

USERS GUIDE



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SECTION 1 - ABOUT THE ANDOR LUCA-S

Thank you for choosing the **Andor Luca-S**. You are now in possession of a revolutionary new **Electron Multiplying Charge Coupled Device (EMCCD)** detector, designed for the most challenging low-light imaging applications. Its unique features and design are discussed in more detail within this User Guide. This guide is designed to help you understand the Luca-S camera and contains information and advice to ensure you get the optimum performance from your new system.

1.1 - TECHNICAL SUPPORT

If you have any questions regarding your Luca-S, please feel free to contact Andor either directly at one of the addresses shown below, via your local representative* or supplier.

<p>Europe</p> <p>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 e-mail: productsupport@andor.com</p>	<p>USA</p> <p>Andor Technology 425 Sullivan Avenue - Suite 3 South Windsor CT 06074 USA Tel. +1 (860) 290-9211 Fax. +1 (860) 290-9566 e-mail: productsupport@andor.com</p>
<p>Asia-Pacific</p> <p>Andor Technology (Japan) 7F Ichibancho Central Building 22-1 Ichiban-Cho Chiyoda-Ku Tokyo 102-0082 Japan Tel. +81-3-3511 0659 Fax. +81-3-3511 0662 e-mail: productsupport@andor.com</p>	<p>China</p> <p>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 e-mail: productsupport@andor.com</p>

***NOTE: THE CONTACT DETAILS FOR YOUR NEAREST REPRESENTATIVE CAN BE FOUND ON OUR WEBSITE.**

1.2 - EMCCD Technology

1.2.1 - 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. **Electron Multiplying Charge Coupled Device (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 2001, 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, readily tunable 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.

1.2.2 - Does EMCCD technology eliminate Read Out Noise?

System noise within modern silicon based detectors has two primary sources, namely 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. And EMCCDs ability to multiply weak signals above the detectors read noise, by applying EM gain, effectively eliminates read noise at any speed by reducing it to less than $1 e^-/p/s$.

1.2.3 - How Sensitive are EMCCDs?

Two parameters significantly influence detector sensitivity namely **Quantum Efficiency (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.

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

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

Andor Technology was the first company to introduce an EMCCD based detector. Since then the company has led the way in the development of EMCCD detectors, introducing the first back illuminated EMCCD in January 2003. Andor have also introduced the world's first dedicated spectroscopic EMCCD camera, and now the first true "mid-range" workhorse EMCCD range in the form of the Luca. Andor now offers the widest range of EMCCD based detectors on the market. The company is also playing a pivotal role in increasing 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 CT, USA. Please go to www.emccd.com for further details.

1.3 - COMPONENTS

Andor's Luca-S exploits the processing power of today's desktop computers. The system's hardware components and its comprehensive software options provide speed and versatility for a range of imaging applications and set-ups.

The Andor Luca-S system is composed of **hardware**, **software choice** and **documentation**. This section of the User's Guide identifies the main hardware components of the system and guides you through the installation procedure. The main components of the Andor Luca-S EMCCD system are as follows:

- Camera containing:
 - EMCCD Sensor
 - Input / Output connectors
- Andor Instruction Manuals on CD-ROM
- **Power Supply Block (PSB)** and power cable
- USB cable
- CS-C mount adapter
- Video cable

NOTE: The Luca-S has a CS-mount as standard. If C-mount is required, use the CS-C mount screw-on adapter provided.

The camera (**figures 1 & 2**) contains the following:

- EMCCD Sensor (commonly referred to as **Detector**) with Pre-Amplifier
- 14 and/or 16-bit analogue to digital converters that digitize data from the analogue controller boards
- Input & output connectors
- CS-mount (CS to C mount adaptor is provided)
- The temperature control components, which regulate cooling of the camera.



Figure 1: Luca-S front view



Figure 2: Luca-S rear view



Figure 3: Luca-S connectors

There are 3x industry-standard **SMB (SubMiniature B)** connectors fitted to the rear of the camera as shown above. These are labeled as follows:

- **Sync**
- **Fire**
- **Ext Trig**

These are used to send or receive **Trigger** and **Fire** signals, which are described later in this manual. They are also **CMOS compatible & series terminated** at source (i.e. in the camera head) for **50Ω** cable.

NOTE: The termination at the customer end should be high impedance (>1KΩ) as an incorrect impedance match could cause errors with timing and triggering. The SMB Ext Trig input is TTL level & CMOS compatible and has 470Ω impedance.

The other connectors are as follows:

- **USB 2.0:** for fast 'plug and play' connection to the controlling PC, via USB 2.0
- **Video output:** the output from the Luca-S can be streamed continuously, at the full frame rate of the camera, through an analogue video output. This can be useful visualization and video recording of extensive processes that would otherwise require large amounts of hardware space if streamed out digitally through the USB 2.0 connector.
- **Power:** connection for the **Power Supply Block (PSB)** shown on the next page.

1.3.3 - Power Supply Block

The PSB uses a proprietary 4-pin connector. The mains connection to the PSB is made via a standard IEC connection.



1.4 - SAFETY PRECAUTIONS**1.4.1 - Working with Electronics**

The circuits used in the camera 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.

The types of equipment that can cause problems are plasma sources, arc welders, radio frequency generators, pulsed discharge optical sources and X-ray instruments. Operation of the system close to intense pulsed sources (lasers, xenon strobes, arc lamps, and the like) may compromise performance, if shielding is inadequate.

1.4.2 - Care of the Camera

YOUR LUCA-S IS A PRECISION SCIENTIFIC INSTRUMENT CONTAINING FRAGILE COMPONENTS. ALWAYS HANDLE WITH THE CARE NECESSARY FOR SUCH INSTRUMENTS. IF THE EQUIPMENT IS USED IN A MANNER NOT SPECIFIED BY ANDOR TECHNOLOGY plc, THE PROTECTION PROVIDED BY THE EQUIPMENT MAY BE IMPAIRED.

1.4.3 - Environmental conditions

- Indoor use only
- Altitudes up to 2000m
- Temperature 5°C to 40°C
- Maximum Relative Humidity 80% for temperatures up to 31°C, decreasing linearly to 50% relative humidity at 40°C
- Other voltage fluctuations as stated by the manufacturer
- Overvoltage category 1
- Pollution Degree 2

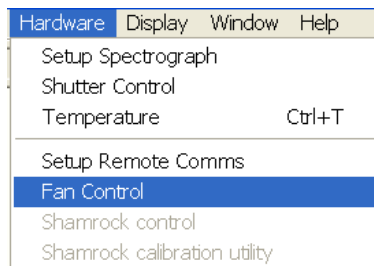
1.4.4 - Head Overheating

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

- The air vents on the sides of the head are accidentally blocked or there is insufficient or no water flow
- The ambient air temperature is over 25°C.

1.4.5 - Fan Settings

The cooling fan can also be switched off, e.g. to minimize vibrations if working on a combined optical/AGM set-up, e.g. in Solis (i). To switch the fan Off or On, select **Fan Control** from the **Hardware** drop-down menu as shown:



The **Fan speed** dialog box will appear:



Select Off or On as required (this may affect the cooling ability of the CCD).

NOTE: We recommend that the fan should not be switched off for more than 2 minutes.

1.5 - SOFTWARE

Luca-S is compatible with a range of Andor software options, offering optimized acquisition control and analysis functionality. Choice of these software options is dictated largely by application demands and budget factors, and is generally a very important part of the purchasing decision. Please go to the following page on our website for further details of our software products: <http://www.andor.com/products/software>

1.5.1 - Andor Solis (i)

Andor **Solis (Solutions for Imaging and Spectroscopy)** is Andor's generic camera control and analysis software platform, with versions specifically designed to run imaging, spectroscopy, time-resolved cameras and associated accessories. It is easy to install and provides "state of the art" acquisition, display and processing capabilities. The Andor Solis (i) software platform is designed for image capture and analysis and is used in a wide range of scientific fields including fluorescence imaging, luminescence imaging, Bose-Einstein Condensation, single molecule detection, upper atmosphere studies, X-ray studies and Raman imaging.

This comprehensive software package has an extensive built-in programming language, **Andor Basic**, which has been specially designed to simplify even the most complex operations. For example, two images can be added and stored in a new data store by simply typing #3 = #1 + #2 - Easy! As Andor Basic can be used to program image acquisitions, automate file storage, communicate with external devices and manipulate data, you can build macros to automate your experiment.

1.5.2 - Andor iQ

Andor iQ is our flagship live cell imaging and microscopy software, designed with flexibility and power in mind. iQ (image and **Quantify**) occupies a central role in our product range and provides optimized control of Andor's award winning EMCCD cameras and automation hardware for a range of bioimaging applications. Continuous development and improvement ensures that Andor iQ represents a powerful and flexible core for live-cell imaging systems.

1.5.3 - Andor Komet 5™

Drivers are available for controlling the Luca-S camera within Komet 5™, Andor's dedicated software for Comet Assay.

1.5.4 - Andor SDK

The Andor **Software Development Kit (SDK)** enables users to control the complete range of Andor cameras. The key part of the SDK is its 32-bit dynamic link library "**ATMCD32D.DLL**" which can be used with a wide variety of programming environments including **C**, **C++**, **Visual Basic** and **LabVIEW**. The library is compatible with Windows 2000 and XP. The SDK provides a suite of functions that allow you to configure the data acquisition process in a number of different ways. There are also functions to control the CCD temperature and shutter operations. To use the SDK effectively the user must develop a software package to configure the acquisition, provide memory management, process the data and create an Andor software interface. Andor also provides an SDK for the Linux operating system.

1.5.5 - Image J

Drivers are available from Andor for acquisition control of the Luca-S camera within Windows version of the popular downloadable imaging software from NIH, Image J. Please go to the following website for further details: <http://rsb.info.nih.gov/ij/download.html>

SECTION 2 - INSTALLATION

2.1 - PC REQUIREMENTS

The minimum recommended computer specification for use with Luca-S is:

- 2 GHz Pentium Processor (or better)
- USB 2.0 compatible
- 1GB of RAM
- Minimum 7,200 RPM HDD
- Operating system: Windows 2000, XP or Vista

To achieve 30+ frames per second the following specification is recommended:

- 3GHz Pentium Processor (or better)
- USB 2.0 compatible
- 2GB RAM
- Minimum 7,200 RPM HDD. For Andor iQ software, a second HDD is considered useful for direct spooling of images
- Hyper-Threading **ENABLED**
- Operating system: Windows 2000, XP or Vista

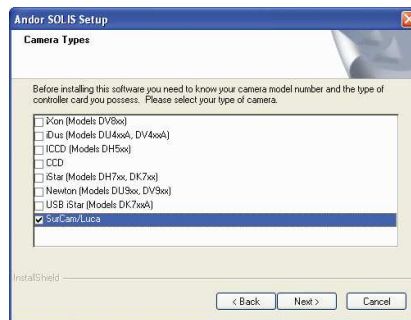
2.2 - INSTALLING THE SOFTWARE & DEVICE DRIVER - WINDOWS OPERATING SYSTEMS (2000/XP)

The Solis software is held on the USB Flash Drive supplied with the Luca-S camera and must be installed before the camera is connected. The install should be carried out as follows:

1. Insert the flash drive in any available USB port and open the **setup.exe** file. The **InstallShield Wizard** for **Andor Solis** will start, e.g.:



2. Click **Next>** and the **Andor Solis Setup** dialog box appears at the **Camera Types** window, e.g.:



3. Tick the **Surcam/Luca** option then click **Next>** and the **Choose Destination Location** window should appear, e.g.:

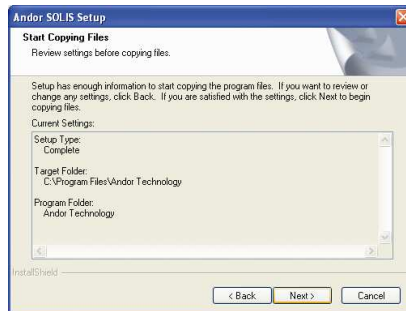


4. Click **Next>** to choose the default destination **C:\Program Files\Andor Technology**. Alternatively, you can click on **Browse...** and choose your own destination folder.

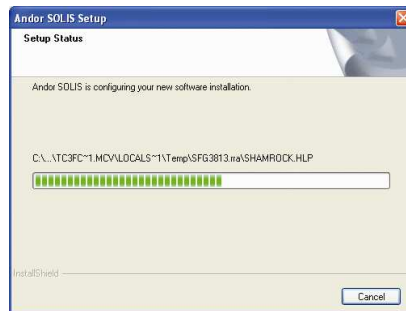
5. The **Select Program Folder** window should appear, e.g.:



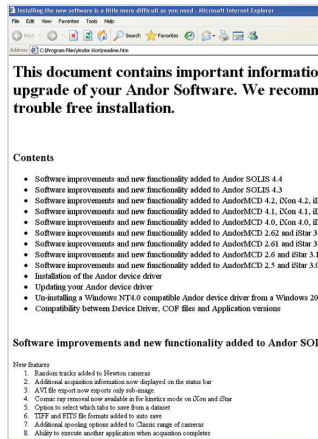
6. Select the **Andor Technology** option then click **Next>**. The **Start Copying Files** window should appear, e.g.:



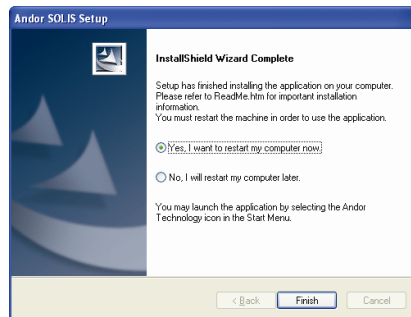
7. Click **Next>** and the **Setup Status** option window should appear with an updating progress bar e.g.:



8. When the progress bar stops, a message similar to the following may appear:



PLEASE READ THE MESSAGES AND OBSERVE ANY IMPORTANT INFORMATION RELEVANT TO YOUR SYSTEM THEN RESTART THE PC AS PER THE FOLLOWING PROMPT:

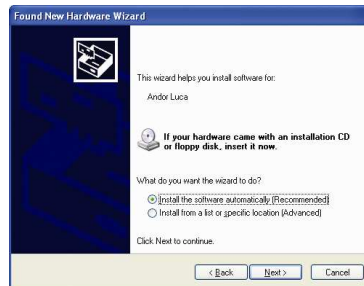


9. Select the **Yes, I want to restart my computer now** option then click **Finish**. Your PC should now reboot automatically.

