel, below which it is still advised to readout through the EM amplifier with Gain applied.

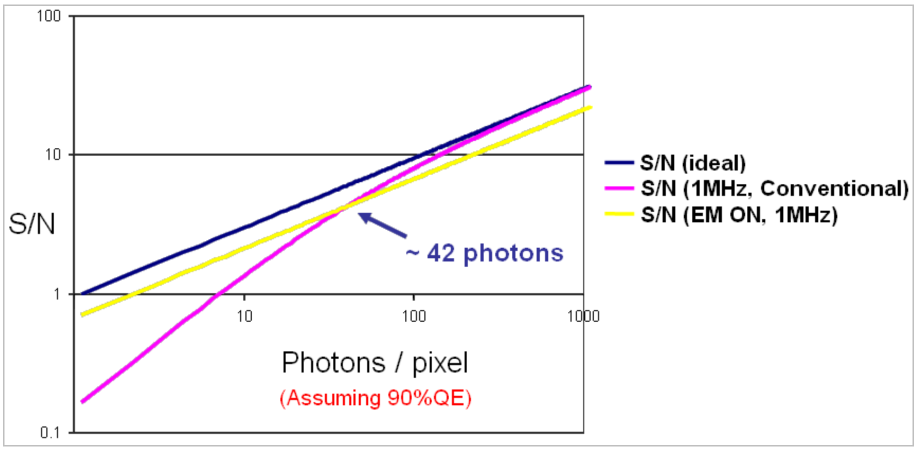


Figure 14: EM Gain ON vs. Conventional Amplifier signal to noise plots for back-illuminated iXon3 EMCCDs at 1MHz readout speed (applies to 897 and 888 models).

3.1.4 - Multiplicative Noise Factor & Photon Counting

It is impossible to know the exact gain a detected signal charge traversing the EM gain register will acquire due to the stochastic nature of the processes which produce EM gain. However it is possible to calculate the probability distribution function of output charge for a given input charge.

At reasonably high gain levels (>30) this uncertainty introduces an additional noise component called **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, only a small probability exists 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 > 590 steps, a very large overall EM gain results. 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 by the gain register from an initial charge packet. 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 $\sqrt{2}$ (or **1.41:1)** for EMCCD technology. **Note: This noise source is significantly greater for the Multi Channel Plate (MCP) of ICCDs than for the gain register of the EMCCD. ICCDs have noise factors typically ranging from 1.5 to >2**.

So, this is an additional form of noise that must be taken into account when calculating Signal/Noise for these detectors. 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 the sensor (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). This additional variation to the signal intensity is represented pictorially below in **figure 15** as a signal intensity profile.

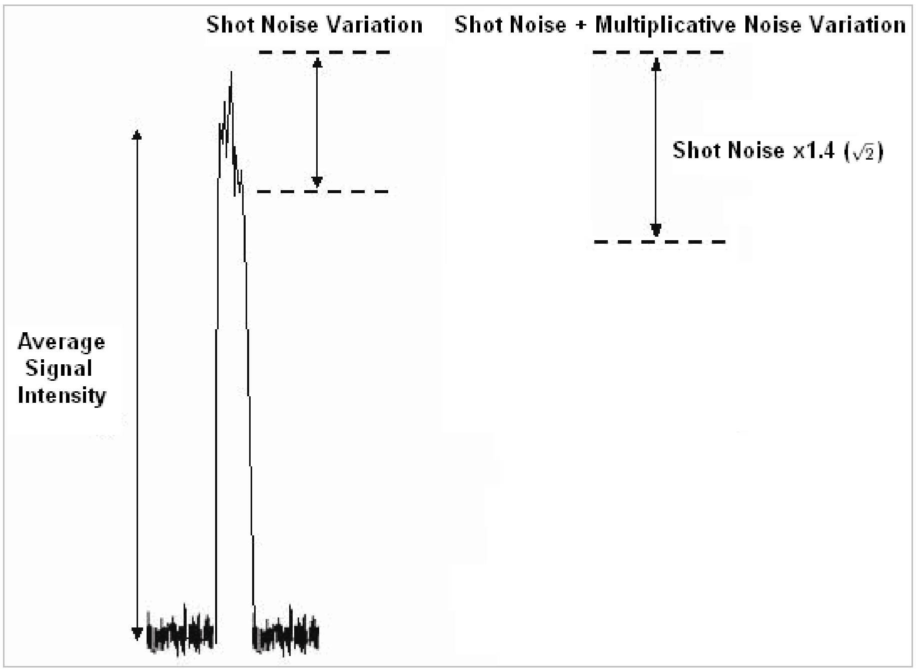


Figure 15: Signal intensity profile

In the limit of when there is less than 1 electron falling on a pixel in a single exposure, the EMCCD can be used in **Photon Counting Mode**. In this mode a threshold is set above the ordinary amplifier readout and all events are counted as single photons. In this mode, with a suitably high gain, a high fraction of the incident photons (>90%) can be counted without being affected by the Noise Factor effect.

3.1.5 - EM Gain dependence and stability

EM gain is a function of the EM voltage and of the sensor operating temperature. When the user applies gain through the software, it is the EM voltage in the gain register that is varied. As can be seen from **figure 16 below**, the dependence of EM gain on EM voltage is sharp (note the logarithmic scaling). This arises because the signal electrons acquire energy as they are accelerated through the EM electric field, and once this field strength reaches the threshold needed to overcome the bandgap energy, the impact ionization rate rises rapidly. This sharp dependence has meant that the software control of EM gain in all EMCCD cameras to date has been via a non-linear scale, with most of the amplification occurring within a relatively small portion at the top of the overall scale. Thus considerable fine tuning by the user to determine an optimal gain setting has been required and even then the actual gain is determined only through measurement of a stable light source, with and without gain applied.

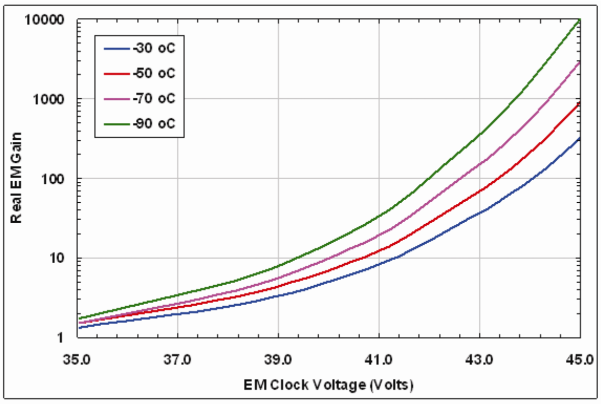


Figure 16: EM gain vs EM clock voltage

Figure 17 shows how the EM gain varies with temperature, this dependence arising primarily from photon scattering of electrons when they are accelerating in the EM electric field. The scattering causes a loss of energy, which increases with temperature. To make up this loss and maintain EM gain, a larger EM electric field must be used at higher temperatures. As can be seen from **Figure 18**, EM gains well in excess of x1000 can be achieved at low temperatures. However it is not recommended that gains above x1000 be used because such high gains can cause significant ageing of the gain register (see **EM Gain Ageing** on **page 32**).

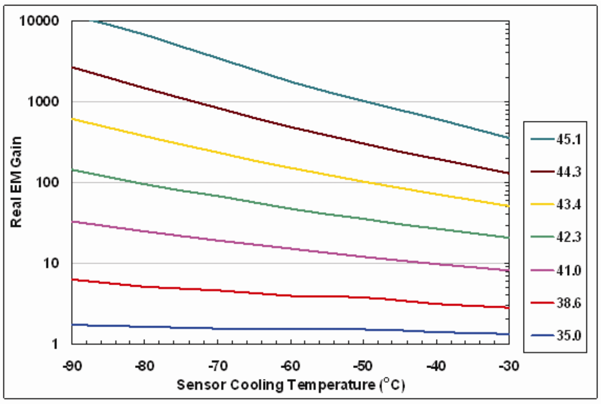


Figure 17: EM gain vs sensor cooling temperature

3.1.6 - RealGain^{TM} : Real and Linear gain

Through a detailed analysis of the complex EM voltage dependence Andor have successfully converted the relationship between EM gain and the EM clock voltage setting into a linear one. Importantly, the actual EM gain can be selected directly from a linear scale displayed in software. No more guesswork with arbitrary gain units on 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. Also, although EM gain is temperature dependent, Andor's linear and real gain calibration extends to any EMCCD cooling temperature. Selecting x300 EM gain @ -50°C, or at -100°C gives the same x300 actual gain! This delivers a new benchmark of simplicity and ease of operation to the user and sets a new precedent in what should be expected from EMCCD technology.

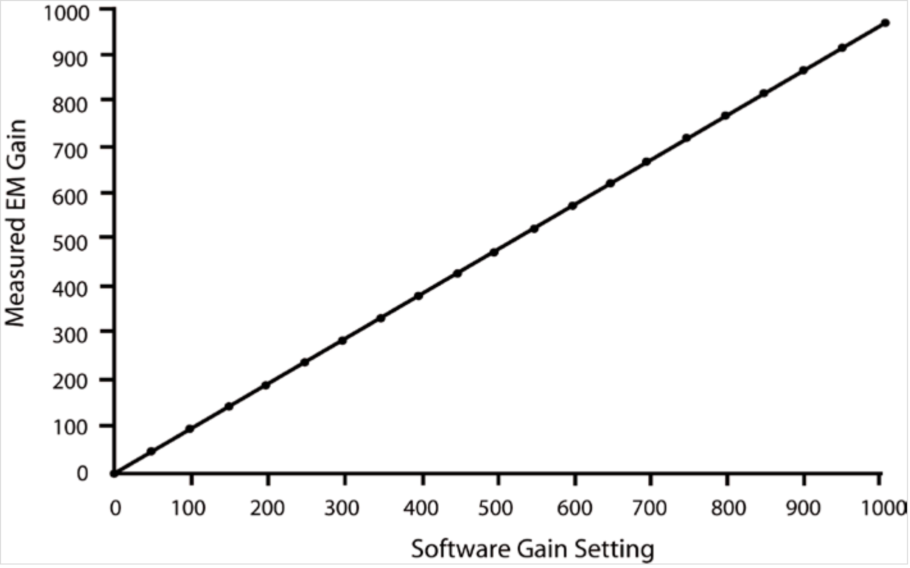


Figure 18: RealGain™ calibration in the iXon3 – the same linear relationship holds across all cooling temperatures

3.1.7 - EM Gain Ageing: What causes it and how is it countered?

As already noted in the discussion on safe camera operation in **Section 2**, EMCCD sensors can suffer from EM gain ageing. This is the phenomenon whereby the EM gain falls off over a period of time when operating at the same clock voltage and cooling temperature. This ageing effect appears to be dependent on the amount of charge that is passed through the gain register, combined with the actual EM electric field strength that it is transferred through. It seems to be very strongly dependent on the EM electric field strength. Therefore when operating at high EM gains the ageing rate can be disproportionately greater. Fortunately, it has been observed that this ageing effect itself decreases with time, meaning that with proper use the device should remain useful for many years. As part of Andor's EMCCD production process all sensors are exposed to conditions that results in much of the "shorter-term ageing" having already occurred prior to calibration and setting of the EM gain.

The explanation for this ageing effect is not fully understood, but it is assumed that accelerating charge through the high electric fields is causing a tiny fraction of that charge to become permanently embedded in the insulator (typically silicon dioxide) between the EM electrode and the active silicon. This slow build-up of charge effectively reduces the field strength produced by the electrode. The signal electrons therefore experience a lower accelerating potential which subsequently produces fewer secondary electrons from the impact ionisation process resulting in less electron multiplication and, in effect, a lower EM gain.

In order to minimise the effect of EM gain ageing it is recommended that the following guidelines are always adhered to:

- Do not use EM gains greater than necessary to overcome the read noise (please refer to **figure 13** on **page 25** and **figure 14** on **page 26**). A rule of thumb is that a gain of x4 or 5 the root-mean-square read noise (accessible from the performance sheet) is more than sufficient to render this noise source negligible. In practice, this can always be achieved with EM Gain of less than x300 (often much less). Pushing gain beyond this value would give little or no extra S/N benefit and would only reduce dynamic range.
- Only select the extended EM gain scale of x 1000 when **single photon counting** and always ensure that the signal falling onto the sensor is indeed within the regime of low numbers of photons per pixel.
- Turn the EM gain OFF when not in use.
- Try not to over-saturate the EMCCD detector.

For simplicity and ease of use many of these guidelines have been uniquely woven into the iXon3 systems, to make it difficult for the user to step outside of them and unwaringly cause accelerated sensor ageing. This defence is two pronged, and makes heavy use of Andor's linear and quantitative gain calibration scale (**RealGain™)** described above.

3.1.8 - Gain & signal restrictions

Part of the measures taken have been to invoke temperature compensated real gain limits, coupled with signal intensity feedback (after EM amplification). This ensures that the user is unable to apply excessive gain and/or signal, any more than is necessary to render the read noise floor negligible for a given signal intensity and readout speed. Secondly, when not actually acquiring data, for example, during “keep clean” cycles or when outside a selected sub-image area, Andor EMCCDs have been internally configured to prevent any unwanted signal entering the EM gain register. Together these measures ensure that the rate of EM gain ageing is significantly reduced.

3.1.9 - EMCAL™

Andor have developed, in the iXon3, a unique and patented method of user-initiated **EM gain self recalibration - EMCAL™**. This is available only in iXon3 cameras that contain L3 vision sensors from E2V, i.e. 897 and 888 models. The 885 range of iXon3 cameras contain sensors from Texas Instruments that show negligible levels of gain ageing, and thus the EMCAL™ function is not required.

Thus for the L3Vision based cameras, even after exercising due care during usage and availing of the above internal restrictions, the EM gain will gradually decrease over an extended period of time. This reduction in EM gain can be rectified by using the EMCAL™ self recalibration process which is very easily initiated by the user. Check on-line for the latest EMCAL™ routine.

This process uses the iXon3 in-built temperature compensated linear gain scales to reset the EM gain calibration to reflect the true values requested on the software scale, in reality giving **RealGain™** values and thus markedly prolonging the operational lifetime and quantitative reliability of the technology, and circumventing the need to return to the factory for recalibration. To the user, this means optimal signal to noise ratio, maximum dynamic range and prolonged system longevity.

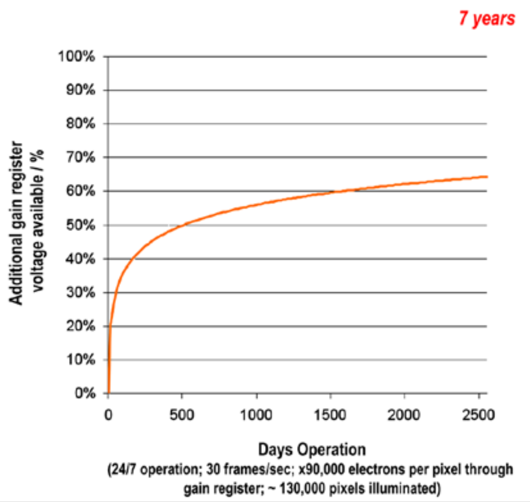


Figure 19: Ageing profile of an Andor backlit EMCCD. Test conditions: 24/7 operation; 30 frames/sec; x90,000 electrons per pixel through gain register; ~ 200,000 pixels illuminated

3.2 - COOLING

The EMCCD sensor is cooled using a **T**hermo**e**lectric (**TE**) cooler. TE coolers are small electrically powered devices with no moving parts, making them reliable and convenient. A TE cooler is actually a heat pump, i.e. it achieves a temperature difference by transferring heat from its "cold side" (the EMCCD sensor) to its "hot side" (the built-in heat sink). Therefore the minimum absolute operating temperature of the EMCCD depends on the temperature of the heat sink. Andor's vacuum design means that we can achieve minimum cooling temperatures unrivalled by other manufacturers. The maximum temperature difference that a TE device can attain is dependent on the following factors:

- Heat load created by the CCD
- Number of cooling stages of the TE cooler
- Operating current

3.2.1 - Cooling options

The heat that builds up on the heat sink must be removed and this can be achieved in one of the two following ways:

1. **Air cooling:** a small built-in fan forces air over the heat sink
2. **Water cooling:** external water is circulated through the heat sink using the water connectors on the head and this can take one of the following forms:
    - Recirculation
    - Chilling

All Andor iXon3 systems support both cooling options. Whichever method is being employed, it is not desirable for the operating temperature of the CCD simply to be dependent on, or vary with, the heat sink temperature. Therefore, a temperature sensor on the CCD (combined with a feedback circuit that controls the operating current of the cooler) allows stabilization of the CCD to any desired temperature within the cooler operating range.

3.2.2 - Fan settings

The speed of the cooling fan can also be controlled, useful if working in experimental configurations which are extremely sensitive to vibration. The vast majority of applications, including optical microscopy, can be used with the default highest fan speed, since the vibrations from the fan are minimal. However, some applications can be extremely sensitive to even the smallest of vibrations (such as when combining an optical set-up with patch clamp electrophysiology or atomic force microscopy) and it can be useful to either select a slower fan speed, or to temporarily turn off the fan altogether, for the duration of the acquisition.

If the fan is being turned off altogether, depending on the cooling temperature selected and on the ambient temperature, the acquisition duration can be as long as 15 - 20 minutes before temperature begins to rise. The fan must then be turned on again to give the head time to re-stabilize (dissipate built-up excess heat from the peltier TE cooler) before the next acquisition is begun.

NOTE: If water cooling is being used, the fan can be turned off and exceptional cooling performance maintained indefinitely.

3.3 - SENSOR READOUT OPTIMIZATION

To allow the camera to be optimized for the widest range of applications it is important to have flexibility in the readout options available, some of these include:

- Cooling (please see **page 34**)
- Sensor preamp settings
- Variable horizontal readout rate
- Variable vertical shift speed
- Output amplifier selection
- Baseline settings
- Binning and sub image settings

These options and an explanation of how to optimize them are explained on **pages 36 - 42**.

3.3.1 - Sensor PreAmp options

An EMCCD sensor can have a much larger dynamic range than can be faithfully reproduced with the Analogue/Digital converters and signal processing circuitry currently available on the market today. To overcome this shortcoming and access the range of signals from the smallest to the largest and to optimize the camera performance it is necessary to allow different pre-amplifier gain settings. However, it is only ever recommended selecting something other than the default highest PreAmp (most sensitive) setting for applications that are extremely challenged by dynamic range concerns. It is very important, however, that for such high-dynamic range applications, the user applies even more care to the amount of EM gain applied (high EM gain can drastically reduce the true dynamic range of the camera). Ideally, for maximum dynamic range whilst maintaining improved **S**ignal to **N**oise (**S/N**), the EM gain setting should be set equal to the read noise at the readout speed selected (value obtainable from the performance sheet that comes with the delivered system).

Pre-amplifier gain selection in CCDs is traditionally used to trade off S/N vs dynamic range. A higher PreAmp setting means fewer electrons/count, resulting in a lower system noise floor, therefore a higher S/N. However, high PreAmp settings may not match well to the pixel well depth of the sensor, therefore a lower setting can be selected to meet the full well depth potential, e.g. a PreAmp setting yielding 1.5 e^-/count may be selected to ensure that the 65536 digitization levels of a 16-bit A/D is closely matched to a 100,000 e^- pixel well depth. A PreAmp setting of 1 e^-/count, while giving a lower noise floor, would not harness the full 100,000 e^- well depth.

The situation is not nearly as straightforward for EMCCDs because:

1. EM gain overcomes readout noise and amplifies signals relative to the digitization noise (which is fixed for a given PreAmp setting).
2. Gain register pixels have a greater well depth than the imaging pixel well depth

The latter point can be particularly confusing and indeed has led to confusion in the field. What this has meant is that we have set some of the lower pre-amp settings associated with the EM-output to match the extended well capacity of the gain register pixels (as reported by the sensor manufacturers E2V or TI). This means that these pre-amp settings are designed to be used **with EM Gain**! Otherwise, the lower well capacity of the imaging pixels will saturate long before the A/D. This is why some users have been confused at not being able to reach the full ~16k counts of the 14-bit A/D channel, when they hadn't applied EM Gain.