10. After the PC has been restarted, connect the Luca-S. The **Found New Hardware** dialog box should appear:



11. Select the **No, not this time** option, then click **Next>** and the following dialog box should appear:



12. Select the **Install the software automatically (Recommended)** option then click **Next>**. The hardware install will commence and the following dialog box may appear:



13. Click on **Continue Anyway** and the install progress should continue as show here:

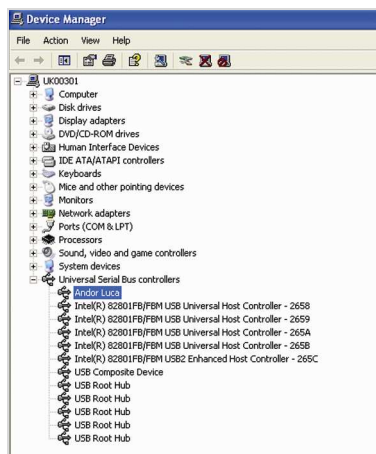


14. After the progress is complete, the following dialog box should appear:



15. Click **Finish** to complete the install.

16. Check the **Device Manager** on the PC to ensure that the **Andor Luca** is on the **Universal Serial Bus controllers** list, e.g.:



17. To start the Solis software, click on the  icon which should now be installed on your desktop.

1. Run Setup.exe on the iQ installation CD root directory
2. Click "**Perform a Full Installation of Andor iQ**" on the screen
3. Follow the steps shown on the new screen.
4. Reboot the computer after the completion of installation

NOTE: The device driver is automatically transferred to the hard drive during iQ software installation. The Luca-S should not be connected to the computer before iQ is installed. After iQ is installed and the computer is rebooted, connect the camera to the computer to enter the device driver installation procedure.

SECTION 3 – HARDWARE 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 **Electron Multiplying Charged Coupled Devices (EMCCDs)**. At the heart of your Luca-S 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 **Charged Coupled Device (CCD)** but with the **shift register** extended to include an additional section, the **Multiplication** or **Gain Register** as shown below.

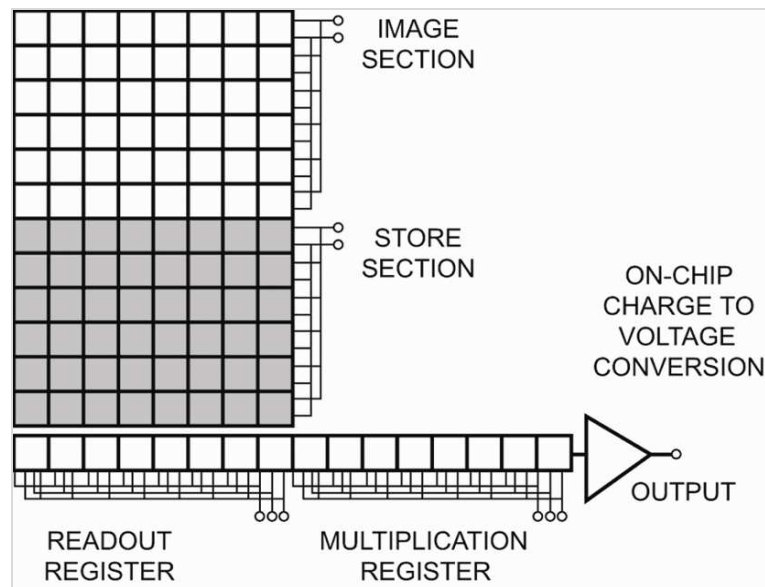


Figure 4: EMCCD structure

During an acquisition using a conventional **Frame Transfer 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 5**.

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.

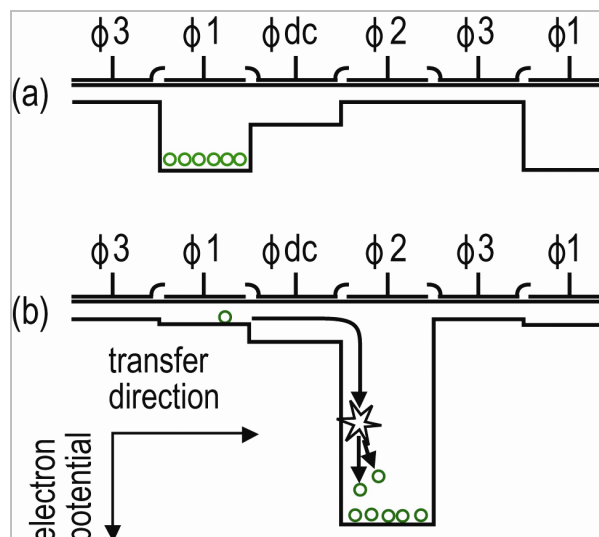
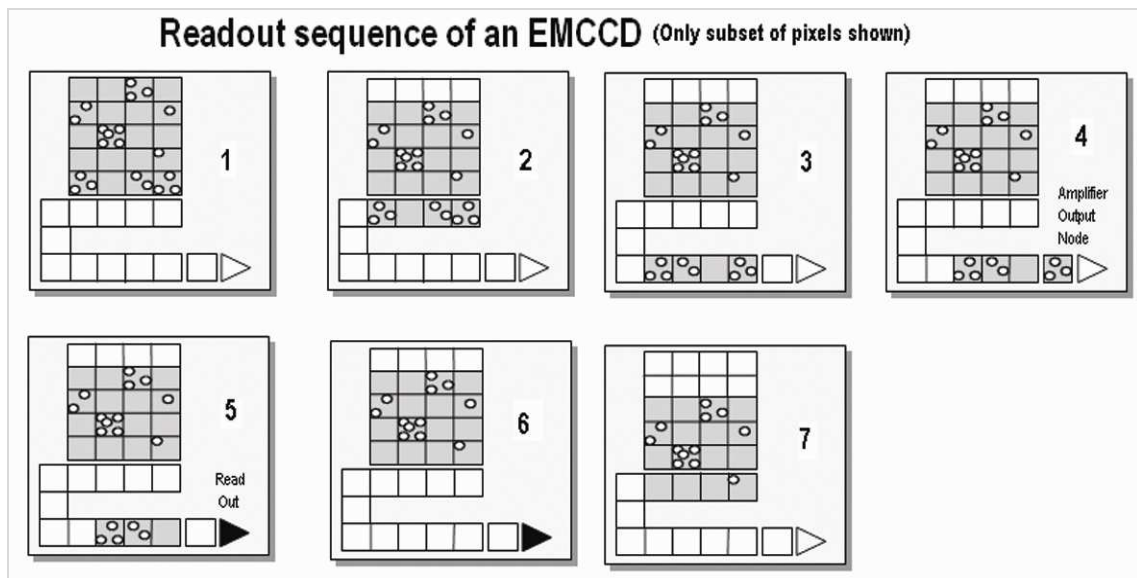


Figure 5: Gain register operation

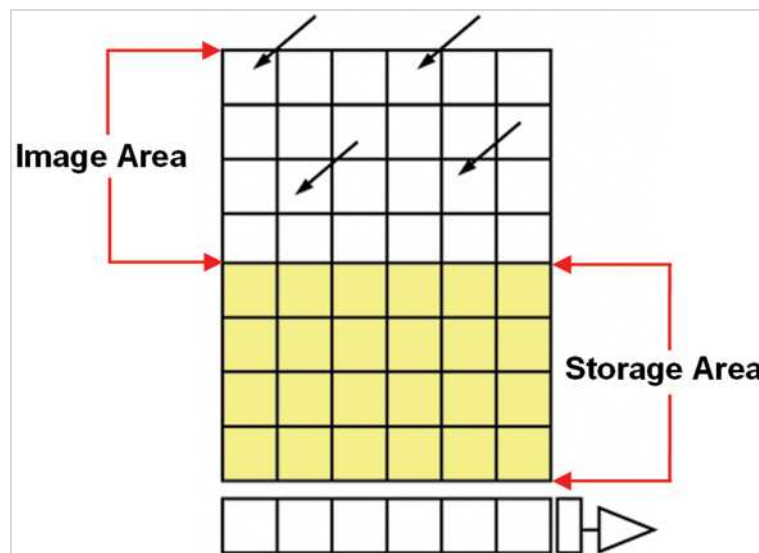
In the course of **readout**, charge is moved vertically into the shift register then horizontally from the shift register into the output node of the amplifier. The simple readout sequence illustrated below (which corresponds to the default setting of the Full Resolution Image binning pattern) allows 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 (please see **Vertical & Horizontal Binning** on pages 28 - 29).



- 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.

Frame transfer is a special acquisition mode that is only available if your system contains a Frame Transfer CCD, (FT CCD) as is the case with Luca-S. An FT CCD differs from a standard CCD in 2 ways.

- Firstly, a FT CCD contains 2 areas, of approximately equal size (see diagram below). 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. The second section is the Storage Area, and is located between the Image Area and the read-out 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 the 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 while the next image is being acquired. It also allows a FT CCD to be used in imaging mode without a shutter.



Binning is a process that allows charge from two or more **pixels** to be combined on the **EMCCD**-chip prior to readout (please see **page 24**). Summing charge on the EMCCD and doing a single readout gives better noise performance than reading out several pixels and then summing them in the computer memory. This is because each act of reading out contributes to noise.

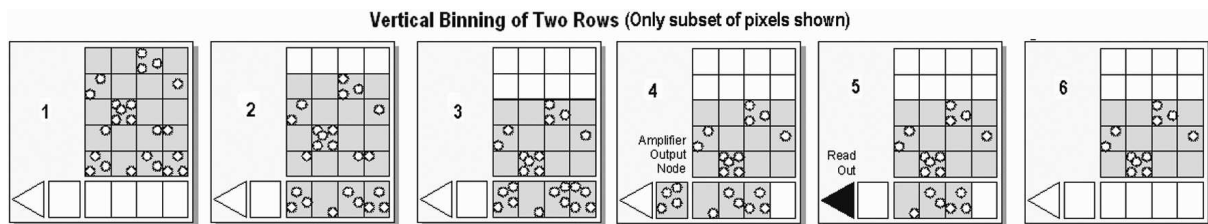
There are two main variants of the binning process:

- **Vertical binning**
- **Horizontal binning**

In addition there are several **binning patterns** that tailor the main binning variants to typical application usage.

3.2.1 - Vertical Binning

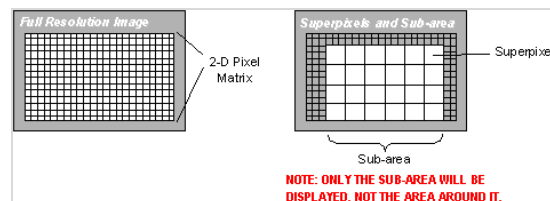
In Vertical Binning, charge from two or more rows of the EMCCD-chip is moved down into the shift register before the charge is read out. The number of rows shifted depends on the binning pattern you have selected. Thus, for each column of the EMCCD-chip, charge from two or more vertical elements is **summed** 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 node of the amplifier and read out. The figure below illustrates readout of data from adjacent tracks, each track comprising two binned rows of the EMCCD-chip.



- 1** Exposure to light causes a pattern of charge (an electronic image) to build up on the frame (or “image area”) of the CCD-chip.
- 2** Charge in the frame is shifted vertically by one row, so that the bottom row of charge moves down into the shift register.
- 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.
- 4** 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.
- 5** Charge is shifted into the output node of the amplifier.
- 6** The charge in the output node of the amplifier is passed to the analog-to-digital converter and is read out. Steps 4 and 5 are repeated until the shift register is empty. The process is repeated from Step 2 until the whole frame is read out.