Basically, Andor recommend using the default highest value PreAmp setting (e.g. x4.8 setting of the iXon3 897E giving ~ 11.3 e^-/count @ 10MHz) for most low-light applications. Most genuinely low light applications are not limited by well capacity, as long as sensible EM gain settings are applied (we recommend not exceeding x300 EM gain, except for single photon counting experiments). At this highest PreAmp setting, the 14-bit A/D would saturate @ 16,380 x 11.3 e^-/count = 180,800 e^-. In our opinion, this is a resaonable range to cover the majority of low light measurements. For example, with an EM gain of x300 (RealGain™), it would take 600 electrons in a pixel of the sensor to reach this A/D saturation limit. Say the QE is 80% at the wavelength of interest then this corresponds to maximum of 750 photons falling onto that pixel. That is perfectly satisfactory dynamic range for the vast majority of low light imaging applications.

The core reason for us wishing to recommend this PreAmp setting, even over the middle (~x2.4) PreAmp setting, is that it implements an additional restriction as to how much charge is allowed to build up in the sensor. This in turn will help **minimize the rate of EM gain ageing** (please see **page 31** for further details on measures against gain ageing). However, some applications can be very demanding of dynamic range, and for those we recommend using a lower PreAmp setting such as x2.4. This will ensure the A/D capacity is more closely matched to the well capacity of the gain register pixels, thus affording maximum dynamic range. Also as mentioned above, to maximize the true dynamic range of the camera we recommend tuning the RealGain™ gain setting to a value close to the value of the readout noise at the selected readout speed (e.g. if readout noise is ~ 50 electrons @ 10 MHz, set the EM gain to x50 for maximum dynamic range).

3.3.2 - Variable Horizontal Readout Rate

The **Horizontal Readout Rate** defines the rate at which pixels are read from the shift register. The faster the horizontal readout rate the higher the frame rate that can be achieved. The ability to change the pixel readout speed is important to achieve the maximum flexibility of camera operation, particularly in terms of dynamic range. Slower readout typically allows lower read noise and higher available dynamic range, but at the expense of slower frame rates. There are number of different horizontal readout rates available on all iXon3 models. Please refer to the performance sheet for readout rates available on your particular model.

3.3.3 - Variable Vertical Shift Speed

The vertical shift speed is the time taken to vertically shift all pixels one row down, with the bottom row entering the shift register. The ability to vary the vertical shift speed is important for several reasons. It is possible using the different vertical speeds to better synchronize the frame rates to external events such as a confocal spinning disc. Faster vertical shift speeds also have benefits such as lower **C**lock **I**nduced **C**harge (**CIC**). A drawback with faster vertical shift speeds is that the charge transfer efficiency is reduced, effectively reducing the pixel well depth. This is particularly important for bright signals as a pixel with a large signal is likely to have some charge left behind if the vertical shift speed is too fast. This will result in degraded spatial resolution.

Slower vertical clocks ensure better charge transfer efficiency giving maximum pixel well depth but result in a slower maximum frame rate. To improve the transfer efficiency the clocking voltage can be increased using the vertical clock voltage amplitude setting. However, the higher the voltage, the higher the clock-induced charge. Thus the user must make a measured judgement as to which setting works best for their situation, for example:

- **For low CIC:** Use the fastest vertical shift speed that still transfers charge correctly (no image distortion), without having to select excess vertical shift voltage amplitude
- **For maximum pixel well depth:** Use the slowest vertical shift speed, which will give an increase in CIC
- **For maximum frame rate:** Use the fastest vertical shift speed and increase the vertical shift voltage amplitude to the minimum value that regains the full pixel well depth.
- **To reduce vertical smearing during very short exposure**: use a faster vertical shift speed. This vertical smearing is due to the fact that light is still falling on the image area during the short time taken to transfer the charge from the image area into the storage area. If the actual exposure time is of a similar magnitude to this transfer time then as pixels are shifted vertically through brighter regions of the image they will collect "extra" charge which will manifest itself as vertical streaking. **NOTE: For extremely short exposure times, a fast external shutter or pulsed light source may be required.**
- **For short exposures (e.g. 1ms):** with high signal count and DC illumination, it may be necessary to increase the vertical clock voltage to ensure that the keep clean cycle can fully remove the extremely high (saturated) signal that may have accumulated during the sensor readout phase.

3.3.4 - Output amplifier selection

A number of the EMCCD sensors in the iXon3 range have dual output amplifiers, an electron multiplying output amplifier and a conventional output amplifier. This increases the versatility of the camera as the EM amplifier can be selected for fast imaging in low light conditions whilst the conventional amplifier can be selected where more light is available and a slower readout with its associated lower read noise and higher dynamic range is preferred.

Figure 20 below details schematically the readout structure on sensors with both output amplifiers present. From this it can seen that when reading out through the EM amplifier accumulated charge will move to the right along the serial register and then into the EM gain register. When the conventional output amplifier is selected the charge to be read out will move along the serial register to the left then be transferred directly into the conventional output amplifier. This change in direction has the effect of producing mirror images when comparing raw data from the two output amplifiers. Some software packages will automatically reverse the image orientation of one of the output amplifiers to allow direct comparison of images. The user should consult their software manual to verify if this is the case.

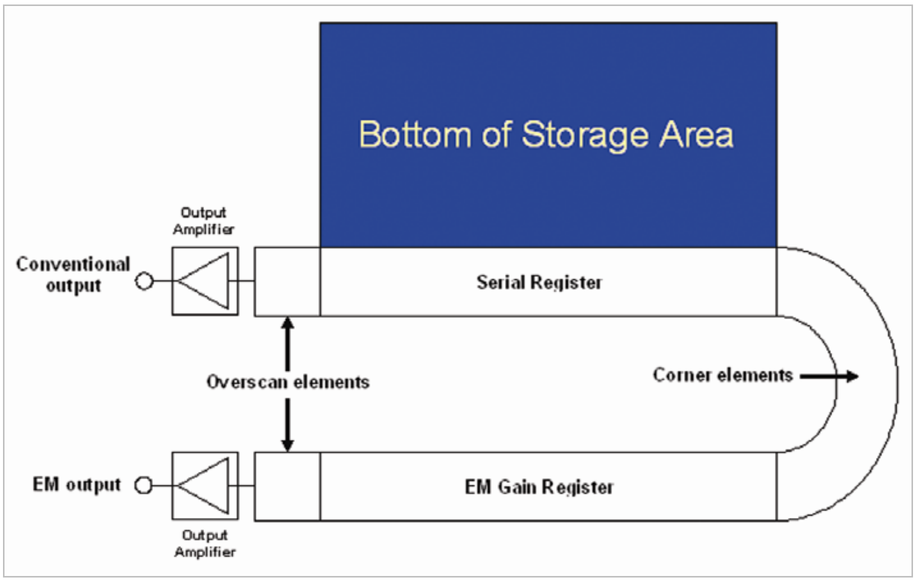


Figure 20: Sensor readout structure

3.3.5 - Baseline Optimization

3.3.5.1 - Baseline Level and Baseline Offset

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. This baseline level often tends to increase with decreasing sensor cooling temperature. For all iXon3 cameras it is factory calibrated to approximately 400 counts at a cooling temperature of -75°C.

NOTES:

- **At warmer temperatures the baseline level will decrease and may move below zero resulting in a signal of zero counts being displayed. This can be overcome either by moving to a lower cooling temperature or by using the baseline offset option which adds up to 1000 counts to the baseline level.**
- **Conversely at colder temperatures than the calibration temperature the baseline may increase slightly and this can be countered by using the baseline offset option to subtract up to 1000 counts from the baseline level.**

3.3.5.2 - Baseline Clamp

When acquiring data small changes in the ambient temperature and/or in the heat generation of the driving electronics within the camera may cause some drift in the baseline level. This is most often observed during long kinetic series.

Any drift in the baseline level can be corrected by using the **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 100 counts to ensure that the displayed signal level is always a positive number of counts.

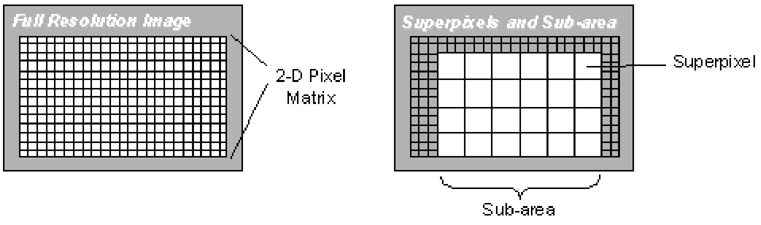
NOTE: Before activating Baseline Clamp, please ensure that the baseline level is above zero counts for the cooling temperature selected.

3.3.6 - Binning and Sub Image options

Binning is a process that allows charge from two or more **pixels** to be combined on the **EMCCD**-chip prior to readout. Summing charge on the EMCCD and doing a single readout gives better noise performance than reading out several pixels and then summing them in computer memory. This is because each act of reading out contributes to noise. There are two types of the binning as follows:

- **Vertical Binning:** Where charge from two or more rows of the EMCCD-chip are moved down into the shift register before the charge is read out. The number of rows shifted depends on the binning pattern selected. Thus, for each column of the EMCCD-chip, charge from two or more vertical elements is **summed** into the corresponding element of the shift register. The charge from each of the pixels in the shift register is then shifted horizontally to the output amplifier and read out.
- **Horizontal Binning**: Where charge from two or more pixels in the serial register are transferred into the output amplifier and read out as one combined data value. Thus the charge from two or more of the horizontal elements is effectively summed into the output amplifier before being readout.

Combining both the vertical and horizontal binning methods produces “**Superpixels”**. These consist of two or more individual pixels that are binned and read out as one large pixel. Thus the whole CCD, or a selected sub-area becomes a matrix of Superpixels, e.g.:



NOTE: ONLY THE SUB-AREA WILL BE DISPLAYED, NOT THE AREA AROUND IT.

The horizontal and vertical binning parameters determine the dimensions of any superpixels created. On the one hand superpixels result in a loss of spatial resolution when compared to single pixel readout, but on the other hand they offer the advantage of summing data on-chip prior to readout thereby producing a better signal to noise ratio and a higher frame rate. All iXon3 models offer completely flexible binning patterns which are user-selectable from software.

For the purpose of initial focusing and alignment of the camera, or to increase the readout speed, the user may wish to only readout a particular sub-area of the CCD to produce a **Sub Image**.

When a sub image has been defined, only data from the selected pixels will be digitized. Data from the remaining pixels will be discarded. The flexible configuration of the iXon3 allows the user to set the **Sub Image** area to any size and location on the CCD chip.

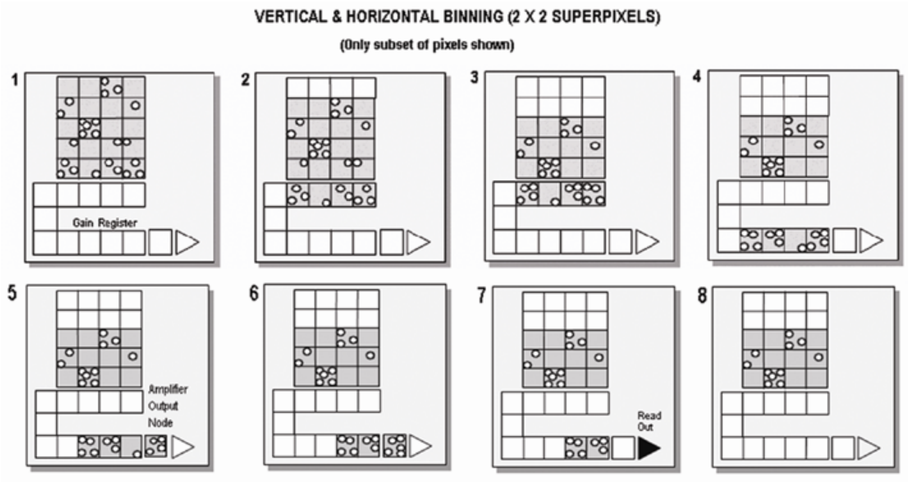


Figure 21: Vertical & Horizontal binning of two rows

Step 1	Charge is built up on the sensor
Step 2	Charge in the frame is shifted vertically by one row, so that the bottom row of charge moves down into the shift register.
Step 3	Charge in the frame is shifted vertically by a further row, so that the next row of charge moves down into the shift register, which now contains charge from two rows - i.e. the charge is vertically binned.
Step 4	Charge in the shift register is moved horizontally (through the EM gain register, if using the EM output amplifier) until the first data pixel is just about to enter the output node of the amplifier.
Step 5	Charge in the shift / EM gain register is moved horizontally by one pixel, so that charge on the endmost pixel of the shift register is transferred into the output node of the amplifier
Step 6	Charge in the shift register is again moved horizontally, so that the output node of the amplifier now contains charge from two pixels of the shift register - i.e. the charge has been horizontally binned.
Step 7	The charge in the output node of the amplifier is passed to the analog-to-digital converter and is read out
Step 8	**Steps 5 - 7** are repeated until the shift register is empty. The process is repeated from **Step 2** until the whole frame is read out.

3.4 - ACQUISITION OPTIONS

3.4.1 - Capture Sequence in Frame Transfer Mode

A number of acquisition modes are available for the iXon3 range to best suit your experimental demands. In **F**rame **T**ransfer **(FT)** acquisition mode, the iXon3 can deliver its fastest performance whilst maintaining optimal Signal to Noise. It achieves this through simultaneously acquiring an image onto the image area whilst reading out the previous image from the masked frame storage area. Thus there is no time wasted during the readout and the camera operates with what is known as a 100% ‘duty cycle’.

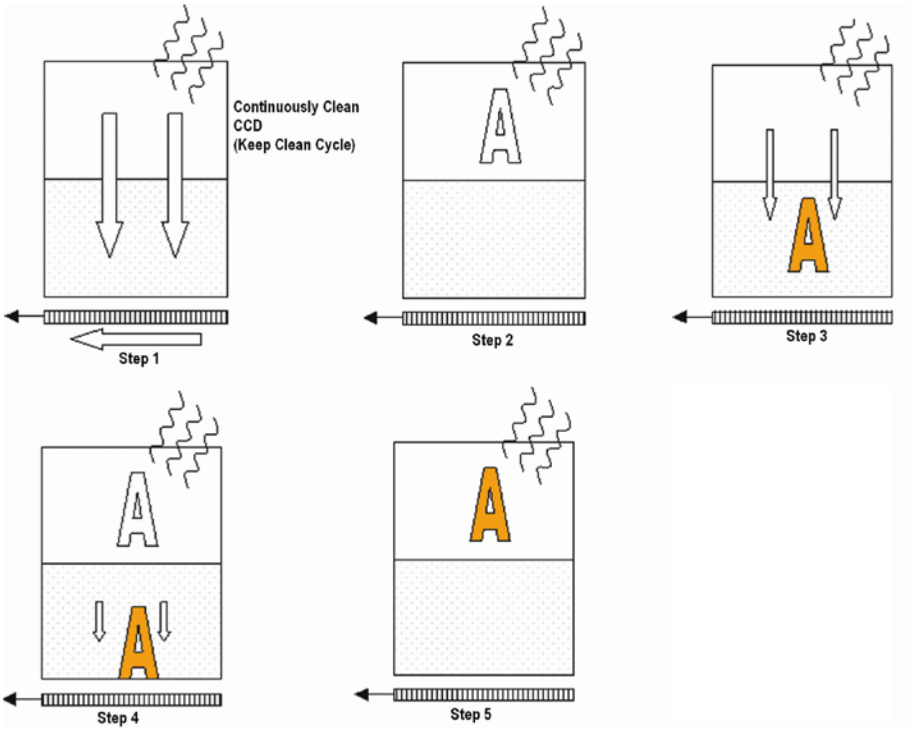


Figure 22: Capture sequence (FT mode)

Step 1	Both **Image** and **Storage** areas of the CCD are fully cleaned out. This is known as a "**Keep Clean Cycle**". Keep Clean Cycles occur continuously to ensure that the camera is always ready to start an acquisition when required. Further details of the keep clean cycle are given later.
Step 2	On receipt of a Start acquisition command the CCD stops the Keep Clean Cycle. This allows the image (photoelectric charge) to build up in the **Image** area of the CCD. The CCD remains in this state until the exposure time has elapsed, at which point the read-out process starts.
Step 3	The first phase of the readout process is to quickly shift the charge, built up in the **Image** area, into the **Storage** area. The time required to move the charge into the **Storage** area is approximately calculated as follows: (**No. of rows in the Image area) x (vertical shift rate).**
Step 4	Once the **Image** area has been shifted into the **Storage** area the **Image** area stops vertically shifting and begins to accumulate charge again, i.e. the next exposure starts. While the **Image** area is accumulating charge the **Storage** area is being read out. This readout phase can take tens of milliseconds to seconds depending on the image size, readout pattern and readout speed.
Step 5	On completion of the readout, the system will wait until the exposure time has elapsed before starting the next read-out (i.e. returning to **Step 3**).

- In this mode, there are no keep cleans between images during an accumulation or kinetics series as they are not necessary
- This mode gives the fastest way to continually take images; however, the minimum exposure time is restricted to the time taken to read-out the image from the Storage area.
- The accumulation cycle time and the kinetic cycle time are fully dependent on the exposure time and hence cannot be set via software.
- In external trigger mode there are no keep cleans and the External trigger starts the "read out" phase. The exposure time is the time between external triggers and hence the user cannot set the exposure or cycle times. However, the user can define the amount of time between the external trigger event occurring and the readout starting. This can be useful in those situations where the TTL trigger occurs before the light event you are trying to capture. This effectively moves the exposure window in time but the exposure time is still the period between trigger events.
- There is no need for a mechanical shutter. The exposure time is long, compared to the time required to shift the image into the Storage area and therefore image streaking will be insignificant.

3.4.2 - Capture Sequence in Non-Frame Transfer Mode with an FT CCD

It is also possible to operate an FT CCD in a **N**on-**F**rame **T**ransfer (**NFT**) mode. In this mode of operation, an FT CCD acts much like a standard CCD. The capture sequence for this mode is illustrated here:

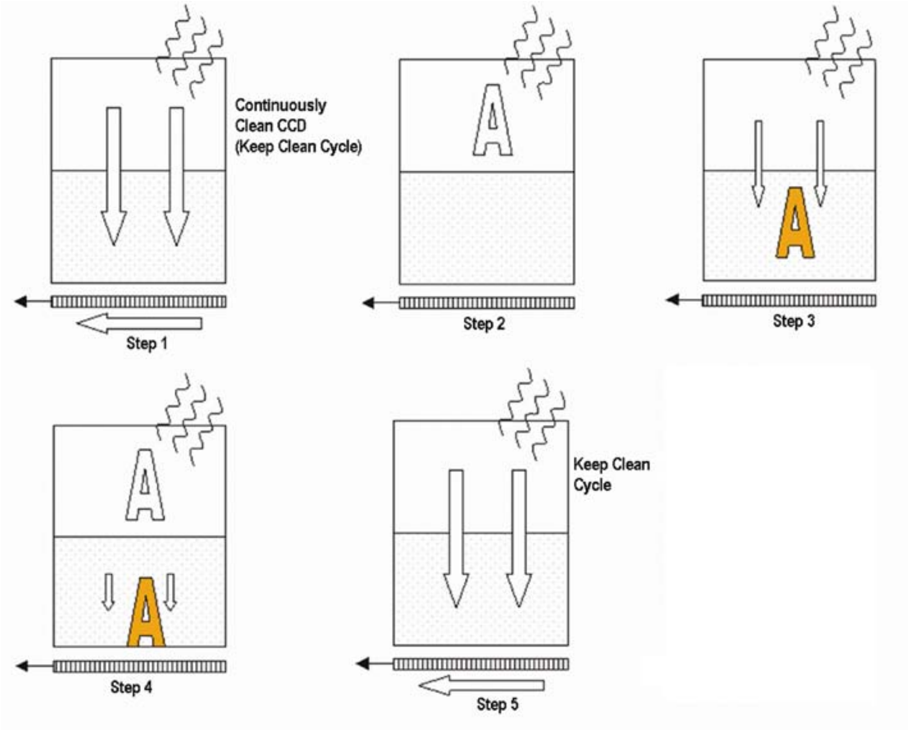


Figure 23: Capture sequence (NFT mode)

Step 1	Both **Image** and **Storage** areas of the CCD are fully cleared out.
Step 2	On receipt of a start acquisition command the CCD stops the **Keep Clean Cycle** and an acquisition begins. The image builds up in the **Image** area of the CCD until the exposure time has elapsed, at which point the read-out process starts.
Step 3	The first phase of this process is to quickly shift the charge built up in the **Image** area into the **Storage** area. The time required to move the charge into the **Storage** area is the same as in Frame Transfer mode.
Step 4	With the image now in the **Storage** area the captured image is read out. The time taken to read out the image is again the same as in the **Frame Transfer** mode.
Step 5	On completion of the read-out, the CCD is completely cleared, ready to acquire the next image. The CCD will remain in the Keep Clean Cycle until the end of the accumulation or kinetic cycle time, depending on the acquisition mode, i.e. back to **Step 1**. As at least one Keep Clean Cycle is performed between each exposure, the minimum exposure time is no longer set by the time to read out the image.