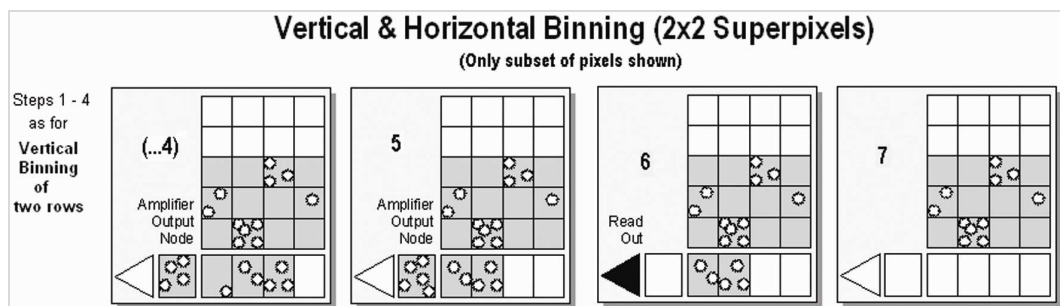
3.2.2 - Horizontal Binning (Creating Superpixels)

Shifting the charge horizontally from several pixels at a time into the output node is known as Horizontal Binning. Horizontal binning in combination with Vertical binning allows you to define so-called “Superpixels”, which consist of two or more individual pixels that are binned and read out as one large pixel. The whole CCD, or your selected sub-area, becomes a matrix of superpixels. The horizontal and vertical binning parameters determine the dimensions of any superpixels you may choose to create.




On the one hand, superpixels (by comparison with single pixels) result in a more coarsely defined image when the data are displayed in Image Display Mode; on the other hand, superpixels offer the advantages of summing data on-chip prior to readout, including better Signal to Noise ratio. In the example on page (where each superpixel is of dimensions 2 x 2 pixels), charge from two rows is first binned vertically into the shift register; then charge from two pixels of the shift register is binned horizontally into the output node of the amplifier. The effect of the combined binning processes is a summed charge equating to a 2 x 2 superpixel.

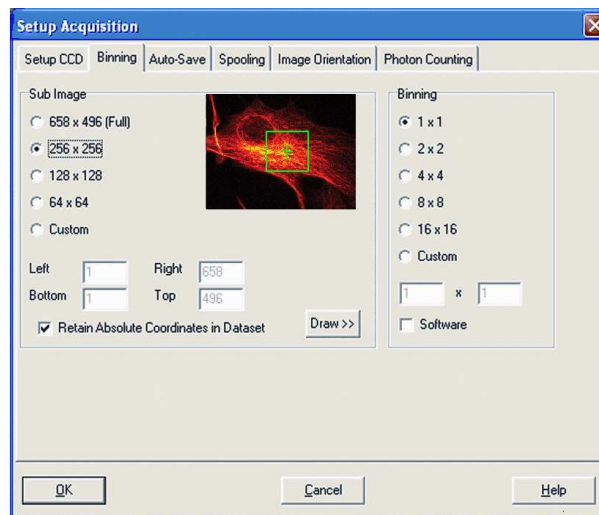
Since this example initially involves binning charge from two rows, the process begins in the same way as the previous example (please see **Steps 1 - 4 of Vertical Binning of Two Rows** on **page 28**) then horizontal binning begins.



- ...4 Charge from two rows has already been vertically binned into the shift register (see Vertical Binning of Two Rows previously). Now 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, and subsequently into the output node of the amplifier.)
- 5 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.
- 6 The charge in the output node of the amplifier is passed to the analog-to-digital converter and is read out.
- 7 Steps 4 to 6 are repeated until the shift register is empty. The process is repeated from Step 2 (again please see **Vertical Binning of Two Rows** on the previous page) until the whole frame is read out.

For the purpose of initial focusing and alignment of the camera, or to increase the readout speed, you may use the software to select a **Sub Image** of the chip.

- In Solis (i), to select Sub Image mode, click on the  button. When the Luca-S is running in Sub Image mode, only data from the selected pixels will be digitized. Data from the remaining pixels will be discarded.
- In Solis (i), to read out data from a selected area (or **Sub Image**) of the CCD use the radio buttons to select the resolution, which you require, e.g.:

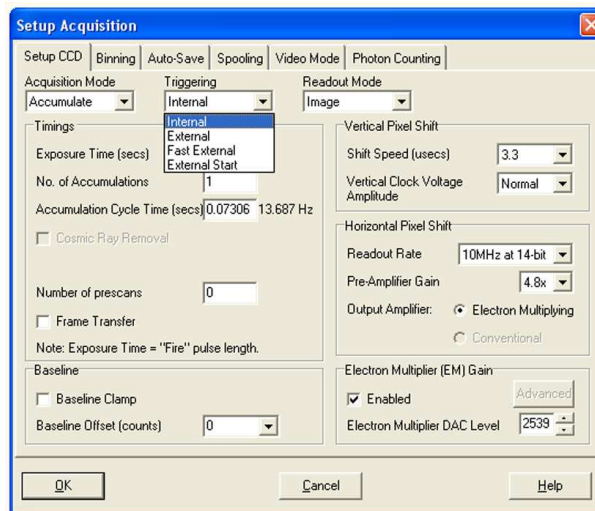


Depending on the type of sensor in use, the software offers a choice of up to four defined sub images, i.e.:

- 256 x 256 pixels
- 128 x 128 pixels
- 64 x 64 pixels
- **Custom:** there is also an option for you to define a Custom Sub Image. This function allows you to set the Sub Image to any size and location on the CCD chip. To define a Custom Sub Image tick the **Custom** button, then use the co-ordinate entry dialogue boxes to select the size and location of your sub image.

3.3 - TRIGGERING OPTIONS

The **Triggering** modes are selected from the Setup Acquisition dialog box of the software used. This is shown here for Solis (i):



The options available are as follows:

- **Internal**
- **External**
- **Fast External**
- **External Start**

3.3.1 - Internal

In **Internal** mode, once you issue a data acquisition command, the system determines when data acquisition begins.

You can use Internal mode when you are able to send a trigger signal or “**Fire Pulse**” to a short-duration, pulsed source (e.g. a laser). In this case starting data acquisition also signals the pulsed source to fire. The **Fire Pulse** is fed from the **Fire SMB** connector on the detector.

Internal Trigger Mode is also used with “**Continuous Wave**” (**CW**) sources (e.g. a steady-state fluorescence signal) where incoming data, for the purposes of your observation, are steady and unbroken. This means that acquisitions can be taken at will.

3.3.2 - External

In **External** mode once you issue a data acquisition command, an exposure will not be acquired until your system has received an External Trigger signal generated by an external device (e.g. a laser). The External Triggering signal is fed to the **Ext Trig** SMB connector on the rear of the camera.

NOTE: If you have a shutter connected and are using external triggering, you must ensure that the shutter is open before the optical signal you want to measure occurs.

Normally, when using **External Trigger** the system will only enable the triggering after a complete Keep Clean Cycle has been performed. This is to ensure that the CCD is always in the same known state before it is triggered. This is particularly important when the system is in **Accumulation** or **Kinetics** mode. In cases where repetition rate is paramount, and slight variation in the base background level is less important, it is possible to remove this restriction by using **Fast External** triggering.

NOTES:

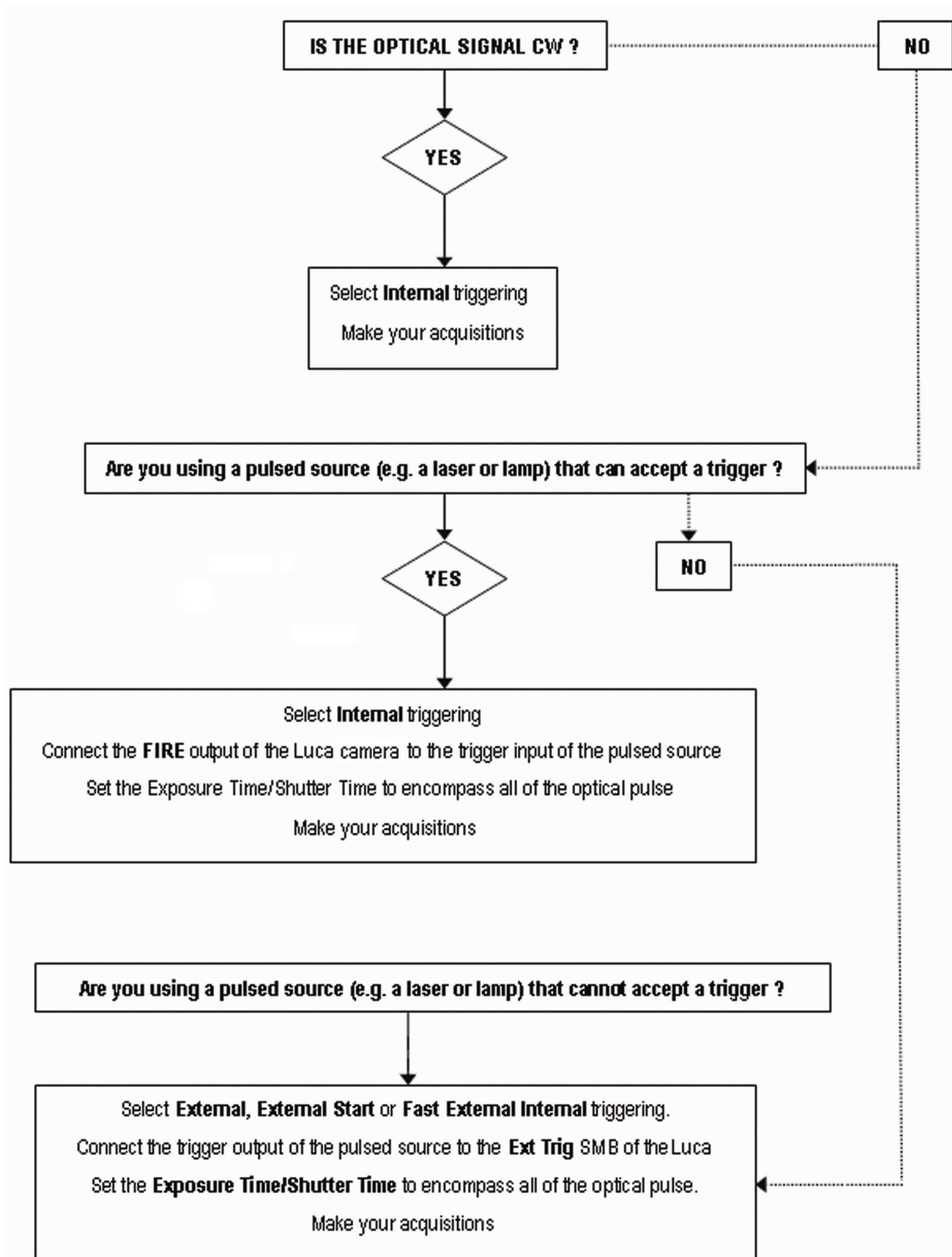
1. **The Keep Clean process is continuous on the Luca-S and any delay is negligible.**
2. **If you need maximum repetition rate, have a shutter connected and are using fast external triggering, you must ensure that the shutter is open before the optical signal you want to measure occurs.**

With **External Start** triggering, once you issue a data acquisition command, data series will not be acquired until your system has received an external trigger signal generated by an external device. The system will then continue to acquire data based on user options set within the Acquisition Dialog.

This means that an External Start Trigger could be used to commence acquisition of a Kinetic series, but with the parameters of that series being controlled by internal software options. The External Start trigger signal is fed to the camera head via the **Ext Trig** SMB on the back of the camera.

NOTE: If you have a shutter connected and are using an external trigger, you must ensure that the shutter is open before the optical signal you want to measure occurs.

The following flowchart will help you decide whether you should use **Internal**, **External**, **External Start** or **Fast External** triggering.



An acquisition is taken to be the complete data capture process that is executed whenever you select Take Signal, Take Background, or Take Reference from the Acquisition Menu or whenever you click the Take Signal button. By contrast, a scan (an “**Acquired Scan**” in the definitions that follow) is **1x readout** of data from the CCD-chip. Several scans may be involved in a complete data acquisition.

The minimum time required for an acquisition is dependent on a number of factors, including the **Exposure Time** (i.e. the time in seconds during which the EMCCD collects light prior to readout) and the **Triggering** mode. Triggering modes are described in more detail in **pages 31 - 33**.

Fast Kinetics allows exposure times on a microsecond timescale. Use Fast Kinetics when you need an exposure time that is smaller than the minimum Kinetic Cycle Time in a standard Kinetic Series. In Fast Kinetics mode the image to be recorded is imaged across a certain section of the CCD. The non-illuminated part of the CCD is used for storage of image before readout. You must ensure that light does not fall on this storage part of the CCD. By way of explanation, take the example of an illuminated sub-area of height 8 rows. An image is imaged along this sub-area and the resulting image is then shifted down 8 rows into the non-illuminated area, thus sampling the image in time. In this way the image is temporarily stored on the CCD itself, rather than in the computer.

The process is repeated until the frame of the CCD is filled with time-sampled images or until the number of images you have specified for your series has been acquired. Next each image is transferred into the shift register in turn, and read out in the normal way. With a CCD-chip of height 512 pixels and a sub-area height of 8 rows, 64 discrete images can be stored on the CCD.

From the Setup Acquisition dialog box, you can change the following parameters:

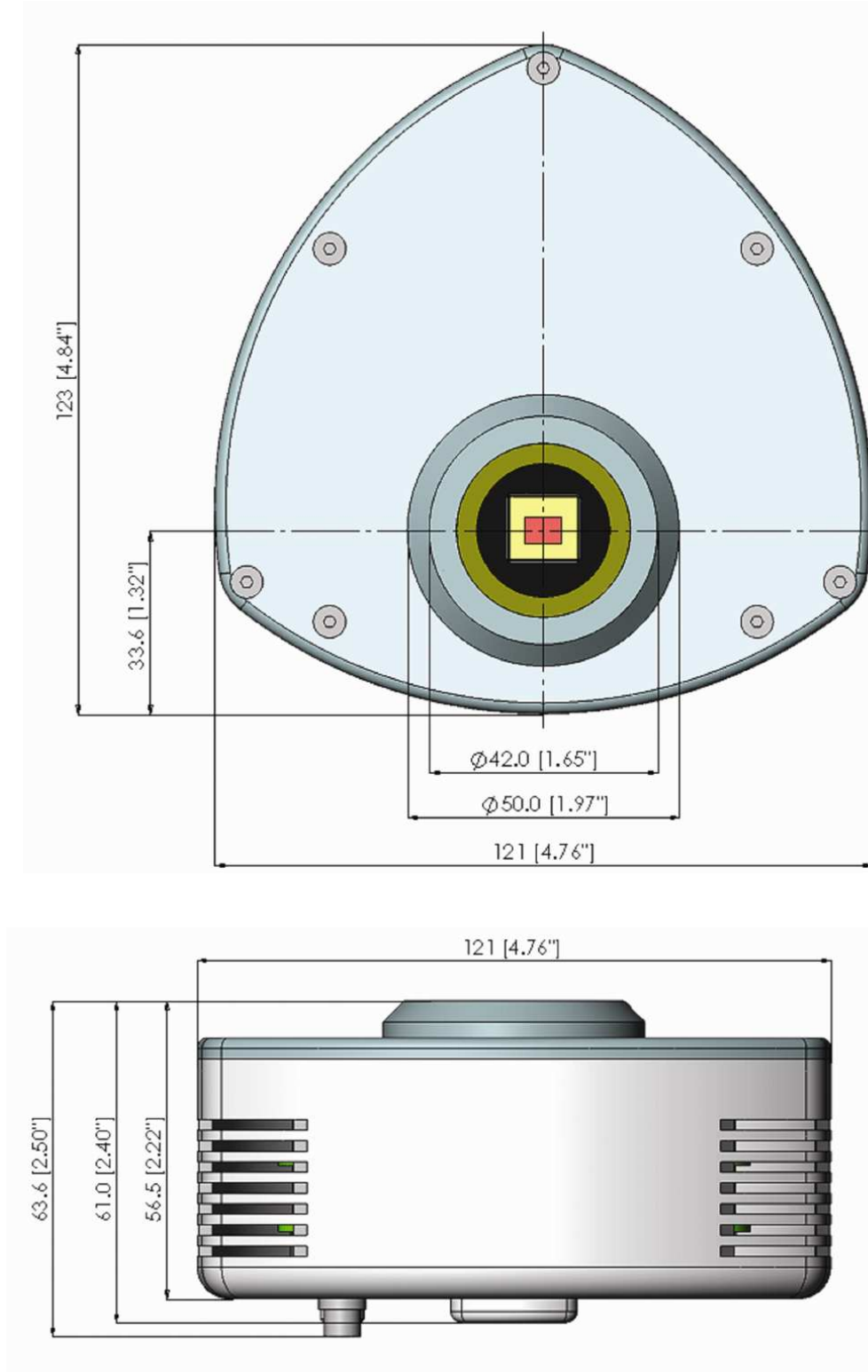
- **Exposure Time:** the Exposure Time also represents the cycle time of the Fast Kinetics series. There is no separate parameter for a Fast Kinetics cycle time
- **Sub Area Height** in rows
- **Number in Series:** the number of time-sampled images you want to acquire
- **Binning** of the images
- **Offset** of the active area from the bottom of the detector can also be specified

The data from each of the images in the **Fast Kinetics** series is stored as an image. Select **Image** from the **Readout Mode** drop down list on the **Setup Acquisition** Window. Fast kinetics is not available in multi-track mode.

With Fast Kinetics you may use the following Trigger Modes, namely: Internal, External, Fast External and External Start.

- In **Internal** mode, the system determines when the acquisition begins, and then uses the acquisition settings defined by the user. This mode is equivalent to the internal triggering mode for Single Scan etc.
- In **External** mode, a trigger pulse is required to start each scan in the series. The rising edge of the trigger starts the exposure time. After the exposure time has elapsed, the numbers of rows 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.
- In **External Start** mode, data will not be acquired until the system receives an initial external trigger signal from an external device, like a laser. From that point on, the system alone determines when data are acquired based on the user settings, as in the case of Internal Trigger. As the system changes from an external trigger mode to internal trigger mode on receipt of the initial trigger signal the exposure time of the first scan in the series will not be the same as the subsequent scans. The exposure time is defined as the time between vertical shifts.

Dimensions shown in mm (inches):



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 Limited.

'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.

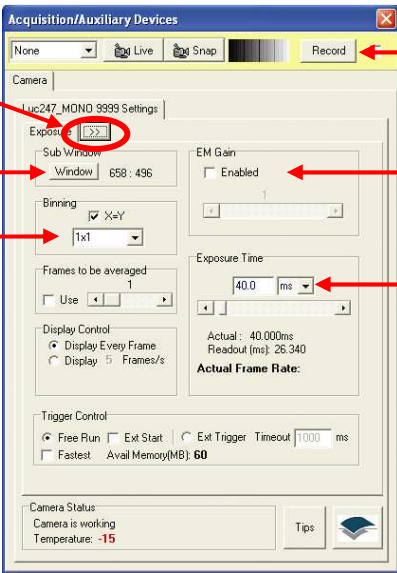
3.7 - WARRANTIES & LIABILITY

1. Subject to these Conditions set out below, the Seller warrants that the Goods will correspond with their specification at the time of delivery and will be free from defects in material and workmanship for a period of 12 months from the date of delivery.
2. The above warranty is given by the Seller subject to the following conditions:
 - 2.1 the Seller shall be under no liability in respect of any defect in the Goods arising from any drawing, design or specifications supplied by the Buyer;
 - 2.2 the Seller shall be under no liability in respect of any defect arising from fair wear and tear, wilful damage, negligence, abnormal working conditions, failure to follow the Seller's instructions (whether oral or in writing), misuse or alteration or repair of the Goods without the Seller's approval;
 - 2.3 the Seller shall be under no liability under the above warranty (or other warranty, condition or guarantee) if the total price for the Goods has not been paid by the due date for payment;
 - 2.4 the above warranty does not extend to parts, material or equipment not manufactured by the Seller, in respect of which the Buyer shall only be entitled to the benefit of any such warranty or guarantee as is given by the manufacturer to the Seller.
3. Subject as expressly provided in these conditions, and except where the Goods are sold to a person dealing as a consumer (within the meaning of the Unfair Contract Terms Act 1977), all warranties, conditions or other terms implied by statute or common law are excluded to the fullest extent permitted by law.
4. Any claim by the Buyer which is based on any defect in the quality or condition of the Goods or their failure to correspond with specification shall (whether or not delivery is refused by the Buyer) be notified in Writing to the Seller within 7 days from the date of delivery or (where the defect or failure was not apparent on reasonable inspection) discovery of the defect or failure. If delivery is not refused, and the Buyer does not notify the Seller accordingly, the Buyer shall not be entitled to reject the Goods and the Seller shall have no liability for such defect or failure, and the Buyer shall be bound to pay the price as if the Goods had been delivered in accordance with the Contract. In no event shall the Buyer be entitled to reject the Goods on the basis of any defect or failure which is so slight that it would be unreasonable for him to reject them

5. Where any valid claim in respect of the Goods which is based on any defect in the quality or condition of the Goods or their failure to meet specification is notified to the Seller in accordance with these Conditions, the Seller shall be entitled to replace the Goods (or the part in question) free of charge or, at the Seller's sole discretion, refund to the Buyer the price of the Goods (or a proportionate part of the price), but the Seller shall have no further liability to the Buyer.
6. Except in respect of death or personal injury caused by the Seller's negligence, the Seller shall not be liable to the Buyer by reason of any representation (unless fraudulent), or any implied warranty, condition or other term, or any duty at common law, or under the express terms of the Contract, for any indirect, special or consequential loss or damage (whether for loss of profit or otherwise), costs, expenses or other claims for compensation whatsoever (whether caused by the negligence of the Seller, its employees or against otherwise) which arise out of or in connection with the supply of the Goods, or their use or resale by the Buyer and the entire liability of the Seller under or in connection with the Contract shall not exceed the price of the goods, except as expressly provided in these Conditions.
7. The Seller shall not be liable to the Buyer or be deemed to be in breach of the Contract by reason of any delay in performing, or any failure to perform, any of the Seller's obligations in relation to the goods, if the delay or failure was due to any cause beyond the Seller's reasonable control. Without prejudice to the generality of the foregoing, the following shall be regarded as causes beyond the Seller's reasonable control:
- 7.1 Act of God, explosion, flood, tempest, fire or accident;
 - 7.2 war or threat of war, sabotage, insurrection, civil disturbance or requisition;
 - 7.3 acts, restrictions, regulations, bye-laws, prohibitions or measures of any kind on the part of any governmental, parliamentary or local authority;
 - 7.4 import or export regulations or embargoes;
 - 7.5 strikes, lock-outs or other industrial actions or trade disputes (whether involving employees of the Seller or of third party);
 - 7.6 difficulties in obtaining raw materials, labour, fuel, parts or machinery;
 - 7.7 power failure or breakdown in machinery.

APPENDIX 1 - ACQUIRING IN IQ

A1.1 - DEVICE SET-UP DIALOGUE BOXES (LIVE / FOCUSING MODE)



Clicking here opens the Advanced settings dialogue shown in figure 7 below

Record to channels: Define your acquisition channel settings as-you-go

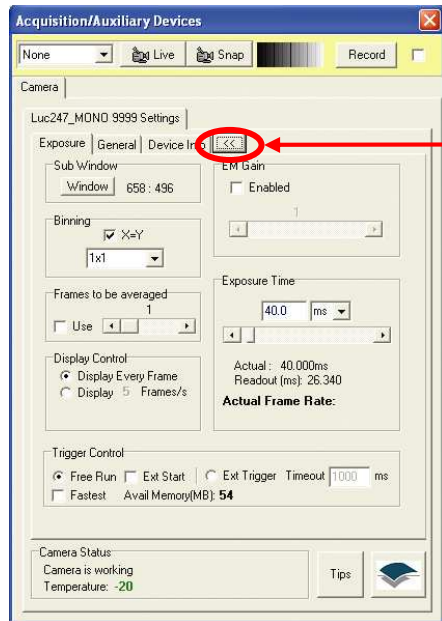
EM Gain:
Disabled = EM gain off
Enabled = EM gain activated

One exposure window:
Down to μ s

Sub array selection: User-defined

Standard binning selection:
From 1x1 to 16x16

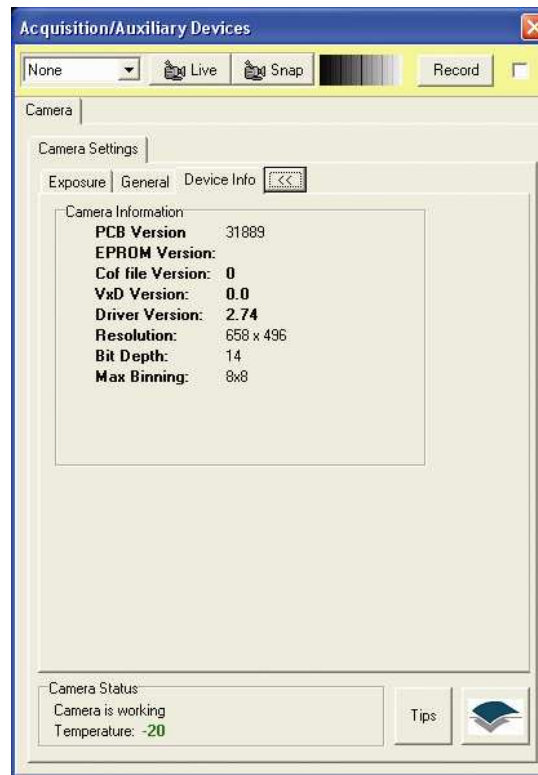
Figure 6: Basic settings dialog (default)



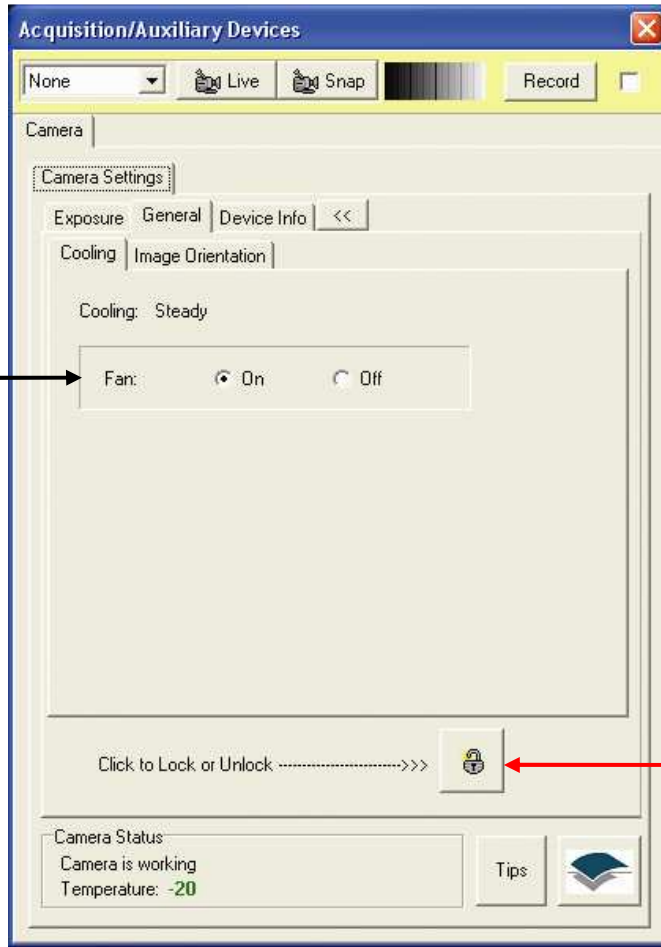
Clicking here switches back to the Basic settings dialogue