3.4.2.1 - Points to note about using an FT CCD as a standard CCD

- The exposure time, accumulation cycle time and the kinetic cycle time are independent.
- The minimum exposure time is not related to the time taken to readout the image.
- External trigger operates as if the CCD was an NFT CCD.
- As the captured image is quickly shifted into the Storage area, even in NFT mode, the system can still be used without a mechanical shutter.
- For short exposure times the image may appear streaked as the time taken to shift the Image area into the Storage area and the exposure time may be of similar magnitude, but much less than a ral non-frame transfer.
- Light falling on the Image area while the Storage area is being read out may contaminate the image in the Storage area due to charge spilling vertically along a column from the Image area. The slower the read-out rate or the shorter the exposure time the greater the possibility of corruption. To see why this is the case consider the following situation:

"During a 100us exposure enough light has fallen on a pixel to register 10000 counts, or 100,000 electrons assuming 10e/count. The image is then shifted into the Storage area. To read out the image, assuming 1000x1000 pixels, it would take approximately 100ms at 10MHz readout rate. This means that during the reading out of the image 10 million counts (10000 * 1000) will have been acquired into the pixel described above. As a pixel saturates at approximately 160,000 electrons this means that the pixel will over saturated by 60 times. All the excess charge has to go somewhere, and spreads vertically along the CCD column. As the clocks in the Image area are not actively shifting the charge, the mobility of the charge will be low and you may not see any effect. However, when you consider that more than one pixel in any given column could be exposed to 10000 counts per 100µs. The chance of corrupting data is correspondingly increased. Reducing the amount of light falling on the CCD outside of the exposure period and increasing the exposure time accordingly will reduce the possibility of data corruption".

3.4.3 - Capture Sequence for Fast Kinetics with an FT CCD

Fast **K**inetics (**FK**) is a special readout mode that uses the actual CCD as a temporary storage medium and allows an extremely fast sequence of images to be captured. The capture sequence is illustrated here:

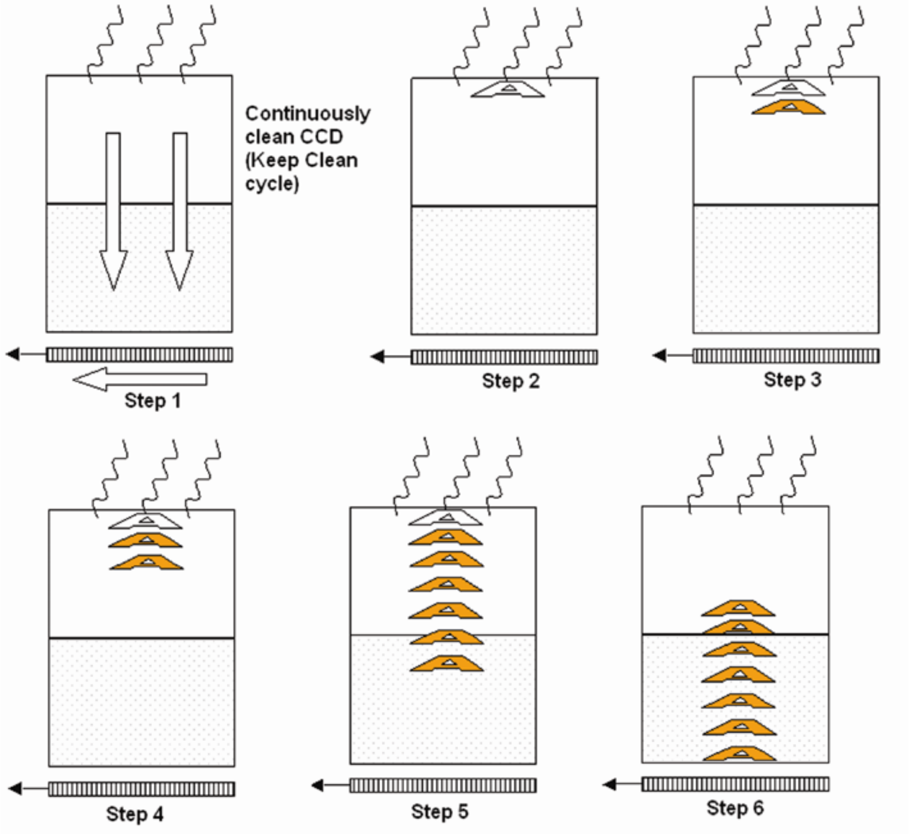


Figure 24: Capture sequence (Fast Kinetics mode)

Step 1	Both the Image and Storage areas of the CCD are fully cleaned (the **Keep Clean Cycle**).
Step 2	The CCD stops the Keep Clean Cycle and the acquisition begins. The image builds up on the illuminated sub-area of the CCD.
Step 3	The CCD remains in this state until the exposure time has elapsed, at which point the complete CCD is clocked vertically by the number of rows specified by the user.
Step 4 & 5	The process is continued until the number of images stored equals the series length set by the user.
Step 5	At this point the sequence moves into the readout phase by first vertically shifting the first image to the bottom row of the CCD. The CCD is then read out in the standard method.

3.4.3.1 - Points to consider when using Fast Kinetics mode

- Light **<u>must</u>** 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.
- Due to the very short exposure times, streaking may be evident.

3.4.4 - Keep Clean Cycles

iXon3 cameras have a range of different **Keep Clean Cycles** that are run depending on the actual model and the state the camera is in. The first keep clean cycle to be discussed is the one that runs while the camera is in an idle state, i.e. waiting for the PC to tell it to start an acquisition sequence. We will then look at the keep clean cycle running during an internal trigger kinetics series sequence. Finally, we will look at the keep clean cycle running while the camera is waiting for an external trigger event to occur.

When the camera is idle, i.e. not actively capturing images, it is repeatedly running the **Idle Keep Clean Cycle**. This cycle is composed of a vertical shift, followed by a series of horizontal shifts. The number of horizontal shifts is dependent on the actual CCD.

When the **Start** command is received from the PC, the camera will complete the current keep clean cycle and then perform a sufficient number of vertical shifts to ensure both **Image** and **Storage** regions are completely charge free, see the figure below. On completion of this sequence the camera is ready to run the exposure sequence. The exact exposure sequence will depend on several factors including the trigger and the readout modes selected. These will be discussed later in this document.

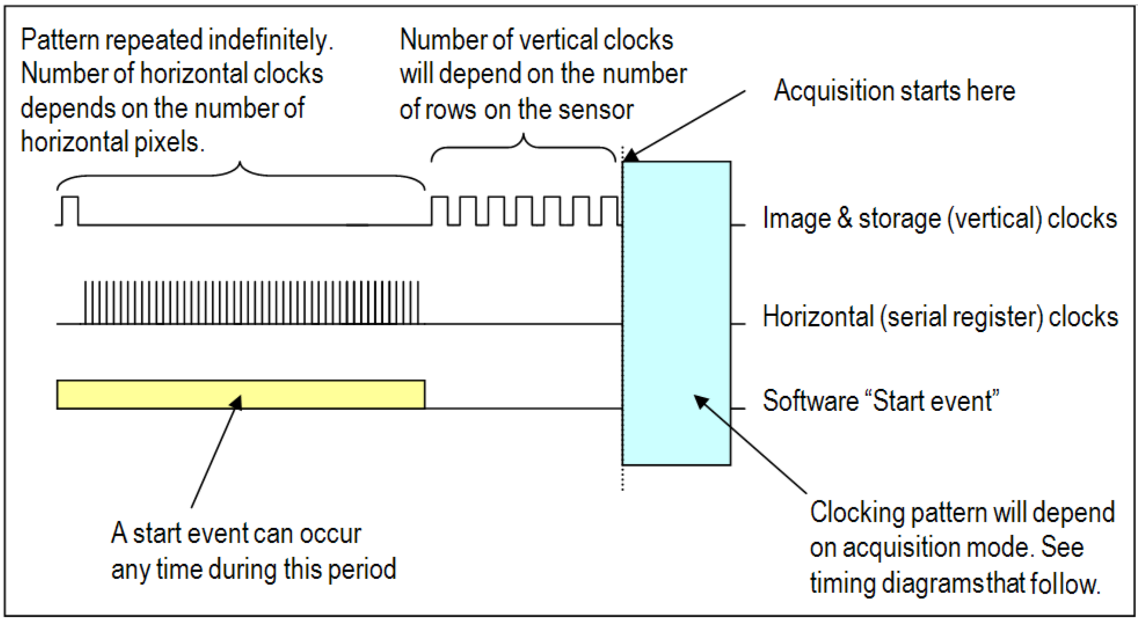


Figure 25: Idle Keep Clean Cycle

The second type of keep clean is executed between individual scans in a kinetic series, and is relevant to Non-Frame Transfer Mode combined with either Internal or Software Trigger. It is called the **Internal Keep Clean Cycle**. When the user configures a kinetics series acquisition as well as defining the exposure time and the readout mode, they also define the number of scans to capture and the time between the scans. During the time between individual scans the sensor must be kept free of charge to ensure the data captured is a true reflection of the light that fell on it during the exposure period. The keep clean cycle run during this time is very similar to that described in the **Idle Keep Clean Cycle** on the previous page in that the cycle is one vertical followed by a series of horizontals. In this mode however the number of times the cycle is repeated is determined by the cycle time set by the user. The keep clean is completed with a sufficient number of vertical shifts to ensure both the **image** and **storage** areas are charge free.

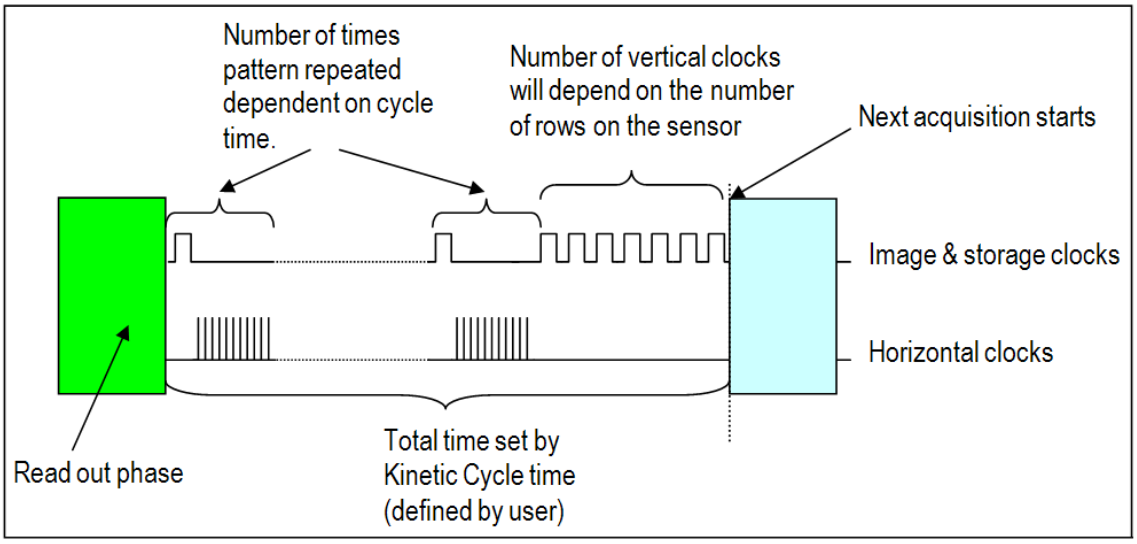


Figure 26: Internal Keep Clean Cycle

The third keep clean is the **External Keep Clean Cycle.** This cycle uses a different sequence of horizontal and vertical clocking as it must be able to respond to external events extremely rapidly, but at the same time keep the **Image** area of the sensor charge free. As can be seen from the figure below, the **external keep clean cycle** consists of a continuous cycle of one vertical shift, both **Image** and **Storage**, followed by one horizontal shift. When an external trigger is detected the current cycle will complete before the exposure phase starts. It is worth noting that although the external keep clean cycle will complete the current cycle, this will not result in the total loss of signal as only one vertical shift will have occurred. For pulsed light of very short time duration, microseconds (i.e. of the order of one vertical shift), the resultant image may appear to have shifted one row.

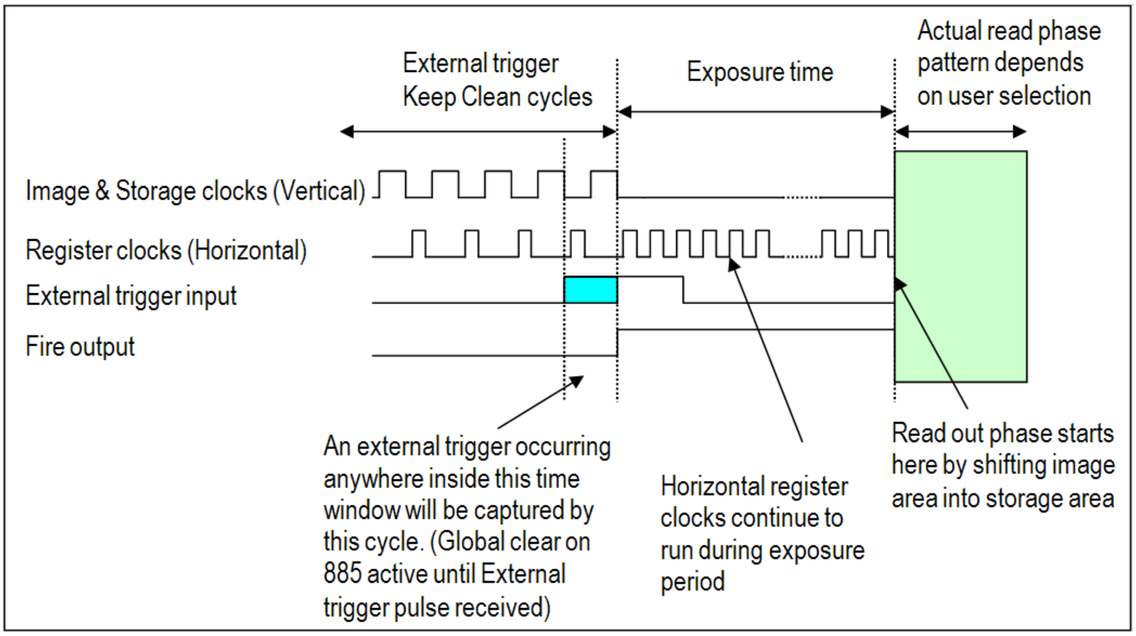


Figure 27: External Trigger Keep Clean Cycle

NOTE: The 885 range of iXon3 cameras have a global clear feature which can be used to remove all the charge accumulated in the whole Image section of the sensor simultaneously. This feature is enabled in all 885 models when external trigger is selected. It is particularly useful in external trigger mode when the light level pre-trigger is quite bright as it will reduce the effect of vertical streaking.

3.5 - TRIGGERING OPTIONS

The iXon3 range of cameras have several different triggering modes. These include **Internal, External** (and **Fast External**), **External Start, External Exposure and Software Trigger.** It should be noted that not all the triggering options are available on all cameras. Note also that many of these features require iCam technology within the camera, fuller details of which can be viewed through www.andor.com

- In **Internal Trigger** the camera determines the exact time when an exposure happens based on the acquisition settings entered by the user. This is the most basic trigger mode and requires no external intervention.
- In **External Trigger,** once an acquisition has been started, the camera is placed into a special cleaning cycle called **"External Keep Clean Cycle"** which ensures that charge build up on the CCD is kept to a minimum while waiting for the external trigger event. The external keep clean consists of a continuous sequence of one vertical shift followed by one horizontal shift. Once the External Trigger is received the current keep clean sequence is completed and the exposure phase initiated. The exact nature of the acquisition will depend on the user settings and is explained in more detail in a subsequent section. The external trigger is fed via the Ext Trig input on the camera head.
- **Fast External Trigger** is for the most part identical to External Trigger. It differs in only one key aspect. In Fast external trigger the camera will not wait for a sufficient number of keep clean cycles to have been completed to ensure the image area is completely clean of charge before accepting an external trigger event, but instead will allow a trigger event to immediately start the acquisition process. As a result, Fast External trigger allows a higher frame rate than standard external trigger. **NOTE: Once a "sufficient" number of keep cleans have been performed, External and Fast External Trigger are identical.**
- **External Start** is a mixture of External and Internal Trigger. In this mode the camera will perform a sequence of External Keep Clean Cycles while waiting for one external trigger event to occur and then start the acquisition process going. Once this external trigger event has occurred the camera will switch to internal trigger and the acquisition will progress as if the camera was in internal trigger mode.
- **External Exposure Trigger** is a mode of operation where the exposure time is fully controlled by the external trigger input. While the trigger input is high the CCD is accumulating charge in the **Image** area. When the external trigger goes low, the accumulated charge is quickly shifted into the **Storage** area and then read out in the normal manner.
- **Software Trigger** is a mode whereby the camera and software are in a high state of readiness and can react extremely quickly to a trigger event issued via software. This mode is particularly useful when the user needs to control other equipment between each exposure and does not know in advance how long such control will take or if the time taken changes randomly.

These modes are explained and illustrated in more detail in the following sections.

3.5.1 - Triggering options in Frame Transfer (FT) mode

3.5.1.1 - Internal (FT)

This is the simplest mode of operation in that the camera determines when the exposure happens. By monitoring the **FIRE** output the user can determine exactly when the camera is “**exposing**”.

When the camera is idle, it is running the **Idle Keep Clean Cycle** described previously. On receipt of the Start command from the PC, the camera will complete the current keep clean cycle and then perform sufficient vertical shifts to ensure that the **Image** and **Storage** regions are completely free of charge. The camera then starts its real exposure sequence for which the timing sequence is illustrated in the figure below.

The first thing to notice is that the **FIRE** output is high for much of the time. This is because there are no keep cleans running between each acquisition and hence the exposure time starts on completion of the transfer of the **Image** area into the **Storage** area. This also has the consequence that the exposure time and the cycle time are closely linked. We have defined the exposure time as the time during which there are no vertical shifts occurring, which also corresponds to the time during which the **FIRE** output will be high. The other point to note is that the exposure time overlaps the read out of the image.

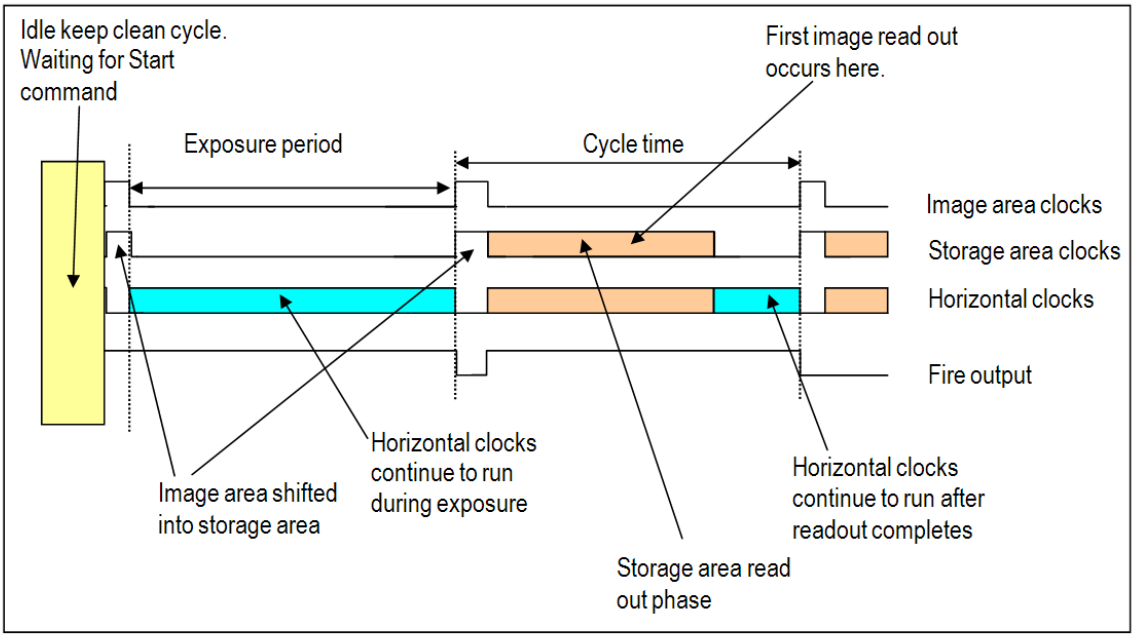


Figure 28: Internal Trigger in Frame Transfer mode

3.5.1.2 - External (FT)

When the camera is idle, it is running the **Idle Keep Clean Cycle** described previously. On receipt of the Start command from the PC the camera goes into its **External Keep Clean Cycle**. This cycle consists of one vertical followed by one horizontal shift, repeated continuously. The camera will repeat this cycle **X** times, where **X** is the number of image rows on the sensor, before it will accept any external trigger events. Once this period is over the camera will continue running the External Keep Clean cycle until an External Trigger is received. At that point the current external keep clean cycle is completed and 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 readout 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 readout phase. The camera continues in this cycle, until the number of images requested has been captured. Because the **Image** area is not cleaned between trigger events the effective exposure time is the time between events. The **User Defined Delay** is to allow for the capture of events which occur after the trigger pulse. In the case of the first trigger the effective exposure time is given by the user defined delay since keep clean cycles have been running up until the first trigger. This is in contrast to the subsequent exposure periods which are defined the time between the external trigger events. Thus, for experimental protocols that involve **C**ontinuous **W**ave (**CW**) light the first image will be dimmer, some protocols may require that this image is discarded.

NOTE: In the case of the 885 camera range the global clear feature will be ON until the first trigger is received.

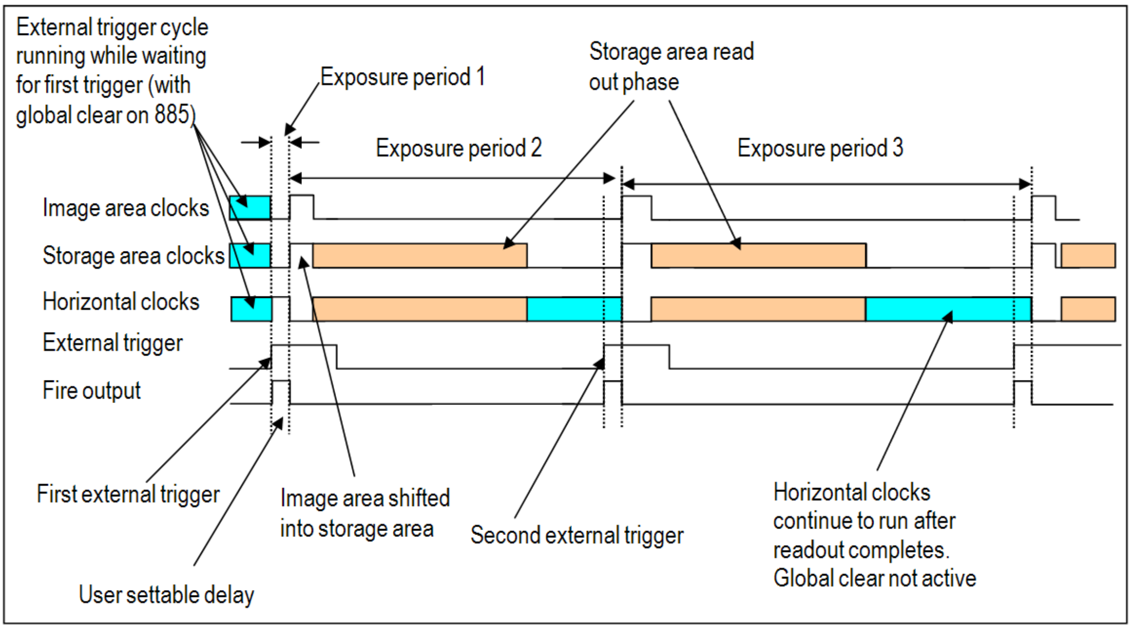


Figure 29: External Trigger in Frame Transfer mode

Since all X3 cameras have iCam technology, the rising edge of the external trigger can occur before the end of the previous read out provided that the falling edge of the Fire pulse occurs after the readout has completed, i.e. the external trigger is only accepted up to the "user defined delay period" before the end of the readout. This enhanced trigger mode will result in a higher frame rate (see **figure 30** below):

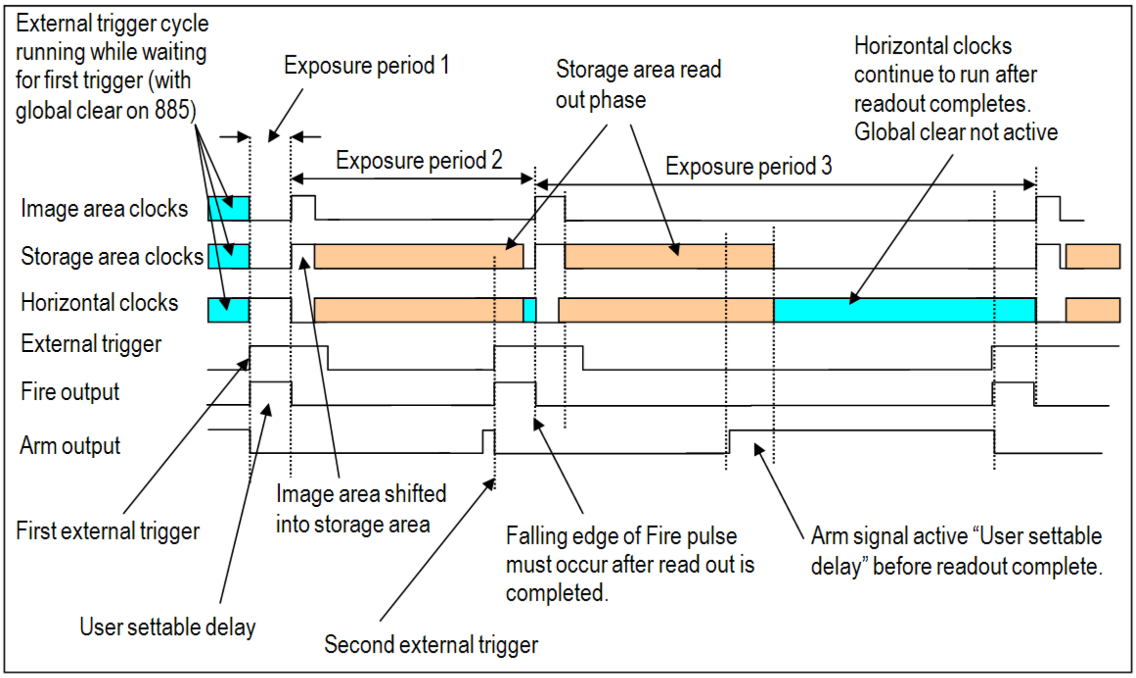


Figure 30: 'iCam-enhanced' External Trigger in Frame Transfer mode

External Exposure combined with Frame Transfer readout is available <u>only</u> on iXon+ 885 cameras. It is not available for other iXon3 models, since this mode requires an 885 Global Clear function (please see **page 57**). This mode is distinct from the triggering 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, 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 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.**

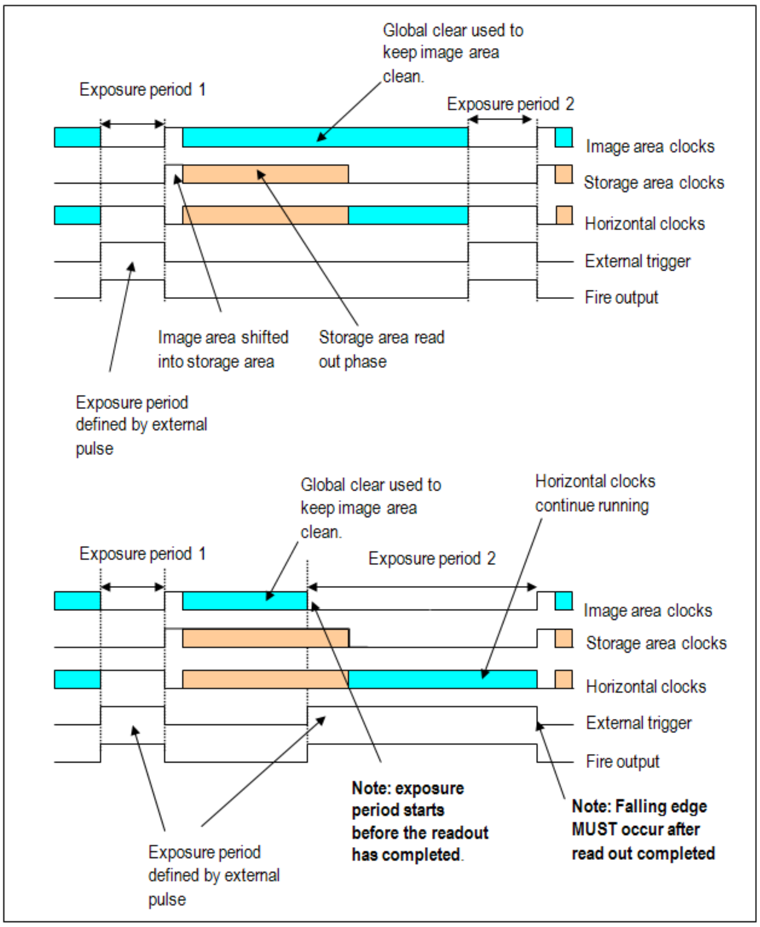


Figure 31: External Exposure Trigger in Frame Transfer mode (885 model only)

3.5.2 - Triggering options in Non-Frame Transfer (NFT) mode

3.5.2.1 - Internal (NFT)

When the camera is idle, i.e. not actively capturing images, it is repeatedly running the **Idle Keep Clean Cycle**. When the **Start** command is received from the PC, the camera will complete the current keep clean cycle and then perform sufficient vertical shifts to ensure the **Image** and **Storage** regions are completely free of charge. The camera is now ready to start the real exposure sequence.

The timing sequence is illustrated in the figure below. During the exposure the **FIRE** output will be high and there will be no vertical clocking. However, the horizontal register will keep running. At the completion of the exposure time the **FIRE** pulse will go low and the **Image** area of the CCD will be shifted into the **Storage** area. As the acquired signal is now safely placed in the masked off region of the CCD light still falling on the CCD will not contaminate the acquired image while it is being read out. On completion of the readout the camera will perform the **Internal keep clean cycle** until the user specified cycle time has elapsed. This process is continued until the complete series of acquisitions has taken place.

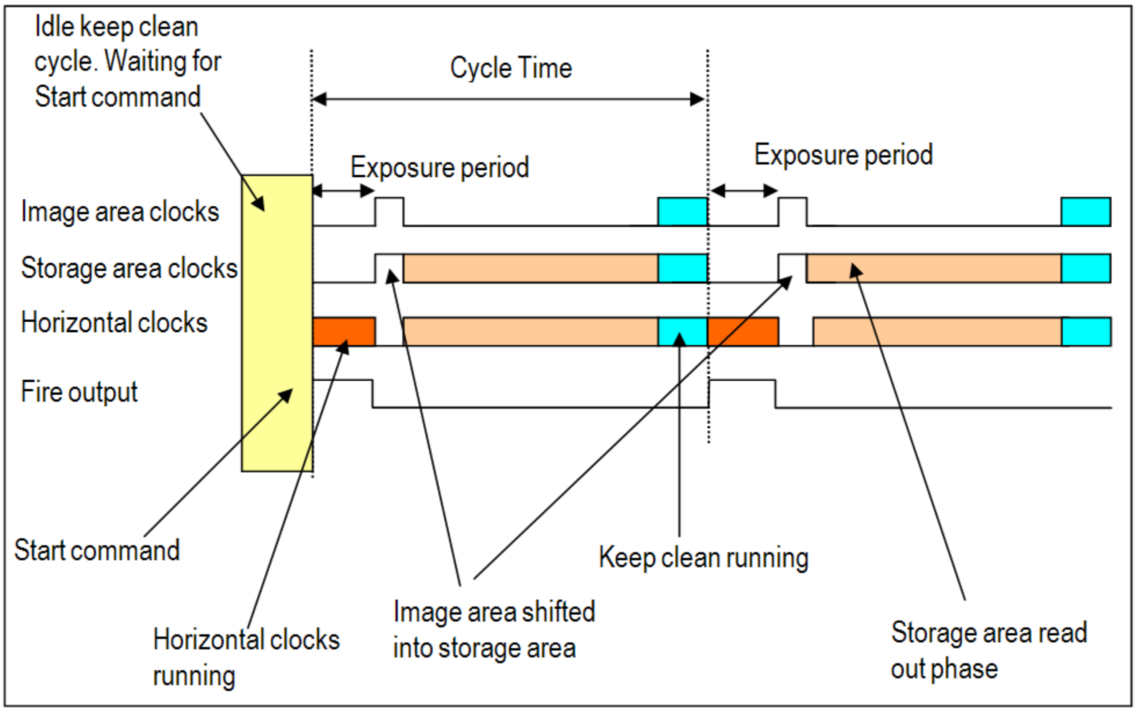


Figure 32: Internal Trigger in Non-Frame Transfer mode

In **External Trigger** modes once an acquisition has been started, the camera is placed into the special clearing cycle called "**External Trigger Keep Clean'** which was discussed previously. As can be seen from the figure below the external keep clean cycle runs continuously until the first external trigger event is detected at which point the current cycle series will complete before the exposure phase starts. During the exposure there are no vertical clocks running, however, there will be horizontal clocks to ensure that the shift register continues to be kept clean. 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 readout the camera restarts the external keep clean cycle.

If the camera is in **Fast External Trigger** mode it will accept a trigger event immediately and start the next exposure. If, however, the camera is in normal external trigger the camera will perform sufficient **External keep clean cycles** to ensure the **Image** area is fully cleaned before it will accept an external trigger. Once this period has passed, **Normal** and **Fast External Trigger** operate the same.

Fast External Trigger is useful in those cases where there is very little background light and the user is looking for the fastest frame rate. With Fast External Trigger, you may see variation in the background contribution to the signal from light that may have been allowed to fall on the sensor during the readout of the previous image. Fast external trigger does not mean that when a trigger is accepted that the system will respond quicker than in normal external trigger mode.

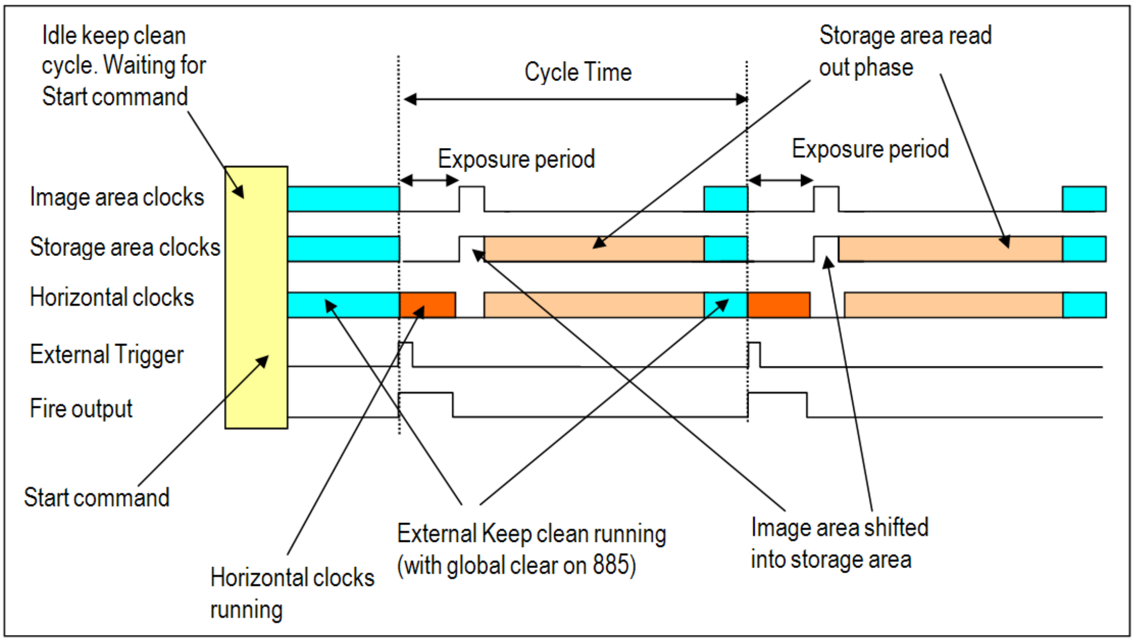


Figure 33: External Trigger in non-Frame Transfer mode

NOTE: There is no need to worry if the trigger occurs at the early phase of the keep clean cycle as the light signal will not be lost during the completion of the cycle, since only one vertical shift will have occurred. For pulsed light of very short duration (of the order of one vertical shift), the resultant image may appear to have shifted one row.

For the 885 range of cameras, the Global Clear functionality is used to keep the image area free of charge prior to the external trigger pulse. This reduces the pre-trigger image smear which might be seen on other frame transfer sensors.