Figure 7: Advanced settings dialog

Lists information about camera type, its drivers, on-board software versions and other technical specifications, e.g.:



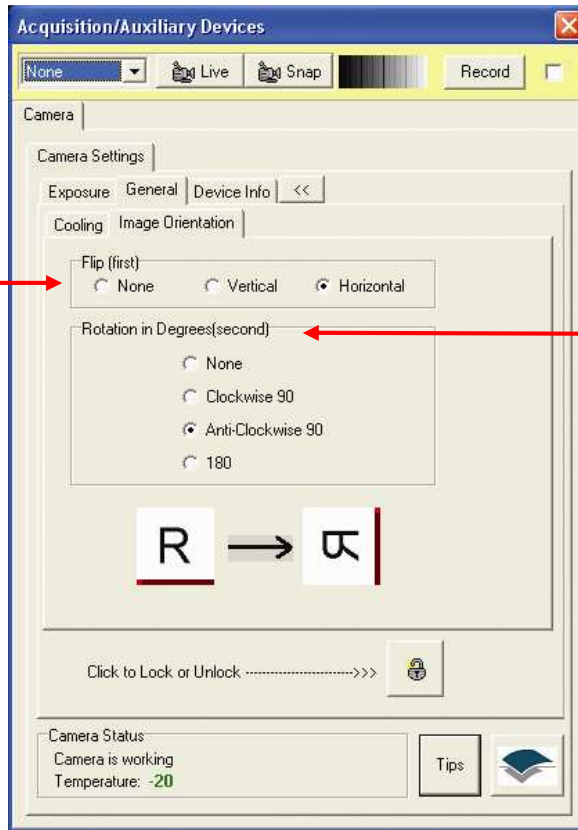
Fan Settings: On or Off



Click here to lock settings

Flip (first):

- None
- Vertical
- Horizontal

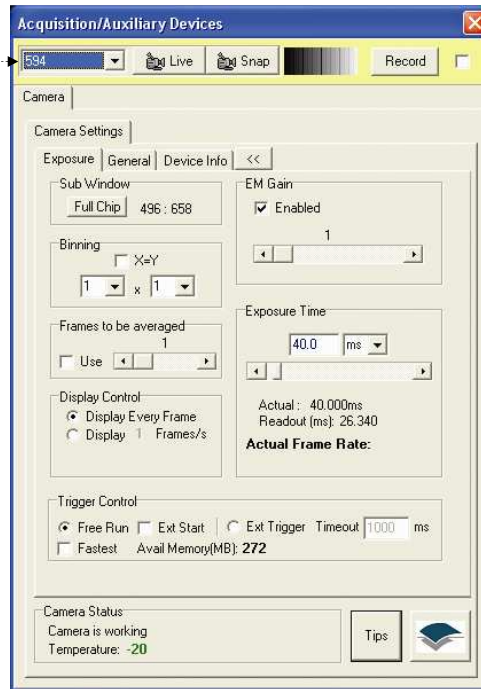


Rotation in Degrees (second):

- None
- Clockwise 90
- Anti-Clockwise 90
- 180

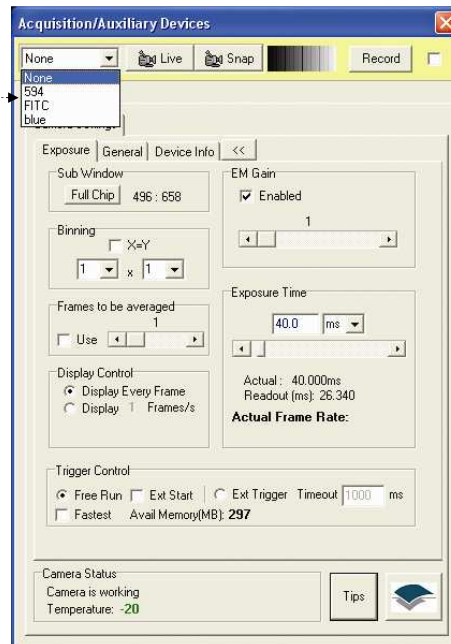
A1.4 - USING ACQUISITION CHANNELS

This feature is used to call existing channels into dialogue set-up, e.g. to test and modify existing channel settings with a new sample in 'live acquisition/focus mode'

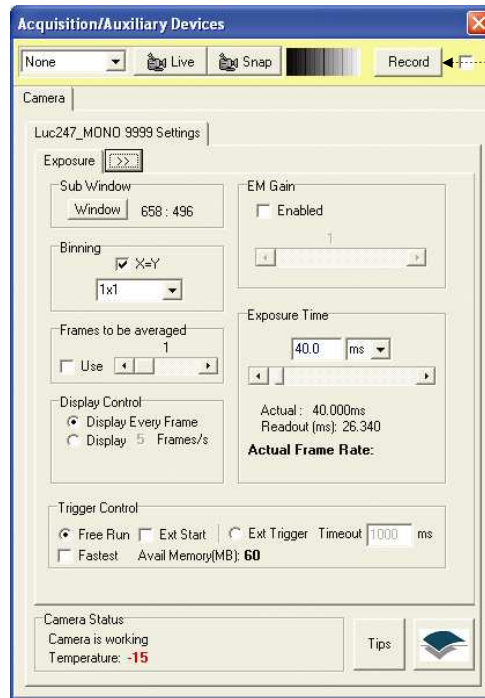


A1.5 - SELECTING ACQUISITION CHANNELS

Drop-down list of all pre-defined channels to choose from for your current acquisition

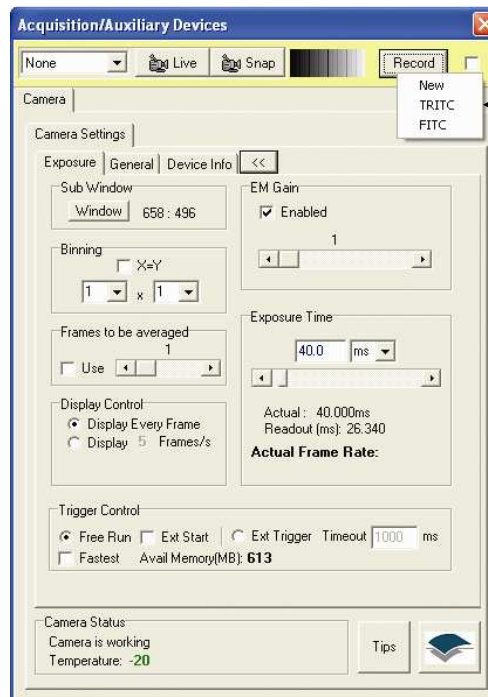


A1.6 - RECORDING ACQUISITION CHANNELS



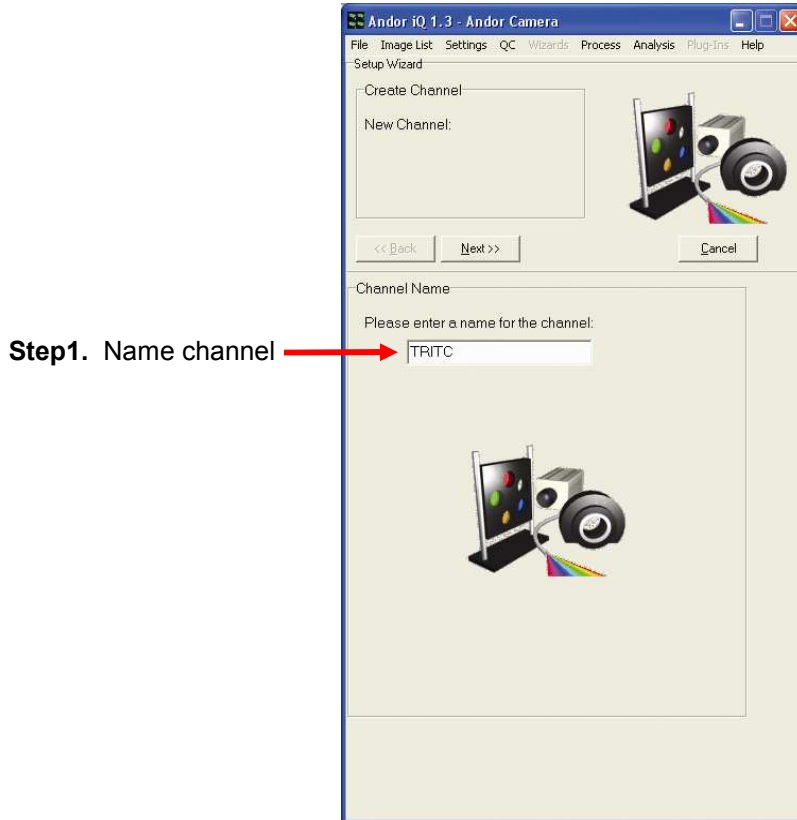
This saves your new live settings to an existing, pre-defined acquisition channel **or** to a new channel

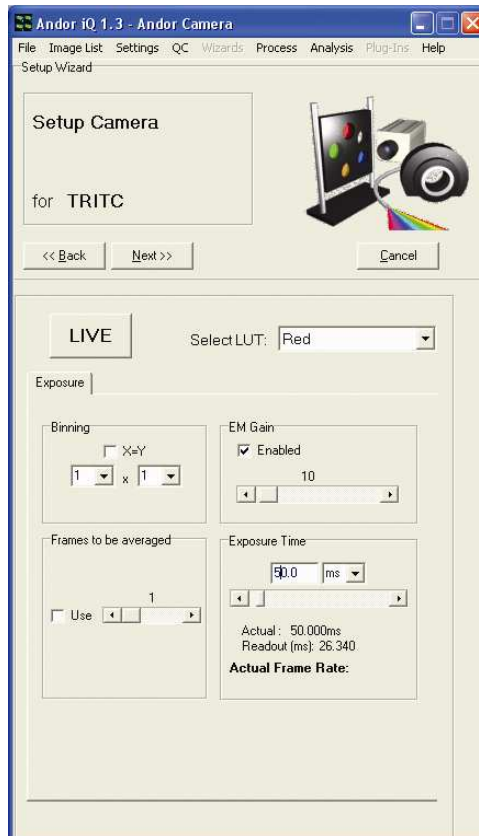
A1.6.1 - Recording/modifying acquisition channels



You can now record your settings to a new or an existing acquisition channel

Acquisition channels can also be set up in a create channel wizard:

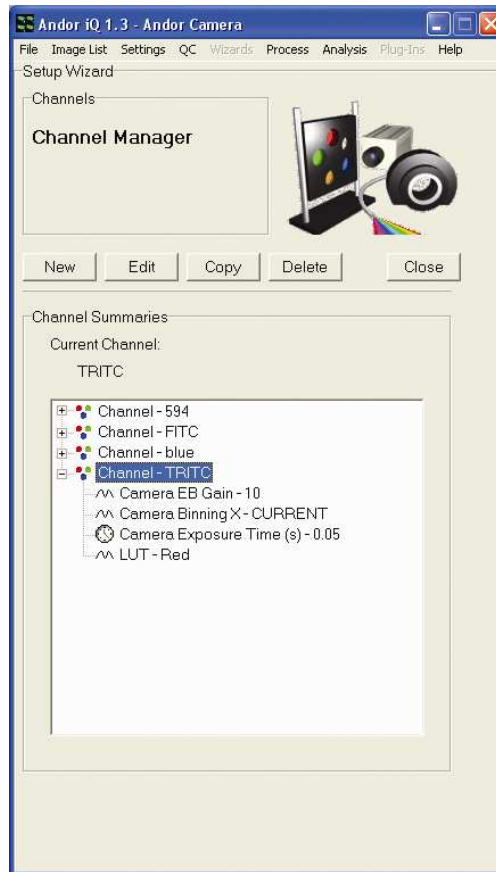




Step 2. Here you can define:

- LUT settings
- Binning
- EM gain (enabled/disabled and its scaling)
- Exposure time
- Other settings (e.g. frame averaging)

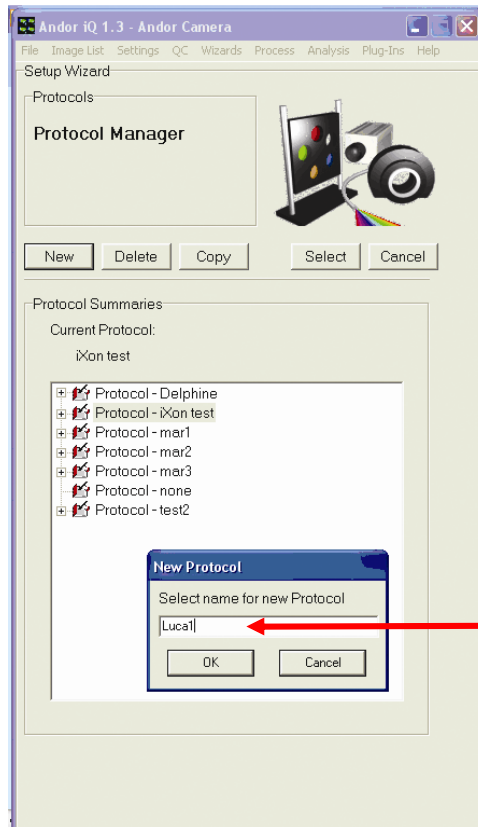
The user may previously have decided upon these in 'live mode'