Also, due to the 'iCam' functionality of the 885, this feature can be used to allow the overlap of exposure and readout but still maintain a charge free sensor prior to the external trigger pulse, **see figure 34** below:

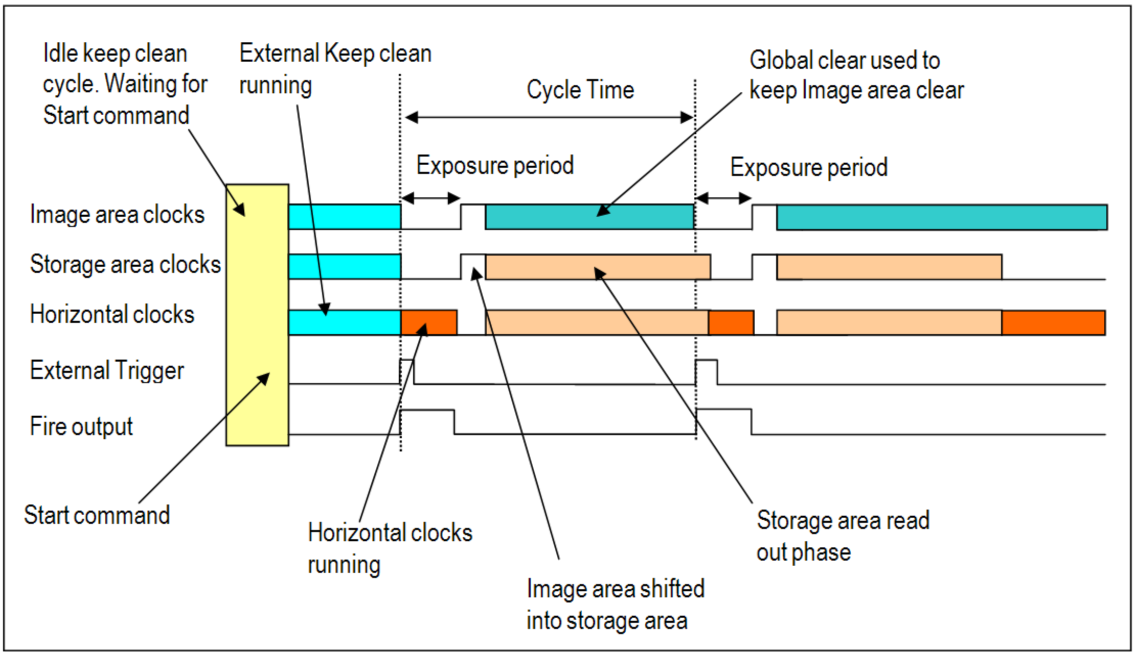


Figure 34: 'iCam-enhanced' External Trigger in non-Frame Transfer mode (885)

External Exposure combined with Non-Frame Transfer readout is available to all iXon3 cameras. This mode is distinct from the triggering 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. The exposure is physically ended by shifting the **Image** area into the **Storage** area. The **Storage** area is then readout in the normal manner.

On completion of the readout, the external keep clean cycle is started again.

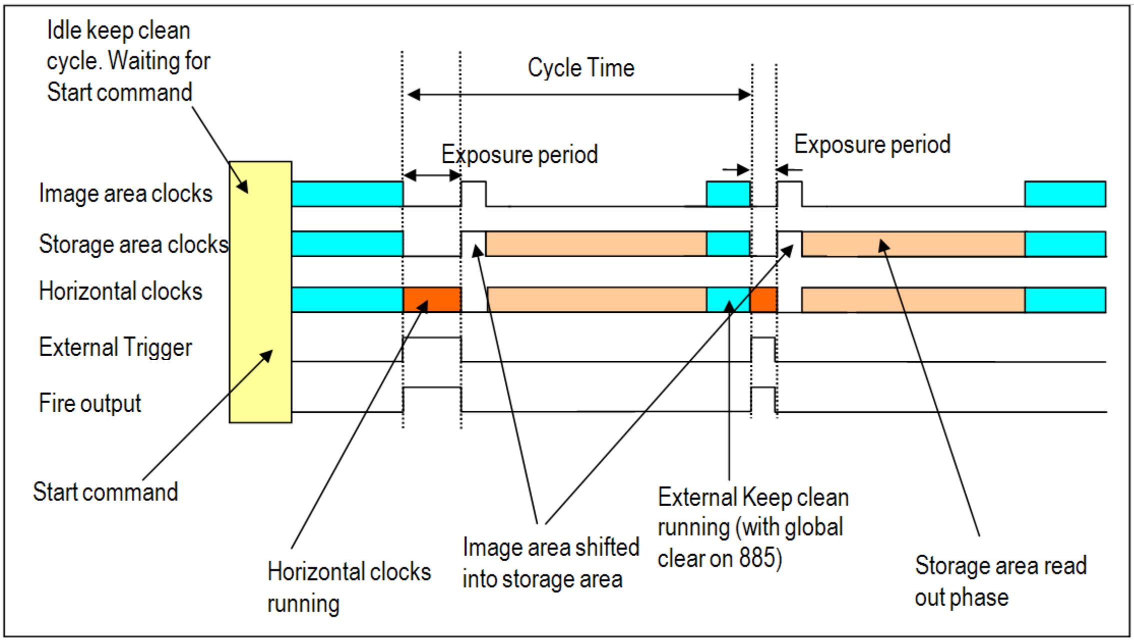


Figure 35: External Exposure Trigger in Non-Frame Transfer mode

This mode is particularly useful when the user needs to control other equipment between each exposure and does not know in advance how long such control will take or if the time taken changes randomly. With **Software Trigger** the camera and software are in a high state of readiness and can react extremely quickly to a trigger event issued via software.

In this mode the camera will run the **Idle Keep Clean Cycle** until the Start command is issued by the PC which is identical to all the modes previously discussed. On receipt of this command the camera will switch to running the normal **Internal Keep Clean** until a **Software Trigger** command is issued by the PC. This event will start the exposure and readout sequence. On completion of the readout the camera will return to the **Internal Keep Clean** until the next **Software trigger** is issued.

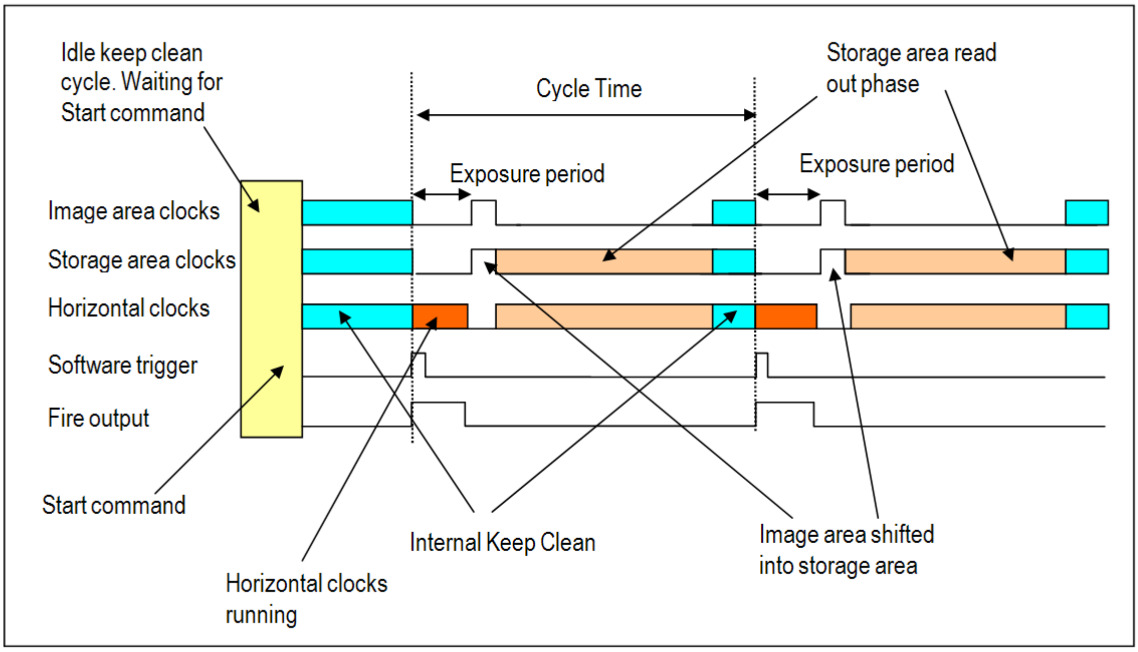


Figure 36: Software trigger in non-frame transfer mode

3.5.3 - Trigger options in Fast Kinetics (FK) mode

3.5.3.1 - Internal (FK)

As Fast Kinetics uses both the **Image** and **Storage** areas as temporary storage areas the number of options available is quite limited. The simplest mode is again **Internal Trigger** and as with the internal trigger modes described previously the system determines when the acquisition begins and then uses the exposure time defined by the user. On completion of the exposure period the cameras performs the number of vertical shifts defined by the user and then again waits for the exposure period before the next set of vertical shifts.

This process is repeated until the number in the series has been captured at which point the read out starts. The timing sequence is shown below and as before the **Fire** output envelopes the period when no vertical clocking is occurring. You will also see there are no readout cycles or keep clean cycles running during the sequence, hence the very fast kinetic cycle period but limited number of exposures in the series.

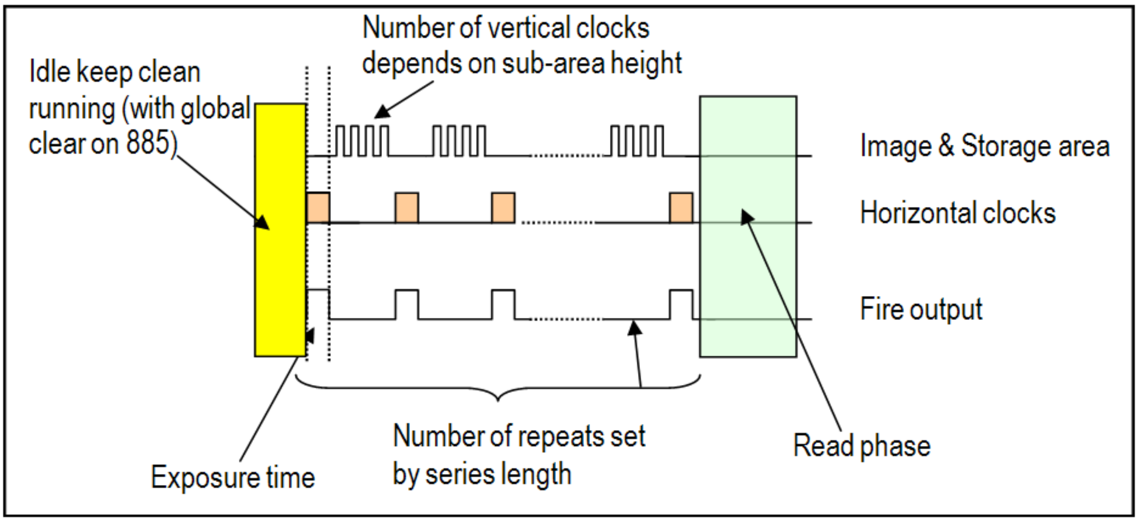


Figure 37: Internal Trigger in Fast Kinetics mode

In **External Trigger** mode, a trigger pulse is required to start each scan in the series. The rising edge of the trigger defines the trigger event. The user can delay the start of the vertical shifting relative to the trigger event. After the delay has elapsed, the number of rows (as specified by the user) are vertically shifted. The system then waits for the next trigger to start the next scan. As there is no keep clean cycle running while waiting for the external trigger the "**real**" exposure time is the time between each trigger. A consequence of this is that if your experiment has a constant background signal, but your trigger period is not fixed, you may see different background levels in your signal. As with internal trigger the data is only read off the sensor when the capture sequence has completed.

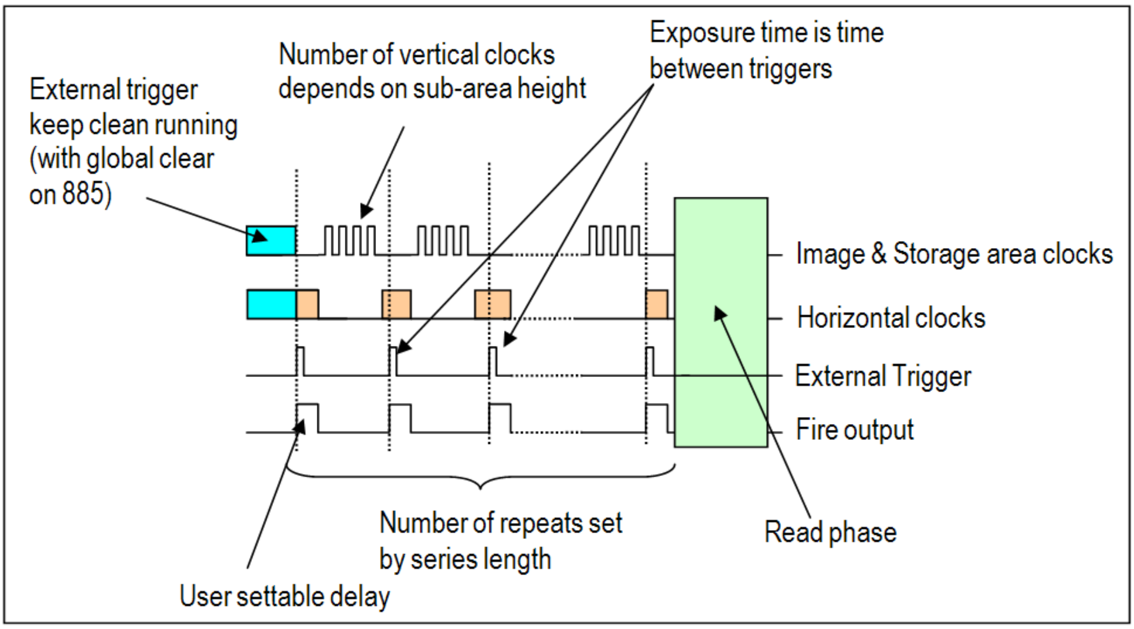


Figure 38: External Trigger in Fast Kinetics mode

External Start trigger mode is a combination of **external** and **internal** trigger. At the start of the capture process the camera is running the external keep clean cycle waiting of a trigger pulse to be applied to the External Trigger input. On receiving the trigger the exposure starts. The exposure period is defined by the user. On completion of the exposure period, the camera performs the number of vertical shifts, defined by the sub-area height (set by the user) and then again, wait for the exposure period before the next set of vertical shifts. This process is repeated until the number in the series has been captured at which point the readout starts.

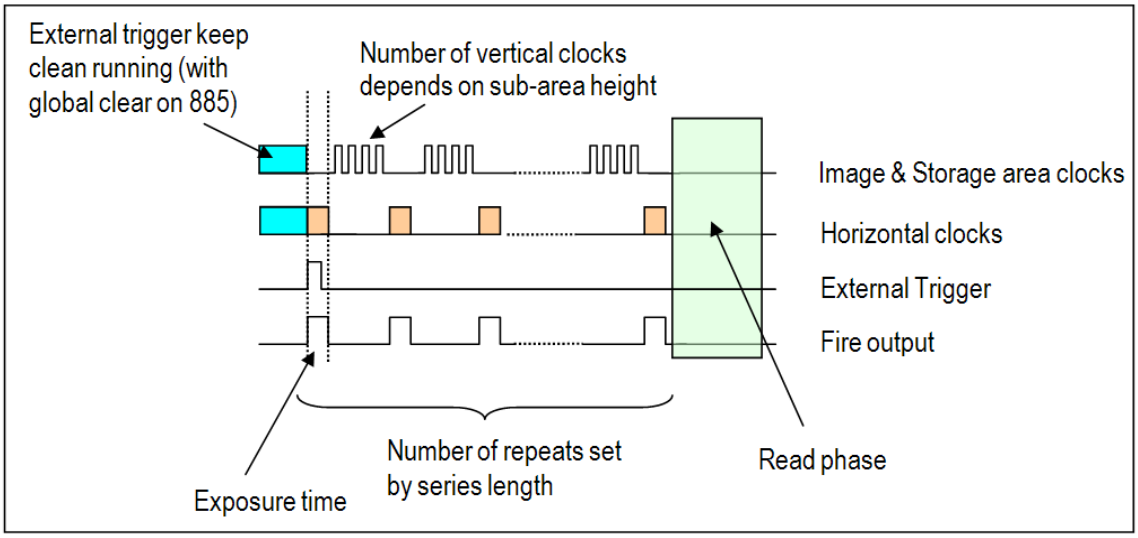


Figure 39: External Start trigger in Fast Kinetics mode

3.6 - SHUTTERING

The iXon range of cameras can be supplied with a built in shutter. The shutter is intended for taking background images and protecting the camera from excessive light and dust. It is not designed to operate at the high frame rates the camera is capable off. Under normal operation the shutter should be set to **Permanently Open** and the shutter open and close times to 0 seconds. If you do need the shutter to open and close automatically during your experiment then set the opening and closing times to 50ms.

3.7 - COUNT CONVERT

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, 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 **figure 40** below:

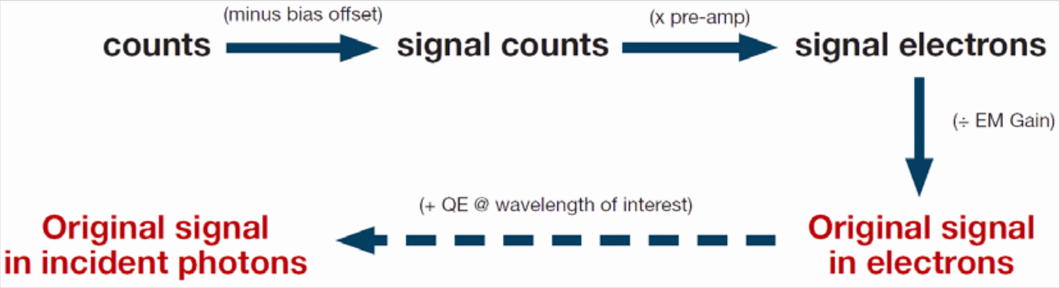


Figure 40: Count Convert calculation path

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.

3.8 - OptAcquire

OptAcquire is a unique control interface, whereby a user can conveniently choose from a pre-determined list of set-up configurations, each designed to optimise 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 tuneable, meaning the camera can be adapted and optimized for a wide variety of quantitative experimental requirements, ranging 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 OptAquire menu. Furthermore, advanced users may wish to create their own additional OptAcquire. 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 10 MHz and 1MHz. 10MHz for faster frame rates, 1 MHz for 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)** – Overlapped 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.

3.8.1 - OptAcquire modes

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 < 3ms.
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 < 10sec.
Photon Counting with Long Exposures (> 10 sec)	Configuration recommended for photon counting with individual exposures > 10sec.

3.9 - 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.
- 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.

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 which 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.

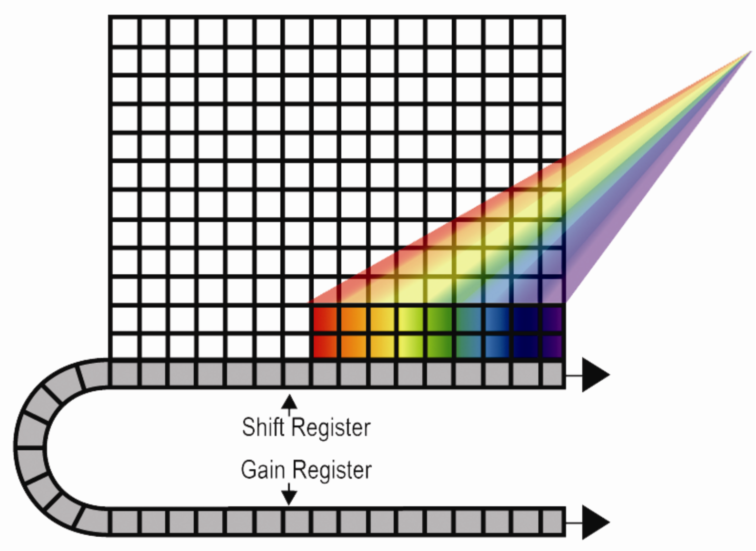


Figure 41: Cropped Sensor Mode. The active imaging area of the sensor is defined in a way that only a small section of the entire chip is used for imaging. The remaining area has to be optically masked to prevent light leakage and charge spill-over that would compromise the signal from the imaging area. 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.

3.9.1 - Cropped Sensor Mode Frame Rates

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 sufficiently small crop area. Series of measurements done on Andor iXon3 885 EMCCD camera have demonstrated that Cropped Sensor Mode in conjunction with binning has pushed the speed beyond 4000 frames per second.

The table below shows the Imaging frame rate potential of the Andor iXon3 885 EMCCD camera under conditions of Cropped Sensor Mode readout:

	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	-

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 > 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 approx 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.

3.10 - ADVANCED 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 low noise floor can perform photon counting. The approach can be further enhanced through innovative ways to post process kinetic data. The industry-leading dark current and **C**lock **I**nduced **C**harge (**CIC**) specification of the Andor's back-illuminated iXon3 897 model renders it uniquely suited to imaging by Photon Counting.

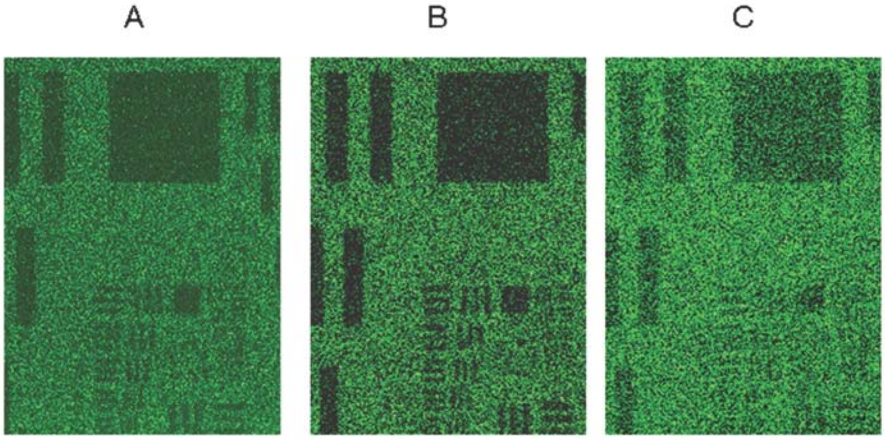
Photon Counting can only be successfully carried out with very weak signals, because as the name suggests, it involves counting only single photons per pixel. If more than one photon falls on a pixel during the exposure, an EMCCD (or an ICCD for that matter) cannot distinguish the resulting signal spike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

Key Fact – To successfully photon count with EMCCDs, there has to be a significantly higher probability of seeing a 'photon spike' than seeing a dark current/CIC 'noise spike'. The iXon3 897 has the lowest dark current/ 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 known 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.

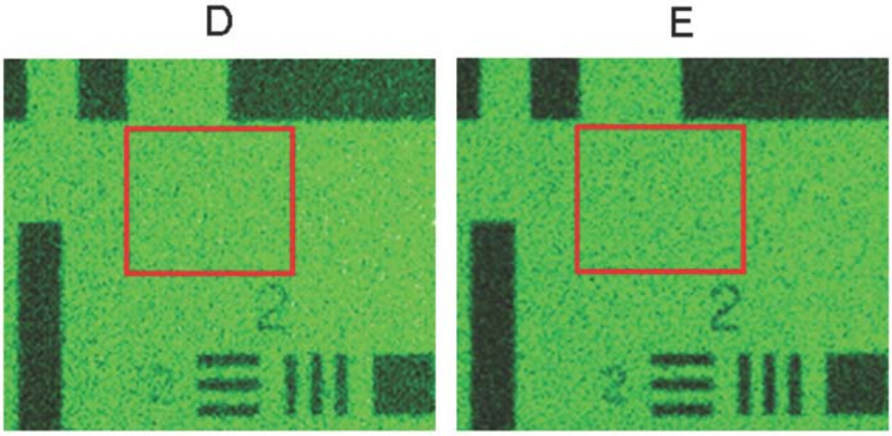
To successfully photon count with EMCCDs, there has to be a significantly higher probability of seeing a ‘photon spike’ than seeing a dark current/CIC ‘noise spike’. The lower the contribution of this ‘spurious’ noise source to a single exposure within the accumulated series, the lower the detection limit of photon counting and the cleaner the overall image will be, as demonstrated in Figure 43 below:



Standard EMCCD Image - Low CIC

Photon Counting - Low CIC

Photon Counting - High CIC



Standard EMCCD Image

Photon Counting

🟥 *ROI Signal to Noise =* ***3.86***

🟥 *ROI Signal to Noise =* ***6.02***

Figure 42: ‘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) is 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.

3.10.1 - 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.

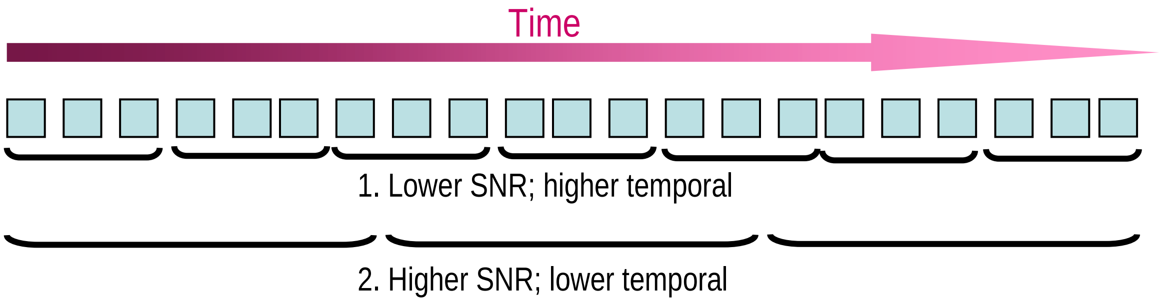


Figure 43: Schematic illustration of how photon counting can be applied to a kinetic series as a post processing step, affording increased flexibility in 'trail and error' trading SNR vs temporal resolution.

SECTION 4 - HARDWARE

4.1 - EMCCD TECHNOLOGY

4.1.1 - What is an Electron Multiplying CCD?

Current trends in photonics are placing unprecedented demands on detector technology to perform at significantly greater levels of sensitivity and/or speed. **E**lectron **M**ultiplying **C**harge **C**oupled **D**evice (**EMCCD**) technology has been designed to respond to this growing need and in turn is opening up new avenues of novel experimental design.

EMCCD technology, sometimes known as “on-chip multiplication”, is an innovation first introduced to the digital scientific imaging community by Andor Technology in 2000, with the launch of our dedicated, high-end iXon platform of ultra-sensitive cameras. Essentially, the EMCCD is an image sensor that is capable of detecting single photon events without an image intensifier, achievable by way of a unique electron multiplying structure built into the chip.

EM gain can be increased to a degree. It is readily adjustable in real time through the software, where extremely weak signals may be detected above the read noise of the camera at any readout speed. This is important because the traditional problem of combining sensitivity with speed in standard CCDs is that the two are mutually exclusive, i.e. greater read noise detection limits result from faster pixel readout.

4.1.2 - Does EMCCD technology eliminate Read Out Noise?

System noise within modern silicon based detectors has two primary sources: dark current noise and read noise. The higher the noise floor on a detector the less able it is to read out the extremely weak signals associated with ultra low light imaging.

With thermoelectric cooling, dark current noise can be reduced to negligible levels. An EMCCDs ability to multiply weak signals above the detector’s read noise floor, by applying EM gain, effectively eliminates read noise at any speed by reducing it to **<< 1 e^-/p/s**.

4.1.3 - How sensitive are EMCCDs?

Two parameters significantly influence detector sensitivity, namely **Q**uantum **E**fficiency (**QE**) and system noise. QE is a measure of a camera’s ability to capture valuable photons. A high QE results in more photons being converted to photoelectrons within the CCD pixels.

Once converted, the photoelectrons in a given pixel must overcome the detection limit or noise floor of the camera, which is set by the system noise. EMCCDs deliver superior sensitivity by maximizing QE and minimizing system noise, through the unique gain control feature. Single photon events are now well within the capabilities of super sensitive EMCCD technology.

4.1.4 - What applications are EMCCDs suitable for?

EMCCD based detectors have been designed for the most demanding of low light, dynamic applications. These detectors have redefined the sensitivity expectations of scientific grade cameras, with a detection limit as low as single photons.

These levels of sensitivity are vital for low light, life science and physical science imaging applications such as single molecule detection, live cell microscopy, weak luminescence detection, or demanding astronomy applications (to name only a few).

4.1.5 - What is Andor Technology's experience with EMCCDs?

Andor Technology was the first company to introduce an EMCCD based detector in 2000. Since then the company has led the way in the development of EMCCD detectors, introducing the first back illuminated EMCCD in January 2003.

Andor now offers the widest range of EMCCD based detectors on the market. The company is also playing a pivotal role in increasing our understanding of this ground-breaking technology.

In September 2003 it hosted the 1st International EMCCD Symposium in Limavady, Northern Ireland, a dedicated conference which looked at the current usage and future development of EMCCDs. This was followed in April 2005 by the 2nd International EMCCD Symposium, held in Connecticut, USA. Please go to **www.emccd.com** for further details.

4.2 - EMCCD SENSOR

The EMCCD sensor is the core enabling technology of the system. Everything else in the camera has been designed to extract the absolute best operational performance from this sensor. All EMCCD sensors in the iXon3 range have a frame transfer architecture. The frame-transfer EMCCD uses a two-part sensor in which one-half of the array is used as a storage region and is protected from light by a light-tight mask. Incoming photons are allowed to fall on the uncovered portion of the array and the accumulated charge is then rapidly shifted into the masked storage region for transfer to the serial output register. While the signal is being integrated on the light-sensitive portion of the sensor, the stored charge is read out. Frame transfer devices have typically faster frame rates than full frame devices and have the advantage of a high duty cycle i.e. the sensor is always collecting light.

A potential disadvantage of this architecture is the charge smearing during the transfer from the light-sensitive to the masked regions of the CCD, (although they are significantly better than full frame devices).The smearing being more prevalent when exposure times are closer to the time taken to shift the charge under the mask (in the order of milliseconds).

The Andor iXon3 is the best camera on the market for minimizing such smear, as the vertical clock speeds can be tuned via the software to deliver the fastest parallel shifts in the industry, which has the further advantage of faster overall frame rates (especially when using sub-array and/or pixel binning readout options).

Essentially, the EMCCD sensor is capable of detecting single photon events without an image intensifier, achievable by way of a unique electron multiplying structure built into the chip. Traditional CCD cameras offered high sensitivity, with readout noises in single figure $< 10e^-$ but at the expense of slow readout. Hence they were often referred to as ‘slow scan’ cameras. The fundamental constraint came from the CCD charge amplifier. To have high speed operation the bandwidth of the charge amplifier needs to be as wide as possible but it is a fundamental principle that the noise scales with the bandwidth of the amplifier hence higher speed amplifiers have higher noise.

Slow scan CCD’s have relatively low bandwidth and hence can only be read out at modest speeds typically less than 1MHz. EMCCD cameras avoid this constraint by amplifying the charge signal before the charge amplifier and hence maintain unprecedented sensitivity at high speeds. By amplifying the signal the readout noise is effectively by-passed and as such, EMCCD readout noise is no longer a limit on sensitivity (and can often be considered negligible).

Please see pages 23 & 24 for further details on EMCCD technology and sensor architecture.

4.3 - VACUUM HOUSING

Unless protected, cooled CCD sensors will condense moisture, hydrocarbons and other gas contaminants that will attack the CCD surface. If that happens, CCD performance will decline proportionally and will eventually fail. Fortunately, the integrity of the sensor can be preserved by housing it in a protective enclosure. However, it is important to understand that all such environments are not the same and the underlying technology used can seriously impact camera life (and performance).

A permanent hermetic vacuum head is an essential component of high-end imaging and spectroscopy EMCCD cameras. A permanent vacuum requires not only a hermetic seal, but also low outgassing. These criteria are what Andor's UltraVac^{TM} vacuum process uniquely ensures. It is the low **outgassing** (see **page 78**) that is the real challenge and, in reality, what sets the **real** limit on long-term performance. Andor has developed and utilized the UltraVac^{TM} process over more than 10 years, so it is proven with 1000's of systems in the field and a measured **M**ean-**T**ime-**B**etween-**F**ailure (**MTBF**) of 100 years; that means it will take 100 years for half of them to fail!

Furthermore, Andor's rigorous, proprietary vacuum process is carried out in a Class 10,000 clean room; this means less than 10,000 particles of less than 0.5 micron dimension per cubic meter. The air is fully replenished every minute. We welcome visitors to inspect our state-of-the-art facility.

- ✓ Sustained vacuum performance over many years operation – proprietary process to minimize outgassing.
- ✓ Benefit from a thoroughly proven solution - UltraVac^{TM}. 10 years of shipping vacuum systems to the field and a negligible failure rate (an MTBF of 100 years).
- ✓ Performance improves because the temperature of the chip can be reduced significantly. Better cooling (down to -100°C with an enhanced thermoelectric Peltier design) translates into substantially lower darkcurrent and fewer blemishes.
- ✓ Such darkcurrent performance is particularly critical to EMCCD technology, where even a single thermal electron is detected as a spurious noise spike.
- ✓ Elimination of condensation and outgassing means that the system can also use only a single entrance window, with antireflection coating – you can believe the QE curve.
- ✓ The permanent hermetic vacuum ensures that peak quantum efficiency and cooling will not degrade, even after years of operation.

4.3.1 - Thermoelectric cooler

The iXon3 range makes use of a four-stage (see **NOTE** below), Peltier cooling assembly, which utilizes the thermoelectric effect to rapidly cool the sensor down to the stable operating temperature. TE coolers have a cold end (in contact with the sensor) and a hot end. Heat must be efficiently dissipated from the TE cooler for effective cooling of the sensor.

The iXon3 is expertly designed to yield maximum heat dissipation, via either forced air cooling (in-built fan) or water cooling, which in combination with Andor's UltraVac$^{\textbf{TM}}$ vacuum process, results in market-leading cooling performance. A recirculator or a chiller can be purchased from Andor Technology to provide convenient and effective heat dissipation through water cooling.

The iXon3 camera also contains temperature control components, which regulate the cooling of the camera and ensure that a stable temperature is maintained between and throughout measurements.

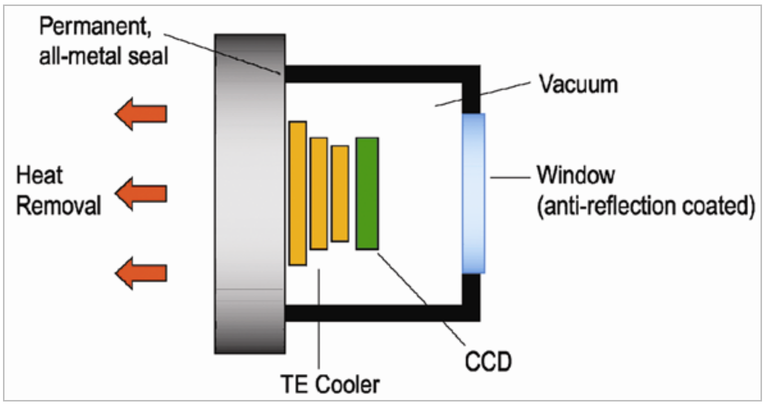


Figure 44: Ultravac^{TM} metal hermetic vacuum sealing

NOTE: DV885 models have a three-stage peltier cooler; DU885 models have a four-stage peltier cooler.

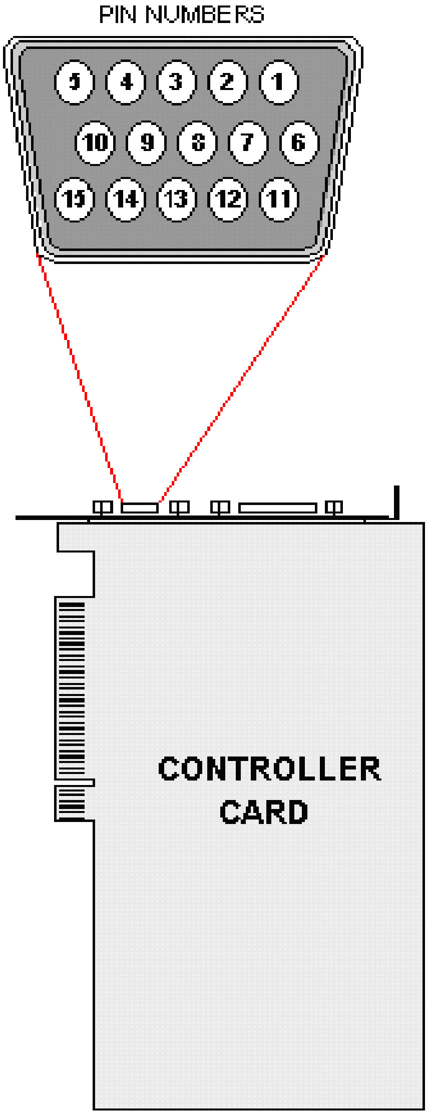
4.4 - OUTGASSING

Outgassing is the release of a gas trapped in material. It is a problem encountered in high-vacuum applications. Materials not normally considered absorbent can release enough molecules to contaminate the vacuum and cause damage to optical sensors, window coatings, etc.

Even metals and glasses can release gases from cracks or impurities, but sealants, lubricants, and adhesives are the most common cause. Left unchecked, cooling performance would steadily degrade and therefore lead to increased dark current. Furthermore, resulting electrochemical reactions would eventually destroy the sensor.

4.5 - CONTROLLER CARD PINOUTS

CONTROLLER CARD: AUXILIARY CONNECTOR DESCRIPTION



1 = Aux In 1
2 = Aux In 2
3 = Aux In 3
4 = I2C DATA
5 = Aux In 4
6 = Aux Out 2
7 = Aux Out 1
8 = Aux Out 3
9 = I2C Clock
10 = GROUND
11 = Reserved
12 = Reserved
13 = Reserved
14 = Aux Out 4
15 = +5V

WARNING: Pins 11, 12 & 13 are reserved. These pins are NOT available for auxiliary use. DO NOT make electrical connections to these pin locations when attaching external devices via the Controller Card Auxiliary Connector Port. Doing so may cause damage to the Controller Card, the Camera or your external device.