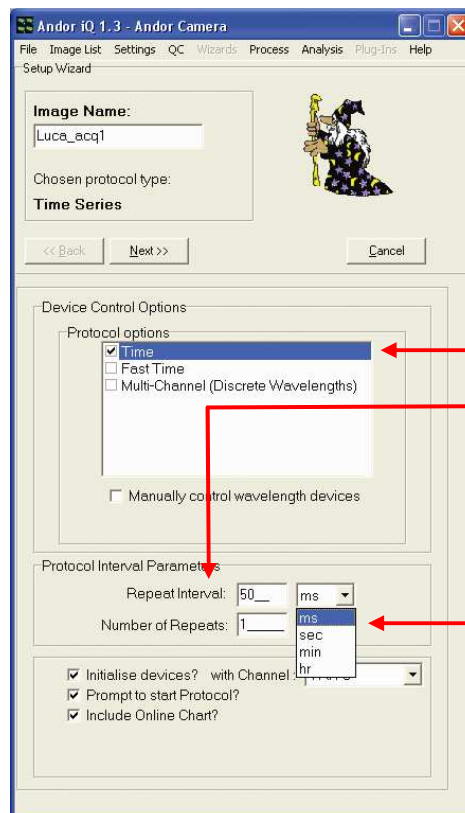
Step 3. Complete

If we were controlling an external filter wheel, we would also have attributed this channel to filter position through this wizard. **NOTE: Sub-Array (window) settings selected in device set-up dialogue are global, i.e. apply to all channels.**

We need to understand how these channels are then used in different types of acquisition protocols. Firstly let us consider a time-lapse protocol (single channel/colour) using the Protocol Manager



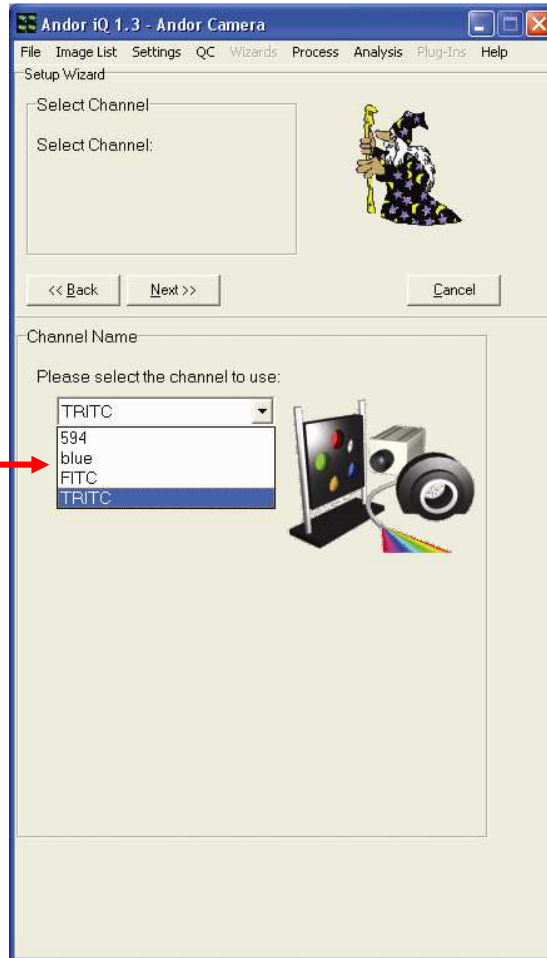
Step 1. Name protocol



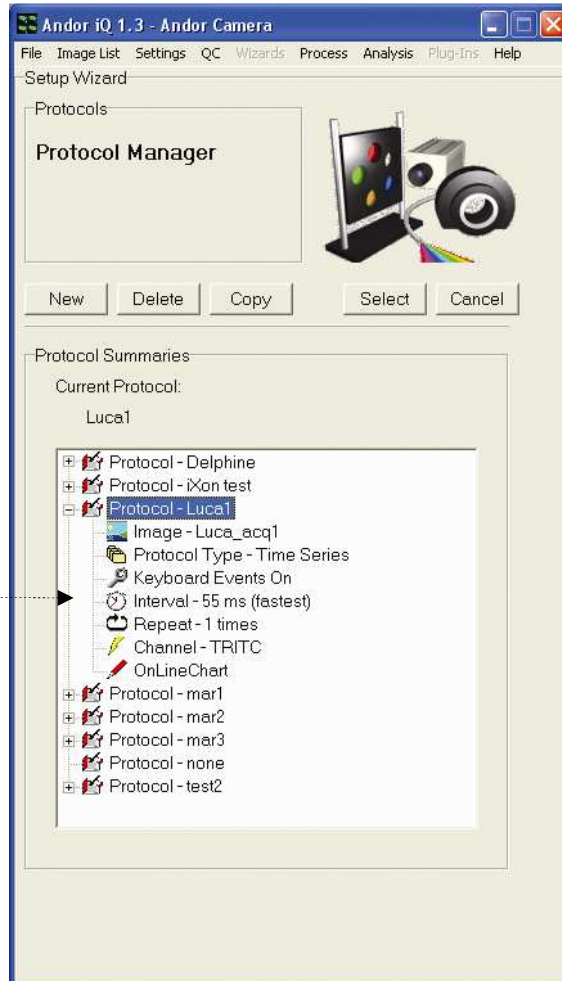
Step 2. Select:

- Time
- Repeat interval between acquisitions
- Number of repeats

Step 3. Select channel →

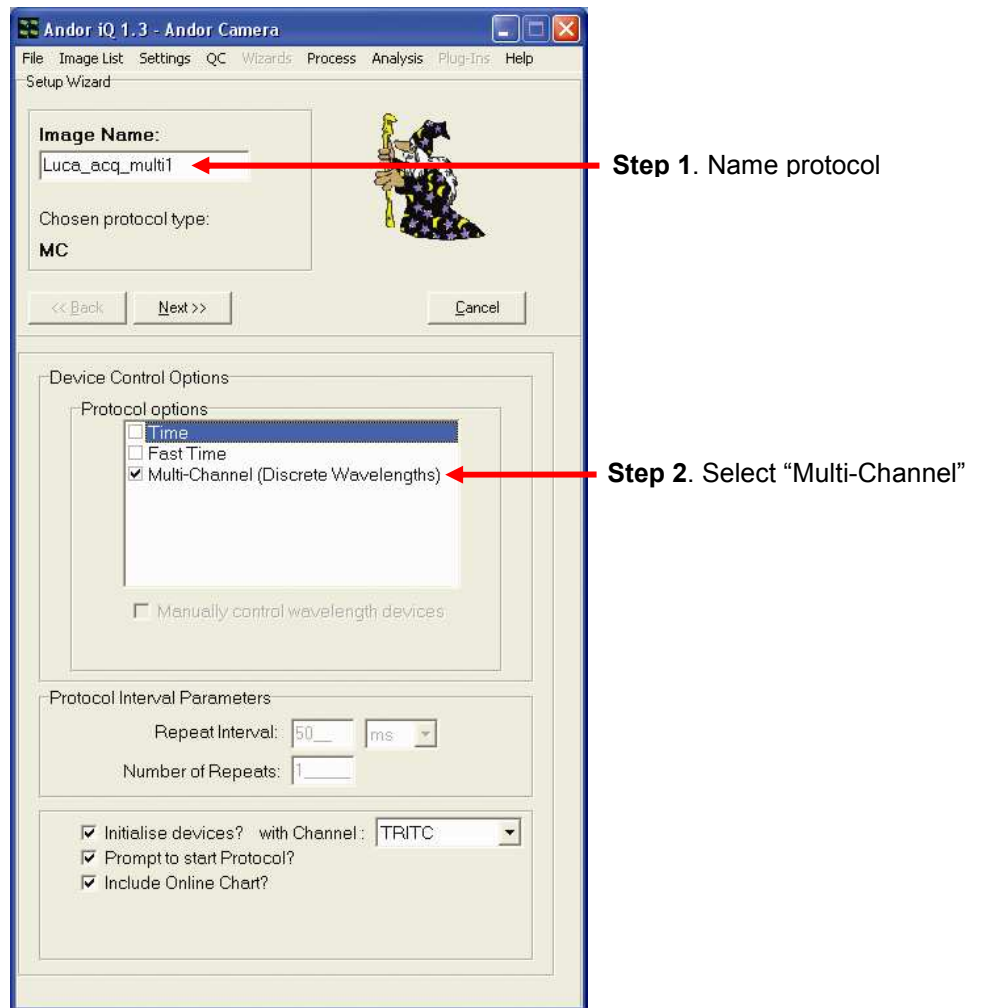


NOTE: Channels remain Non-Frame Transfer in this mode and exposure times can be selected that are shorter than the readout time.



You can get a summary of your protocol through the **protocol tree**

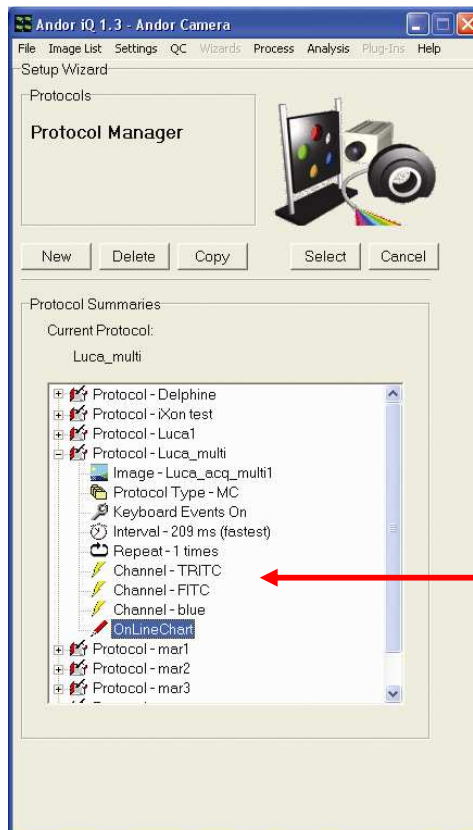
Now let us set up a multi-channel protocol (i.e. multi-colour):



Step 3. Select number of channels and pick them.



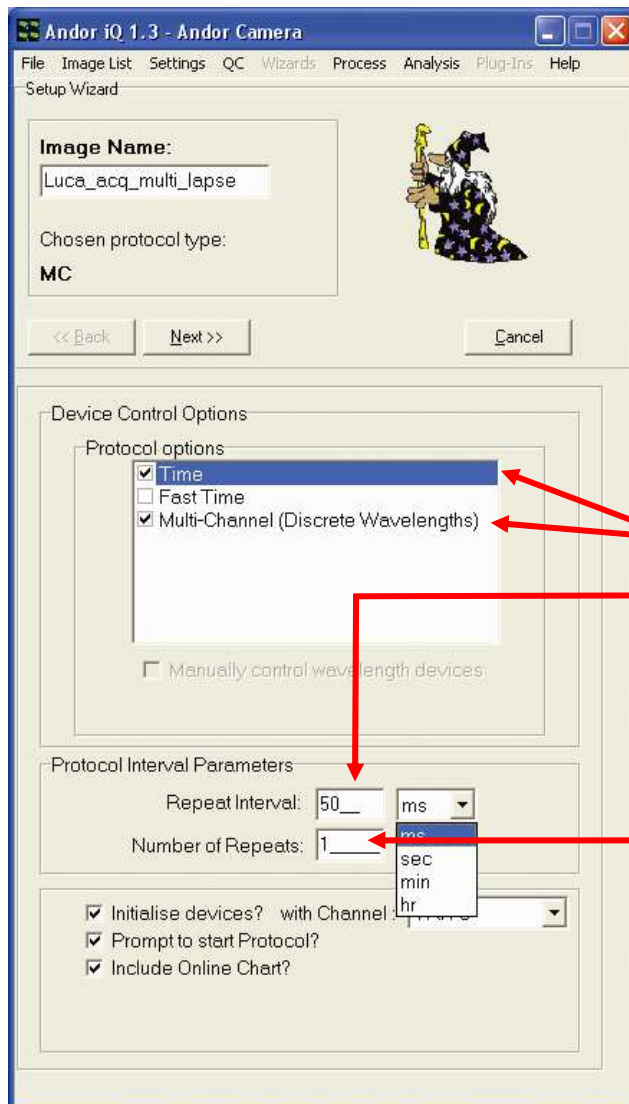
NOTE: Again, channels remain non-frame transfer. You do not need frame transfer operation when you have to change filters between exposures.