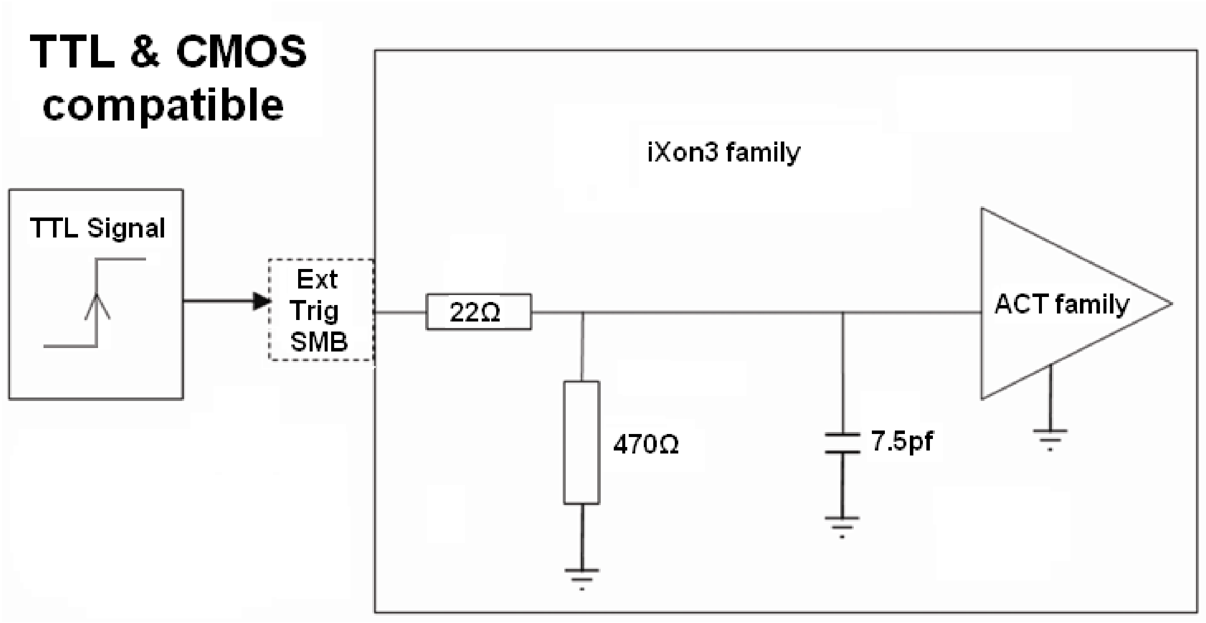


Figure 45: External Trigger SMB

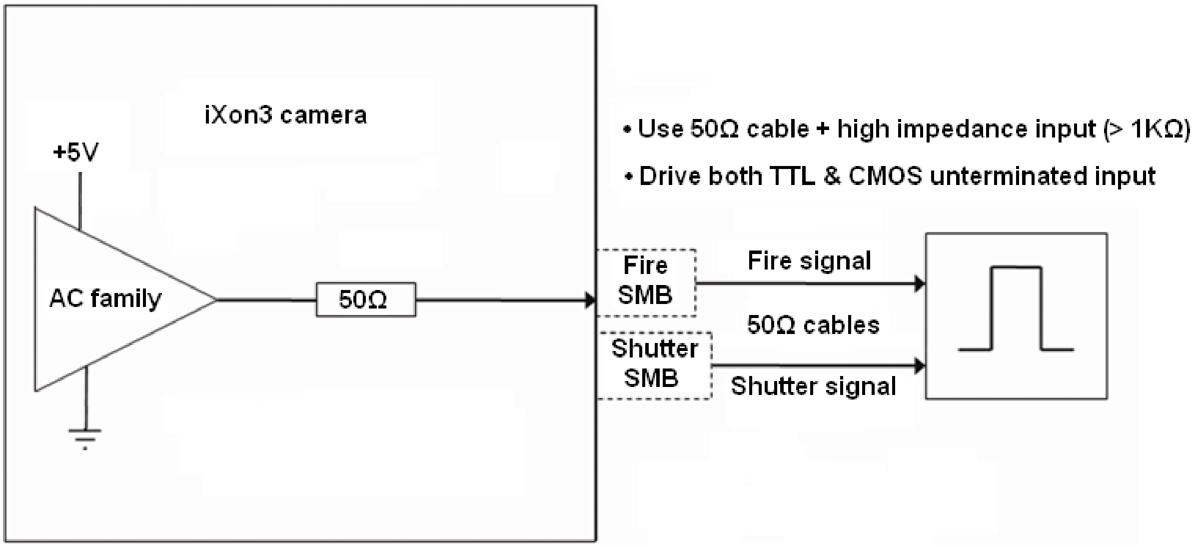


Figure 46: Fire SMB & Shutter SMB

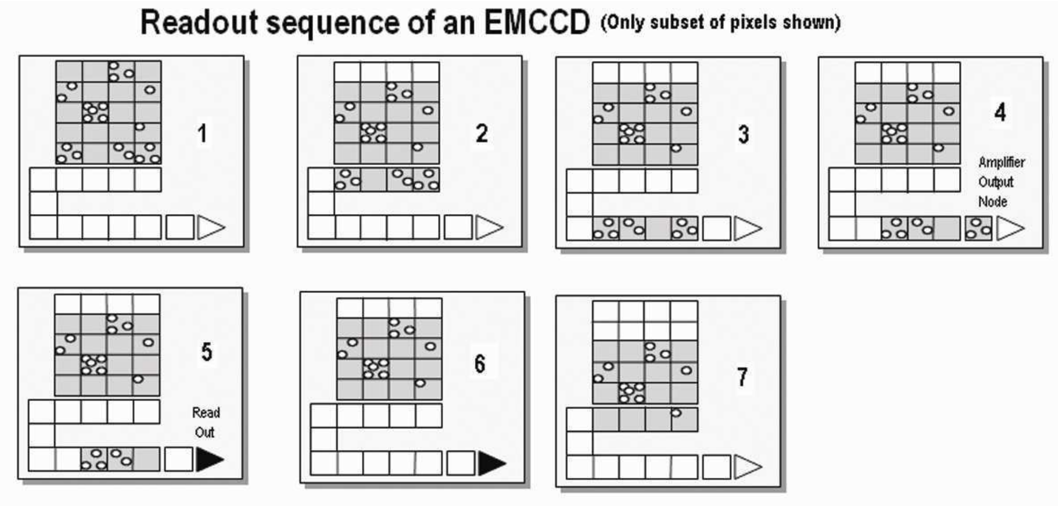
APPENDIX

A1.1.- GLOSSARY

If this is the first time you have used Andor's EMCCD, the glossary that follows will help familiarize you with its design philosophy and some of its key terminology.

A1.1.1 - Readout sequence of an EMCCD

In the course of readout, charge is moved vertically into the shift register then horizontally from the shift register into the output node of the amplifier. The simple readout sequence illustrated below (which corresponds to the default setting of the Full Resolution Image binning pattern) allows data to be recorded for each individual element on the EMCCD-chip. Other binning patterns are achieved by summing charge in the shift register and/or the output node prior to readout. For further information on Binning, please refer to **pages 41 - 42**).



1. Exposure to light causes a pattern of charge (an electronic image) to build up on the frame (or Image Area) of the EMCCD-chip.
2. Charge in the frame is shifted vertically by one row, so that the bottom row of charge moves into the shift register.
3. Charge in the shift register is moved horizontally by one pixel, so that charge on the endmost pixel of the shift register is moved into the Gain register.
4. Charge is shifted into the output node of the amplifier.
5. The charge in the output node of the amplifier is passed to the analog-to-digital converter and is read out.
6. **Steps 3** and **4** are repeated until the shift register is emptied of charge.
7. The frame is shifted vertically again, so that the next row of charge moves down into the shift register.

The process is repeated from **Step 3** until the whole frame is read out.

A1.1.2 - Accumulation

Accumulation is the process by which data that have been acquired from a number of similar scans are added together in computer memory. This results in improved signal to noise ratio.

A1.1.3 - Acquisition

An Acquisition is taken to be the complete data capture process.

A1.1.4 - A/D Conversion

Charge from the CCD is initially read as an analogue signal, ranging from zero to the saturation value. A/D conversion changes the analogue signal to a binary (digital) number which can then be manipulated by the computer.

A1.1.5 - Background

Background is a data acquisition made in darkness. It is made up of fixed pattern noise, and any signal due to dark current.

A1.1.6 - Binning

Binning is a process that allows charge from two or more pixels to be combined on the EMCCD-chip prior to readout.

A1.1.7 - Counts

Counts refer to the digitization by the A/D conversion and are the basic unit in which data are displayed and processed. Depending on the particular version of the detection device, one count may, for example, be equated with a charge of 10 photoelectrons on a pixel of the CCD.

A1.1.8 - Dark Signal

Dark signal, a charge usually expressed as a number of electrons, is produced by the flow of dark current during the exposure time. All CCDs produce a dark current, an actual current that is measurable in (typically tenths of) milliamps per pixel. The dark signal adds to your measured signal level, and increases the amount of noise in the measured signal. Since the dark signal varies with temperature, it can cause background values to increase over time. It also sets a limit on the useful exposure time. Reducing the temperature of the CCD reduces dark signal (typically, for every 7ºC that temperature falls, dark signal halves). CCD readout noise is low, and so as not to compromise this by shot noise from the dark signal, it is important to cool the detector to reduce the dark signal. If you are using an exposure time of less than a few seconds, cooling the detector below 0ºC will generally remove most of the shot noise caused by dark signal.

A1.1.9 - Detection Limit

The Detection Limit is a measure of the smallest signal that can be detected in a single readout. The smallest signal is defined as the signal whose level is equal to the noise accompanying that signal, i.e. a Signal to Noise ratio (S/N) of unity. Sources of noise are as follows:

- Shot noise of the signal itself
- Shot noise of any dark signal
- Readout noise

If the signal is small, we can ignore its shot noise. Furthermore, if a suitably low operating temperature and short exposure time can be achieved, the lowest detection limit will equal the readout noise.

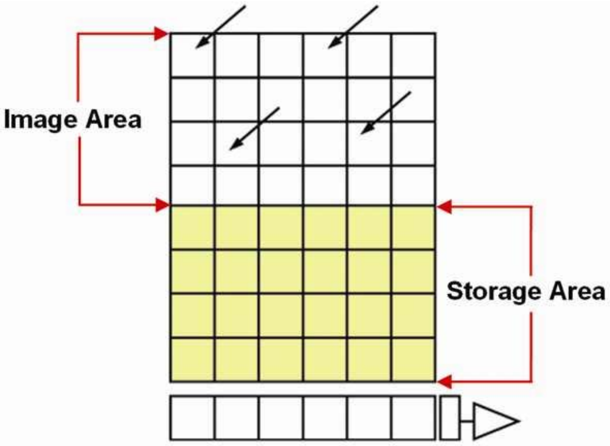
A1.1.10 - Exposure Time

The Exposure Time is the period during which the CCD collects light prior to readout.

A1.1.11 - Frame Transfer

Frame transfer is a special acquisition mode that is only available if your system contains a **F**rame **T**ransfer **CCD** (**FT CCD**). An FT CCD differs from a standard CCD in 2 ways. Firstly, it contains 2 areas, of approximately equal size as follows:

1. The first area is the **Image Area**, which is located at the top and farthest from the read-out register. This is the light sensitive area of the CCD.
2. The second section is the **Storage Area**, and is located between the Image Area and the Readout Register. This section is covered by an opaque mask, usually a metal film, and hence is not sensitive to light



The second way in which a FT CCD differs from a standard CCD is that the Image and Storage areas can be shifted independently of each other. These differences allow a FT CCD to be operated in a unique mode where one image can be read out will the next image is being acquired. It also allows a FT CCD to be used in imaging mode without a shutter. **Note: This is only applicable when the camera is running in Accumulate or Kinetic mode.**

A1.1.12 - Noise

Noise is a complex topic, the full exploration of which is beyond the scope of this glossary. However noise may be broken down into two broad categories, as follows:

1. Pixel Noise
2. Fixed Pattern Noise

A1.1.12.1 - Pixel Noise

Let us first attempt to define pixel noise. Assume that a light signal is falling on a pixel of the CCD. If the charge on the pixel is read, and the read process is repeated many times, the noise may be taken as the variation in the values read. The Root Mean Square (rms) of these variations is often used to express a value for noise. As a rule of thumb, the rms is 4x to 6x smaller than the peak to peak variations in the count values read from the pixel. Pixel noise has three main constituents:

- Readout noise
- Shot noise from the dark signal
- Shot noise from the light signal itself

Shot noise cannot be removed because it is due to basic physical laws. Most simply defined, shot noise is the square root of the signal (or dark signal) measured in electrons

A1.1.12.1.1 - Readout Noise

Readout noise (which in our detectors is, in any case, low) is due to the amplifier and electronics. It is independent of dark signal and signal levels and is only very slightly dependent on temperature. It is present on every read, as a result of which it sets a limit on the best achievable noise performance. Shot noise from the dark signal is dependent on the exposure time and is very dependent on the temperature; shot noise from the signal is additionally dependent on the signal level itself. If either the signal or the dark signal falls to zero, their respective shot noise also falls to zero. The total pixel noise is not, however, simply the sum of the three main noise components (readout noise, shot noise from the dark signal, and shot noise from the signal). Rather, the rms gives a reasonable approximation - thus:

$$\textbf{total} = \textbf{sqrt}\,(\textbf{readnoise}^2 + \textbf{darkshot}^2 + \textbf{sigshot}^2)$$

where:

- **total** is the pixel noise
- **readnoise** is the readout noise
- **darkshot** is the shot noise of the dark signal
- **sigshot** is the shot noise of the signal

A1.1.12.2 - Fixed Pattern Noise

Fixed **P**attern **N**oise (**FPN**) consists of the differences in count values read out from individual pixels, even if no light is falling on the detector. These differences remain constant from read to read. The differences are due in part to a variation in the dark signal produced by each pixel, and in part to small irregularities that arise during the fabrication of the CCD. Since fixed pattern noise is partly due to dark signal, it will change if the temperature changes, but because it is fixed, it can be completely removed from a measurement by background subtraction.

A1.1.13 - Quantum Efficiency/Spectral Response

The glossary refers to signals as a number of electrons. More strictly speaking these are “photoelectrons”, created when a photon is absorbed. When a UV or visible photon is absorbed by the detector it can at best produce only one photoelectron. Photons of different wavelengths have different probabilities of producing a photoelectron and this probability is usually expressed as **Q**uantum **E**fficiency (**QE**) or **Spectral Response**.

QE is a percentage measure of the probability of a single photon producing a photoelectron, while spectral response is the number of electrons that will be produced per unit photon energy. Many factors contribute to the QE of a CCD, but the most significant factor is the absorption coefficient of the silicon that serves as the bulk material of the device.

A1.1.14 - Readout

Readout is the process by which data are taken from the pixels of the CCD and stored in computer memory. The pixels, which are arranged in a single row, are read out individually in sequence. Readout involves amplifying the charge on each pixel into a voltage, performing an analog to digital conversion then storing the data in computer memory. The time taken to perform this operation is known as the “read time”.

A1.1.15 - Saturation

Saturation is the largest signal the CCD can measure. A signal is measured in terms of the amount of charge that has built up in the individual pixels on the CCD-chip. A number of factors determine the maximum amount of charge that the CCD can handle

A1.1.16 - Scans (Keep Clean & Acquired)

The CCD is continually being “scanned” to prevent its becoming saturated with dark current (see **Dark Signal** on **page 80**).

- If the Scan is being used simply to “clean” the CCD (i.e. it is a keep-clean scan), the charge from the CCD is discarded.
- In an acquired scan however, the charge undergoes analog to digital conversion and is acquired into computer memory so that it can be used for subsequent processing and display: it is “read out” (see **Readout** above).

Unless the context specifically indicates otherwise, “scan” in this User Guide generally refers to an acquired scan.

A1.1.17 - Shift Register

The Shift Register usually consists of a single row of elements (or pixels) running parallel to and below the bottom row of light-gathering pixels (the image area) on the CCD-chip. The shift register is protected from light by an aluminium mask. The elements in the shift register have a greater capacity to store charge (i.e. a greater “well depth”) than the other pixels on the CCD-chip

A1.1.17 - Shot Noise

Shot Noise is due to basic physical laws and cannot be removed. Any signal, whether it is a dark signal or a light signal, will have shot noise associated with it. Most simply defined:

If the signal or dark signal = **N** electrons, the shot noise is the square root of N.

You can do nothing about the shot noise of your signal, but by choosing minimum exposures and operating the CCD at suitably low temperatures, the dark signal, and hence the noise from the dark signal, can be reduced.

A1.1.18 - Signal To Noise Ratio

The **Signal to Noise Ratio** (commonly abbreviated as **S/N** or **SNR**) is the ratio between a given signal and the noise associated with that signal. Noise has a fixed component, and a variable component (shot noise) which is the square root of the signal. Thus, the S/N usually increases (improves) as the signal increases.

The maximum S/N is the ratio between the maximum signal (i.e. the saturation level) and the noise associated with that signal. At near saturation levels the dominant source of noise is the shot noise of the signal.

A1.2 - MECHANICAL DIMENSIONS

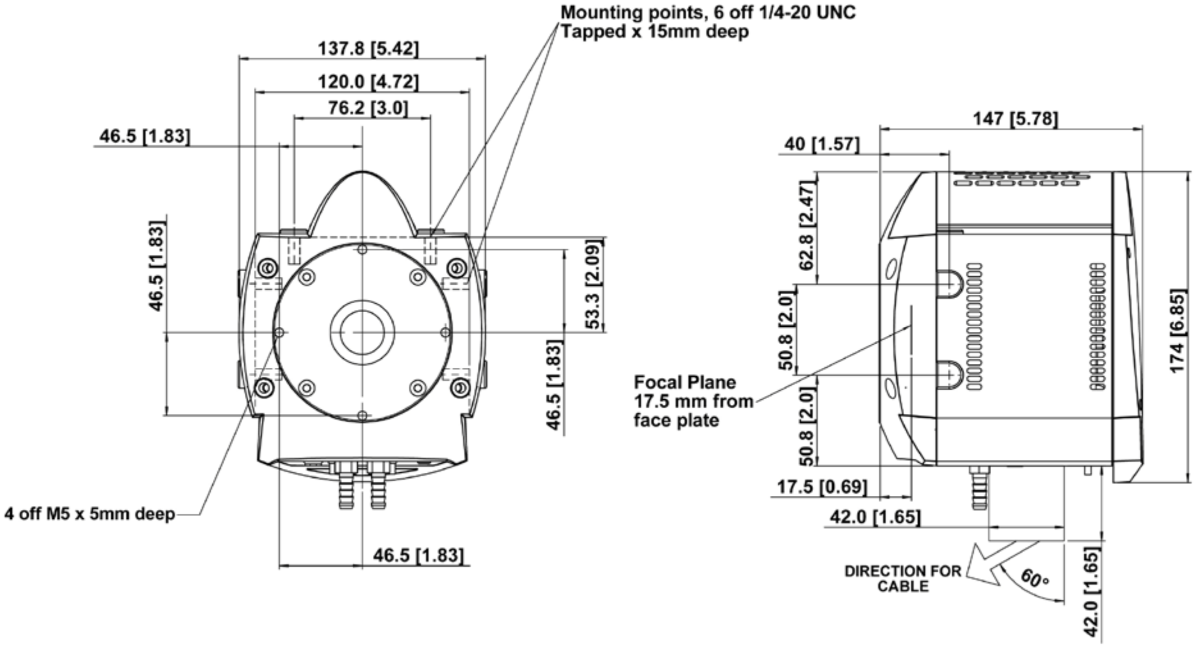


Figure 47: Dimensions of 860, 885 & 897 models

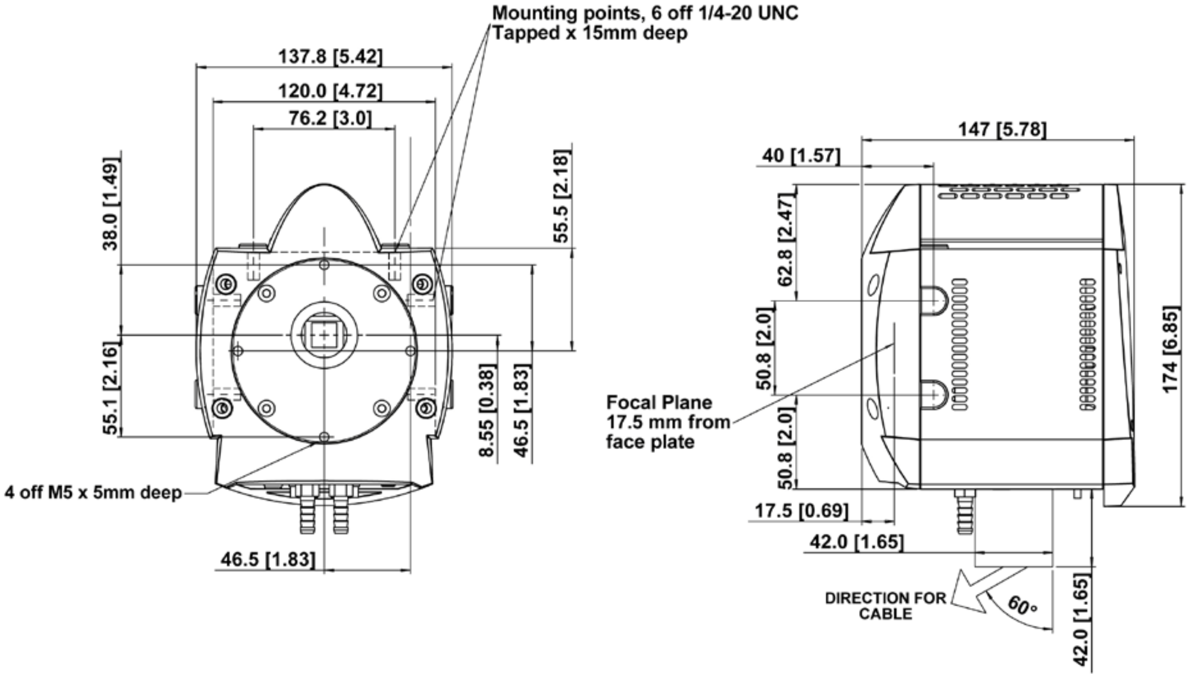
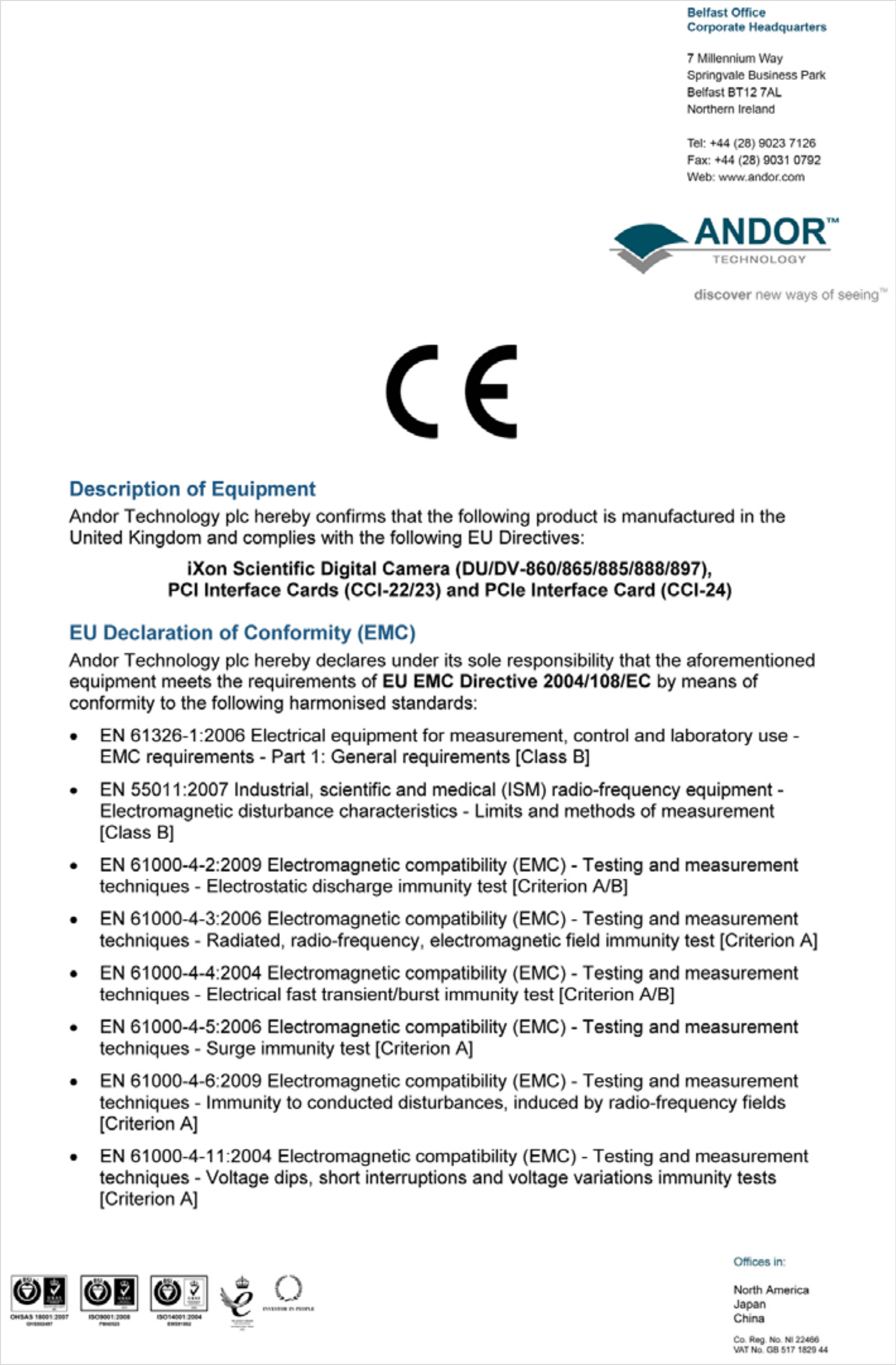


Figure 48: Dimensions of 888 mode

A1.3 - DECLARATION OF CONFORMITY



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CE

Description of Equipment

Andor Technology plc hereby confirms that the following product is manufactured in the United Kingdom and complies with the following EU Directives:

iXon Scientific Digital Camera (DU/DV-860/865/885/888/897),
PCI Interface Cards (CCI-22/23) and PCIe Interface Card (CCI-24)

EU Declaration of Conformity (EMC)

Andor Technology plc hereby declares under its sole responsibility that the aforementioned equipment meets the requirements of **EU EMC Directive 2004/108/EC** by means of conformity to the following harmonised standards:

- EN 61326-1:2006 Electrical equipment for measurement, control and laboratory use - EMC requirements - Part 1: General requirements [Class B]
- EN 55011:2007 Industrial, scientific and medical (ISM) radio-frequency equipment - Electromagnetic disturbance characteristics - Limits and methods of measurement [Class B]
- EN 61000-4-2:2009 Electromagnetic compatibility (EMC) - Testing and measurement techniques - Electrostatic discharge immunity test [Criterion A/B]
- EN 61000-4-3:2006 Electromagnetic compatibility (EMC) - Testing and measurement techniques - Radiated, radio-frequency, electromagnetic field immunity test [Criterion A]
- EN 61000-4-4:2004 Electromagnetic compatibility (EMC) - Testing and measurement techniques - Electrical fast transient/burst immunity test [Criterion A/B]
- EN 61000-4-5:2006 Electromagnetic compatibility (EMC) - Testing and measurement techniques - Surge immunity test [Criterion A]
- EN 61000-4-6:2009 Electromagnetic compatibility (EMC) - Testing and measurement techniques - Immunity to conducted disturbances, induced by radio-frequency fields [Criterion A]
- EN 61000-4-11:2004 Electromagnetic compatibility (EMC) - Testing and measurement techniques - Voltage dips, short interruptions and voltage variations immunity tests [Criterion A]

OHSAS 18001:2007 ISO9001:2008 ISO14001:2004 INVESTOR IN PEOPLE

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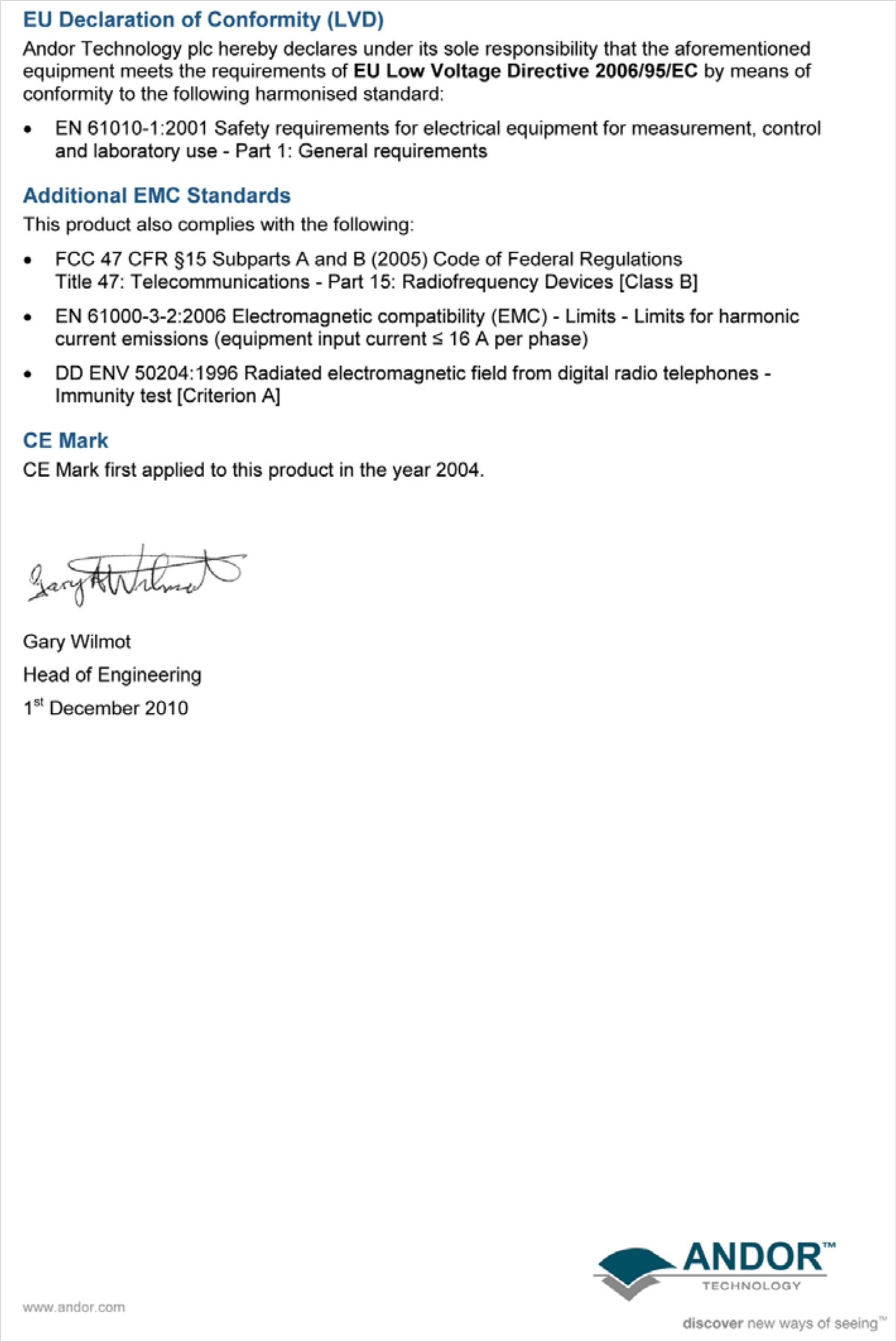

EU Declaration of Conformity (LVD)

Andor Technology plc hereby declares under its sole responsibility that the aforementioned equipment meets the requirements of **EU Low Voltage Directive 2006/95/EC** by means of conformity to the following harmonised standard:

- EN 61010-1:2001 Safety requirements for electrical equipment for measurement, control and laboratory use - Part 1: General requirements

Additional EMC Standards

This product also complies with the following:

- FCC 47 CFR §15 Subparts A and B (2005) Code of Federal Regulations
  Title 47: Telecommunications - Part 15: Radiofrequency Devices [Class B]
- EN 61000-3-2:2006 Electromagnetic compatibility (EMC) - Limits - Limits for harmonic current emissions (equipment input current ≤ 16 A per phase)
- DD ENV 50204:1996 Radiated electromagnetic field from digital radio telephones - Immunity test [Criterion A]

CE Mark

CE Mark first applied to this product in the year 2004.

Gary Wilmot

Head of Engineering

1st December 2010

www.andor.com

discover new ways of seeing™

A1.4 - TERMS & CONDITIONS

1. In these Conditions:

‘**BUYER**’ means the person who accepts a quotation of the Seller for the sale of the Goods or whose order for the Goods is accepted by the Seller.

‘GOODS’ means the goods (including any instalment of the goods or any parts for them) which the Seller is to supply in accordance with these Conditions.

‘SELLER’ means Andor Technology plc.

‘CONDITIONS’ means the standard terms and conditions of sale set out in this document and (unless the context otherwise requires) includes any special terms and conditions agreed in writing between the Buyer and Seller.

‘CONTRACT’ means the contract for the purchase and sale of the Goods.

‘WRITING’ includes telex, cable, facsimile transmission and comparable means of communication.

2. Any reference in these Conditions to any provision of a statute shall be construed as a reference to that provision as amended, re-enacted or extended at the relevant time.

3. The headings in these Conditions are for convenience only and shall not affect their interpretation.

A1.5 - STANDARD WARRANTY AND WARRANTY SERVICES

1.1 Introduction

1.1.1 This document describes the general Andor Standard Warranty policy and procedures as it relates to services obtained by a Customer under warranty. It does not replace or supersede any Product or Customer specific Warranty terms and conditions.

1.1.2 Warranty is a guarantee of quality of supplied goods (Software & Hardware). It is an obligation to rectify or replace product defects during an agreed period of time.

1.2 Standard Warranty Policy

1.2.1 Andor's Limited Standard Warranty Policy on Hardware warrants all new products to be free from defects in materials and workmanship for 12 months from the date of dispatch. (Exceptions to the standard hardware warranty offer exist and are shown in 1.2.2, 1.2.3 below). Within this period, Andor will, at its sole option, repair or replace any components which fail in normal use. Such repairs or replacements will be made at no charge to the Customer for parts or labour; however, the Customer shall be responsible for any transportation and insurance costs from the Customer premises to the Andor designated point of return. Andor shall be responsible for transportation and insurance costs related to returning the repaired or replacement unit to the Customer. The customer shall be responsible for any import duties, taxes and customs clearance fees associated with the return of the unit. This warranty does not cover failures due to abuse, misuse, accident, or unauthorized alterations or repairs. Andor assumes no responsibility for special, incidental, punitive, or consequential damages, or loss of use.

1.2.2 The following hardware components have warranties greater than 12 months:

Vacuum Warranty: Andor provides a 7 Year warranty on its UltraVac™ seal for iXon3 cameras. The UltraVac™ seal provides a permanent hermetic vacuum head, which protects the CCD sensor. Should the vacuum fail during the warranty period Andor will repair the vacuum and the components protected by it (including the CCD sensor). If you suspect a vacuum failure, typically shown by degradation in quantum efficiency or cooling performance then you should stop using the camera immediately and contact your seller support representative. Continued use of the unit once vacuum failure is suspected will void the warranty on the sensor and the components held inside the vacuum. Components protected by the vacuum, but damaged or defective due to any cause other than vacuum failure, are subject solely to the standard warranty terms.

ICCD: Andor provides a 2 year warranty for its ICCD products. Any damage caused by laser burn, bleaching of the photocathode (brought about by over illumination of the cathode) or ion damage of the cathode (brought about by excessive numbers of photoelectrons in the Multichannel plate) will not be covered by the warranty.

1.2.3 The following products and parts have specific warranty limitations:

X-RAY Cameras: Andor's standard warranty terms apply to x-ray cameras except for the sensor and other exposed parts, which are not covered in those models a) where the sensor is openly exposed (typically DO/DX models) and/or b) where the sensor is used for the direct detection of x-ray photons.

Sensor: Andor does not warrant sensors to be completely free from defects.

Items considered as consumables are not covered under this warranty, including but not limited to the following items: cables, fibre optics, filters.

Third party products not manufactured by Andor are not covered under this warranty. The customer will only be entitled to the benefit of any such warranty or guarantee as is given by the manufacturer to Andor unless specifically agreed in writing by both parties.

1.2.4 Andor guaranties that the supplied Software substantially conforms to published specifications - original license. Andor does not warrant software to be error free or that Customers will be able to operate software without problems or interruptions. The standard software warranty period is 12 months from dispatch.

1.2.5 During Warranty, customers have access to Andor support to report product defects only. Warranty does not include training or consultancy services.

1.2.6 The Warranty period is deemed to start at the date of dispatch from Andor's manufacturing facility.

1.2.7 Customers must notify the Andor customer service centre within 30 days of taking delivery of a product or part they believe to be defective. Andor will refund all fees associated with the return of any product that has been reported as defective within 30 days of delivery.

1.2.8 Failure to pay invoices when due may result in the interruption and/or cancellation of the standard warranty.

1.2.9 Andor warrants the replacement or repaired parts/components to be free from defects in materials and workmanship for twelve months from the date of dispatch or for the reminder of the warranty period, whichever is longer.

1.2.10 For products returned under warranty Andor will extend the complete product warranty by 30 days.

Hardware and Software WARRANTY SERVICE

1.3 Service Description

1.3.1 The Andor Repair service provides a repair and return service for defective products supplied by Andor under a supply contract. Using this service the original, defective part sent in by the Customer will be, where possible, returned after repair or will be replaced. Any warranty obligation contained in an Andor supply contract will be carried out in accordance with this Repair Service.

1.3.2 In order to be eligible for warranty repair or replacement, the equipment must be suffering a defect which meets the criteria set out in the supply contract and must be within its specified warranty period. Services such as upgrades to Hardware and Software are excluded from the scope of this service description and should be ordered separately.

1.4 Access to Service

1.4.1 A Customer who has purchased their product via a reseller or third party and who believes they have a warranty defect should in the first instance contact a representative of their seller's product support team. Customers who have bought products directly from Andor can access the Service Desk at www.andor.com/contact_us/support_request .

1.4.2 The Customer should indicate that they are pursuing a warranty claim and specify the equipment type and the contract under which it was supplied. The Service Desk representative will then work with the Customer to establish the nature of the defect and to determine whether the reported defect is one which meets the criteria under the supply contract for warranty remediation. This process will comprise question and answer between Service Desk and Customer and the Service Desk operative may, at their sole discretion, ask the Customer to perform some basic diagnostic actions in relation to the problem item.

1.5 Hardware Remediation

1.5.1 If the issue cannot be resolved remotely and a fault has been diagnosed, a Return Materials Authorization ("RMA") number will be issued. This RMA number will be valid for 30 days from the date of issue. An RMA number must be obtained from Andor prior to the return of any material. The RMA number must appear clearly on the outside of the shipping container and on return paperwork included inside the package.

1.5.2 Following allocation of a RMA number by Andor, the Customer shall ship the PART to Andor at customer expense. The customer is responsible for return shipping and insurance costs. Any products returned without an RMA number may be refused and returned to the customer at their expense. Andor shall provide a single point of return for all products.

1.5.3 On receipt of the part at the Andor repair facility, Andor shall carry out the necessary fault diagnosis and repair and return the part to the Customer.

1.5.4 The method of shipment and choice of courier for the return will be at Andor’s discretion. Delivery Duties Unpaid (DDU) Incoterms 2000. Andor does not guarantee the arrival time of the part.

1.5.5 Customer must adhere to Andor packing instructions (including anti-static precautions) when shipping the defective unit as any damage incurred during shipment to Andor will not be covered under warranty. The packing instructions can be obtained from Andor as part of the part request procedure.

1.5.6 If the part is not economically repairable then a replacement part (new or refurbished) will be supplied at Andor discretion and expense.

1.5.7 In case of replacement the replacement unit becomes the property of the Customer on an exchange basis.

1.5.8 In case of misuse the Customer will be contacted to decide the course of action. These actions may include:

- Scrapping the part
- Return of the defective unrepaired part to the Customer
- Replacement with a new or refurbished part. Andor will invoice the customer the full merchandise contracted customer price of the unit.

1.5.9 Unless elsewhere agreed between the Customer and Andor, this service does not include root cause analysis, the provision of fault reports or lead-time and performance metrics.

1.6 Software Remediation

1.6.1 During Warranty Customers have access to the Service Desk at www.andor.com/contact_us/support_request to report product defects. A Customer who has purchased their product via a reseller or third party and who believes they have a software warranty defect should in the first instance contact a representative of their seller’s product support team.

1.6.2 Where as a result of the process described in 1.3.2 above it is determined that the defect relates to software, a trouble ticket will be logged in respect of the software issues observed.

1.6.3 Under the warranty provisions of the supply contract we will not provide the customer with a guaranteed SLA (service level agreement) for their problem.

A1.6 - THE WASTE ELECTRONIC AND ELECTRICAL EQUIPMENT REGULATIONS 2006

Where appropriate, Andor has labelled its electronic products with the WEEE label (crossed out wheelie bin) to alert our customers that products bearing this label should not be disposed of in a landfill or with municipal waste. If you have purchased Andor-branded electrical or electronic products in the EU after August 13, 2005, and are intending to discard these products at the end of their useful life, Andor are happy to assist.

The cost for the collection, treatment, recycling, recovery and sound environmental disposal of these goods at the end of its useful life has not been included in the price. If you require help/assistance regarding the disposal of this equipment please refer to our website, or contact our sales team at which point instructions and a quotation can be provided.

A copy of the Company's WEEE Policy can be viewed at the Company website www.andor.com .