Protocol tree summary

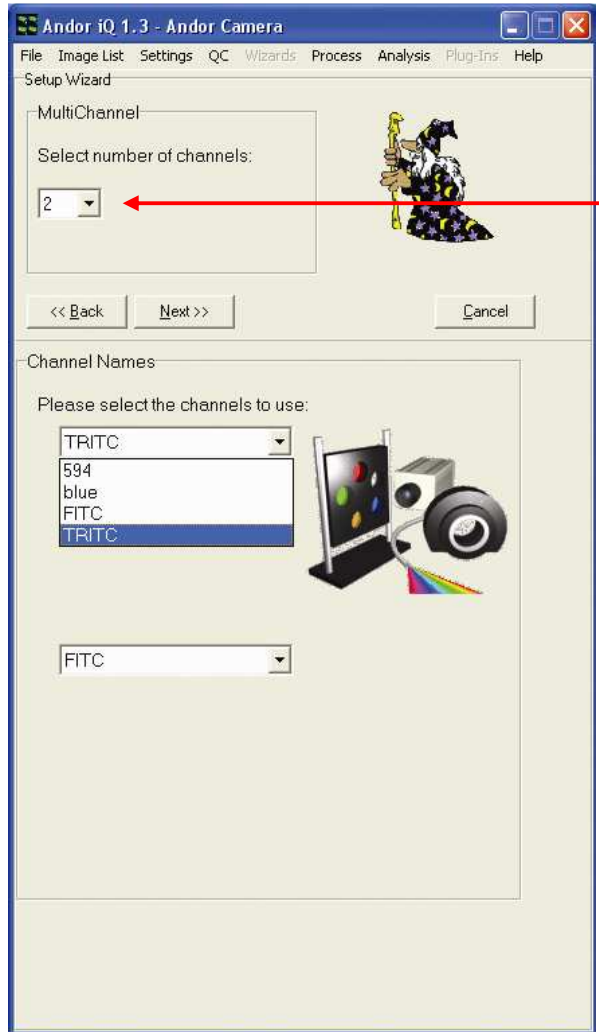
We can perform combined multi-channel + time-lapse experiments.

Step 1. Name protocol



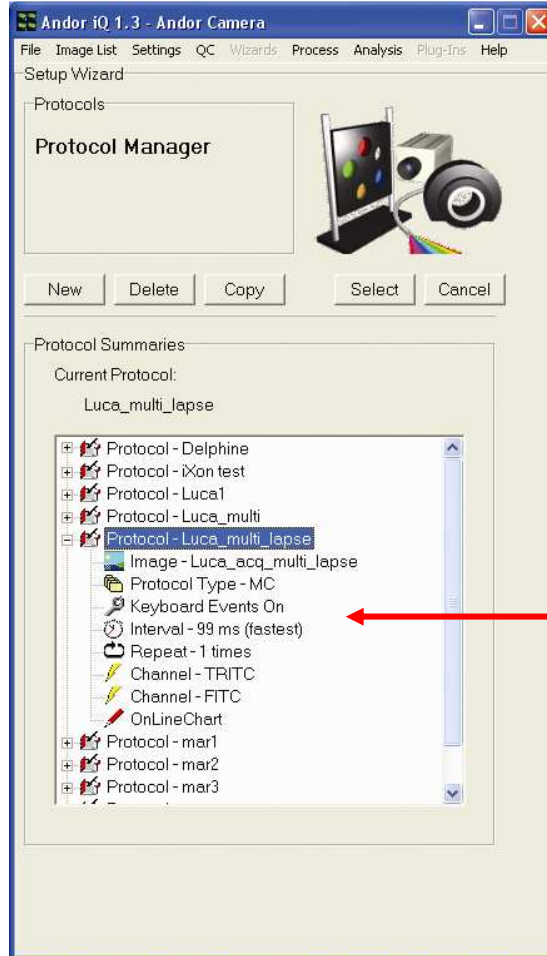
Step 2. Select:

- Multi-Channel and Time
- Repeat interval between acquisitions group
- Number of repeats



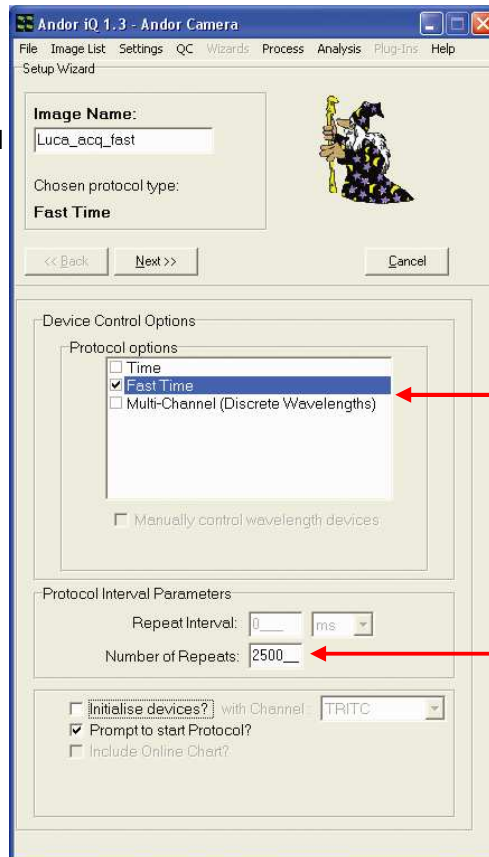
Step 3. Select number of channels and name.

NOTE: Again, Channels remain Non-Frame transfer



Protocol tree

Step 1. Name the protocol

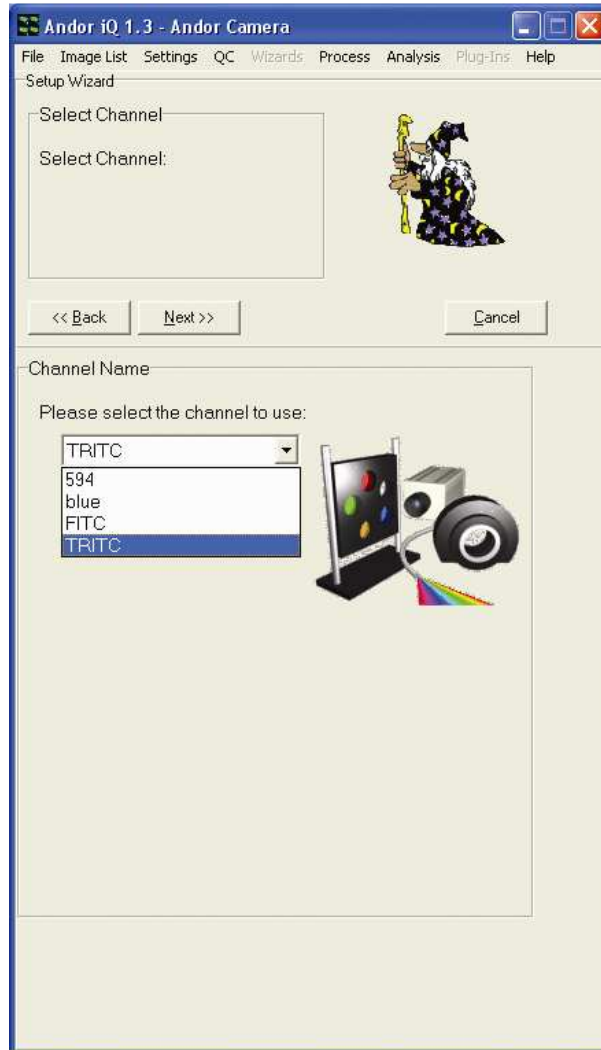


Step 2. Select:

- “Fast Time”
- Number of repeats, i.e. no. of frames in the kinetic series)

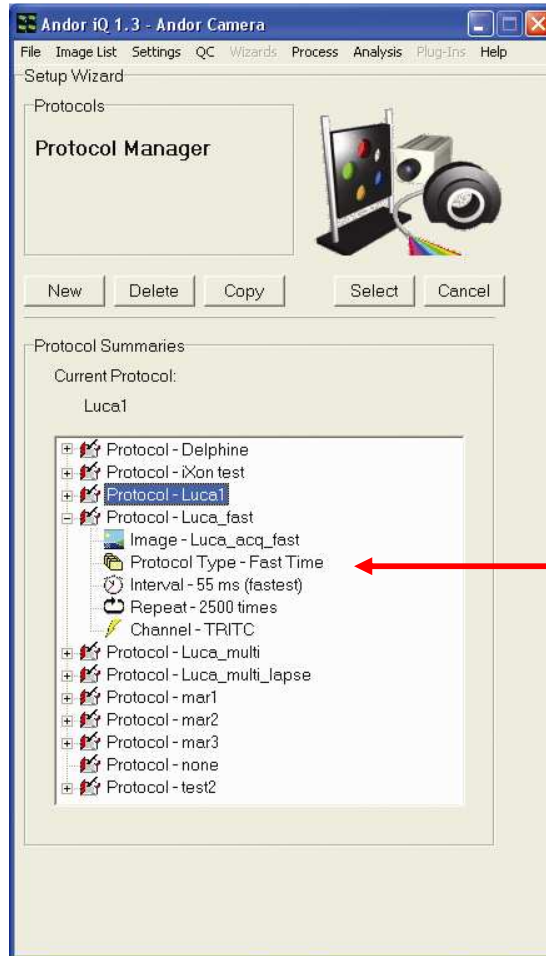
Fast Time uses only a single channel, and is designed to deliver fastest time resolutions from the camera. In the case of Luca-S this is with Frame Transfer mode.


Step 3. Select the channel you wish to use.



NOTES:

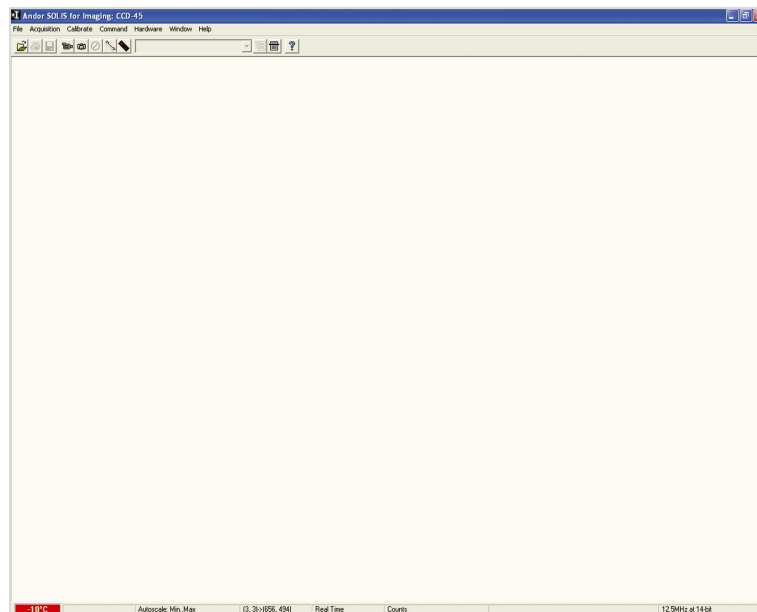
1. All channels used for fast time revert to Frame Transfer readout!
2. If this channel setting has exposure time less than the readout time for the binning and sub-array selection used, the exposure time is automatically extended to match that readout cycle (and the user is informed). In Frame Transfer mode, exposure time cannot be shorter than readout time.



To start the Solis software, click the  icon which should have been placed on the PC desktop. The **Solis (i) splash screen** should then appear:



The **Main Window** then appears:

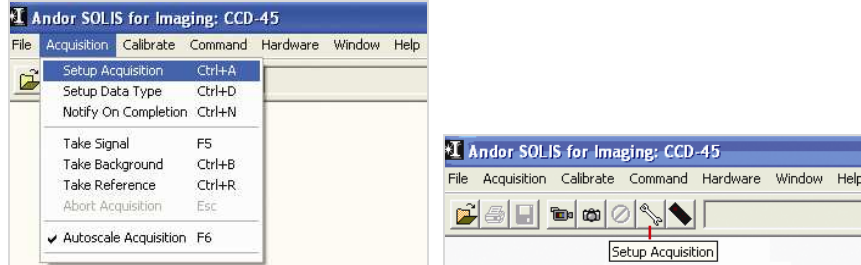


The Main Window is your “entry point” to the system. The menu options that you select from either execute functions directly, or launch further windows/dialog boxes that let you select the functionality you require.

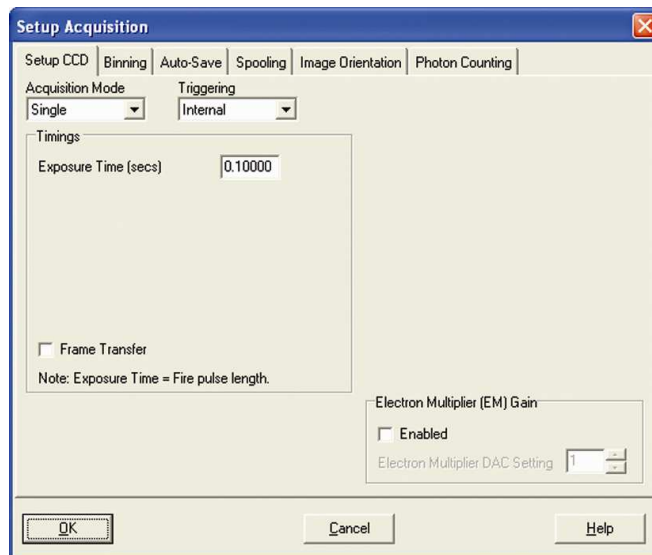
A2.2 - SETUP ACQUISITION DIALOG BOX

This is the primary settings area you will require to use to set the camera up to take acquisitions.

From the menus, select **Setup Acquisition** from either the dropdown menu or by clicking the “**Spanner**” icon as shown :



The **Setup Acquisition** dialog box will then appear, e.g.:

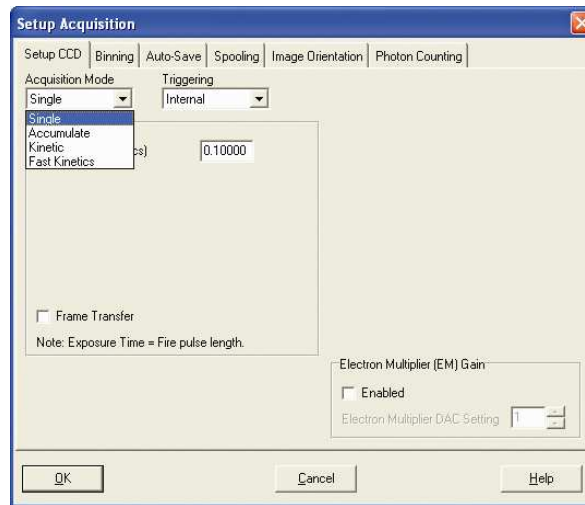


From this area, you can then select the parameters required for your acquisition. These are described briefly in the next few pages. To further guide you through the functions and facilities of the software, please select the **Help** function at any time.

Should you have any problems working with our software, please do not hesitate to contact us via your local contact shown in **Technical Support** on **page 7**.

A2.3 - SETUP CCD TAB

A2.3.1 - Acquisition mode

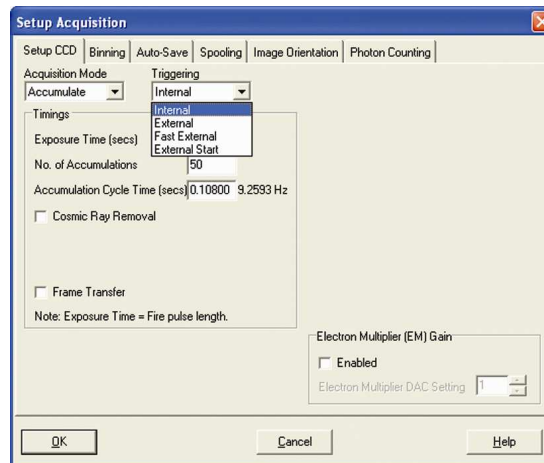


The options available are as follows:

- **Single**
- **Accumulate**
- **Kinetic**
- **Fast Kinetics**

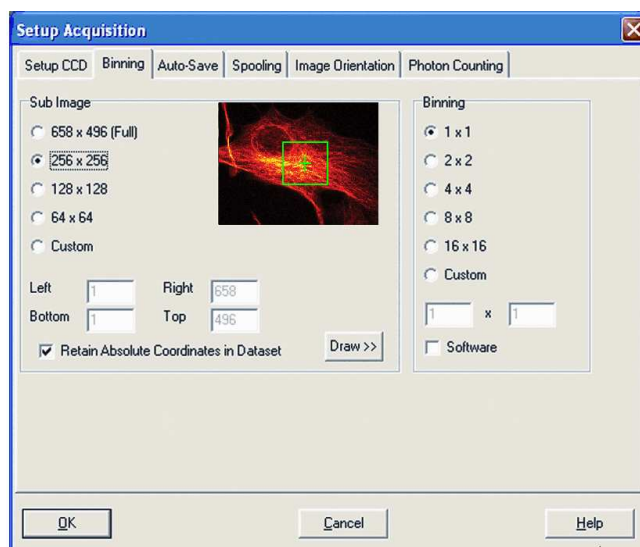
As you select an Acquisition Mode you will notice that you are able to enter additional exposure-related parameters in a column of text boxes. Appropriate text boxes become active as you select each Acquisition Mode.

NOTE: The value you enter in one text box may affect the value in another text box.



The options available are:

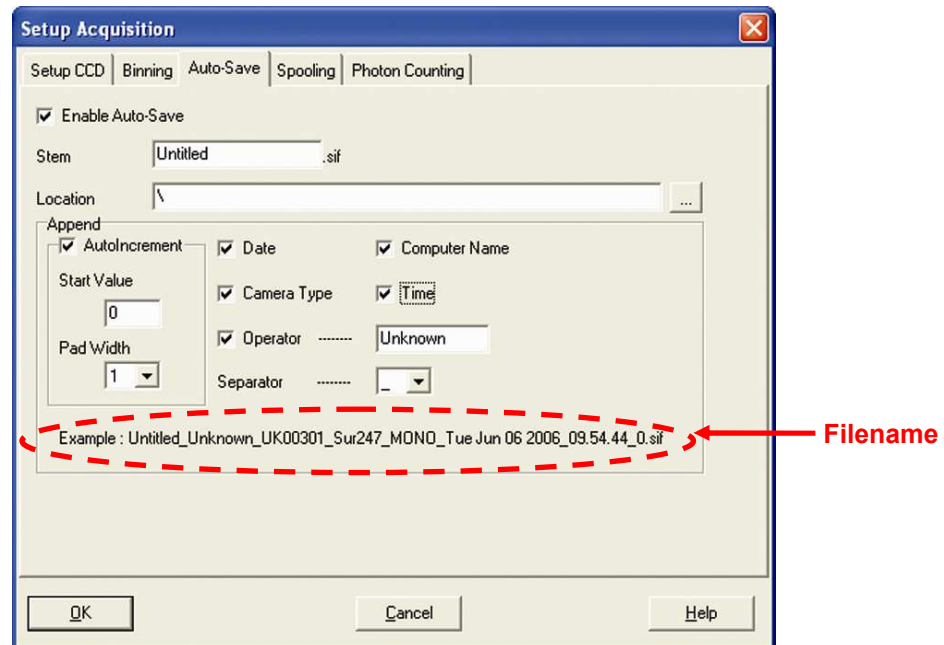
- Internal:** Once you issue a data acquisition command, the system determines when data acquisition begins. You can use **Internal** mode when you are able to send a trigger signal or '**Fire Pulse**' to a short-duration, pulsed source (e.g. a laser). In this case starting data acquisition also signals the pulsed source to fire. The **Fire Pulse** is fed from the **Fire SMB** connector on the camera.
- External:** Once you issue a data acquisition command, data will not be acquired until your system has received an **External Trigger** signal generated by an external device (e.g. a laser). The External Triggering signal is fed to the **Ext Trig SMB** connector on the rear of the camera.
- Fast External:** Normally, when using **External Trigger** the system will only enable the triggering of the system after a complete **Keep Clean** Cycle has been performed. This is to ensure that the CCD is always in the same known state before it is triggered. This is particularly important when the system is in Accumulation or Kinetics mode. In cases where repetition rate is paramount, and slight variation in the base background level is less important, it is possible to remove this restriction by using **Fast External** triggering.
- External Start:** With **External Start** triggering, once you issue a data acquisition command, data will not be acquired until your system has received an external trigger signal generated by an external device. The system will then continue to acquire data based on user options set within the Acquisition Dialog. This means that an External Start Trigger could be used to commence acquisition of a Kinetic series, but with the parameters of that series being controlled by internal software options. The External Start trigger signal is fed to the camera head via the **Ext Trig SMB** on the back of the camera.



From the binning tab you can select the following parameters:

- **Full image (658 x 496 pixels)**
- **Sub Image areas:**
 - 256 x 256 pixels
 - 128 x 128 pixels
 - 646 x 64 pixels
 - Custom (allows you to set the Sub Image to any size and location on the CCD chip)
- **Binning patterns:**
 - 1 x 1
 - 2 x 2
 - 4 x 4
 - 8 x 8
 - 16 x 16
 - Custom (as required)

You can also use the **Draw>>** option to select the size and location of your Sub Image. In order to use the Draw Option, you must first acquire a full resolution image. This will be the template on which you will draw your Sub Image.



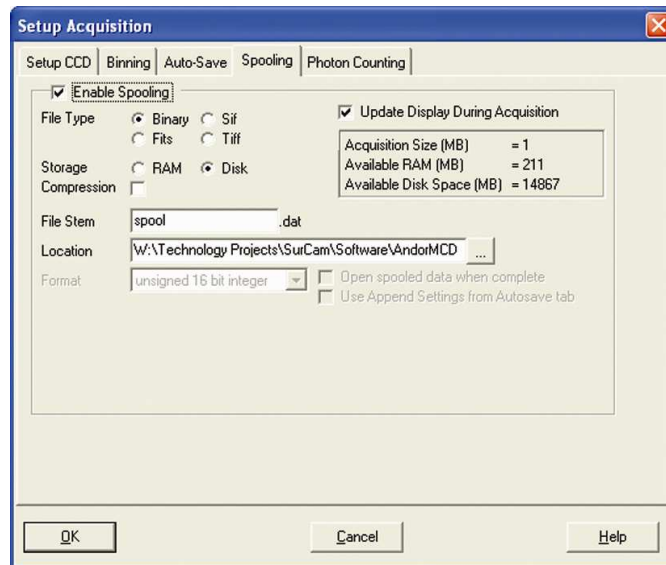
Auto-Save allows you to set parameters and controls for the auto saving of acquisition files thus removing the worry of lost data and files. Tick the **Enable Auto-Save** box. If selected, acquisitions will be saved automatically when each one is completed. **NOTE: Each subsequent auto-saved file will over-write the previously auto-saved one.**

There is also an **Auto-Increment** On/Off tick box. This allows a number to also be appended to the main **Stem Name**. This number is automatically incremented each time a file is saved. This time the auto-saved files will not overwrite any previous auto-saved files. In the Auto-Save dialog box, a Stem Name may be entered. This is the main root of the name that the acquisition is to be saved as. The Stem Name can be appended with a number of details :

- **Operator name (supplied by user)**
- **Computer name**
- **Camera type**
- **Date**
- **Time**

Any combination of these may be selected by activating the relevant tick box.

NOTE: This function will only auto-save Single Scan, Kinetic Series, Fast Kinetics or Accumulated images, not data acquired in Video mode.



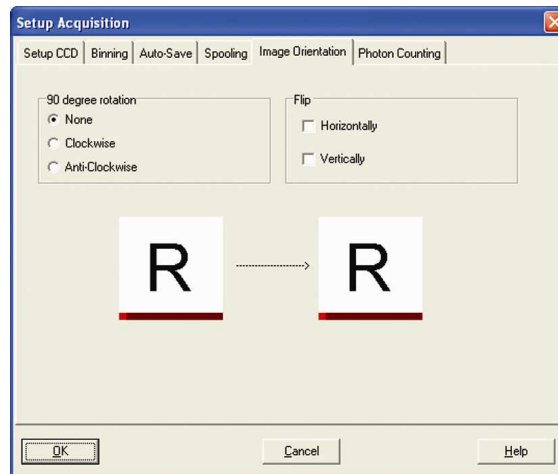
The Andor Solis software allows you to spool acquisition data direct to the hard disk of your PC. This is particularly useful when acquiring a series of many images. The amount of data generated by a Kinetic Series of, for example 1000 acquisitions, is huge and more than most PC RAM can handle. With the spooling function enabled, data is written directly to the hard disk of you PC, as it is being acquired.

To enable the spooling function on your software, tick **Enable Spooling** in the Spooling dialog box. You must also enter a stem name, and also select a location for the for this spooled data file.

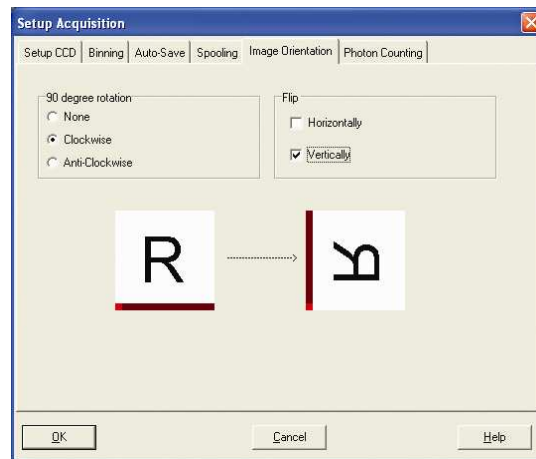
NOTES:

1. **Spooling large amounts of data at 30+ frames per second straight to hard disk for later retrieval, requires a hard disk of sufficient read-write speed. Andor recommends only very high-speed hard disk drives be used for this type of operation.**
2. **Turning off the option to display date during acquisition can result in faster acquisition speeds being reliably spooled to hard disk.**

A2.7 - IMAGE ORIENTATION TAB




The **Image Orientation** tab allows you to change the direction in which data is read out. Simply click the required tick boxes, e.g.:




A2.8 - TAKE SIGNAL

Once you have setup the required parameters, you can carry out one of the following actions to obtain an acquisition or acquisition series:

- Click the  button on the Main Window
- Press **F5** on the keyboard
- Select the **Take Signal** option from the **Acquisition** drop-down menu as shown:

Acquisition	Command	Hardware
Setup Acquisition	Ctrl+A	
Setup Data Type	Ctrl+D	
Notify On Completion	Ctrl+N	
Take Signal	F5	
Take Background	Ctrl+B	
Take Reference	Ctrl+R	
Abort Acquisition	Esc	
<input checked="" type="checkbox"/> Autoscale Acquisition	F6	

You can also click the  button to acquire data continuously (in Live / Video Mode).

An example of an image display is shown here:

