



A USER'S GUIDE TO **ANDOR** *ixon*

ELECTRON MULTIPLYING CCD

Version 1A



A USER'S GUIDE TO
ANDOR *ixkon*

ELECTRON MULTIPLYING CCD

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Version 1A

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Congratulations on the purchase of your new Andor iXon camera.

You are now in possession of revolutionary new 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 as a road map for the iXon camera, and contains information and advice to ensure you get the optimum performance from your new system.

As well as general advice on installation, handling electronics, and some background to the unique EMCCD technology, the manual provides instructions on operating the iXon software.

You can also make use of the Online Help for advice and instructions on getting the best out of your iXon camera.

Towards the back of the manual there are a number of carefully prepared Tutorials that will allow you to quickly demonstrate the unique capabilities of this revolutionary detector.

Please feel free to contact Andor Technology directly, or your local representative or supplier, if you have any questions regarding your iXon system.

Andor Technology Ltd

Using The Manual

In the software, all the controls you need for an operation are grouped and sequenced appropriately in on-screen windows. As far as possible, the descriptions in this **User's Guide** are laid out in sections that mirror the **Windows Interface**.

The following type-styles are used in the right-hand margin of the **User's Guide** to pick out key features of the menus:-

- **Menu**
- **Menu Option**

These styles are reflected in the format of the main texts. In addition, windows and dialog boxes are identified as:

- *Active Window / Dialog Box*
- ***Dialog Box - functional area***
- ***Dialog Box - option, parameter, etc***
- **CCD Sensor - other significant features**

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

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INSTALLATION

Working with Electronics

The computer equipment that is to be used to operate the **iXon CCD Detector** 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 **Detector Head**. The computer manufacturer's safety precautions should be followed when installing the **Interface Card** into the computer.

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

Looking after the Detector Head

THERE ARE NO USER-SERVICEABLE PARTS INSIDE THE DETECTOR HEAD. A NUMBER OF SCREWS ON THE DETECTOR HEAD HAVE BEEN MARKED WITH RED PAINT TO PREVENT TAMPERING. IF YOU ADJUST THESE SCREWS YOUR WARRANTY WILL BE VOID.

NEVER USE WATER THAT HAS BEEN CHILLED BELOW THE DEW POINT OF THE AMBIENT ENVIRONMENT TO COOL THE DETECTOR.

You may see condensation on the outside of the detector body if the cooling water is at too low a temperature 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 detector head with a soft, dry cloth. It is likely there will already be condensation on the cooling block and cooling fins inside the detector head. Set the detector head aside to dry for several hours before you attempt reuse. Before reuse blow dry gas through the cooling slits on the side of the detector head to remove any residual moisture. Use warmer water or reduce the flow of water when you start using the device again.

IMPORTANT: See also SECTION 4.12 **SETTING THE TEMPERATURE - Cooling Performance and Operation** for further operating characteristics and safety features of your system.

Your detector is a precision scientific instrument containing fragile components. **ALWAYS HANDLE THE DETECTOR WITH CARE.**

Additional statement regarding Equipment Operation

If the equipment is used in a manner not specified by Andor Technology Ltd, the protection provided by the equipment may be impaired.

Environmental Conditions

- Indoor use
- Altitude up to 2000m
- Temperature 5°C to 40°C
- Maximum relative humidity 80% for temperature up to 31°C, decreasing linearly to 50% relative humidity at 40°C
- Over voltage category 1
- Pollution Degree 2

Electrical Ratings

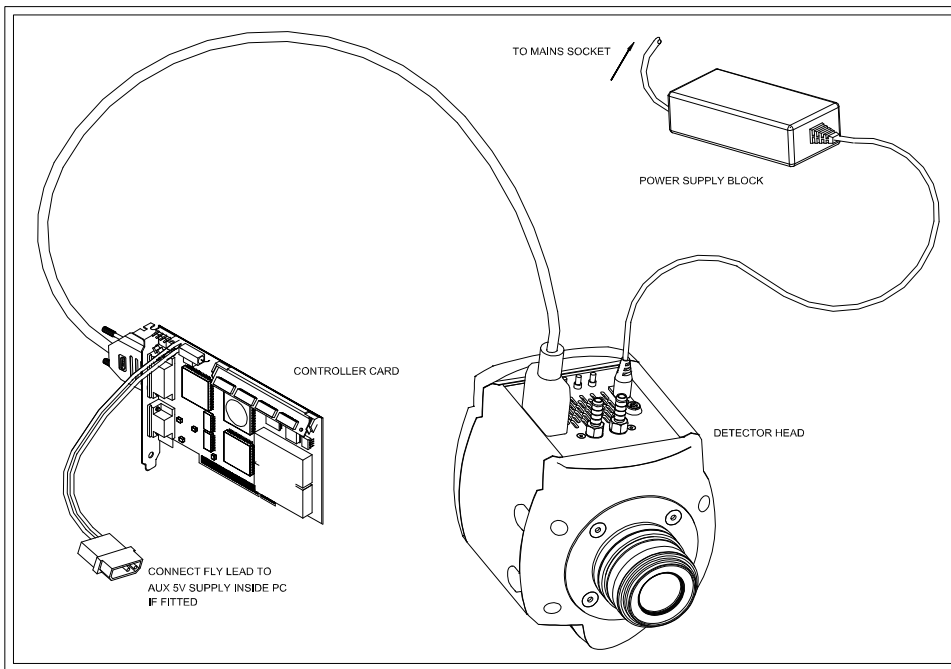
- 5Vdc 20 Watts
- The external Power Supply Block will supply a maximum additional 7.5V at 30 Watts

The Andor iXon CCD system is composed of **hardware** (notably the detector head and the card), the **software**, and **documentation** (including on-line help, the **User's Guide to Andor iXon CCDs**, and the **Programmer's Guide to Andor Basic**). This section of the User's Guide identifies the main components of the system and guides you through the installation procedure.

Andor's **iXon CCD (Charge Coupled Device)** exploits the processing power of today's desktop computers. The system's hardware components and its comprehensive software provide speed and versatility for a range of imaging applications and set-ups.

The main components of the Andor iXon CCD are:

- **Detector Head**
- **Plug-In Card:** PCI format
- **Cable:** Detector Head to Controller Card
- **iXon Software:** CD format
- **iXon CCD User's Guide** (this manual)
- **Andor Basic Programmer's Guide**
- **Power Supply Block (PSB) and cable**
- **C-Mount Lens** (Optional)
- **C-Mount Lens Adaptor** (Optional)
- **F-Mount Lens** (Optional)
- **F-Mount Lens Adaptor** (Optional)



**Main Components of
iXon CCD System**

The Detector Head

The **Detector Head** contains the **CCD Sensor** and its **Pre-Amplifier**. It also contains the **Temperature Sensor**, the pre-amplifier for the temperature sensor, and the **Thermoelectric Cooler**. The head can be attached to a **microscope** or other optical device for acquiring data.

The Detector Head also contains one or more analog-to-digital converters that digitize data from the analogue controller boards. Under software control this data is transferred to the computer, where it is stored in computer memory.

The temperature control components, which regulate cooling of the camera, are also stored in the detector head.

The **EMCCD Sensor** is in an evacuated, sealed housing. Thus there is no need to worry about condensation inside the sealed housing if the sensor is cooled to a temperature below the dew point.

EMCCD Sensor

Two connectors allow water to be passed through the head to assist cooling as required.

The Controller Card

The **Controller Card** buffers data from the detector head, before transfer to the computer memory, via the PCI bus.

The card works only with IBM AT compatible computers, and requires one PCI slot.

Detector Head to Controller Card Cable

The 3m Cable that connects the detector head to the Controller Card uses a proprietary 36-way connector. It is well shielded against electrical interference.

Power Supply Block (PSB)

See SECTION 4.12 **SETTING THE TEMPERATURE - Cooling Performance and Operation** for details of temperatures that can be achieved when using the supplied PSB. A 2.1 mm Jack connector, links the detector head to the power PSB.

Please note - cooling is only available when the PSB is connected to the detector.

Software

Software is supplied on CD. The system requires Windows 95, 98, 2000, NT, ME, or XP

Manuals

The following manuals are supplied with each Andor CCD system:

- The **User's Guide to Andor iXon CCD** (this manual)
 - The **Programmer's Guide to Andor Basic**
-

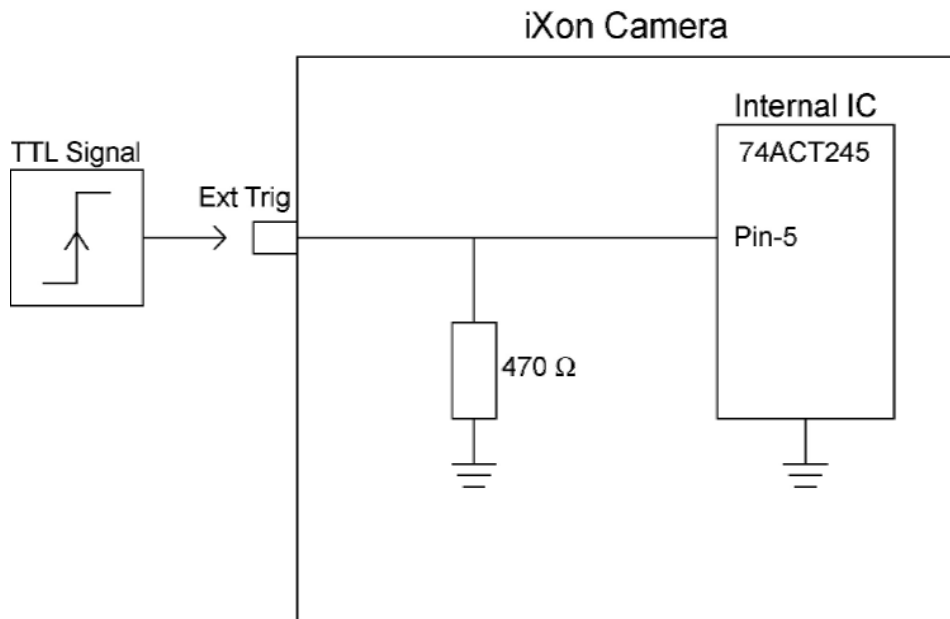
SMB Connectors

The SMB outputs (Fire, Shutter and Arm) on the iXon head:

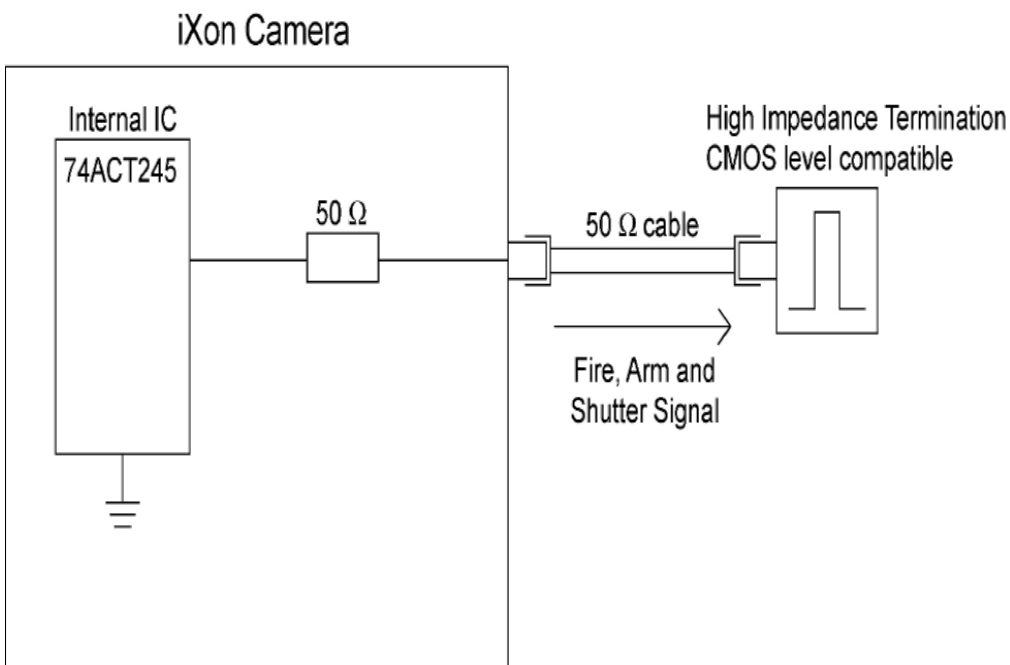
- Are CMOS level compatible
- Are series terminated at source (i.e. in camera head) for 50 ohm cable
- Termination impedance at customer end should be high impedance (*NOT* 50 ohm)

The SMB input (External Trigger) is TTL level compatible and has a 470 ohm impedance. (See diagrams on following page for further explanation of triggering)

External Trigger Signal



Fire, Arm and Shutter Signal



Computer Requirements

IBM AT Compatible Computers offer considerable computing power at relatively modest cost.

The system requires a PCI-compatible computer. The PCI slot you use must have bus master capability. The minimum recommended specification is a 800 MHz Pentium, with 256 Mbytes of RAM, and a hard disk, and a CD-ROM for installation of the software. The computer must be running Windows 95, 98, 2000, NT, ME, or XP.

A few recommended computer manufacturers are: HP-Compaq, Dell, and IBM.

A **Hard Disk**, with at least 10MB free storage capacity, is required.

Hard Disk

Software is provided on CD.

For **Video Output**, 256 colors and resolution of at least 800 x 600 are recommended. High quality video cards give clearer and faster graphics.

Video Mode

Use any **Monitor** compatible with your graphics card. Picture quality is, however, usually a function of price, and dot pitch sizes of 0.28 mm or smaller are recommended.

Monitors

Installing the Controller Card

Install the **PCI Controller Card** as you would other slot-in cards - such as graphics cards. Consult the manual supplied with your personal computer to ensure correct installation of the Controller Card for your particular PC.

Perform the installation as follows:

- Exit the computer's operating system and switch off the computer and its accessories (see above).
- Unplug the computer and its accessories from the wall outlet(s).
- Unplug all cables from the rear of the computer.
- Unscrew the cover mounting screws on the computer and set them aside safely.
- Gently remove the cover of the computer.
- Situated inside the personal computer are a number of **Expansion Slots**. The Controller Card can be installed in any PCI slot that has bus master capability. Having decided which slot you are going to use, remove the **Metal Filler Bracket** that covers the opening for the slot at the back of the computer. Keep the retaining screw safe - you will need it later in the installation procedure. You are now ready to install the Controller Card.
- While observing appropriate static control procedures (see SECTION 2.1, **Working with Electronics**), firmly press the connector on the long edge of the card into the chosen expansion slot, so that the card's metal mounting bracket (located at the end of the card and bearing the connectors for the detector head and the multiple I/O adapter) is next the opening on the back panel of the computer.
- Making sure that the card's mounting bracket is flush with any other mounting brackets or filler brackets to either side of it, use the screw that you removed from the filler bracket earlier to secure the **Controller Card** in place.
- Replace the cover of the computer and secure it with the mounting screws. Reconnect any accessories you were using previously (see below).

Connecting the System

Connect the elements of your system as follows:

- Plug your PC into the mains outlet to ensure grounding, but keep the power switched off.
- Connect the **Detector Head** to the **Controller Card** using the **Cable** provided. The 36-way connectors on the cable are polarized so that there is only one way of installing the cable. It is important that this cable is securely fastened to provide a good grounding between the detector head and Controller Card.

Your system has been supplied with a **Power Supply Block (PSB)** for cooling. The PSB connects to the detector head via a 2.1mm Jack plug, and to the mains electricity supply with a standard three-pin plug, or the equivalent plug for your location. There is only one socket on the detector head that the PSB can be connected to. For best performance the PSB should be plugged into the same power source as the computer.

Installing the Software

◇ With **Windows Operating Systems (95/98/2000/XP/ME)**

- During the start up sequence the operating system will detect the Andor plug-in card and a Dialog box will prompt you for the location of the device driver. Insert the **Andor CD** and navigate from the Dialog box to the **Setup Information File** (atmcd.inf). Select the device driver file and click **OK**.
- The 'installation wizard' now starts. (If it does not start automatically, run `\setup.exe` on the CD.) Follow the on-screen prompts. Remove the CD and then restart the computer to complete the installation.
- Run the Andor application: from the PC's desktop select **Start...Programs...Andor iXon. ...Andor iXon. .**
- You are ready to make a data acquisition....

◇ With **Windows NT...**

- Insert the Andor **CD**. The ‘installation wizard’ now starts. (If it does not start automatically, run `\setup.exe` on the CD.) Follow the on-screen prompts.
 - Now restart the computer.
 - Run the Andor application: from the PC’s desktop select **Start...Programs...Andor iXon. ...Andor iXon.**
 - You are ready to make a data acquisition....
-

Acquiring your First Data

To acquire your first data click the **Take Signal** button (marked with a camera icon) on the *Main Window*. The acquired data appear in a *Data Window*. The **User’s Guide** gives full details of the ways in which data can be acquired and manipulated.

The *Main Window* is your ‘entry point’ to the system.

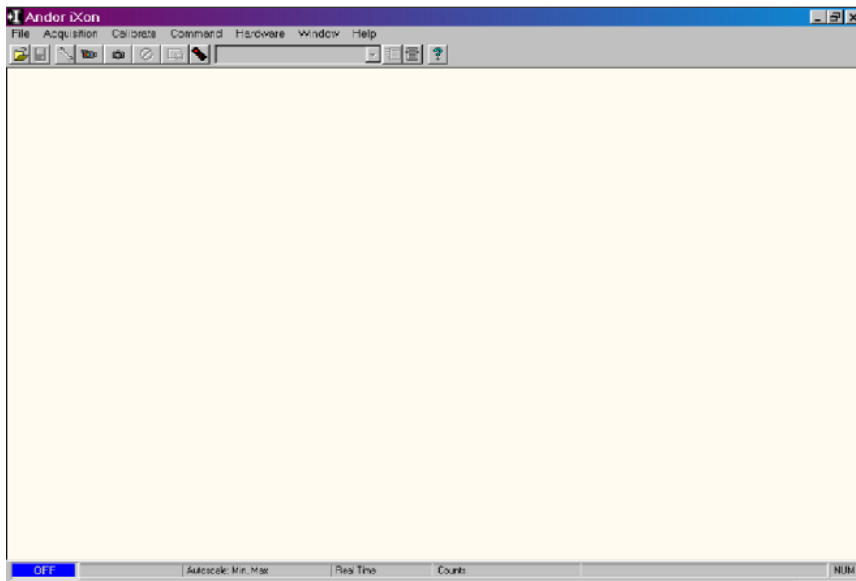
The menu options that you select from the *Main Window* either execute functions directly, or launch further windows and dialog boxes that let you select the functionality you require.

Some menu options are also represented as easy-to-use buttons on the *Main Window*. If you allow the ‘arrow’ cursor to rest on a button, a short explanatory caption will appear below the button. Each menu, with its associated buttons, is discussed in depth in the appropriate section of the **User’s Guide**.

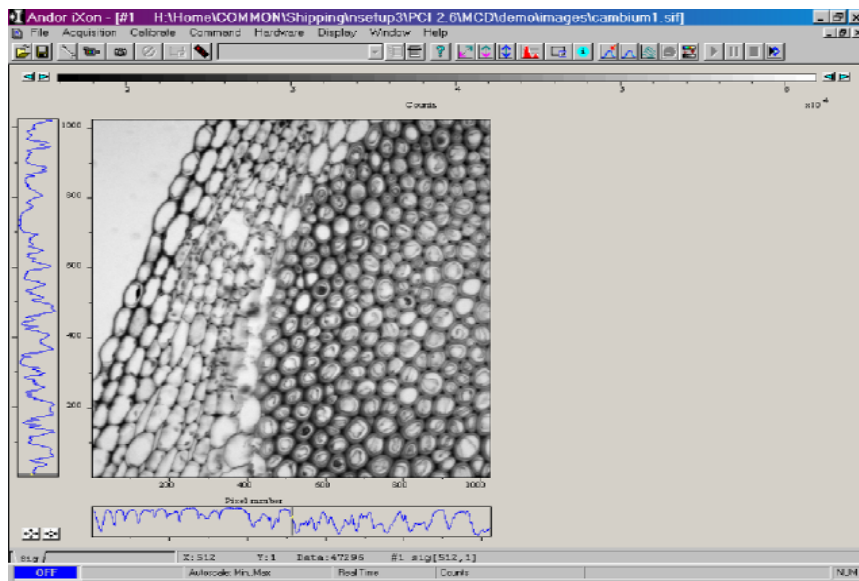
Note: Some menu titles and buttons appear on the *Main Window* only under particular circumstances:

- The *Display Menu* and its associated buttons will not appear until you open a *Data Window*. (See SECTION 4.7 **DISPLAYING DATA**.)
- The *Edit* and *Search Menus* and their associated buttons appear only when a *Program Editor Window* is active. (See SECTION 6 **WORKING WITH PROGRAMS**.)

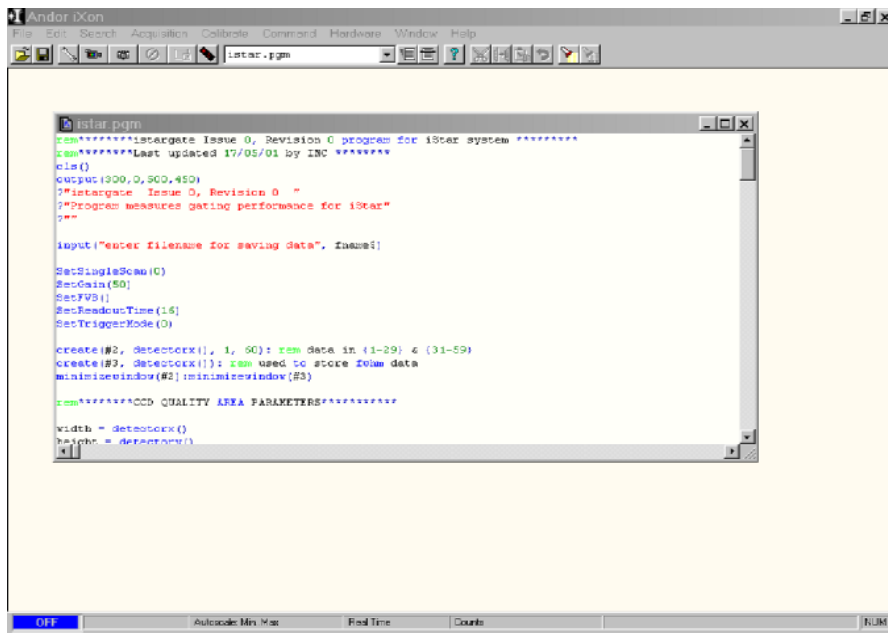
The following pages show you the *Main Window* that appears by default and the windows that appear when data are being displayed or when you are working with programs.



Main Window (Default)



Main Window (Displaying Data)



Main Window (Working with Programs)

If you have an active *Data Window* on screen, clicking the **File Information** button on the *Main Window* launches a *File Information Window*. The *File Information Window* displays file details and lets you enter your own notes.

Information



The **Filename** associated with the active *Data Window*. If the data has not yet been saved this field will be blank.

Filename

The **Date and Time** at which the acquisition was made.

Date and Time

The **Temperature** to which the detector had been cooled.

Temperature

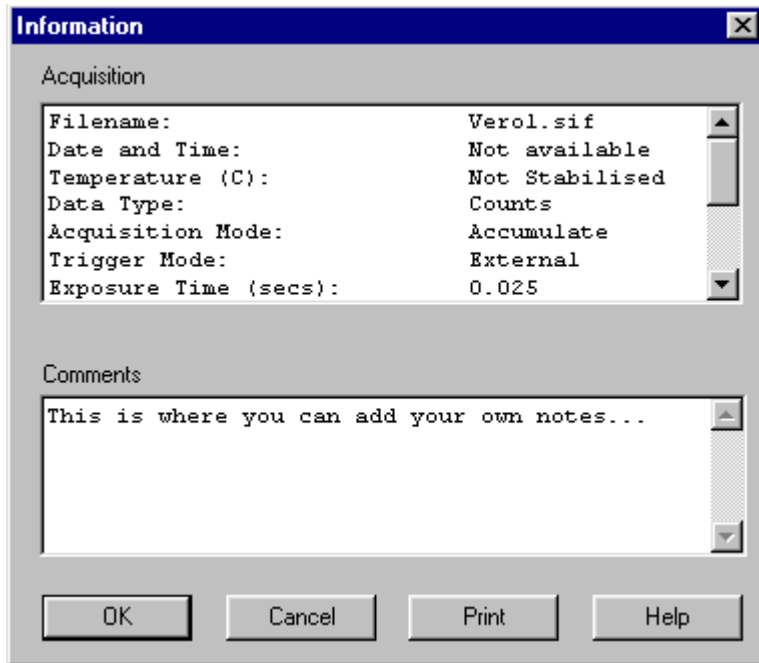
The **Model** number of the detector.

Model

The *File Information Window* also provides the following information on the data acquisition itself. (For more detailed descriptions of the modes and parameters which are associated with data acquisition and which appear on the *File Information Window* see SECTION 4.4 **DATA ACQUISITION**, SECTION 4.5 **SETTING THE ACQUISITION**, and SECTION 4.6 **CHOOSING THE DATA TYPE**.)

- **Data Type** - Counts, % Transmittance, etc.
- **Mode** - i.e. Single Scan, Real Time, Integrate, Kinetic Series.
- **Readout Time per Pixel (μs)** - value given in microseconds.
- **Trigger Source** - i.e. Internal, External.
- **Exposure Time (secs)**.

Depending on the mode in which the data acquisition was made, further information may also appear - e.g. the **Accumulation Cycle Time** for an accumulated acquisition.



File Information Dialog Box

Hot keys (or shortcuts) let you work with the system directly from the keyboard, rather than via the mouse.

F1 provides **Help**.

The following hot keys relate to data acquisition:

Key Strokes	Description
F5	Take signal scan
F6	Autoscale Acquisition
Ctrl + B	Take background
Ctrl + R	Take reference
Esc	Abort Acquisition

Many of the hot keys relate to the *Data Window*.

Key Strokes	Description	Display Mode		
		2D	3D	Image
+	Expand ('Stretch') data-axis	•	•	•
-	Contract ('Shrink') data-axis	•	•	•
Ins	If maintain aspect ratio off, Expand ('Stretch') x-axis If maintain aspect ratio on, Expand ('Stretch') x-axis and y-axis	•	•	•
Del	If maintain aspect ratio off, Contract ('Shrink') x-axis If maintain aspect ratio on, Contract ('Shrink') x-axis and y-axis	•	•	•
/	On image, if maintain aspect ratio off, Expand ('Stretch') y-axis On image, if maintain aspect ratio on, Expand ('Stretch') x-axis and y-axis			•
Home	Move cursor farthest left	•	•	•
End	Move cursor farthest right	•	•	•

Key Strokes	Description	Display Mode		
		2D	3D	Image
PgUp	Scroll up through tracks	•	•	
PgDn	Scroll down through tracks	•	•	
Shift + PgUp	Move to next image in series	•	•	•
Shift + PgDn	Move to previous image in series	•	•	•
Left Arrow	Move cursor left	•	•	•
Right Arrow	Move cursor right	•	•	•
Up Arrow	Scroll trace up / On image: move cursor up	•	•	•
Down Arrow	Scroll trace down / On image: move cursor down	•	•	•
Shift + Left Arrow	Scroll trace / image left	•	•	•
Shift + Right Arrow	Scroll trace / image right	•	•	•

Key Strokes	Description	Display Mode		
		2D	3D	Image
Ctrl + Left Arrow	Peak search left	•		
Ctrl + Right Arrow	Peak search right	•		
F7	Toggle Palette			•
F8	Reset	•	•	•
F9	Rescale	•	•	•
Alt + F9	Toggle Rescale Mode	•	•	•
Ctrl + F9	Scale to Active (see Displaying Data)	•		
F10	File Information	•	•	•

The following hot keys relate to the Andor Basic programming language:

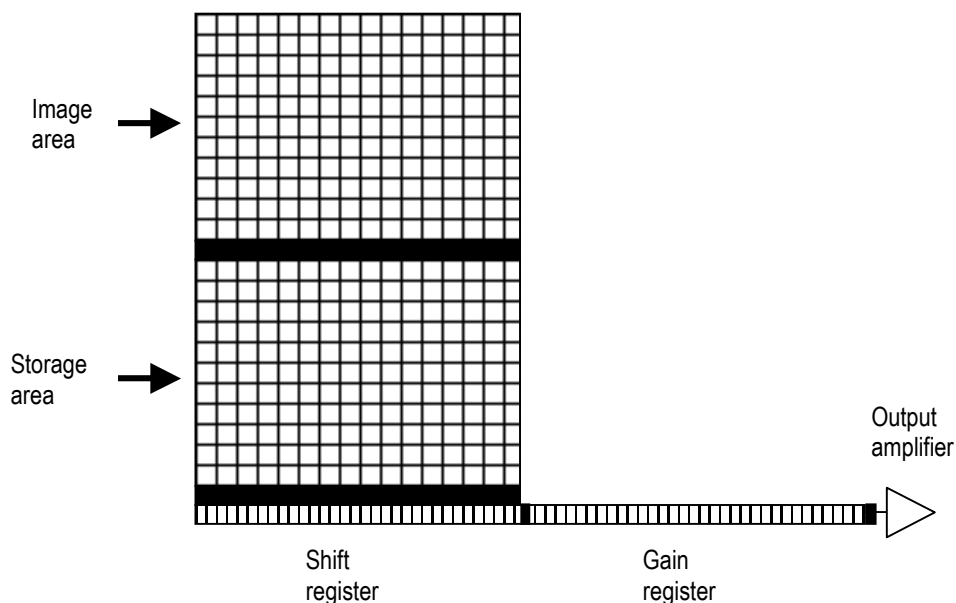
Key Strokes	Description
Ctrl + P	New program
Ctrl + E	Run program
Esc	Abort acquisition / program
Ctrl + L	Command line
Ctrl + F1	Context sensitive help on reserved words in the Andor Basic programming language is available if you are using the <i>Program Editor Window</i> .

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Typical EMCCD Chip

EMCCD Structure

Specification (no./size of pixels etc.) varies according to the chip model



Advances in sensor technology have led to the development on a new generation of ultra-sensitive, low light CCDs. At the heart of your iXon detector is the latest **Electron Multiplying Charged Coupled Device**, 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 iXon detector is identical in structure to a conventional **CCD** but with the **shift register** extended to include an additional section, the Gain Register.

Impact Ionization

One of the electrodes (phases) in the Gain Register is replaced with two electrodes, the first is held at a fixed potential and the second is clocked as normal, except that much higher voltages (between 40V and 60V amplitude) are used 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 ionisation' as they transfer.

The impact ionisation causes the generation of new electrons, i.e. **multiplication or Gain**. The multiplication per transfer is actually quite small, only around X1.01 to X1.015 times maximum, but when done over a large number of transfers substantial gain is achieved.

By inserting the electron amplification stage before the output amplifier (see Figure 1), the signal may be increased above the readout noise, hence effectively reducing the readout noise. See SECTION 3.2 – **EMCCD GAIN** for further explanation of EMCCD gain.

The **shift register** runs below and parallel to the light collecting rows. It has the same number of pixels as a light-collecting row, but is masked, to prevent light from falling on it. The gain register is also masked.

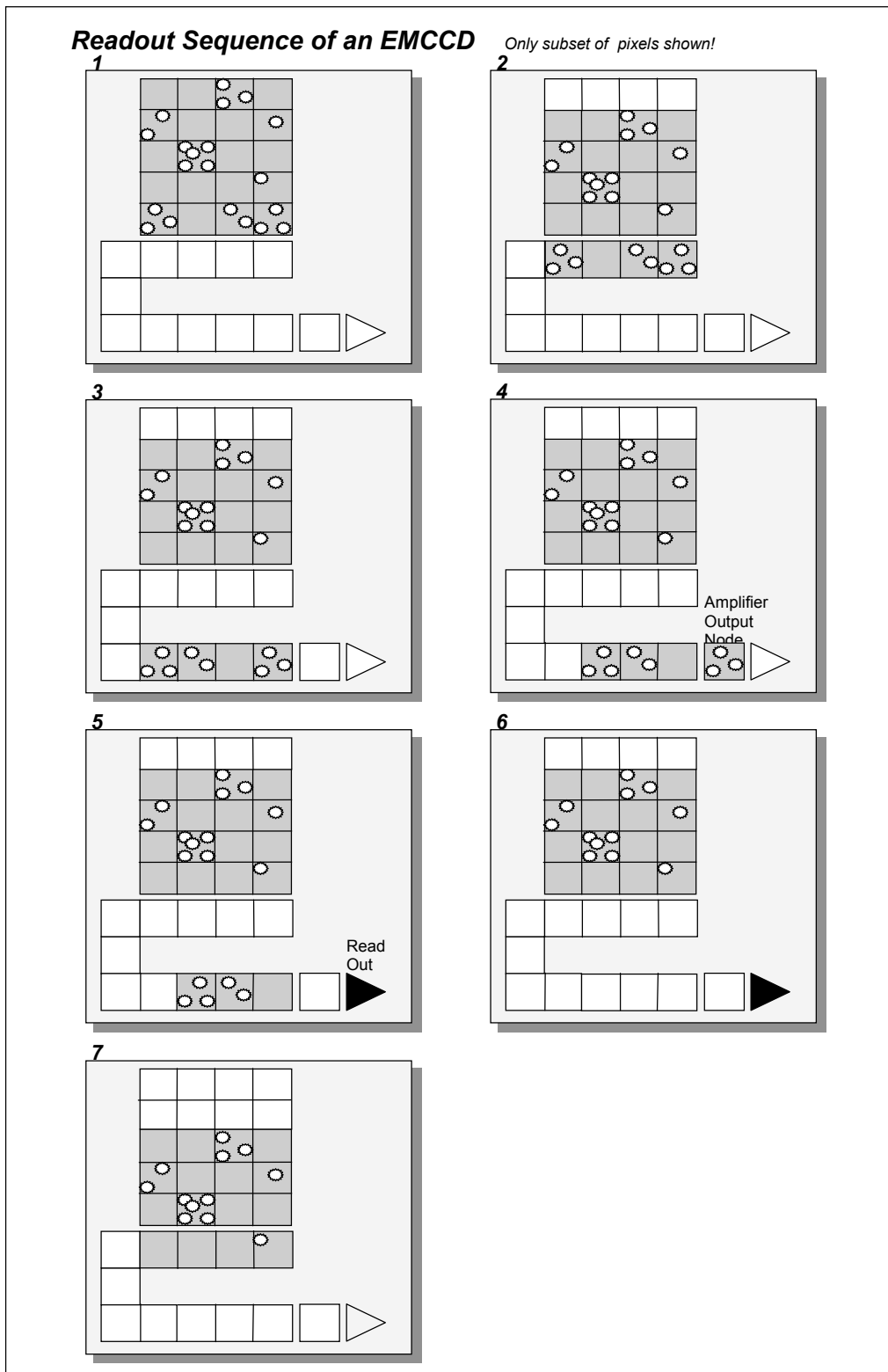
When light falls on an element, electrons (photoelectrons) are produced and, in normal operation, these electrons are confined to their respective elements. Thus, if an image (or any light pattern) is projected on to the array, a corresponding charge pattern will be produced. To capture the image pattern into computer memory, the charge pattern must be transferred off the chip, and this is accomplished by making use of a series of horizontal (i.e. parallel to the rows/shift register) transparent electrodes that cover the array. By suitable 'clocking', these electrodes can be used to shift (transfer) the entire charge pattern, one row at a time, down into the shift register.

The shift register also has a series of **electrodes** (which are vertical, i.e. parallel to the columns) that are used to transfer the charge packets, one element at a time, into the output node of the ‘on-chip’ **amplifier**.

The output of the amplifier feeds the **analog-to-digital converter** (A/D), which in turn converts each charge packet into a 14 / 16-bit binary number. Unlike Andor’s standard CCD detectors, the A/D converter on the iXon is located inside the camera head

Readout Sequence of an EMCCD

In the course of **readout**, charge is moved vertically into the shift register, and 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 CCD-chip. Other binning patterns are achieved by summing charge in the shift register and/or the output node prior to readout. See **Horizontal and Vertical Binning** below.



- 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, and subsequently into the output node of the amplifier.
- 4 The charge in the output node of the amplifier is passed to the analog-to-digital converter and is read out.
- 5 Steps 3 and 4 are repeated until the shift register is emptied of charge.
- 6 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.

Vertical and Horizontal Binning

Binning is a process that allows charge from two or more **pixels** to be combined on the EMCCD-chip prior to readout (see **Readout Sequence of a EMCCD** above.)

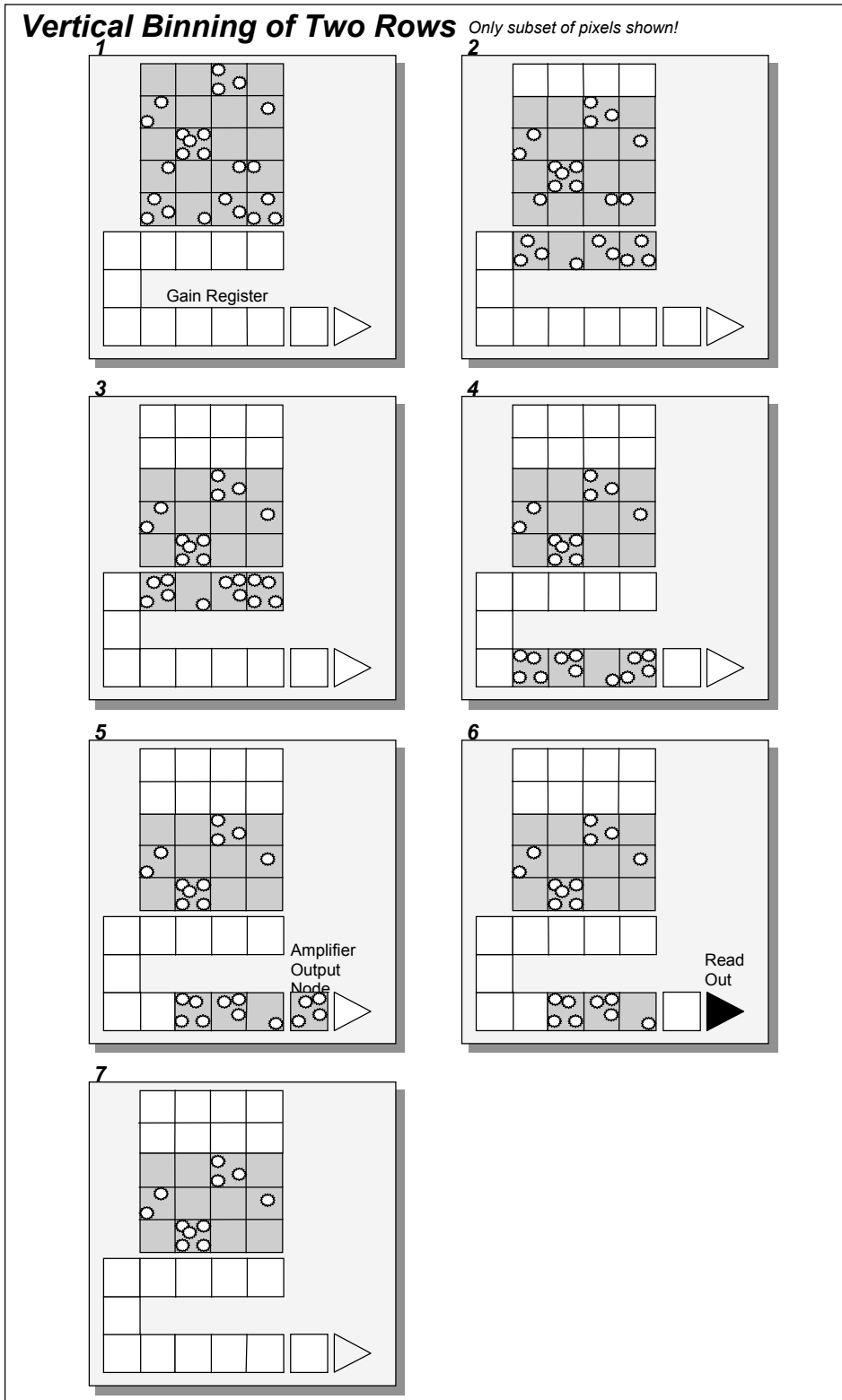
Summing charge *on* the EMCCD, and doing a single readout gives better noise performance than reading out several pixels and then summing them in the computer memory. This is because each act of reading out contributes to noise.

There are two main variants of the binning process: **vertical binning** and **horizontal binning** (described below). In addition there are several **binning patterns** that tailor the main binning variants to typical application usage.

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 following example 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, and then 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 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.

Horizontal Binning

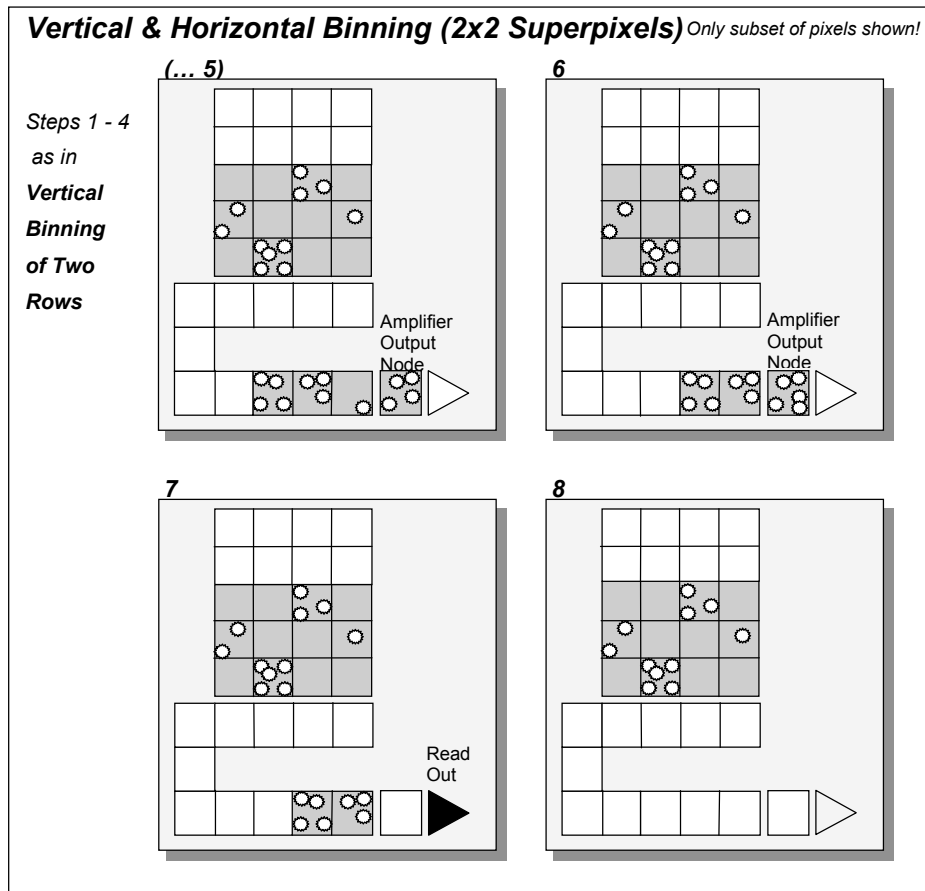
Shifting the charge horizontally from several pixels at a time into the output node is known as **horizontal binning**.

Horizontal binning in combination with **vertical binning** allows you to define so-called superpixels that in **Image Display Mode** (see SECTION 4.7 **DISPLAYING DATA**) represent as a single picture element charge that has been binned from a group of pixels. For example, charge that is binned vertically from two rows and horizontally from two pixels before being read out is displayed as a superpixel of dimensions 2 x 2 pixels. You can define these superpixels from the *Imaging Dialog Box*, which is accessible from the *Setup Acquisition Dialog Box* (see SECTION 4.5 **SETTING THE ACQUISITION**).

On the one hand, superpixels (by comparison with single pixels) result in a more coarsely defined; on the other hand, superpixels offer the advantages of summing data on-chip prior to readout.

In the following example, where each superpixel is of dimensions 2x2 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 2x2 'superpixel'.

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



- 4 Charge from two rows has already been vertically binned into the shift register (see ‘Vertical Binning of Two Rows’, above). 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, see ‘Vertical Binning of Two Rows’, above) until the whole frame is read out.

The output from the gain register is fed into a conventional CCD output amplifier. This amplifier, even in a scientific CCD, will have a readout noise of a few electrons rms and around 10 or 20 electrons rms at MHz readout rates. However this noise will effectively be reduced by the multiplication factor of the gain register which, when high enough, will achieve noise levels below 1 electron rms. So by using the gain you can effectively reduce the noise to insignificant levels at any readout speed. For example, the iXon 87 has a readout noise of a few to tens of electrons, depending on read out speed

Gain and Noise

Using gain will itself add some noise to a measured signal due to the statistical nature of the multiplication process. A similar effect exists in ICCDs and is referred to as the Noise Factor. The amount added is dependent on the signal level and the gain. If there is no gain, then there is no extra noise. At high gain (tens of times higher than unity) it is calculated as the square root of N , where N is the signal in electrons. This will add to the shot noise of the signal to become the square root of $2N$. So if the signal is large enough to be above the readout noise then there is probably no need for gain and it should be reduced or turned off. Conversely, if the signal is being lost in the readout noise then increasing the gain is the only way to detect it. If the gain is set high enough, then detection of single electron events will be possible. These events will appear on an image as a spike several hundred counts high. In Andor's iXon CCD cameras the gain is normally factory set to a maximum of 1000 times, depending on the type of system. The gain performance of the iXon is comparable to high end ICCDs.

As the gain is increased it will at some point begin to cause a decrease in Dynamic Range. This occurs when the gain equals the readout noise, in electrons. If higher sensitivities, and hence higher gains, are required then there will have to be a trade off for Dynamic Range. To maintain as much Dynamic Range as possible it is advisable not to use a higher gain than is necessary to measure a signal.

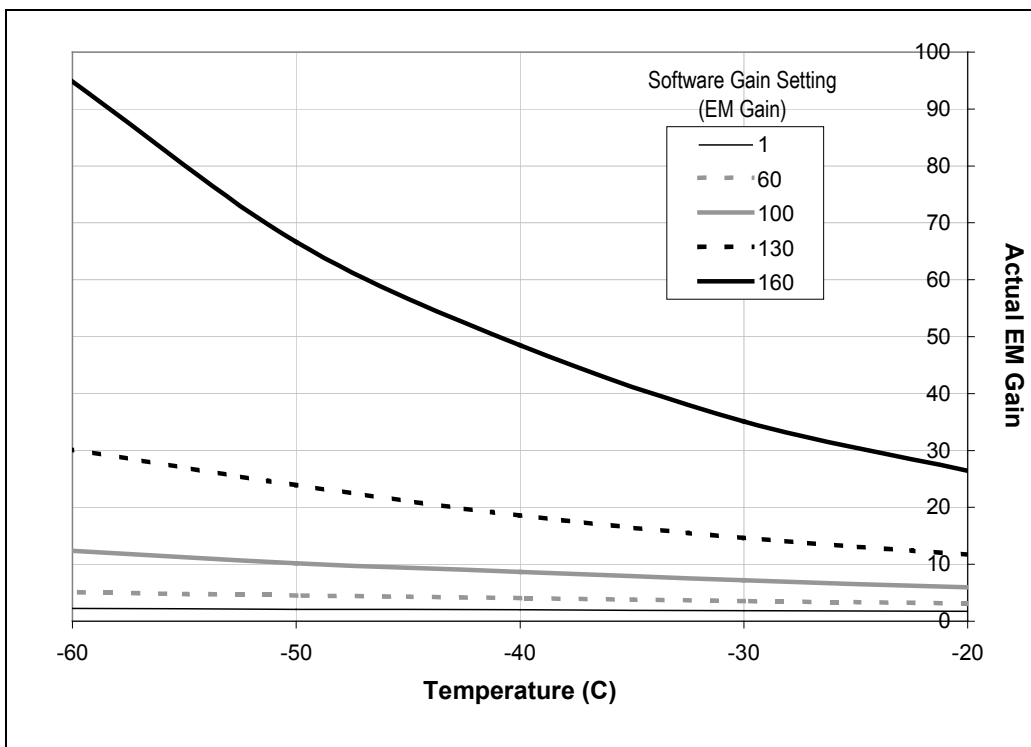
Gain and Dynamic Range

Darksignal or dark current is just the same as in conventional CCDs. When judging the required operating temperatures to eliminate the darksignal contribution, usually a temperature where the darksignal shot noise is comfortably below the readout noise is selected. In this case, further cooling provides no benefit. For the EMCCD, however, where there can be essentially no readout noise and single electron events can be detected, ideally no darksignal is desired. This does not mean that more cooling is needed to see the benefits of the EMCCD, it is just that yet more sensitivity through yet longer exposures can be obtained with more cooling than usual.

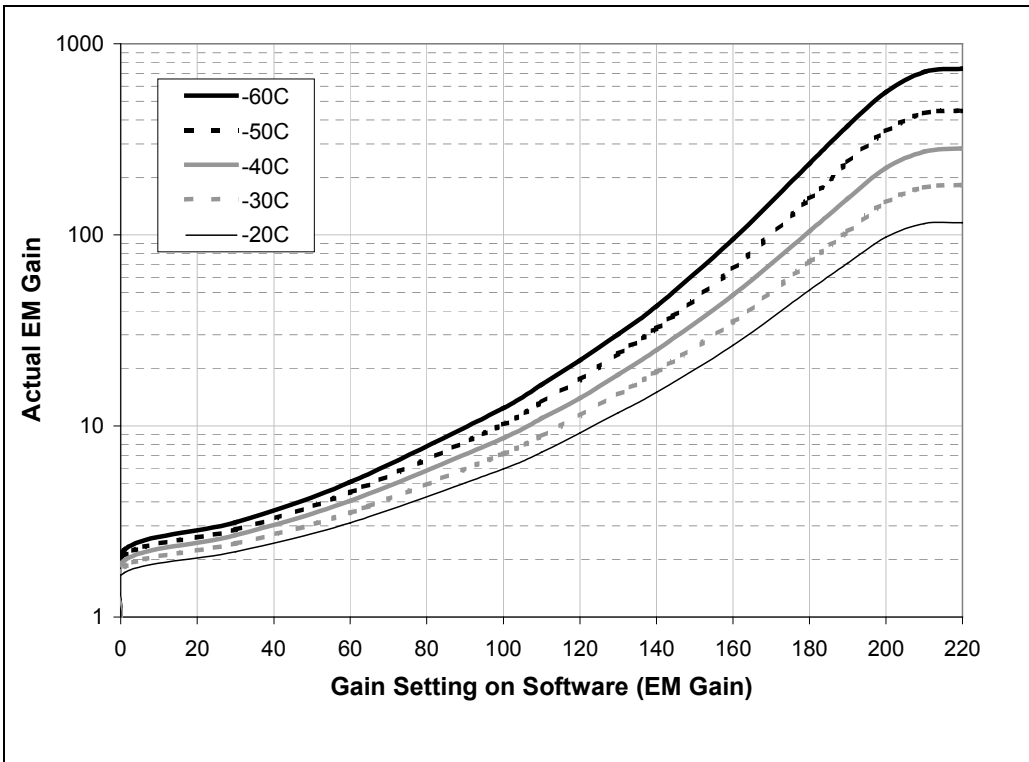
The EMCCD and Darksignal

The gain of an EMCCD system varies with temperature. The graph below shows how the gain multiplication increases as the temperature decreases. Curves are shown for various software gain settings and the figures are typical values. So if a system is operated at room temperature it will have reduced gain. Because of this temperature dependence it is recommended that the system is cooled, so that the temperature, and hence the gain, is stabilized.

Gain Temperature Dependence



This graph shows how the EM Gain setting on the software is related to the actual electron multiplication factor for various temperature settings. Again the figures are typical.



Note: At gain setting 0, the Electron Multiplication process is disabled, and amplification does not take place

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The rich functionality of the software package is described in detail in the remainder of the **User's Guide**. However, if this is the first time you have used an Andor detector, the short descriptions that follow will help familiarize you with its design philosophy and some of its key terminology.

When you acquire data, by reading out a scan or a series of scans of the CCD-chip at the heart of the detector, the data are stored together in a **Data Set**, which exists in your computer's **Random Access Memory (RAM)** or on its **Hard Disk**. You can also create a data set via the **Andor Basic** programming language.

Data Set and Data Window

Whenever a data set is in RAM (e.g. you have acquired new data, have created a new data set using the Andor Basic programming language, or have retrieved a data set from file), it is displayed in a **Data Window**. The data set is identified on the title bar of the *Data Window* by a *name* (the Windows filename, if you have explicitly saved the data; otherwise the default name 'Untitled Document') and a number, *#n*.

#n uniquely identifies the data set while the data set is being displayed (i.e. is in RAM). *#n* is also temporary: it ceases to be associated with the data set once you close all *Data Windows* bearing the same *#n* (and so remove the data set from RAM). *#n* may be regarded as a temporary shorthand for the *name* of the data set you are displaying.

The data set may be viewed (in different **Display Modes**) as **2D** or **3D** traces or as a false color **Image**.

You can open several *Data Windows* to look at the same data set (possibly in different levels of detail or in different Display Modes): each *Data Window* has the same *name* and *#n* (which identify the data set), but a unique number *:x* (following the data set *name*) to identify the window itself.

Using a *Data Window* in 2D Display Mode you can overlay data traces from the same and from other data sets (e.g. to compare data, possibly in different levels of detail.) However, data can be modified only in a *Data Window* labeled with the *name* and the *#n* of the data set to which the data belong. If you modify a data set and attempt to close the data window, you will be prompted to save the data set to file.

To initiate a data acquisition (and so create a data set of newly acquired data) you must either click the **Take Signal** button on the *Main Window* or select one of the **Take...** menu options on the **Acquisition** Menu. The system opens a *Data Window* labeled with the number *#0* and the name *Acquisition*, and displays the acquired data. To view signal, background and reference data, select the appropriate tab on the bottom edge of the *Data Window*. If you have selected **Video** as the **Acquisition Mode** (this is the default setting) new data will continue to be acquired and displayed until you press the <ESC> key or click the **Abort** button on the *Main Window*.

Acquisition

The *Command Line* gives you ready access to all functions and arithmetic data processing of the Andor Basic programming language - without the need to write programs! However, to process the contents of a data set, the data set must first be in memory (RAM), and a corresponding *Data Window* will therefore be on screen. As an example, the following entry on the command line adds together the data in the data sets #1 and #2, and stores the result in a third, possibly new, data set labeled #100. Thus **#100= #1 + #2**

The Command Line

The software provides **On-Line Help** typical of Windows applications. For details of how to use On-Line Help, Consult your Windows **User's Guide**.

On-Line Help can generally be launched from a **Help** button on the window or dialog box about which you require help. Alternatively, press **F1** on your keyboard.

On-Line Help will provide you with information that is relevant to the part of the application from which help was called. Thus, calling help from the *Main Window* will present you with an overview of all the available help topics - the *Main Window* itself being the 'entry point' for the application as a whole. By contrast, calling help from the Setup Acquisition Dialog Box will present you with topics and subtopics particularly relevant to that dialog box. However, from whatever point you enter the on-line help service, it is possible to navigate to any other help topic.

In addition to the main On-Line Help, the system provides help that relates specifically to the Andor Basic programming language. If you are working in a *Program Editor Window*, context sensitive help is available on the 'reserved words' of the programming language: with the cursor on or immediately after a reserved word, press *Ctrl + F1*. See also SECTION 6 **WORKING WITH PROGRAMS**.

File

The **File** Menu makes available typical Windows facilities to let you **Open** and **Save** files, as well as facilities specific to Andor's Software to let you create or run programs. For further details of Windows file handling dialog boxes (e.g. the *Open* and *Save as* dialog boxes) consult your **Windows User's Guide**. The *File Menu* offers the following options:

Open launches an '*Open*' *Dialog Box*. If you open a data file (.sif), the system launches a *Data Window* (see SECTION 4.7 **DISPLAYING DATA**). If you open a program file (.pgm), the system launches a *Program Editor Window* and makes available a selection of *editing tools* on the *Main Window* (see SECTION 5.2 **EDITING PROGRAMS**).

Open...



Close removes the active *Data Window* or the active *Program Editor Window* from the *Main Window*. You will be prompted to save any unsaved data.

Close

Save stores the contents of an active and previously saved *Data Window* or *Program Editor Window* under the current filename.

Save



Save as... launches a '*Save As*' *Dialog Box*. The '*Save As*' *Dialog Box* lets you save the contents of an active *Data Window* or *Program Editor Window* under a filename and in a directory of your choice.

Save As...

Virtual Memory sets parameters for the disk storage system in the application. In the same way that the **Spool To Disk** option allows new acquisitions to be written directly to a storage space on the hard drive (which allows acquisition data larger than the physical memory of the computer to be acquired), so **Virtual Memory** allows you to pre-allocate a buffer on the hard drive in which large files (e.g. long kinetic series) are held as you open them. Only the most recent few frames at a time from the series are held in RAM as you work on the data, the rest of the data is read and written at high speed from the virtual memory buffer.

Virtual Memory

This is faster and more reliable than making use of the virtual memory feature of the operating system (which simulates extra physical memory using a buffer on the hard drive to hold data larger than would normally fit in the RAM available) because Andor's Virtual Memory system is specifically designed to handle the large file you are attempting to load and work with in the MCD application.

Whereas the operating system's virtual memory management is attempting to "guess" which data should be held in physical memory, and which should be cached to the buffer, based on data traffic, Andor Virtual Memory knows exactly how to handle the large file being opened in MCD.

There are three simple choices for you to make when the Virtual Memory dialog opens. First, a tick box enables/disables Andor Virtual Memory.

Secondly, you may set a **Threshold** value. This is the file size in megabytes above which Virtual Memory will be used. Many of your data series might well fit normally into the physical memory of your computer, in which case using Virtual Memory carries no speed advantage. Set this value to reflect the size of your computer's RAM initially. You may find that you need to lower the threshold if you often have other applications running, or open multiple data files in MCD at one time, as these activities can cause there to be less physical memory available than you expect. Generally, the lower the threshold value, the faster large files will open. However, if a large kinetic series is held in virtual memory it may be somewhat slower to step through or 'play' as a movie, compared to the same file opened without virtual memory.

Finally, you must choose a hard drive **Location** where the Virtual Memory buffer will reside. It is important that you choose a location on a LOCAL hard drive (ideally an uncompressed volume), because the speed of file access across a network connection may be much slower than to a local hard drive – thus negating the speed advantage of using Virtual Memory.

Batch Conversion

Batch Conversion allows conversion of multiple .sif files, stored on disk, into various formats. There are three main sections to the dialog: Source, File list and Destin *Source*

To convert multiple files they must be in the same directory on disk. This directory is selected in the upper section of the dialog, either by typing in the path or by selecting the browse button and navigating to the appropriate directory.

File List

All .sif files in the directory specified by the source will be displayed in the center section of the dialog. The files that are to be converted can now be selected by dragging the mouse around them or by holding the Ctrl key while clicking on the files. Files can be deselected by clicking on the file whilst holding down the Ctrl key.

Destination

Once the Source files have been selected, the directory where the output files will be placed needs to be chosen. This is done in a similar way to selecting the source directory. If the files are to be placed in a new folder, this can be done by selecting the new folder button and typing the desired path into the dialog. Having selected the source files and destination, the type of conversion should be selected using the 'Convert To' combo box. The available options are: ASCII (text file), BMP (windows bitmap), GRAMS (Graphic Relational Array Management System), JPEG (image format), RAW (binary file) or TIFF (Tagged Image File Format).

[**Note:** By Clicking the 'More >>' button, dialog box is extended to show further options which can be set. While these extra options are displayed the button changes to show 'Less <<'. Clicking the 'Less <<' button hides these extra options. The extra options allow you to select which of the datasets (if present in your .sif file) should be converted. A separate file, or sequence of files will be created for each dataset in the file if, the dataset has been selected and, the dataset exists in the file being converted. Files will be distinguished by the postfixes _sig, _bgd and _ref. If only one dataset is selected these postfixes will not be added.]

When the above selections have been made, the Convert button at the bottom of the dialog should be enabled (if not check that the source and destination directories are valid and that there is at least one file selected in the list of sifs). Pressing the convert button may bring up another dialog box depending on which conversion type was chosen. For the image formats (i.e. BMP, JPEG, TIFF), the dialog will prompt you to select the image format and data range. For the ASCII format the separator to be used between data values is requested and also whether acquisition information should be added to the end of the data. The RAW file type dialog requests the number of bits to be used to store each data value. There is no dialog for the GRAMS data type. Select the required options, if available, and click the OK button to begin the conversion.

For .sif files containing a series of images or spectra, the conversion process will create either a single file containing all the images or a sequence of files for each image. The ASCII, RAW and GRAMS types result in a single data file, whereas the BMP, TIFF and JPEG types will create a separate image file for each. In the latter case the files are distinguished from each other by a postfix ‘_X’ where X is the number of the image in the original .sif file.

The ‘Export As’ Dialog Box allows you to store displayed data in a selection of formats, including:-

Export As...

ASCII (.asc);

BMP (.bmp);

TIFF - Tagged Image File Format (.tif);

Raw Data (.dat).

JPEG (.jpg).

AVI (.avi).

MPEG (.mpeg).

The available export options will vary depending on the Display Mode you are currently using, and the Readout Mode in which you acquired your data. See also SECTION 4.7 **SETTING THE ACQUISITION**. The table below indicates the combinations available.

Display Mode	Readout Mode	Export as... Options						
		BMP	TIFF	ASCII	DAT	JPEG	AVI	MPEG
<i>Image</i>	<i>Image</i>	✓	✓	✓	✓	✓	✓	✓
<i>2D / 3D</i>				✓	✓			

ASCII

Exporting Data as **ASCII** text, means you can subsequently import your data into other applications (such as spreadsheets) that use the ASCII format. When you click the **OK** button on the *'Export As' Dialog Box*, having first selected *ASCII XY* from the *'Save file as type' Drop Down List*, the system launches a *'Save Data as ASCII XY' Dialog Box*. A series of radio buttons on the dialog box allows you to choose whether you want to save the signal, background or reference data (see SECTION 4.1 **WORKING WITH DATA**) from the live data set.

A further series of radio buttons allows you to choose a character that will serve as a *'Separator'* between the numeric values in your raw data. You can then configure the importing application to recognize this separator (or delimiter) and – in the case of a spreadsheet – display the data in a suitable configuration of rows and columns. In a Windows 95 application such as Excel you can perform this configuration by means of a wizard, launched automatically as you import the ASCII file.

Exporting data as ASCII text causes all the data associated with the data set to be exported – not just the portion of the data that is currently being displayed. Data which have been acquired through **Full Vertical Binning** or from a **Single Track** will typically be displayed on a spreadsheet as a single column. Data from acquisitions involving **Multi-Track**, **Imaging** or **Kinetic Series** are normally displayed in rows and columns, the columns representing the height (and/or the member of the kinetic series) and the rows the width of the CCD-chip. (See SECTION 4.5 **SETTING THE ACQUISITION**).

BMP

BMP exports data in Microsoft Windows bitmap (.bmp) format. Bitmap files can be embedded into documents created in Microsoft word processing and presentation packages, etc. If you adjust the image displayed (e.g. zoom in), it is the displayed image that is exported: *What You See Is What You Get* (WYSIWYG)!

Tagged (or Tag) Image File Format (TIFF, .tif) is used for storing bitmapped images and is widely supported by commercial publishing packages. When you save data as a .tif file, the system automatically creates a file containing a number of labels (tags), which describe key properties of the data (gray levels, byte format, etc.) As with BMP, the image is exported WYSIWYG fashion.

TIFF

The 'Export Raw Data' Dialog Box allows the user to save the active data set to a .dat file located on disk. The .dat file comprises data only and has *no header information* of any kind. The original data set remains unchanged.

Raw Data

A 'data set' refers to a collection of data comprising one or more of the following subsets, selectable from the *Export as .dat* Dialog box

Signal (*sig*)

Background (*bg*)

Reference (*ref*)

Cal (*Lamp Calibration*)

A .dat file, however, will contain *only one* of the above data subsets. (Please note that the *cal* subset is used in radiometry/colorimetry measurements. It does not refer to the x-axis calibration of *sig*, *bg* or *ref* data.

The .dat file format

The .dat file format reflects the CCD sensor format, e.g., with a CCD sensor of 1024 columns * 256 rows then the first 1024 data values in the .dat file correspond to the first row of the CCD, the second 1024 data values correspond to the second row, etc.

The .dat data type

	Data Type	No. of Bytes	Range
1)	16 bit integer	2	-32,768 to 32,767
2)	32 bit integer	4	-2,147,483,648 to 2,147,483,647
3)	32 bit float	4	3.4×10^{-38} to $3.4 \times 10^{+38}$

Option 1) saves a data set to a 16 bit integer .dat file. Note: if a data value exceeds the limits of a 16 bit integer (<-32,768 or > 32,767), that data value is truncated to the corresponding limit (e.g. if a data value is 36,000 then the value is truncated to 32,767).

Option 2) saves a data set to a 32-bit integer .dat file. The limits for the 32 bit integer are handled in similar fashion to the 16 bit integer above.

Option 3) saves a data set to a 32-bit floating point .dat file.

When using your own software to handle a .dat file, you have to work out how many bytes to read in. Each 32-bit value requires 4 bytes to handle the value. Thus, for example, to read in a 32-bit .dat file consisting of 1024 data values, you would have to read in 4096 bytes in total.

Internally data is stored as 32-bit float. Saving data to integer data files will result in a loss of precision. Original data remains unaffected.

A configuration file contains the values that appear on the system's dialog boxes whenever the application is launched, or whenever a configuration file is newly loaded. Using configuration files is an easy way to tailor the overall application set-up to suit particular experiments. Configuration files reside in the same directory as the executable (.exe) of the application itself. The factory-supplied configuration file (.cfg) contains typical default settings. Each time you start up the system, the iXon .cfg is loaded automatically.

Configuration Files

The **Configuration Files** menu item opens a further submenu with options **Load...** and **Save...** that let you select or create configuration (.cfg) files:

Use **Save...** to store your current settings under a filename and in a directory of your choosing. You can, if you wish, overwrite an existing configuration file.

Use **Load...** to select the configuration file you currently want to use. The system will immediately use the settings in the newly loaded file.

New Program launches an empty *Program Editor Window* and makes available a selection of *editing tools* on the *Main Window* (see SECTION 6 **WORKING WITH PROGRAMS**).

New Program

Run Program executes the contents of an active *Program Editor Window* (see SECTION 5.3 **RUNNING PROGRAMS**).

Run Program

Run Program by Filename launches an '*Open*' *Dialog Box*. Select the file that contains the program you want to run. The program begins execution as soon as the file is selected (see SECTION 6.3 **RUNNING PROGRAMS**).

**Run Program by
Filename**

Print Preview... launches a *Print Preview Window*

Print Preview

Print launches a standard *Print Dialog Box*.

Print

Print Setup launches a standard *Print Setup Dialog Box*.

Print Setup

Exit lets you leave the application.

Exit

Preparing to Acquire Data

Once you have installed the software and hardware, you are in fact able to acquire data (as **Counts**) simply by clicking the **Take Signal** button on the *Main Window*.

Acquisition

However, to help you prepare to acquire data, the **Acquisition Menu** on the *Main Window* provides the following options:

Setup Acquisition launches a *Setup Acquisition Dialog Box* where you can select the manner in which data are acquired. See SECTION 4.5 **SETTING THE ACQUISITION**

Setup Acquisition



Setup Data Type launches a *Data Type Dialog Box* where you can select the type of information (for example, **Counts** or **Bg Corrected Counts**) that you want the system to compute and display.

Setup Data Type

Data are computed and displayed automatically in your selected data type each time you perform **Take Signal**.

The data type you select will determine whether you need to take a background and/or a reference scan using the **Take Background** and/or **Take Reference** options.

The system acquires data in accordance with the parameters you have selected on the *Setup Acquisition Dialog Box*. Data are displayed in the *Acquisition Window*. See SECTION 4.7 **DISPLAYING DATA**.

From the **Acquisition** Menu on the *Main Window*, you may make the following data acquisitions: **Take Signal**, **Take Background**, **Take Reference**.

You may also: **Abort Acquisition**.

If you select **Autoscale Acquisition** (the ‘tickable’ item on the *Acquisition Menu*) the system will configure the *Acquisition Window*, if necessary adjusting its scales in real time, so that all data values are displayed as they are acquired. The manner in which the data are displayed corresponds with the selection you make under **Rescale Data Mode** on the **Display** Menu, where you may choose to display values between zero and maximum (0...max); between minimum and maximum (min...max); or between zero and 16384 (0...16384). For more detail see SECTION 4.7 **DISPLAYING DATA - Rescale**.

Autoscale Acquisition

Take Signal on the *Acquisition Menu* instructs the system to acquire data. The acquired data, displayed under the **Sig** tab of the *Acquisition Window*, are of the type selected via *Setup Data Type* on the **Acquisition** Menu. Your choice of data type may mean that you have to perform Take Background and/or Take Reference before you perform **Take Signal**.

Take Signal



Take Background on the **Acquisition** Menu instructs the system to acquire raw background data, which are then displayed as **counts** under the **Bg** tab of the *Acquisition Window*. No calculations are performed on these data.

Take Background

The data type you select via **Setup Data Type** on the Acquisition Menu may require you to perform Take Background before you perform Take Signal.

Note: that you do not necessarily have to take background data prior to each acquisition of signal data: if the data acquisition parameters remain unchanged since you last performed Take Background, then no new background data are required.

Take Reference on the Acquisition Menu instructs the system to acquire background corrected data that will be used subsequently in calculations that require a reference value. Before executing this function you must therefore perform Take Background.

Take Reference

The data you acquire using Take Reference are displayed as **counts minus background** under the **Ref** tab of the *Acquisition Window*.

The data type you select via Setup Data Type on the Acquisition Menu may require you to perform Take Reference before you perform Take Signal.

Note: that you do not necessarily have to take background data prior to each acquisition of reference data: if the data acquisition parameters remain unchanged since you last performed Take Background, then no new background data are required. Similarly, you may not have to acquire new reference data each time you perform Take Signal.

Video Mode instructs the system to immediately acquire data, and to continue to acquire until Abort Acquisition is pressed. The acquired data, displayed under the **Sig** tab of the *Acquisition Window*, are based on the parameters selected via *Video Mode* on the **Acquisition** Menu.

Video Mode



(camera icon)

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. When the iXon is running in **Sub Image** mode only data from the selected pixels will be collected. Data from the remaining pixels will be discarded. For more information on **Sub Image** mode see SECTION 4 WORKING WITH THE SOFTWARE

Sub Image Mode



Run-time launches the *Run time Control* Dialog box, where you can control the Electron Multiplying (EM) **Gain**, the **Exposure Time**, and the camera's **Binning** pattern. Both controls can be changed either by clicking and dragging with your mouse, or through a choice of Gauges; Single Step, Fine, or Course. The Gauges option should be selected if you are operating your iXon with the Remote Control Unit. See SECTION 4.14 USING THE REMOTE CONTROL UNIT.

Run-time Control



Setting the Acquisition



The *Setup Acquisition Dialog Box* lets you determine the manner in which data are acquired. Launch it either from the *Setup Acquisition* option on the *Acquisition Menu* (see SECTION 4.4 **DATA ACQUISITION - Preparing to Acquire Data**), or by clicking the *Setup Acquisition* button

The Setup Acquisition Dialog Box is divided into the following menus:

Setup CCD

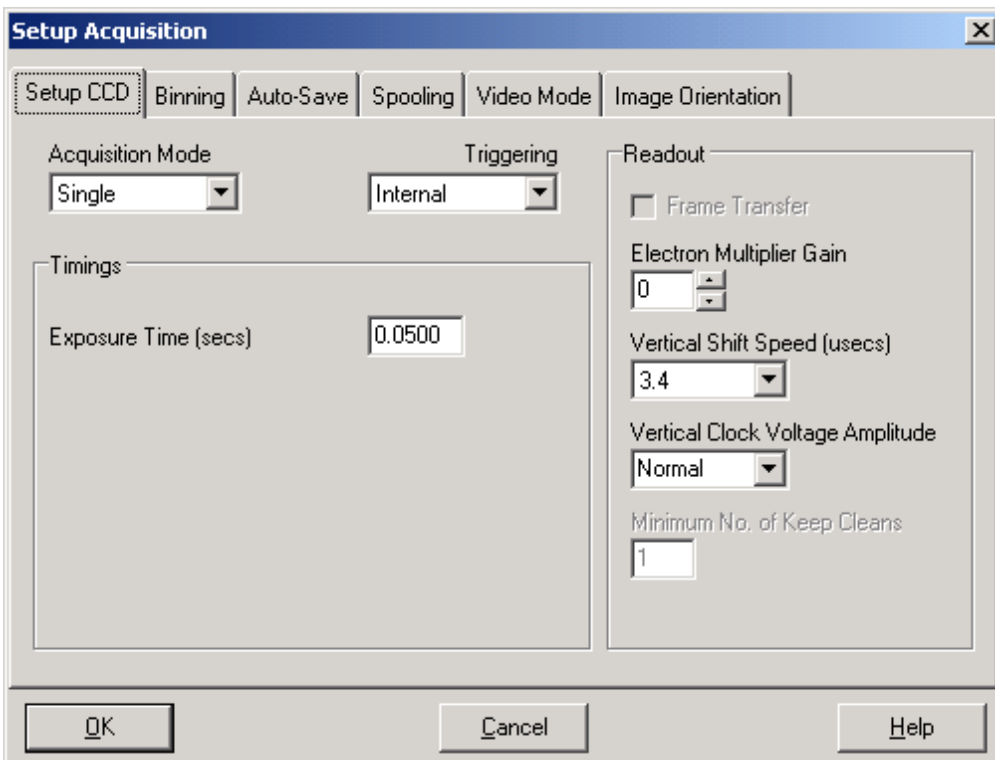
Setup Image

Auto-Save

Spooling

Video Mode

Image Orientation



Setup Acquisition Dialog Box

Setup CCD Menu

When you select *the Setup Acquisition Dialog*, the software will default to **Setup CCD** as the first Dialog menu. This menu will allow you to set the parameters for a number of areas including; **Acquisition Mode**, **Triggering**, Timings for **Exposure** and **Kinetic Cycles**, Electron Multiplying **Gain**, and **Vertical Shift Speed**.

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. The value you enter in one text box may affect the value in another text box. The following matrix lists the Acquisition Modes and for each mode indicates the parameters for which you may enter a value in the appropriate text box.

Acquisition Mode

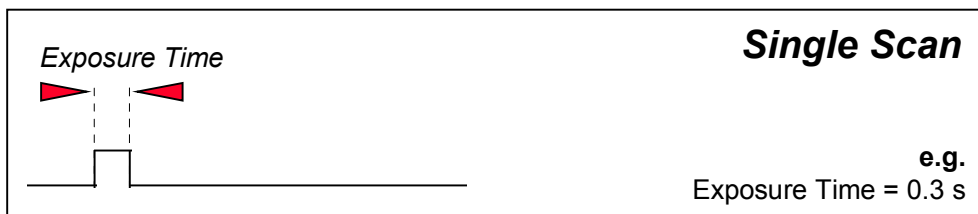
Mode`	Exposure Time	Accumulate Cycle Time **	No. of Accumulations	Kinetic Cycle Time *	No. in Kinetic Series
Single Scan	•				
Accumulate	•	•	•		
Kinetic	•			•	•

The minimum time required for an acquisition is dependent on a number of factors, including the **Exposure Time** and the **Trigger Mode**.

An **acquisition** is taken to be the complete data capture process that is executed whenever you click **Take Signal**, **Take Background**, or **Take Reference** on the **Acquisition** Menu or whenever you click the Take Signal button. By contrast, a **scan** (more properly ‘an acquired scan’ in the definitions that follow) is one readout of data from the CCD-chip - several scans may be involved in a complete data acquisition. (See SECTION 4.4 DATA ACQUISITION - Preparing to Acquire Data.)

In **Single Scan** acquisition mode, the system performs one scan (or readout) of the CCD.

Single Scan

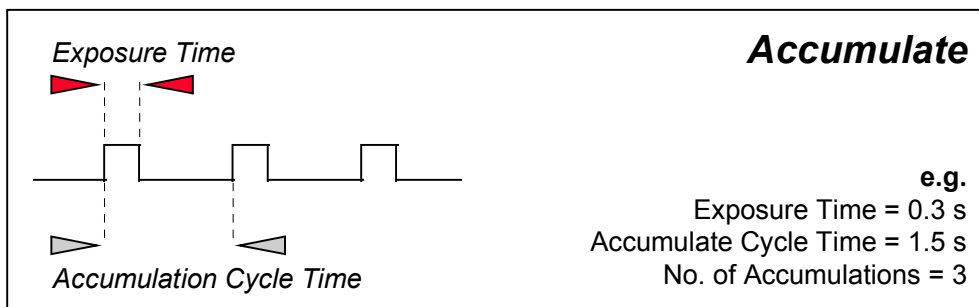


- **Exposure Time** in seconds - i.e. the time during which the CCD collects light prior to readout. The system will default to a minimum Exposure Time should you attempt to enter too low a value.

Note: Single Scan is the simplest acquisition mode.

The **Accumulate** acquisition mode allows you to add together in computer memory, the data from a number of scans to create an 'accumulated scan'.

Accumulate



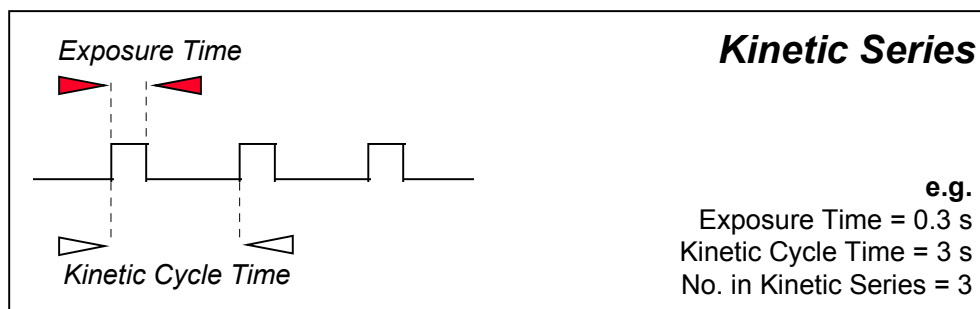
You may key in the following information on the *Setup Acquisition Dialog Box*:

- **Exposure Time** in seconds - i.e. the time during which the CCD collects light prior to readout. The system will default to a minimum Exposure Time should you attempt to enter too low a value.
- **Accumulate Cycle Time** - i.e. the period in seconds between each scan. (This parameter is only available if you have selected the *internal* trigger - see **Trigger Modes** later in this section.)
- **Number of Accumulations** - i.e. the number of scans you want to add together.

Note: This mode is used to improve the signal to noise ratio.

The **Kinetic Series** acquisition mode allows you to capture (into the same data set - see SECTION 4.1 **WORKING WITH DATA**) a sequence of single scans.

Kinetic Series



On the *Setup Acquisition Dialog Box* you may key in the following parameters:-

- **Exposure Time** in seconds - i.e. the time during which the EMCCD collects light prior to readout. The system will default to a minimum Exposure Time should you attempt to enter too low a value.
- **No. of Kinetic Scans** – i.e. the number of scans taken in the Kinetic series.
- **Kinetic Cycle Time** – i.e. the time between the start and finish of each kinetic scan.

The **Kinetic Cycle Time** can also be act as a useful guide to the Frame Rate your camera is operating at. Depending on the Acquisition parameters you set, i.e. Vertical Shift Speed, Binning or Sub Image Patterns, Cycle Time, and whether External Trigger is being used, the **Kinetic Cycle** Dialog box will display a Hz figure. As a guide 1 Hz equals 1 frame per second.

Frame Rates

If External trigger is selected the **Kinetic Cycle** dialog box will indicate the maximum achievable frame rate.

Trigger Modes

The **Trigger Mode** drop-down list box on the *CCD Setup Dialog Box* offers the following options :

Selecting the **Internal Trigger Mode** means that, once you issue a data acquisition command (see SECTION 4.4 **DATA ACQUISITION - Making an Acquisition**), the system determines when data acquisition begins.

Internal

You may use **Internal Trigger Mode** when you are able to send a trigger signal or ‘Fire Pulse’ to a short-duration, pulsed source: 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 head, to the pulsed source.

Internal Trigger Mode is also used with ‘continuous wave’ (CW) sources (an ordinary room light, for instance), where incoming data, for the purposes of your observation, are steady and unbroken: thus you can begin acquisitions ‘at will’.

Choosing the **External Trigger Mode** means that once you issue a data acquisition command (see SECTION 4.4 **DATA ACQUISITION - Making an Acquisition**), data will not be acquired until your system has received an ‘External Trigger’ signal generated by an external device.

External

The External 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.

Choosing the **External Start Trigger Mode** means that once you issue a data acquisition command (see SECTION 4.4 **DATA ACQUISITION - Making an Acquisition**), data will not be acquired until your system has received an ‘External Trigger’ signal generated by an external device. However, the system will 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.

External Start

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 shutters connected, and are using an External Trigger, 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 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. However, this process increases the minimum time between each triggered event. In cases where repetition rate is important, and slight variation in the base background level is less important, it is possible to remove this restriction by using the **Fast External Trigger** option.

Fast External

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 questions and answers will help you decide whether you should use **Internal** or **External Trigger Mode**, **External Start** or **Fast External Trigger Mode**.

1. Is the optical signal CW?
If *yes*, select **Internal Trigger Mode** and make your acquisitions.
If *no*, go to 2.
2. Are you using a pulsed source (e.g. a laser or a lamp) that can accept a trigger?
If *yes*, select **Internal Trigger Mode**. Connect the FIRE output of the Multi I/O Box to the trigger input of the pulsed source. Set the **Exposure Time / Shutter Time** to encompass all of the optical pulse. Make your acquisitions.
If *no*, go to 3.
3. Are you using a pulsed source (e.g. a laser or a lamp) that can send a trigger?
If *yes*, select **External Trigger Mode**, or **External Trigger Start**. Connect the trigger output of the pulsed source to the EXT TRIG input of the camera. Set the **Exposure Time** to encompass all of the optical pulse. Make your acquisitions.

The **Exposure Time** (in seconds) is the time during which the EMCCD collects light prior to readout. The system will default to a minimum Exposure Time should you attempt to enter too low a value.

Exposure Time

The system allows you use the EMCCD-chip at the heart of the detector in a number of ways to collect and read out data. See SECTION 4.5 – **SETTING THE ACQUISITION** to view the various Readout Options and Binning Patterns available with this system.

Readout

Frame transfer is a special acquisition mode that is only available if your system contains a Frame Transfer CCD, (FT CCD).

Frame Transfer

A FT CCD differs from a standard CCD in 2 ways. Firstly, a FT CCD contains 2 areas, of approximately equal size. 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. **Note: This is only applicable when the camera is running in Accumulate or Kinetic mode.**

For an explanation of the **Gain** principle of your iXon CCD, please see SECTION 3 - **INSIDE THE iXON CCD**. In the *Setup CCD Acquisition Dialog* box, you can control the level of **Gain** your iXon CCD is operating at. The **Gain** can be increased from 0 (unity i.e. no gain) to a factory set level of 1000 times. The level of **Gain** required can be entered manually in the control box, or you can use the toggle keys to raise or lower the value to the required level.

Gain

You may select a **Vertical Shift Speed** (the speed with which charge is moved down the CCD-chip prior to readout) from a drop-down list box on the *CCD Setup Acquisition Dialog Box*. The **Vertical Shift Speed** you select will affect the minimum exposure and cycle times available. Note: Using a fast **Vertical Shift Speed** can lower the well depth of your CCD, increase noise, and cause a drop in charge transfer efficiency. Please consult the On Line Help for further details on this, including **Vertical Clock Voltage Amp**.

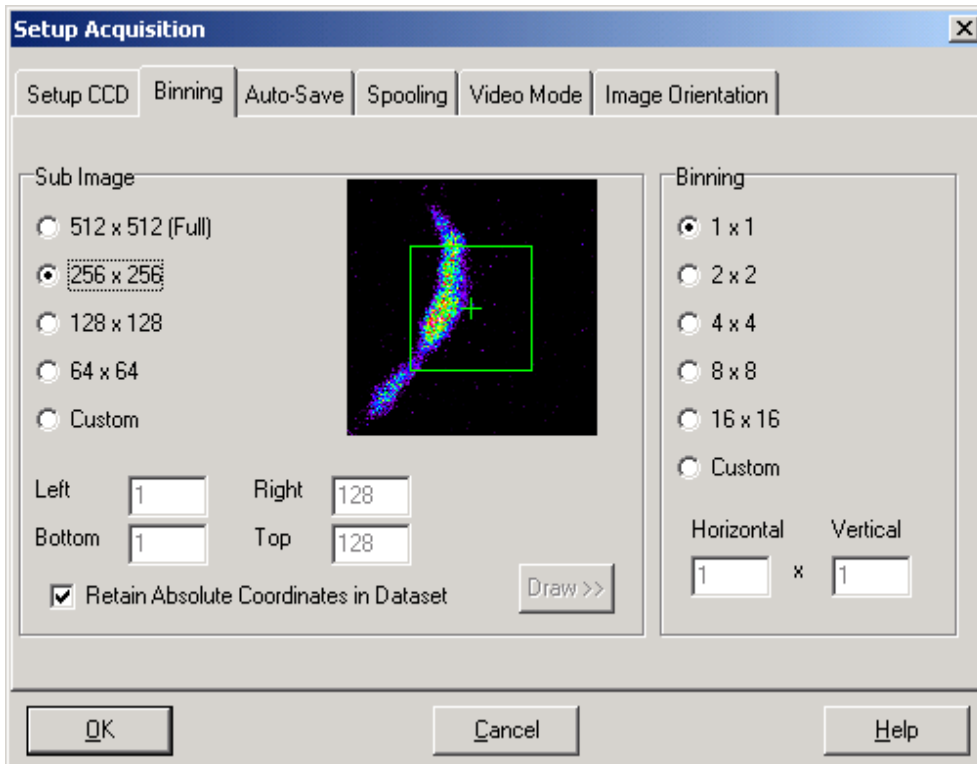
Vertical Shift Speed

The CCD-chip is continually being scanned to prevent its becoming saturated with dark current. Scanning involves moving charge from the CCD-chip into the shift register and then emptying the shift register. If the scan is being used simply to clean the CCD-chip (i.e. it is a keep-clean scan), the charge that is emptied from the shift register is not stored in memory and is, in effect, discarded.

Keep Cleans

While running an Accumulate or Kinetic acquisition, use the Keep Clean Text box to enter a minimum number of Keep Cleans between each scan in the series.

The **Binning** Dialog Box is the adjacent Dialog box to **Setup CCD**. This Dialog box allows you to set and control various **Binning** Patterns, and define **Sub Images** of the iXON CCD.



Binning Dialog Box for iXon 87 CCD (512 x 512 pixels)

By default, taking an Acquisition supplies you with a count from each pixel on the CCD, in effect allowing you to take a picture of the light pattern falling on the pixel matrix of the EMCCD. This default is referred to as a *Full Resolution Image*. The image may be viewed in grayscale or false color, or it may be displayed as 2D or 3D traces.

Sub Image

However, 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. When the iXon is running in **Sub Image** mode only data from the selected pixels will be collected. Data from the remaining pixels will be discarded.

To read out data from a selected area, or **Sub Image**, of the EMCCD use the radio buttons to select the resolution, which you require. The software offers a choice of up to four defined sub images, depending on the format of your iXon CCD camera. These pre-defined sub images are located in the centre of the chip, but can be moved by clicking and dragging the **Sub Image** outline.

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** use the co-ordinate entry Dialog boxes to select the size and location of your sub image.

In addition to the above methods of defining a **Sub Image** on the CCD, 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**. Use the Draw tool to select the size and position of your **Sub Image**. Alternatively, a **Sub Image** can be drawn on the template by positioning your cursor on the image, and dragging out the shape of the **Sub Image** area you require.

Draw

The image that is displayed in the Binning Dialog Box is the last image acquired. If a Region on Interest, or ROI, has been selected it will appear on this image, and you can use your mouse to change the location or size of the ROI. For more detail on using the ROI function see SECTION 4 – **REGION OF INTEREST**.

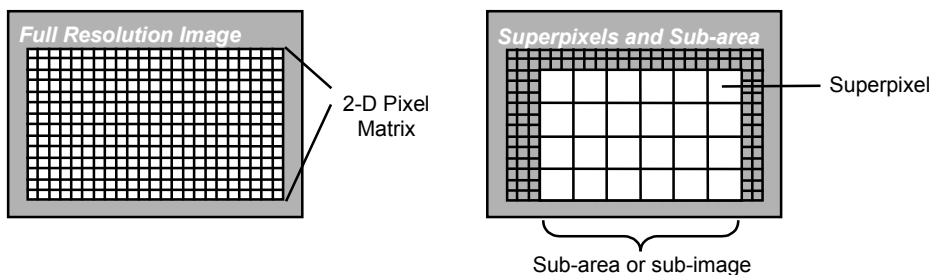
Region of Interest

As well as selecting a **Sub Image** of the CCD, you can also use a system of pixel charge aggregation, known as **Binning**, to create superpixels. Superpixels consist of two or more individual pixels that are binned and read out as one large pixel: the CCD, or your selected sub-area, becomes a matrix of superpixels.

Superpixels

The horizontal and vertical **Binning** parameters determine the dimensions of any **superpixels** you may choose to create.

Binning



The software presents a selection of five of the most common binning patterns; 1x1, 2x2, 4x4, 8x8, and 16x16 pixels. For example, if you enter 4x4 binning the CCD-chip is notionally divided into a matrix of superpixels which each measure 4x4 pixels and provide a signal for readout. There is also an option for you to define a **Custom Binning Pattern**. Check the **Custom** radio button and enter the dimensions of the superpixels (as a number of pixels vertically and horizontally) in the text boxes.

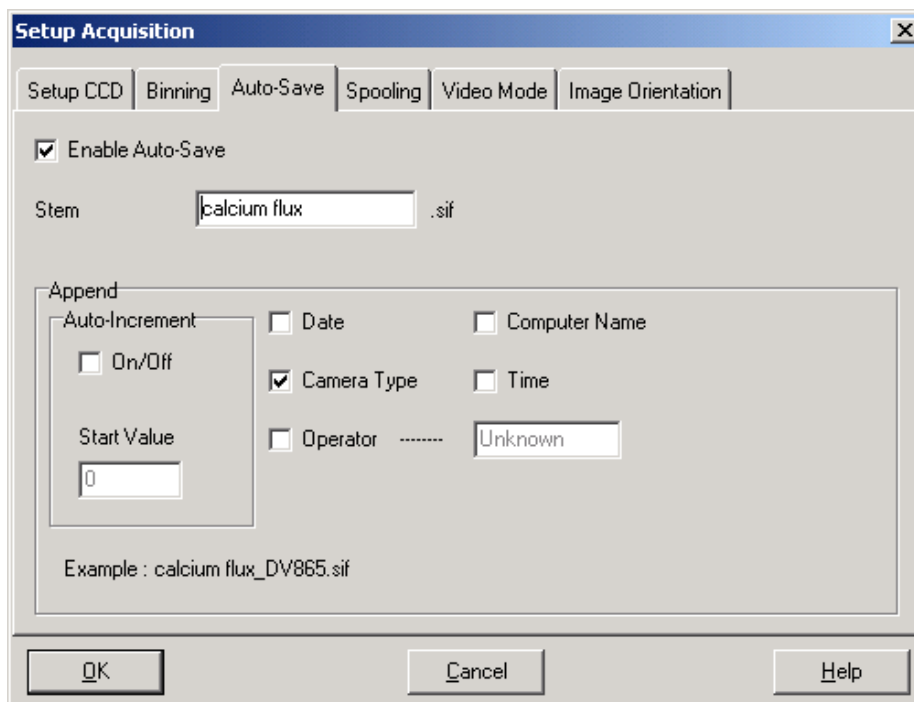
Working with Superpixels

By a process of binning charge vertically into the shift register from several rows at a time (4 rows in the above example, representing the height of your superpixels), and then binning charge horizontally from several columns of the shift register at a time (4 columns in the example, representing the width of the superpixels), the system is effectively reading out charge from a matrix of superpixels which each measure 4x4.

The result is a more coarsely defined image, but faster processing speed, lower storage requirements, and potentially a better **signal to noise ratio**.

Auto Save

The **Auto-Save** Dialog Box is accessed by clicking the **Auto-Save** tab, on the *Setup Acquisition Dialog Box*. This menu allows you to set parameters and controls for the auto saving of acquisition files, removing the worry of lost data and files.



Auto-Save Dialog Box

Enable Auto Save

Auto-Save can be selected by activating the On/Off tick box in the dialog box. If selected, acquisitions will be saved automatically when each one is completed. 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**

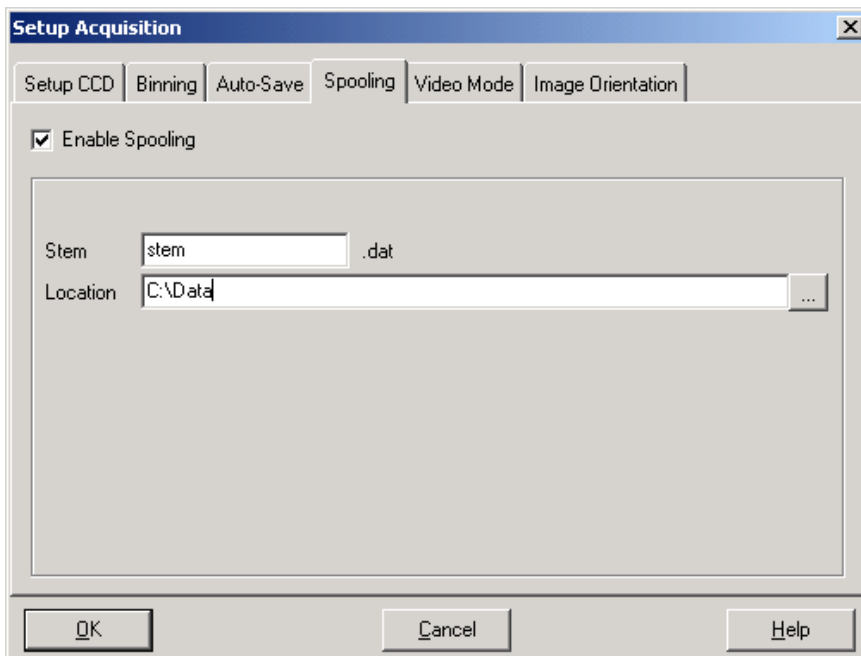
Any combination of these may be selected by activating the relevant tick box.

Please Note: This function will only Auto Save single scan, accumulated, or kinetic images. It will not Auto-Save data acquired under Video Mode.

Spooling

Spooling a computer document or data file is the process of reading it and storing it in a buffer, normally on a hard disk, so it can be printed or otherwise processed at a more convenient time. The Andor MCD software allows you to spool acquisition data direct to the hard disk of your PC. This is particularly useful when taking a large series of Kinetic Images. The amount of data generated by a Kinetic series of say, 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.



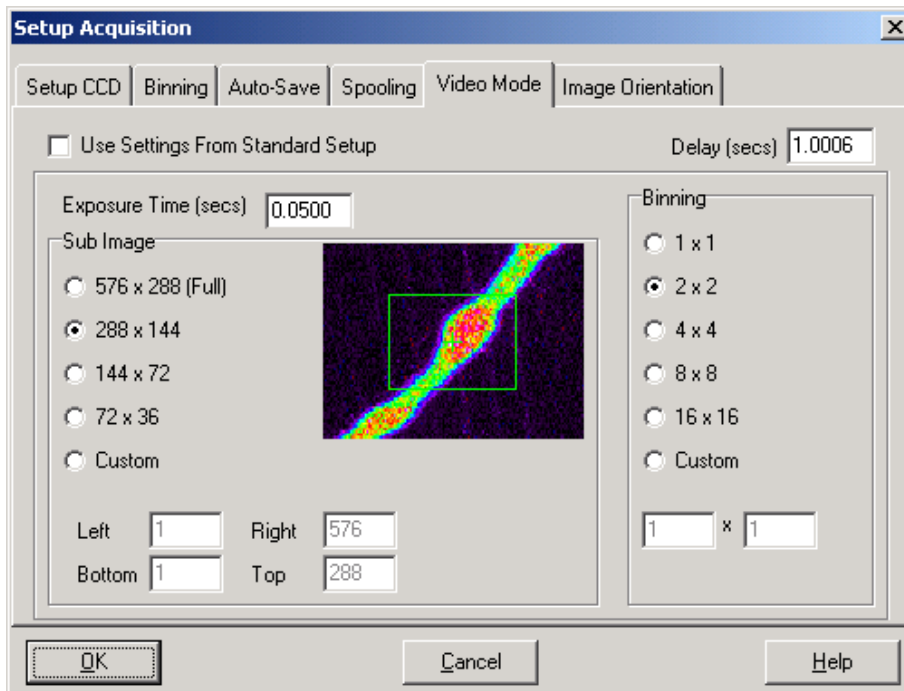
Spooling Dialog Box

To enable the spooling function on your software, check the Enable Spooling button on the **Spooling** Dialog Box. You must also enter a stem name, and also select a location for the for this spooled data file.

Please note: Spooling requires a very fast hard disk, if you are to make full use of this function. We recommend a very fast hard disk for this type of operation.

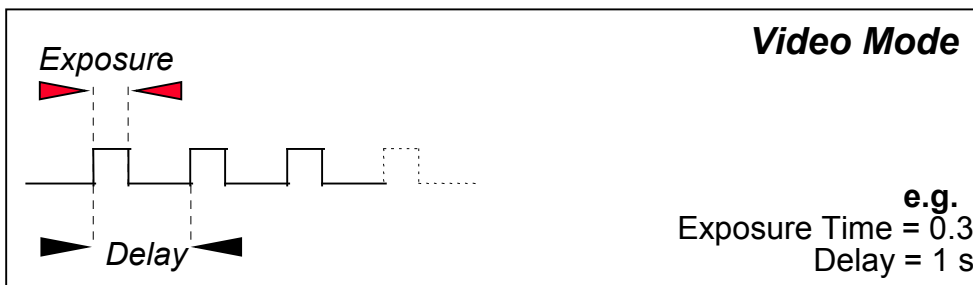
Video Mode

Video Mode is a useful acquisition mode for focusing of your iXon CCD, and for watching experimental events happening in real time. However, this mode will not allow you to save any of the acquired images or data, except for the last frame of the sequence.



Video Mode Dialog Box

By Clicking the **Video** button on the *Main Window*, the system will repeatedly perform a single scan, and update the data display. New data will continue to be acquired and displayed until you press the <ESC> key, or click the *Abort* button on the *Main Window*.



On the *Video Mode Dialog Box* you may enter the following information:

- **Exposure Time** in seconds - i.e. the time during which the EMCCD collects light prior to readout. The system will default to a minimum Exposure Time should you attempt to enter too low a value.
- **Delay** in seconds - i.e. the time you require between scans. The system will default to a minimum delay should you attempt to enter too low a value: in Video Mode the system will actually acquire data at a faster rate than it can be displayed on the monitor.

As with Setup Image, the **Video Mode** Dialog box allows you set and control various Binning Patterns, and define Sub Images of the CCD. These Dialog boxes can only be accessed if the *Use Settings From Standard Setup* radio button is disabled.

Sub Image

By default, taking an Acquisition supplies you with a count from each pixel on the CCD, in effect allowing you to take a picture of the light pattern falling on the pixel matrix of the CCD. This default is referred to as a *Full Resolution Image*. The image may be viewed in grayscale or false color, or it may be displayed as 2D or 3D traces.

However, 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. When the iXon is running in **Sub Image** mode only data from the selected pixels will be readout. Data from the remaining pixels will be discarded.

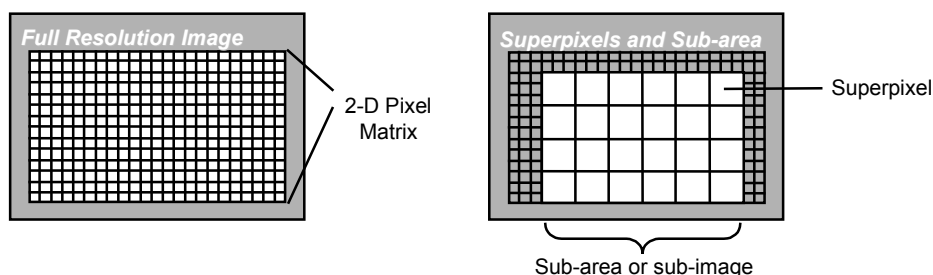
To read out data from a selected area, or **Sub Image**, of the CCD use the radio buttons to select the resolution, which you require. The software offers a choice of three defined sub images: 256 x 256, 128 x 128, and 64 x 64 pixels. There is also an option for you to define a **Custom Sub Image**. This function allows you to set the Sub Image to any size and location on the CCD chip. To define a **Custom Sub Image** use the co-ordinate entry Dialog boxes to select the size and location of your sub image.

As well as selecting a **Sub Image** of the CCD, you can also use a system of pixel charge aggregation, known as **Binning**, to create superpixels. Superpixels consist of two or more individual pixels that are binned and read out as one large pixel: the CCD, or your selected sub-area, becomes a matrix of superpixels.

Superpixels

Binning

The horizontal and vertical **Binning** parameters determine the dimensions of any **superpixels** you may choose to create.



The software presents a selection of five of the most common binning patterns; 1x1, 2x2, 4x4, 8x8, and 16x16 pixels. For example, if you enter 4x4 binning the CCD-chip is notionally divided into a matrix of superpixels which each measure 4x4 pixels and provide a signal for readout. There is also an option for you to define a **Custom Binning Pattern**. Check the **Custom** radio button and enter the dimensions of the superpixels (as a number of pixels vertically and horizontally) in the text boxes.

Working with Superpixels

By a process of binning charge vertically into the shift register from several rows at a time (4 rows in the above example, representing the height of your superpixels), and then binning charge horizontally from several columns of the shift register at a time (4 columns in the example, representing the width of the superpixels), the system is effectively reading out charge from a matrix of superpixels which each measure 4x4.

The result is a more coarsely defined image, but faster processing speed, lower storage requirements, and potentially a better **signal to noise ratio** (since for each element or superpixel in the resultant image, the combined charge from several pixels is being binned and read out, rather than the possibly weak charge from an individual pixel).

Note: A useful mode if you want to capture data as you optimize a hardware set-up, for example.

Image Orientation

The final Dialog box in the *Setup Acquisition Menu* is Image Orientation, which has tick boxes that allow you to flip and rotate image data as they are acquired.

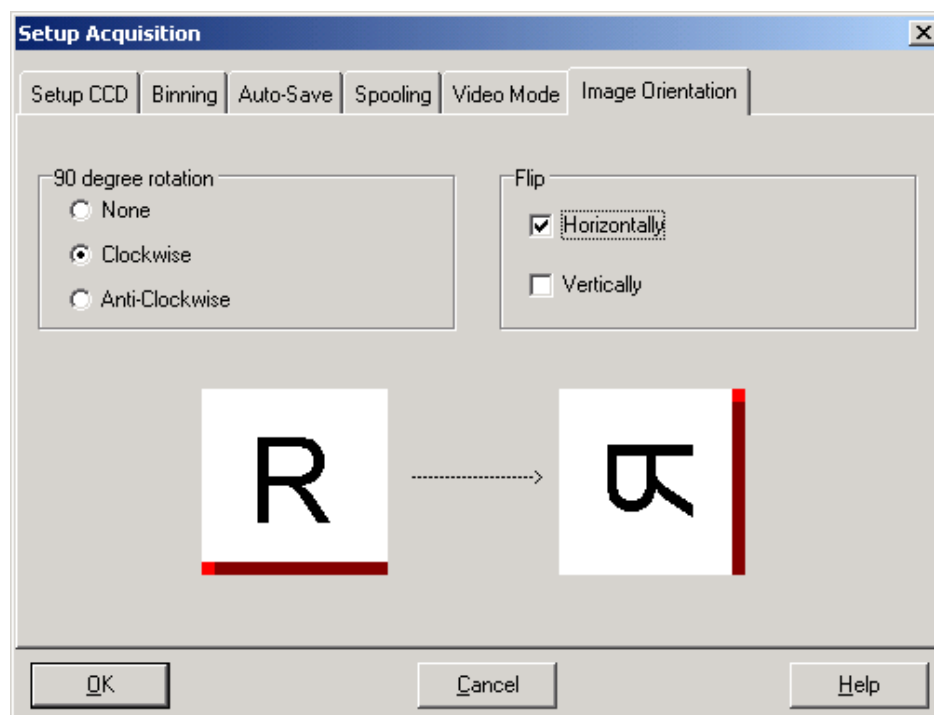


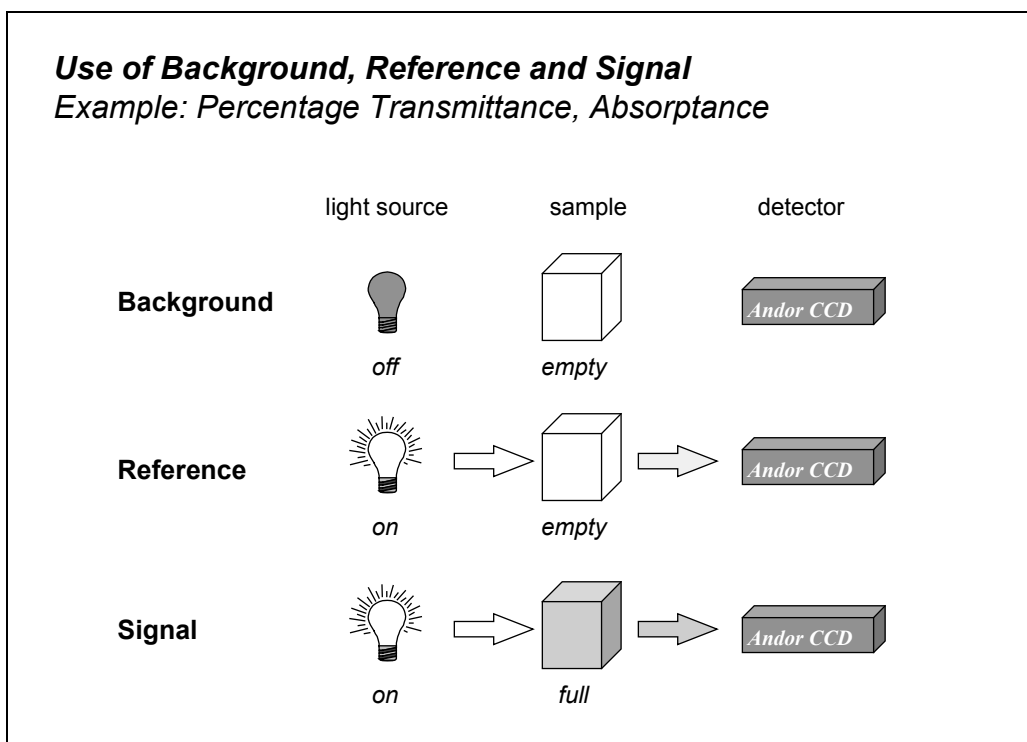
Image Orientation Dialog Box

The Image Orientation Dialog also contains 'R' images, which allow you to easily visualize the various orientation options available.

The *Data Type Dialog Box*, launched from the **Setup Data Type** option on the **Acquisition** Menu, allows you to select the type of data you want the system to compute and display whenever you perform **Take Signal** (see SECTION 4.4 **DATA ACQUISITION**). The acquired data are presented under the *Sig* tab of an *Acquired Data Window* (see SECTION 4.7 **DISPLAYING DATA**).

Use the radio buttons on the *Data Type Dialog Box* to choose the data type you require. For details of the steps needed to calculate certain data types, see **How to Computer Data Types** overleaf.

The illustration below shows typical use of Background, Reference and Signal for computations such as **%Absorbance** or **%Transmittance**.

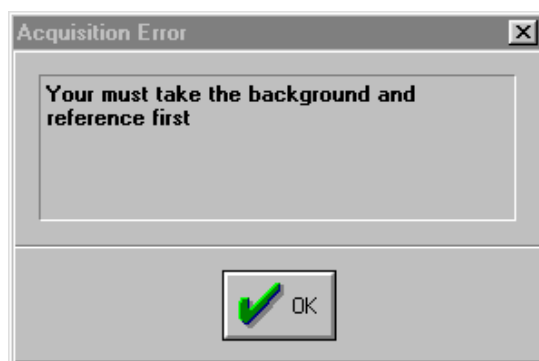


The default data type (used when you capture data and have not explicitly made a selection from the *Data Type Dialog Box*) is **Counts**.

If you select background corrected counts as your data type - **Counts (Bg Corrected)** - you will have to perform **Take Background** before you perform **Take Signal** (see SECTION 4.4 DATA ACQUISITION - Making an Acquisition).

If you select any data type other than Counts or Counts (Bg Corrected) you will have to perform **Take Background** and **Take Reference** (in that order) before performing **Take Signal**.

If you perform an operation ‘out of sequence’, the system will remind you what to do by launching an *Acquisition Error Dialog Box*.



Acquisition Error Dialog Box

Provided you do not change the acquisition parameters, the scans you take for background and reference are automatically used for subsequent data acquisitions whenever you perform **Take Signal**.

The calculations for the various data types assume the following definitions:

- **Signal:** Data in uncorrected Counts, acquired via **Take Signal**.
- **Background:** Data in uncorrected Counts, acquired in darkness, via **Take Background**.
- **Reference:** Background corrected data, acquired (usually for the purpose of computing a material’s reflection, transmission or absorption characteristics) via **Take Reference**. Reference data are normally acquired from the light source, without the light having been reflected from or having passed through the material being studied.

Note: ‘Signal’, as used in the definitions of the calculations, refers to ‘raw’ data from the CCD and should not be confused with the possibly ‘processed’ data to be found under the *Sig* tab of the *Data Window*. See SECTION 4.7 **DISPLAYING DATA.**)

The data types available are listed overleaf.

The data type **Counts** represents raw, digitized data (i.e. no calculations have been performed on the data) from the CCD detector's analog to digital (A/D) converter. Refer to the detailed specification accompanying your particular CCD detector for the number of electrons that correspond to 1 count.

Counts

Counts (Background Corrected) is digitized *Data* from the CCD detector's analog to digital (A/D) converter, where *Background* (or dark signal) has been removed.

**Counts
(Bg. corrected)**

$$\text{Counts (Bg. Corrected)} = \text{Signal} - \text{Background}$$

%Absorptance represents the light absorbed by an object. If *Reference* is the background corrected incident intensity, and *Signal - Background* the transmitted intensity (i.e. the intensity of light which has passed through the material being examined), then:

%Absorptance

$$\% \text{ Absorptance} = 100 \times (1 - (\text{Signal} - \text{Background}) / \text{Reference})$$

Percentage Reflectance represents the light reflected by an object. If *Reference* is the background corrected incident intensity, and *Signal - Background* the reflected intensity (i.e. the intensity of light which has been reflected from the material being examined), then:

%Reflectance

$$\% \text{ Reflectance} = 100 \times (\text{Signal} - \text{Background}) / \text{Reference}$$

Percentage Transmittance represents the light transmitted by an object. If *Reference* is the background corrected incident intensity, and *Signal - Background* the transmitted intensity (i.e. the intensity of light which has been transmitted through the material being examined), then:

%Transmittance

$$\% \text{ Transmittance} = 100 \times (\text{Signal} - \text{Background}) / \text{Reference}$$

Absorbance Units are a measure of light absorbed by an object (i.e. they represent the object's Optical Density (OD)). If *Reference* is the background corrected incident intensity, and *Signal - Background* the transmitted intensity (i.e. the intensity of light which has passed through the material being examined), then $\text{Transmission} = (\text{Signal} - \text{Background}) / \text{Reference}$. Absorbance Units are defined as $\text{Log}_{10} (1 / \text{Transmission})$. Therefore:

Absorbance Units

$$\text{Absorbance Units} = \text{Log}_{10} (\text{Reference} / (\text{Signal} - \text{Background}))$$

The **Absorption Coefficient** indicates the internal absorptance of a material per unit distance (m). It is calculated as $-\log_e t$, where t is the unit transmission of the material and \log_e is the natural logarithm.

Absorption Coefficient
(/m)

If *Reference* is the background corrected incident intensity, and *Signal - Background* the transmitted intensity (i.e. the intensity of light which has passed through the material being examined), then $\text{Transmission} = (\text{Signal} - \text{Background}) / \text{Reference}$, and:

$$\text{Absorption Coefficient} = -\log_e ((\text{Signal} - \text{Background}) / \text{Reference})$$

Attenuation is a measurement, in decibels, of light absorbed due to transmission through a material - decibels are often used to indicate light loss in fiber optic cables, for instance. If *Reference* is the background corrected incident intensity, and *Signal - Background* the transmitted intensity (i.e. the intensity of light which has passed through the material being examined), then

Attenuation (dB)

$$\text{Attenuation} = 10 \times \log_{10} ((\text{Signal} - \text{Background}) / \text{Reference})$$

Data * Ref allows you to ‘custom modify’ the background corrected signal:

Data * Ref

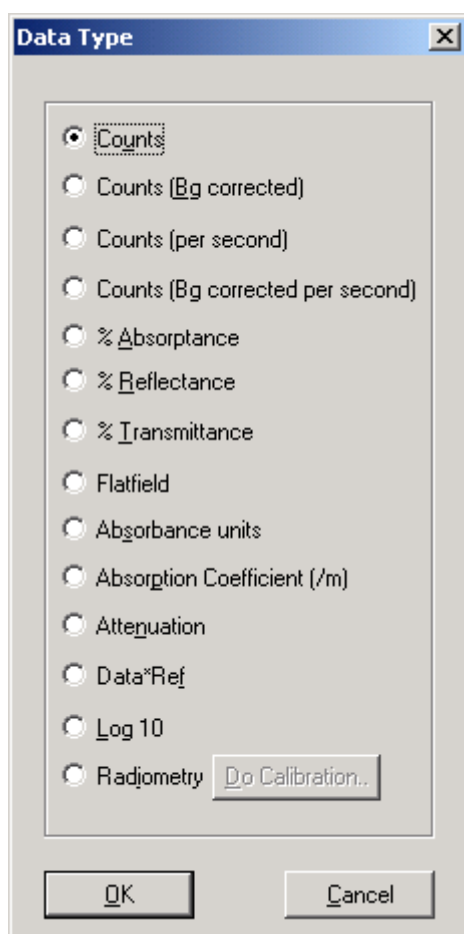
$$\text{Data} \times \text{Ref} = (\text{Signal} - \text{Background}) \times \text{Reference Store Value}$$

See the **Andor Basic** Programming Manual for similar operations.

Log₁₀ simply calculates the logarithm to the base 10 of the background corrected signal counts.

Log₁₀

$$\text{Log Base 10} = \log_{10} (\text{Signal} - \text{Background})$$



Data Type Dialog Box

Functionality for displaying and manipulating data is only available if a Data File has been opened, or if data have been newly acquired, or if you are using the Andor Basic programming language to create a new window in which to display data. In each case data are displayed in a *Data Window*. See also:

SECTION 4.3 HANDLING FILES

SECTION 4.4 DATA ACQUISITION - Making an Acquisition

SECTION 4.9 USING THE COMMAND LINE

SECTION 6 WORKING WITH ANDOR BASIC PROGRAMS

(The Programmer's Guide to Andor Basic gives details of how to use the programming language).

The **Display Menu** offers the following functionality associated with the *Display Window* (the item **Change Display Mode** later in this section also details the buttons associated with displaying data):

Display

Change Display Mode

Add Data Window

Preferences

Axis Setup

Sequence Option

Rescale Data Mode

Data Histogram

Scale to Active

ROI (Region of Interest)

The menu item **Rescale Data Mode** in turn provides five submenu items that allow you choose whether the range of your displayed data covers values between zero and maximum (0...max); between minimum and maximum (min...max); between zero and 65535 (0...65535); between min and 65535; and a Custom option. For a full discussion of this functionality see **Rescale** later in this section, or consult the Online Help.

The menu item **Scale to Active** is only available if you are in **2D** Display Mode and have chosen to overlay a number of traces: see **Overlay** later in this section.

For more details on data displays see **The Data Window** next. For more details on the use of x-, y-, and data-axes in data displays see **Axes** later in this section.

The Data Window

A *Data Window* is launched automatically if you access data that you have previously stored, if you make a new data acquisition, or if you use the **Andor Basic** programming language to create a new *Data Window*.

Newly acquired data will always be displayed in a *Data Window* labeled ‘#0 Acquisition’. This is in effect the ‘live’ *Data Window*. In the **User’s Guide**, this type of *Data Window* is often referred to as an *Acquisition Window*.

Should you decide to save data from the *Acquisition Window*, the *Acquisition Window* will remain on screen and a new *Data Window*, labeled with your new filename, will appear. As with all *Data Windows*, this new *Data Window* is marked with a number, #n, which uniquely identifies at any given time the data set that is being displayed or manipulated in the *Data Window*. The value of #n is temporary (it ceases to be associated with the data when they are no longer being displayed) and its value depends on the order in which you open and close your data sets.

#n is particularly important for identifying sets of data that have not been saved to file and whose data set name is simply ‘Untitled Document’ - a *Data Window* and its contents can, for example, be created directly by means of **Andor Basic**. Moreover, #n remains constant for a given set of displayed data even if you create several views of the data in separate *Data Windows* (see **Add Data Window** later in this section). These multiple views are differentiated by a :x suffix to the data set name, where x is a number.

When you retrieve data which you have previously stored, they will be displayed in a *Data Window* labeled #n and bearing the name of the retrieved file. Along the

bottom of the *Data Window* you will see up to three tabs, labeled **Sig** (Signal), **Bg** (Background), and **Ref** (Reference) which (where appropriate) identify data acquired via the functions **Take Signal**, **Take Background**, and **Take Reference** respectively. Click on the appropriate tab to display the trace you require.

In descriptions of the *Data Window* and in on-screen captions the terms **x-**, **y-**, and **data-axis** are used as follows:-

Axes

- The **x-** and **y-axes** define a pixel's position on the two-dimensional CCD-chip.
- The **x-axis** (or horizontal display axis) corresponds to the breadth of the CCD-chip, and may be calibrated in pixels or in a unit of your choice.
- The **y-axis** corresponds to the height of the CCD-chip and is calibrated in pixels. No y-axis is shown in **2D** Display Mode. In **3D** Display Mode, the y-axis is shown as a diagonal, to lend perspective to the display. In **Image** Display Mode, the y-axis is the vertical display axis.
- The **data-axis** is calibrated in the unit of your choice and has data values plotted against it - see SECTION 4.6 **CHOOSING THE DATA TYPE**. In **2D** and **3D** displays the data-axis is the vertical display axis. In **Image** Display Mode, data are represented by a color or a grayscale tone.

In all display modes, **x-** and **data-**values are displayed on the status bar along the bottom edge of the *Data Window*. When data have been acquired in a mode other than Full Vertical Binning, y-values are also displayed in the status bar.

For a description of acquisition modes, see SECTION 4.5 **SETTING THE ACQUISITION** - Readout Modes.

Change Display

Change Display Mode

The system can display data in a number of ways. The display modes may be accessed from the **Change Display Mode** option on the **Display** Menu or from the corresponding buttons on the *Main Window*, once data have been acquired or retrieved.

The following Display Modes are available: **Image, 2D, 3D**

Image



Data acquired in an imaging mode (see SECTION 4.5 **SETTING THE ACQUISITION**) may be viewed as an image in a *Data Window*. Select the **Image** option on the **Display** Menu or click the **Image** button. For best results you should set your computer display to 256 colors: to remind you, a large X appears across the image if you have your computer configured for less than 256 colors mode. Adjust the color or brightness using the controls associated with the **Palette Bar** (see below).

Reading Off Data Values

The cross-hair (see also **Large Cursor** and **Show 2D Cross Sections** under **Preferences** later in this section) moves to any point on the image that you click. The data value for the point, along with the pixel numbers on the x- and y-axes that identify the point, is displayed on the bottom edge of the *Data Window*.

If your data set contains a kinetic series, each member of which has been acquired in an imaging mode, you will notice a vertical scroll bar that allows you to move between the images that make up the series. The number of the member of the kinetic series is also displayed on the bottom edge of the *Data Window*.

Sub Image Mode

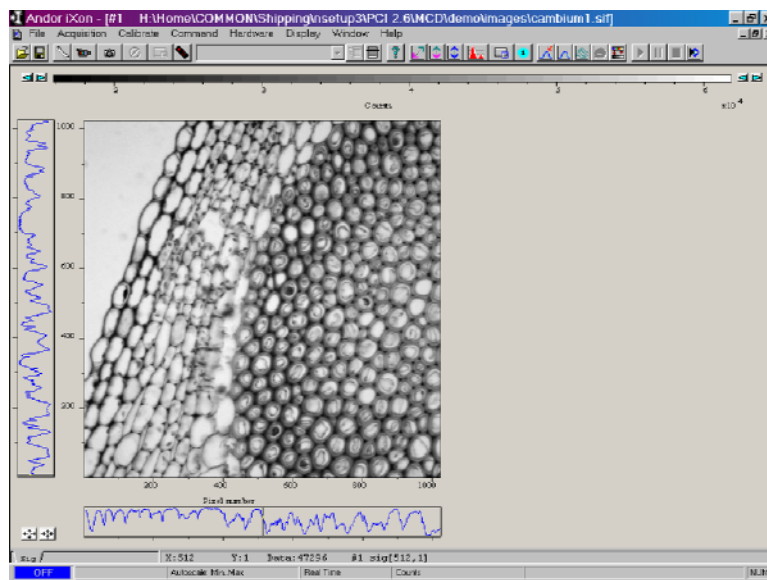


Clicking the **Sub Image** Mode button on the main task bar will launch a Dialog box, with a choice of three options; **Full Image**, **Cancel**, or **Store**. This tool allows you to click and drag a Sub Image on the #0 Window only. This Sub Image region will then be used in subsequent acquisitions. This is an identical function to Draw (See SECTION 4.5 **SETTING THE AQUITION** – Sub Image). Use the Sub Image Mode to select the size and position of your **Sub Image**, by dragging rulers from the X and Y-axis. Alternatively, a **Sub Image** can be drawn on the #0 Window by positioning your cursor on the image, and dragging out the shape of the **Sub Image** area you require.

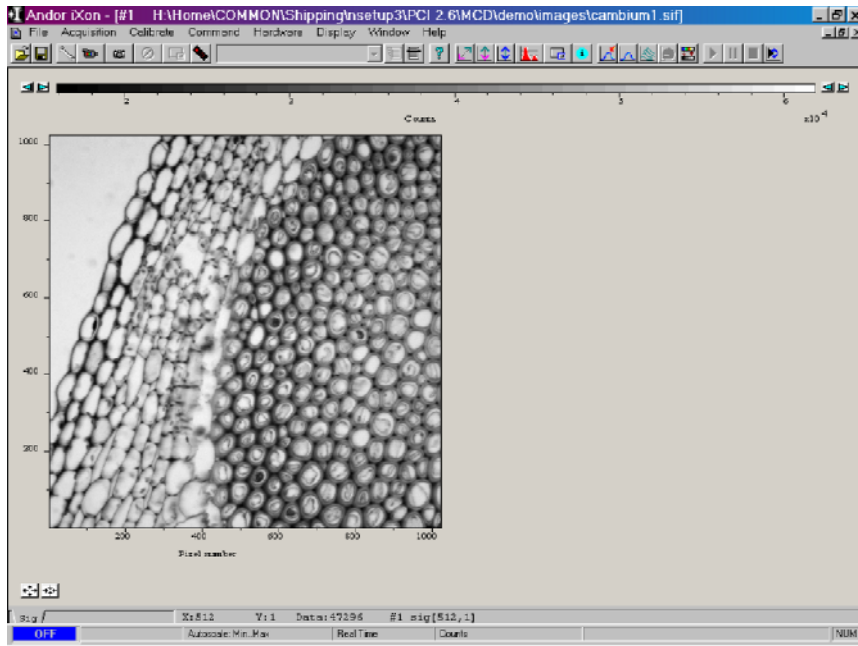
Selecting Color / Grayscale



The display is initially grayscale. Clicking on the Palette button will cause the *Data Window* to cycle through the color modes: **Color**; **Iterated Grayscale** (a small sequence of grays is repeated at intervals to cover the same range of data as grayscale); **Grayscale**, etc.



Data Window (Grayscale Image with side traces)



Data Window (Grayscale Image without side traces)

A **Palette Bar**, running across the top of the window, always shows the full palette of grays or colors available in each color mode. The palette is graded so that lower data values correspond to the darker tones to the left of the palette, and higher data values correspond to the brighter tones to the right of the palette. The scale on the Palette Bar is calibrated in your chosen data units (see SECTION 4.6 **CHOOSING THE DATA TYPE**). By default, the full range of colors/grays is correlated with the full range of data in your data set. This is known as ‘rescaling’ (see Rescale below).

The Palette Bar



Adjusting the Brightness and **Increasing and Decreasing Contrast** later in this section explain how you can use these controls to change the brightness and contrast of the display.

The arrows to the left of the Palette Bar on the *Data Window* allow you to adjust the minimum distinguishable data value (i.e. **min**, the value at which the Palette Bar becomes black); the arrows to the right of the Palette Bar on the Data Window allow you to adjust the maximum distinguishable data value (i.e. **max**, the value at which the Palette Bar becomes white).

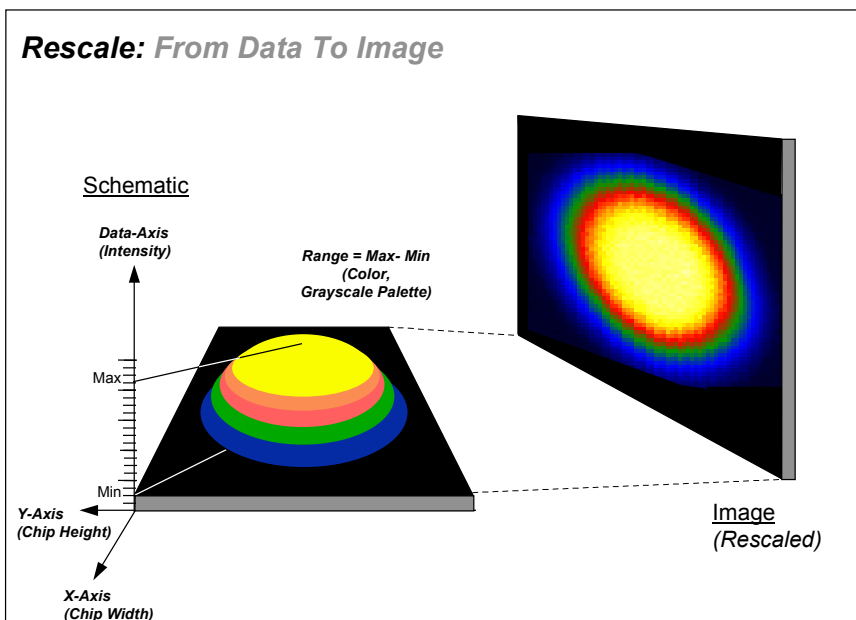
Clicking the **Rescale** button on the button bar of the *Main Window*, when a *Data Window* is open, causes the system to display against an appropriate data scale (and, in the case of **Image Display Mode**, in appropriate colors or grayscale tones) all data that falls within your selected range on the x-axis.

Rescale

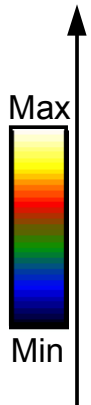


In addition the **Rescale Data Mode** item on the **Display** Menu allows you to select (by means of two further ‘tickable’ sub-menu items) whether your rescaled data distinguishes values between the minimum and the maximum (min...max); between zero and the maximum (0...max); or between 0 and 65535 (0...65535), the maximum number of counts that can be recorded for a single pixel. **Autoscale Acquisition** (see SECTION 4.4 **DATA ACQUISITION - Making an Acquisition**) performs a similar function for the display of data as they are being acquired.

Rescale is available for all the Display Modes. The following comments take rescaling in Image Display Mode as a specific example. Image Display Mode is used to display the members of a Kinetic Series as an image (each member of the series forms a row of the image.)



Data-Axis



Rescale

The computer ‘rescales’ your display by default: the full available palette of colors/grays is correlated with the full range of data you have acquired.

When it rescales in Image Display Mode, the computer finds the maximum (**Max**) and minimum (**Min**) data values, and scales the available number of colors to the range, where the **Range = Max - Min**. Alternatively, depending on the tickable rescale submenu item you have selected (see above), the range may be between 0 and max, or between 0 and 65535.

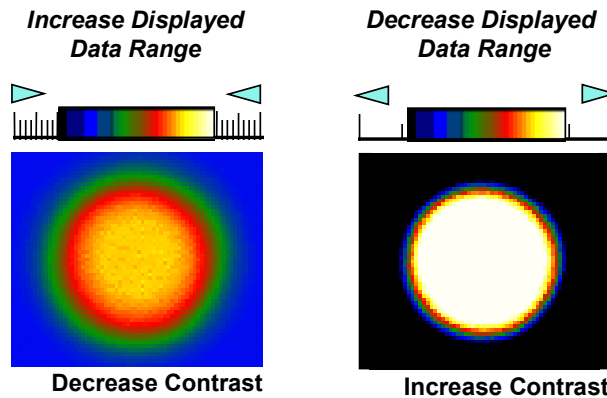
For various reasons (for example, a cosmic ray may produce a data ‘peak’ that is unrepresentative of data levels generally), rescaling may not adequately illustrate the features of the data you are most interested in. To emphasize or de-emphasize particular features, you can adjust the contrast and/or the brightness of the display. See **Increasing and Decreasing Contrast** and **Adjusting the Brightness** below.

Increasing and Decreasing Contrast

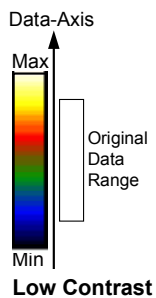
You can use the pairs of left-right arrows at either end of the Palette Bar to increase or decrease the displayed data range (i.e. 'shrink' or 'stretch' the scale on the palette) and thereby alter the contrast of the data shown in Image Display Mode.

Increasing the displayed data range ('shrinking' the scale) decreases the contrast.

Decreasing the displayed data range ('stretching' the scale) increases the contrast.



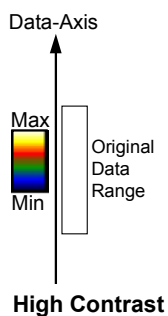
High and Low Contrast: Overview



Assume your original data are 'rescaled' (see Rescale above).

To achieve **low contrast**, the computer scales a larger range of data with the same number of colors as it used for rescaling. Thus the original data are represented by a smaller range of colors.

Increasing the displayed data range produces low contrast.



To achieve **high contrast**, the computer scales a smaller range of data with the same number of colors as it used for rescaling.

Any data value greater than the maximum in the displayed data range is represented by the brightest color (white); any data value lower than the minimum in the displayed data range is represented by the darkest color (black).

Now only a fraction of the original data range is represented by the total number of colors.

Decreasing the displayed data range produces high contrast.

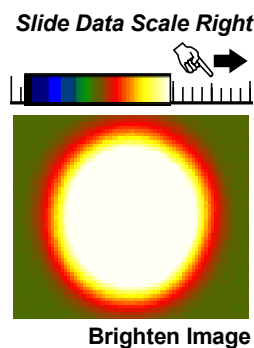
By holding down the left-right arrows at either end of the **Palette Bar** you can alter the minimum and maximum values in the displayed data range independently of each other, and so adjust the contrast to the level you require. As you hold down the arrows (place the cursor over an arrow button and hold down the primary mouse button), observe carefully how the scale changes and how the displayed data range is affected. If you wish to undo your changes, use **Rescale**.

Note: When the left-right arrows are held down for a time, you will see the display flash periodically as the system performs the contrast adjustment. These flashes occur as the system refreshes the color palette to cover your new displayed data range. Hold the arrows down until you reach your required contrast level.

Adjusting the Brightness

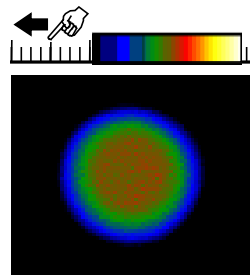
You can alter the **brightness** of the data shown in Image Display Mode by moving the displayed data range so that it covers higher or lower data values. To move the displayed data range, place the cursor on or just below the Palette Bar (the cursor becomes a 'finger') and, holding down the primary mouse button, 'slide' the scale on the palette to the left or right.

Sliding the scale to the right means that the displayed data range covers lower data values (the numbers on the scale decrease). The display becomes brighter.



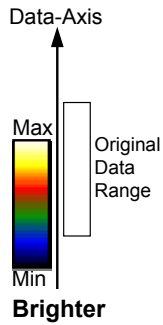
Sliding the scale to the left means that the displayed data range covers higher data values (the numbers on the scale increase). The display becomes darker.

Slide Data Scale Left

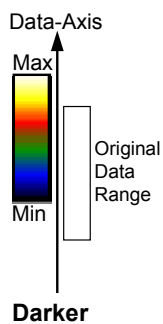


Darken Image

Note: When you slide the scale, you will see the image flash periodically as the system performs the brightness adjustment. These flashes occur as the system refreshes the color palette to cover your new displayed data range. Continue to slide the scale until you reach your required brightness level.



To make the data shown in Image Display Mode **brighter**, the computer uses the same number of colors (as it used for rescaling) to scale lower data values. Thus any data originally represented by darker colors are now represented by brighter colors.



To make the data shown in Image Display Mode **darker**, the computer uses the same number of colors (as it used for rescaling) to scale higher data values. Thus any data originally represented by brighter colors are now represented by darker colors.

If you wish to undo your changes, use Rescale.

Images and Displayed Data Ranges: Overview

In Summary...

- Larger displayed data range: less contrast
- Smaller displayed data range: greater contrast
- Displayed data range covers higher values: darker display
- Displayed data range covers lower values: brighter display

The **Add Data Window** item on the **Display** Menu opens a submenu from which you may create additional views of data that are already on screen in an active *Data Window*. Each of the items on the Add Data Window submenu (see below) launches an additional *Data Window* in which the active data are presented in the display mode of your choice.

Add Data Window

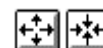
Each additional *Data Window*, which bears the same # number as the active Data Window used to create it, is further identified by an extension (:2, :3, etc.) to the title on the window's title bar. The original Data Window adopts the extension :1. The original and the additional Data Window(s) let you see and manipulate the same underlying set of data: for example, making a new acquisition (see SECTION 4.4 **DATA ACQUISITION - Making an Acquisition**) while a 'family' of Acquisition Windows is on screen, will cause the display in each Acquisition Window to be updated.

Additional Data Windows are available, from the **Add Data Window** submenu, in the following Display Modes: **2D, 3D, Image**.

Preferences for Image Display Mode

If **Always Maintain Aspect Ratio** is selected on the *Display Preference Dialog Box*, special 'fixed aspect' **Zoom In** and **Zoom Out** buttons appear on the *Data Window*. Clicking these buttons causes the scales on both the vertical and horizontal display axes to stretch or shrink proportionately to one another, giving the impression of zooming in towards or zooming out from the image, while maintaining the original proportions of the image.

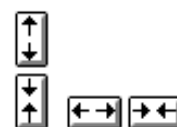
Always Maintain Aspect Ratio



Zoom In / Zoom Out

If **Always Maintain Aspect Ratio** is deselected, you will see separate **Zoom In** and **Zoom Out** buttons for both the vertical and the horizontal axes. The buttons allow you to stretch or shrink the scale on either the x- or y-axis, creating the effect of Zooming In or Zooming Out in either the vertical or horizontal dimension. See also **Zooming In, Zooming Out and Scrolling** previously.

Not Always Maintain Aspect Ratio



Zoom In / Zoom Out

You may also 'zoom' an image by drawing a **Zoom Box** (hold down the primary mouse button and pull the cursor in a diagonal across the screen) around the area that you are interested in. Release the mouse button to perform the zoom operation. The image will zoom to show the selected area in greater detail. The minimum 'zoomable' area is 30 x 30 pixels. If you have selected Always Maintain Aspect Ratio, you may find that an area slightly larger than the zoom box has been

Zoom Box

expanded: the system adds extra area as necessary so that the zoomed image accurately represents the height to width ratio of the individual pixels on your CCD-chip.

Note: To help the system zoom the area you require, draw the Zoom Box in similar proportions to the height and width of the image display.

If **Show 2D Cross Sections** is selected on the *Display Preference Dialog Box*, 2D **Side Traces** are displayed parallel to the vertical and horizontal edges of the display area.

Show 2D Cross Sections

The ‘long edge’ of each Side Trace is calibrated in the same units as the corresponding edge of the display. Plotted against the unmarked ‘short edge’ of the vertical and horizontal Side Traces are the data values taken (respectively) along a vertical or horizontal line running through the cross-hair. (See also **Large Cursor** below.)

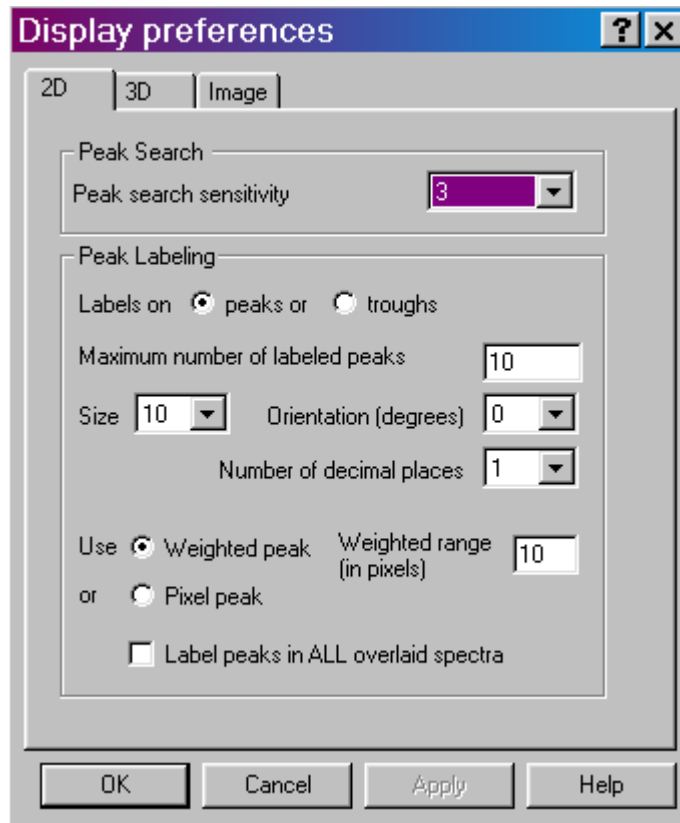
Note: If you are displaying data in Image Display Mode and resize the Data Window, so that it occupies only a small screen area, the system removes the Side Traces and the Zoom In and Zoom Out buttons. In this ‘display only’ mode the Zoom Box is also disabled. ‘Display only’ is of benefit if you want to review many Data Windows simultaneously in Image Display Mode.

If the **Large Cursor** option is selected on the *Display Preference Dialog Box*, the cross-hair runs the full height and width of the display, making it easier to identify the corresponding points on the Side Traces (see **Show 2D Cross Sections** above).

Large Cursor

Show Palette Bar causes the black and white or false color palette to be displayed. Use the arrow buttons at either end of the palette bar to adjust the manner in which values are mapped against colors, and so change the brightness and contrast of the image

Show Palette Bar



Display Preferences Dialog Box (Image Preferences)

Axis Setup

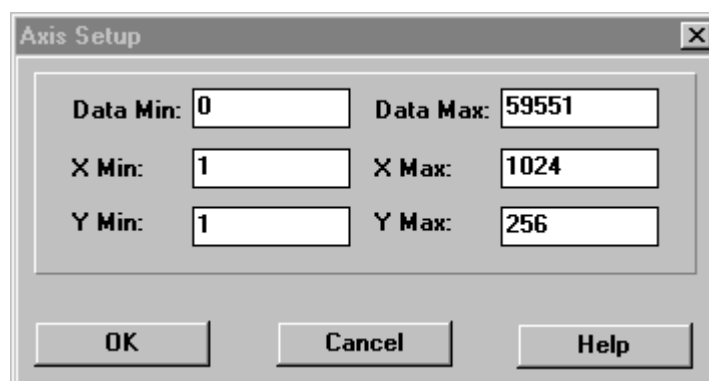
Clicking the **Axis Setup** option on the **Display** Menu launches an *Axis Setup Dialog Box*.

If you select Axis Setup while you are viewing data in **2D** or a **3D** Display Mode, you will be able to enter, in text boxes, the maximum and minimum values you wish to appear on the x- and data-axes (the horizontal and vertical display axes respectively) of your *Data Window*.

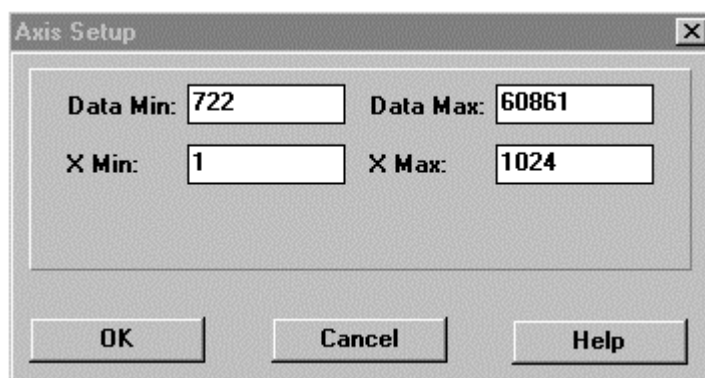
If you select **Axis Setup** while you are viewing data in **Image** Display Mode (see above), you will additionally be able to enter, in text boxes, maximum and minimum values for the y-axis (the vertical display axis) of your *Data Window*. (In a full resolution image, data are represented as a color or a grayscale tone.)

You can enter maximum and minimum x- and y-values of your choice, provided those values (when converted to pixels) do not exceed the width or height of the CCD-chip. However, if you have selected **Always Maintain Aspect Ratio** under Image **Preferences** (see previously), the system may have to resize the ‘plotting region’ in which the image appears: the plotting region then generally occupies a less of the available window space, but the aspect ratio is maintained.

Note: If you want the system to use the maximum available window space, either resize the Data Window or click the Reset button.



Axis Setup Dialog Box (for Image Display Mode)



Axis Setup Dialog Box (for 2D and 3D Display Mode)

The **Data Histogram** Dialog Box can be launched by clicking on the icon on the *Main Window*, or through the **Display** drop down menu. This tool allows you to plot a histogram, or graph, between the maximum and minimum data points in the displayed range. The mode options allow you to select from a number of pre-defined automatic scaling modes, which will assist in setting the correct display range for your experimental set up.

Data Histogram



In **2D** Display Mode the system displays data as an unlabeled trace. To label peaks automatically, click **2D with peak labeling**. While working in labeled mode, you may label a peak manually by double clicking it. To remove a peak label, double click the labeled peak. If you switch off peak labeling, by clicking the 2D button, your manual labeling will be lost. To manually label peaks accurately it is best to **zoom in** on the trace – see below. To change the way in which peaks are labeled and the manner in which you move the cursor between them, use the *Display Preferences Dialog Box*, accessed via the menu bar **Display...Peak Preferences** (see later in this section).

2D



2D with Peak Labeling

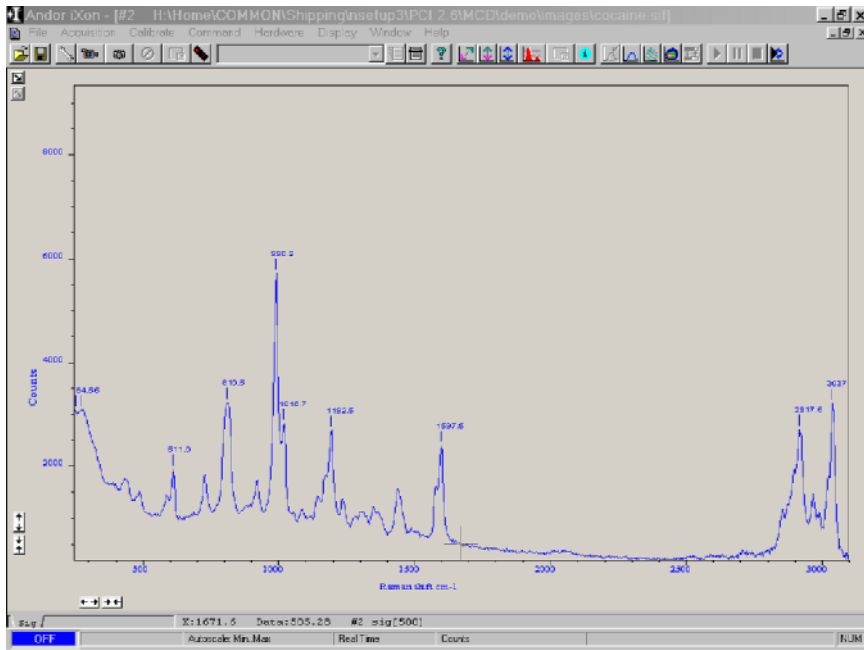


By default, the x-axis will be calibrated in **pixels** ('1' on the x-axis corresponds to the position of the first column of pixels on the CCD-chip, and so on.) The data-axis will by default be calibrated in **counts**.

If you have acquired data in an imaging mode (See SECTION 4.5 **SETTING THE ACQUISITION**), you will be able to view the data from each track on the CCD-chip (or row if you have acquired a **Full Resolution Image**). To view the traces from each track or row individually, use the scroll bar on the *Data Window*. The numeric display on the bottom edge of the *Data Window* will indicate which track or row you are currently viewing.

Note: If there is only one track of data, no track or row number will be displayed, nor will there be a scroll bar.

If you have acquired data as a Kinetic Series, you may also use the scroll bar to move between the members of the series. The display on the bottom edge of the *Data Window* will indicate which member of the series you are currently viewing.



Data Window (2D Display Mode with Peak Labeling) / Display Controls

To read off a data value, click on the trace to position the cursor on the point of interest (you may need to use the left and right arrow keys on your keyboard to position the cursor precisely). The numeric display on the status bar along the bottom edge of the *Data Window* will indicate the corresponding **x**- and **data**-values.

The following functionality is associated with *Data Windows* in 2D Display Mode:

Zooming In, Zooming Out and Scrolling

On a *Data Window* (see above) in **2D** or **3D** Display Mode, pairs of **Zoom In** and **Zoom Out** buttons are provided on both the x- and data-axes of the trace. The buttons allow you to stretch or shrink the scale (to cover a smaller or larger range) on either the x- or data-axis, creating the effect of zooming in or zooming out in either the vertical or horizontal dimension of the display.

Zoom In



Zoom Out



In 2D Display Mode you may also zoom a trace by drawing a **zoom box** (hold down the primary button and pull the cursor in a diagonal across the screen) around the portion of the trace that you are interested in.

Zoom Box

The left and right edges of the zoom box demarcate the range of values that, after zooming, will be shown across the full width of the x-axis; the top and bottom edges of the zoom box demarcate the range of values that will be shown over the full height of the data-axis. Having drawn the zoom box, release the mouse button to perform the zoom operation. The minimum 'zoomable' width is 30 pixels.

Note: You may wish to perform a Rescale (see later in this section) on data you have just zoomed. Rescale will plot all recorded data values that fall within the new, zoomed range of the x-axis against a newly calibrated data-axis. In this way you will be able to see peaks and troughs that may have been clipped off by your zoom box.

See **Preferences** later in this section for details of additional zoom functionality when you display data as an image.

If you have stretched a scale by zooming, you may 'slide' the scale to cover a different range - the display will Scroll in synchrony with the moving scale. Place the cursor arrow over the scale so that it changes to a finger flanked by arrows: now depressing the primary mouse button allows you to 'slide' the scale up and down (or left and right) and scroll the display.

Scrolling

If you place the finger cursor at either end of an axis you will notice that a single arrow appears beside it, indicating the direction in which the scale will slide automatically when you depress the primary mouse button: the display fast scrolls accordingly.

Clicking the Reset button on the button bar of the *Main Window*, when a *Data Window* (see above) is open, returns the displayed data to its original configuration - undoing any adjustment to scale that you may have performed in accordance with the descriptions given in **Zooming In, Zooming Out and Scrolling** above and **Rescale** later in this section. Reset is available for all the Display Modes.

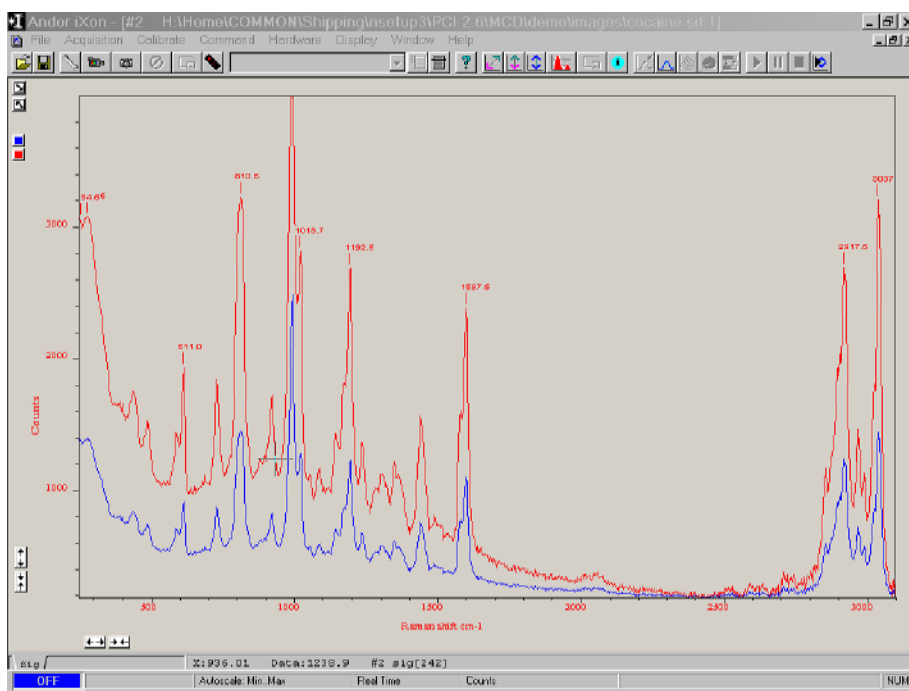
Reset



Overlay allows you to display up to nine **2D** traces simultaneously in the same *Data Window* (see previously), on the same set of axes.

Overlay

The *Data Window* in which you intend to display the overlaid traces must be in **2D** Display Mode. Only the data that was originally in that *Data Window* can be saved or modified when the *Data Window* is active - you cannot, for instance, use the *Data Window* to calibrate traces that have been added as overlays. The *Data Windows* from which the 2D overlays are taken may be in any display mode.



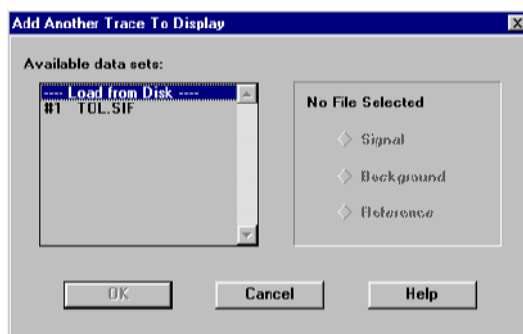
Data Window (2D Display Mode, with overlaid traces)

To add an overlay, click the **Add Overlay** button on the *Data Window*. An 'Add Another Trace to Display' Dialog Box appears on screen. The selection list displays the names of data sets that are already being displayed in a *Data Window*, or you may select data that you have previously stored to disk (a standard Dialog box lets you select the file). Buttons let you specify **Signal**, **Background**, or **Reference** data, if these are contained in the data set you have selected for overlay.

Add Overlay



You may add up to eight overlays to your original data trace. The original data trace is always displayed in blue. Each overlay appears in a unique identifying color.



'Add Another Trace To Display' Dialog Box

Use the color-coded **Active Trace** buttons to choose the trace you want to be able to manipulate in the *Data Window*. The values on the horizontal and vertical axes will change to correspond to the active trace (and will be presented in the same color as the trace itself). Once active, a trace can be manipulated using the **Zoom In**, **Zoom Out**, **Rescale** and **Reset** buttons in the same manner as any **2D Display**.

Active Trace



If you want to remove an overlay, first make sure that it is the active trace (when a trace is active, the values on the display axes match it in color). If necessary use the Active Trace buttons to select a new active trace. Now, to remove the overlay, click the **Remove Overlay** button.

Remove Overlay



The **Overlay and Keep** feature is used only with 'live' data acquired into the *#0 Acquisition Window*. If you have just acquired data that you think you might want to compare with subsequent data, click Overlay and Keep. In the *Acquisition Window* you will see the 'live' trace – which is always colored blue – along with an overlaid copy. In addition, you will see a new *Data Window* containing the selected data. The new *Data Window* is minimized at the bottom of the screen. At a later stage you may choose to save the contents of this new *Data Window* to file.

Overlay and Keep



If you have overlaid a number of traces, the **Scale to Active** item becomes available on the **Display** Menu. Selecting Scale to Active means that all the data traces in your *Data Window* will be plotted against the scales of the active trace. Vertical axes will be rescaled even if the units do not match those of the active trace.

Scale to Active

The ability to overlay data traces is useful if you wish to compare several traces on the same axes. You can even overlay traces from the same data set: you might, for example, want to view different parts of the data in different levels of detail by **Zooming In, Zooming Out and Scrolling** (see previously) and by using **Rescale** (see later in this section).

A Note on Overlays

If you have acquired data in an imaging mode or as a kinetic series (see SECTION 4.5 **SETTING THE ACQUISITION**), you may view the traces taken from all the rows or tracks on one set of axes in a *Data Window*. Select the **3D** option under **Change Display Mode** on the **Display** Menu, or click the **3D** Display button.

3D



Along with the x-axis (calibrated by default to represent pixels across the CCD-chip), and the data-axis (calibrated by default in counts), you now see a third (or y-axis) calibrated in rows or tracks - depending on the acquisition mode you have selected.

To read off a data value on a particular trace, use the scroll bar on the *Data Window* to move the trace into the plane delineated by the x- and data-axes, and click on the trace to position the cursor on the point of interest (you may need to use the left and right arrow keys on your keyboard to position the cursor precisely).

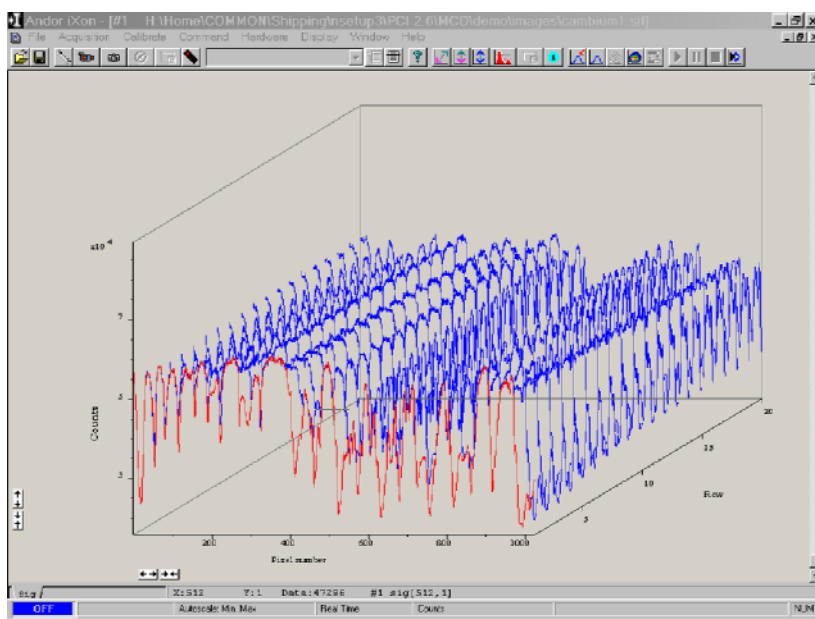
The numeric display on the status bar along the bottom edge of the *Data Window* will indicate the series member on which the cursor is positioned ('Kinetics'), along with the corresponding x- and data-values.

If your data set contains a series of images (each of which may represent data acquired in Kinetics Mode) you will notice that the *Data Window* has two scroll

bars, placed end to end. The upper scroll bar allows you to move between the members of the series, while the lower scroll bar allows you to view the traces that make up the particular member of the series.

For further functionality associated with 3D Display Mode see also:

Zooming In, Zooming Out and Scrolling and Reset under **2D** previously;
and **Rescale** under **Image**, coming up.



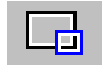
Data Window (3D Display Mode)

The Play, Pause, and Stop Buttons on the *Main Window* are used to control the playback of kinetic series of images. The Play button will commence the sequence of images. Pause will stop and hold the sequence at the point the button is clicked. Stop will terminate the sequence.

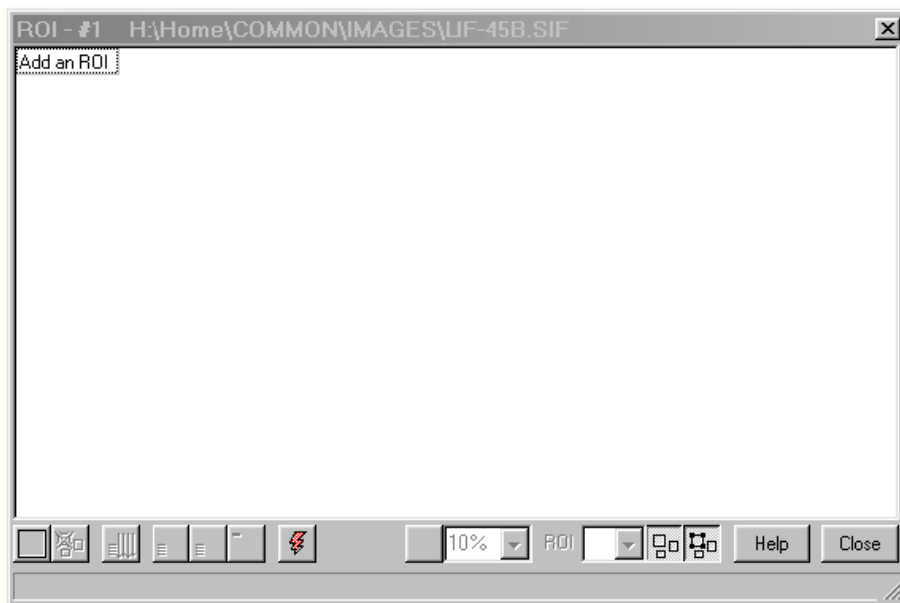
**Play / Pause /
Stop / Play Back
Auto Scale**



Click the **Play Back Auto Scale** button on the bar of the *Main Window* to turn the function On and Off. When the Play Back Auto Scale Function is on, the system will automatically Rescale the display range as each scan in the series is displayed.



Region of Interest is an important post-acquisition tool, used for quantitative analysis of data. **Region of Interest**, abbreviated to **ROI**, can be selected from the **Display** menu on the Main Window, or by clicking the ROI icon the task bar in the Main Window. Remember, the Display menu only becomes active after you have acquired data. Selecting ROI will run the following Dialog box.



Region of Interest (ROI) Dialog Box

The **ROI** display window lists parameters such as mean, standard deviation, maximum etc. for the ROI and also displays a list of ROI manipulation buttons along the bottom.

To select a Region of Interest, click the **Add ROI** button (bottom left), and a red box outlining the ROI will appear on your image. An **ROI** can be drawn on the image by positioning your cursor on the red **ROI**, and dragging out the using the corner handles.

You may use this tool to select and draw multiple ROI's onto your image, and then use the **ROI** data set to compare the values obtained. For further instructions on using the **ROI** tool, please see SECTION 6 – **TUTORIALS** - Extending Dynamic Range in 'Slow Scan' Measurements By Accumulating Signal.

Region of Interest Buttons

Along the bottom of the **ROI** Dialog Window are a series of buttons used for manipulating the **ROI** data. It will be useful to explain the functions of each these buttons.

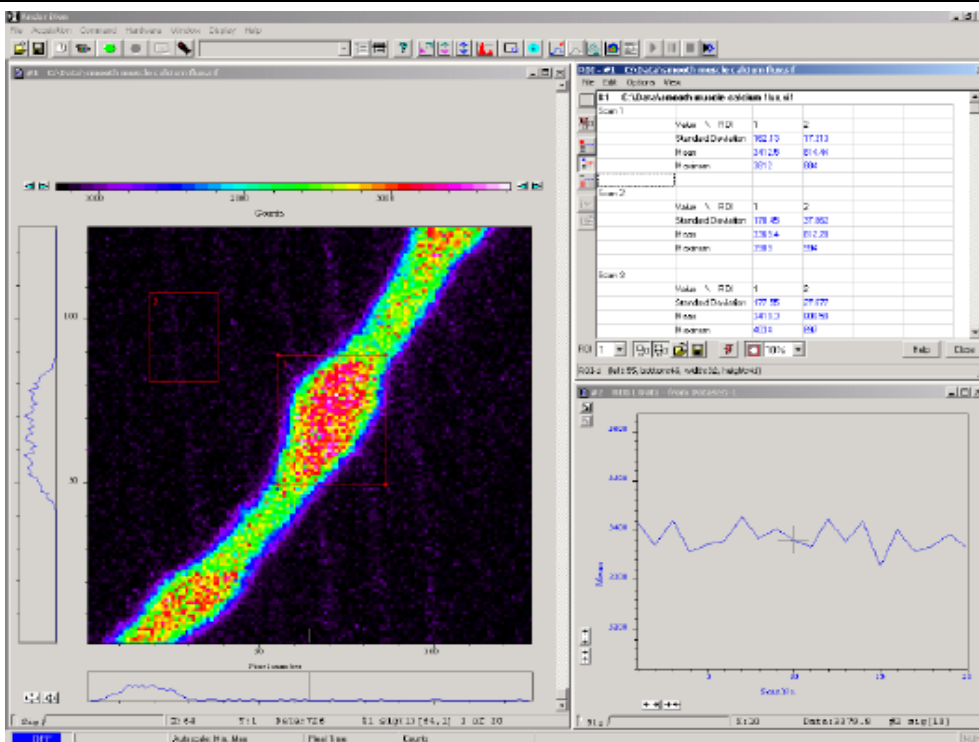
If you click on this button, the red **ROI** box will appear on your image. Use the corner handles to drag out the size and location of your **ROI**. Clicking on this button will also open the **ROI** data tables, which list parameters such as mean, standard deviation, variance etc, along with the pixel co-ordinates for your selected **ROI**

Add ROI



This will delete the current **ROI**, including the **ROI** data set.

Delete Current ROI



ROI Main Window, showing Image, Data Table, and plotted data graph

Clicking this button will present and group your **ROI** data, according to each individual **ROI** region selected on the image. It will also display the pixel co-ordinates for all the ROI's for that scan.

ROI View



Clicking this button will present and group your **ROI** data, according to individual data Scans. See Figure above. It will also display the pixel co-ordinates for all the **ROI's** for that scan.

Scan View



Clicking this button will present and group your **ROI** data, according to value regions – mean, range, variance, and standard deviation. It will also display the pixel co-ordinates for all the **ROI's** for that scan.

Value View



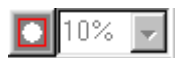
Clicking the Live Update button will allow you to receive and calculate **ROI** data, while the system is running in Real Time Mode. This means you will be able to acquire live data, and at the same time calculate and tabulate **ROI** data.

Live Update



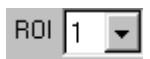
The Hot Spot Approximation tool can be used to take a selected percentage of the highest data values within a given ROI. For example selecting 50% will give you the mean value for the top 50% of pixel values within the **ROI**.

Hot Spot Approximation



The number in the counter identifies the current active **ROI**. The ROI counter can also be used to select and isolate a particular **ROI**, which can be a useful tool if two ROIs are overlapping or are layered on top of each other. By clicking the downward arrow, you can also see how many **ROI's** are currently defined.

ROI Counter



The Show ROI function can be switched on and off by clicking this button. When Show ROI is on, the selected **ROI's** will be shown on the image, and outlined by red boxes. When Show **ROI** is clicked off the red **ROI** boxes are hidden. When this button is On, it is not possible to position the Cross Hair cursor on the image to perform Zoom In or Zoom Out functions.

Show ROI



This tool can only be accessed if Show **ROI** is on (see above). With the Edit **ROI** tool on you are free to change the size and location of your **ROI's**. If the Edit **ROI** tool is off, the red **ROI** boxes become locked and cannot be altered. This is useful tool to prevent accidental interference with **ROI's**.

Edit ROI



Processing Data via the Command Line

The *Command Line Dialog Box* is launched from the **Command Line** option on the **Command** Menu or from the **Command Line** Button on the *Main Window*.

Command Line

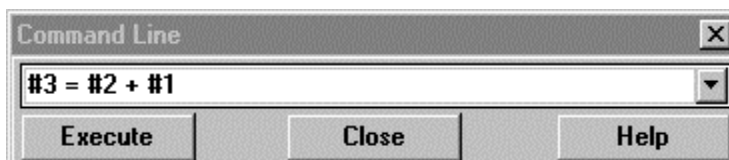


The Command Line allows you to enter one-line commands that are written in the **Andor Basic** programming language. These commands are used to manipulate acquired data.

To run a command, click **Execute**. See the accompanying **User's Guide to Andor Basic** for details of how to use the Andor Basic programming language.

See SECTION 4.1 **WORKING WITH DATA** for a description of the way in which the system stores the data you acquire.

SECTION 6 **WORKING WITH PROGRAMS** explains how to use the text editing facilities that help you write programs in Andor Basic.



Command Line Dialog Box

An alternative method for manipulating acquired data is to use **Arithmetic Operation** function. The *Dialog Box* can be launched by selecting **Operation** from the **Command** drop down menu on the *Main Window*.

Operation

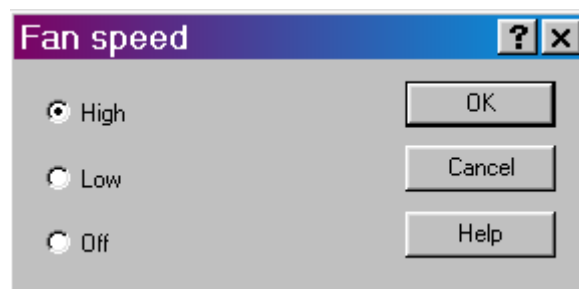
The **Arithmetic Operation** function allows you to manually manipulate data, in a similar manner to that performed by the Command Line.

Configuring the System for your Hardware

Hardware

The **Hardware** Menu allows you to configure the system so that the application software interfaces correctly with its hardware environment. The following options are provided:

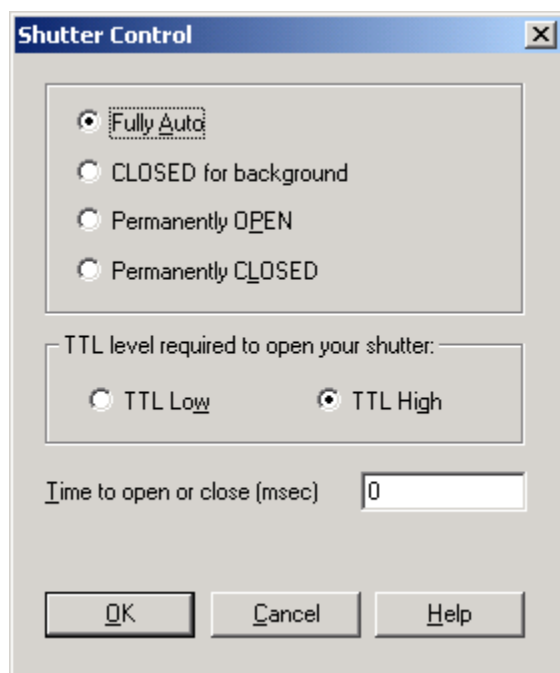
- **Shutter Control** launches a *Shutter Control Dialog Box*, which you can use to indicate when and how a hardware shutter should be used. With a CCD the shutter is used for background shuttering. See SECTION 4.11 **SHUTTER CONTROL**.
- **Temperature** launches a *Temperature Dialog Box* on which you may indicate the temperature to which you wish to cool the detector. See SECTION 4.12 **SETTING THE TEMPERATURE**.
- **Set Up Remote Comms** launches a *Remote Control Dialog Box* on which you can activate the infra-red Remote Control unit for your iXon CCD. You can also select the Communication Port, Com1 or Com2, which the Remote Control receiver will use. See SECTION 4.14 **USING THE REMOTE CONTROL**.
- **Fan Control** launches a *Fan Control Dialog Box* on which you can select the speed of the internal cooling fan – High, Low, or Off. It may be necessary in certain experimental set ups to reduce the speed of the fan, or to turn it off completely, to limit the possible risk of fan vibration affecting the quality of data recorded.



The *Shutter Control Dialog Box* provides you with a selection of options that determine when and how a hardware shutter should be used. Launch it by selecting the **Shutter Control** option on the **Hardware** Menu.

Shutter Control

Certain settings (e.g. **Permanently Open** and **Permanently Closed**) take effect as soon as you close the *Shutter Control Dialog Box* by clicking the **OK** button. Other settings will be applied whenever you acquire data (see: SECTION 4.4 **DATA ACQUISITION - Making an Acquisition**).



Shutter Control Dialog Box

Radio buttons on the *Shutter Control Dialog Box* give you high-level control over the shutter:

Fully Auto is the simplest shutter mode, as it leaves all shuttering decisions to the system. When you perform **Take Signal**, (see SECTION 4.4 **DATA ACQUISITION**) the shutter opens for the duration of the **Exposure Time** you have entered on the *Setup Acquisition Dialog Box* (see SECTION 4.5 **SETTING THE ACQUISITION**).

Fully Auto

Note: This option will automatically provide suitable shuttering for the majority of data acquisitions. The shutter will be closed for background data acquisitions and will be opened for all other data acquisitions.

Custom gives you a greater degree of control over the circumstances in which the shutter opens and closes. Click the **Setup** button to launch a *Customs Settings Dialog Box* (see **Custom Shutter Settings** following) from which you may select more specialized shuttering combinations.

Custom

Permanently Open means that the shutter will be open before, during and after any data acquisition.

Permanently Open

Choose **Permanently Closed** if you wish to take a series of acquisitions in darkness and do not require the shutter to open between acquisitions. You might, for example, wish to capture a sequence of background values. The shutter remains closed before, during and after any data acquisition.

Permanently Closed

On the *Shutter Control Dialog Box*, two radio buttons (**TTL High** and **TTL Low**) let you instruct the system as to how it should control the opening (and closing) of the shutter. (TTL = Transistor-Transistor Logic.)

TTL level required to open your shutter

- If you click the **TTL High** button, the system will cause the output voltage from the controller card to go 'high' to open the shutter. Please Note: If your iXon

CCD has been supplied with a built in shutter, the TTL pulse is factory set to high. Changing this setting will affect operation of the shutter.

- If you click the **TTL Low** button the system will cause the output voltage from the controller card to go 'low' to open the shutter.

Check the documentation supplied by the shutter manufacturer to see whether your shutter opens at a high or a low TTL level.

The shutter pulse is fed from the Shutter out pin on the back of the camera.

Shutters take a finite **Time to open or close**: this is sometimes called the **Transfer Time**. The documentation supplied by the shutter manufacturer should indicate the Transfer Time you can expect from your particular shutter. In the case of a CCD detector, the Transfer Time is used principally to give the shutter enough time to close, before background data are acquired. The system ensures that a period of at least the duration of the Transfer Time elapses between the shutter output signal entering the 'closed' state and commencement of background data acquisition.

**Time for shutter
to open or close**

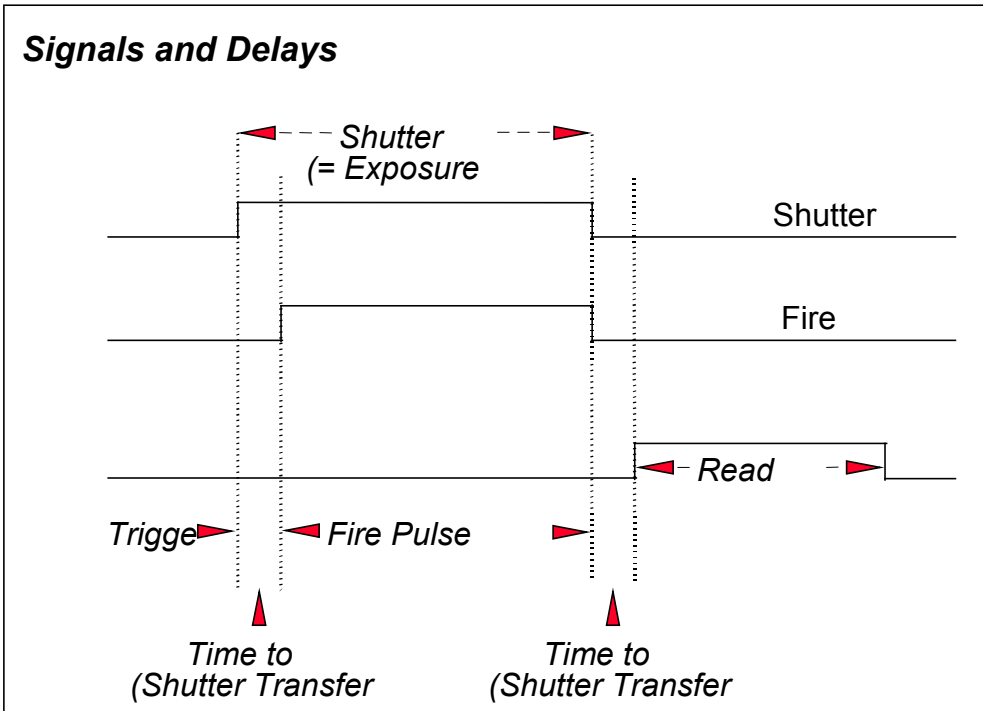
As the iXon contains a Frame Transfer CCD device, a shutter may not be required for most applications. If a shutter is fitted but not required for the experiment, then set it to permanently open, and Time to 0. This will allow the system to operate at its optimum rate. If a Bg is required then close the shutter using Permanently Close, take Bg, and reopen.

Let us look at the **Transfer Time** in the context of the Andor system. By default, the value you enter in the **Exposure Time** text box on the *Setup Acquisition* dialog box determines the length of time the shutter will be in the open state. However, to accommodate the Transfer Time, the rising edge of the **Fire** output signal follows the start of the shutter open state after a delay, equal to the value you enter as **Time to open or close**. You should set this value to the Transfer Time of your shutter.

The system also automatically adds the Transfer Time to the end of the acquisition

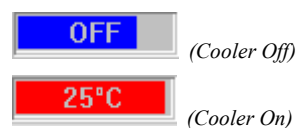
sequence, introducing an appropriate delay between the start of the shutter closed state and the commencement of the data being read out.

If you do not have a shutter connected, set the Time to open or close to **0**. Setting the Time to open or close to any other value will insert extra delays into cycle time calculations. See the diagram below.



Launch the *Temperature Control Dialog Box* by selecting the **Temperature** option on the **Hardware** Menu, or by clicking the **Thermometer** icon at the bottom of the *Main Window*. The *Temperature Control Dialog Box* allows you to:

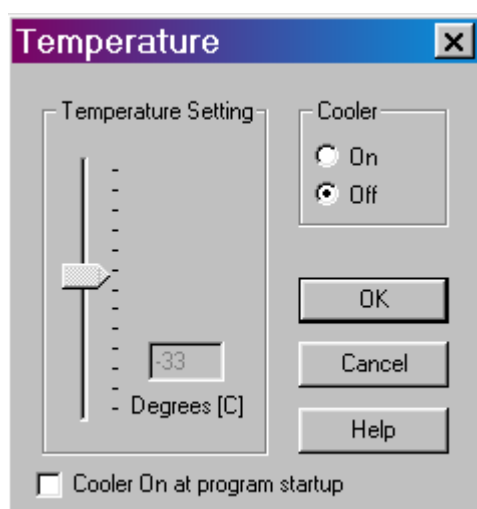
Temperature



- Switch the Cooler **On** or **Off** by clicking the appropriate check box. The caption beside the thermometer icon on the *Main Window* will change accordingly.
- Select a **Temperature Setting** (provided the Cooler is switched On) by entering a value (°C) directly into the text box, or by moving the vertical slider until the required value appears in the text box.
- Close the *Temperature Dialog Box* by clicking the **OK** button. By default, the temperature icon is colored red: when the required temperature has been reached (this may take some minutes), it changes to blue.

SEE **Cooling Performance and Operation** (COMING UP NEXT!) FOR DETAILS OF MINIMUM ACHIEVABLE TEMPERATURES, AND IMPORTANT ADVICE ON AVOIDING OVERHEATING.

Note: Cooling the CCD detector helps you reduce dark signal and its associated shot noise. Cooling will also effect the iXon CCDs Electron Multiplying Gain. For more information on this see SECTION 3 – **INSIDE THE CCD**



Temperature Control Dialog Box

Cooling Performance and Operation: Technical Description

The CCD is cooled using a thermoelectric (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 – that is, it achieves a temperature difference by transferring heat from its ‘cold side’ (the CCD-chip) to its ‘hot side’ (the built in heatsink). Therefore the minimum absolute operating temperature of the CCD depends on the temperature of the heatsink. Our vacuum design means that we can achieve a maximum temperature difference of over 110°C (high end model) – a performance unrivalled by other systems.

The maximum temperature difference that a TE device can attain is dependent on: the heat load created by the CCD; the number of cooling stages of the TE cooler; and the operating current. The heat that builds up on the heatsink must be removed. This can be done in one of two ways:

1. **Air cooling** - a small built-in fan forces air over the heatsink.
2. **Water cooling** – external water is circulated through the heatsink using the water connectors on the top of the head.

All Andor CCD systems support both cooling options. Whichever method is being used, it is not desirable for the operating temperature of the CCD simply to track the heatsink temperature. Therefore a temperature sensor on the CCD, combined with a feedback circuit that controls the operating current of the cooler, allows stabilisation of the CCD to any desired temperature within the cooler operating range. To set the temperature you require, select the **Temperature** option on the Hardware Menu, or click the **Thermometer** icon at the bottom of the *Main Window*.

In order to operate the cooling on your iXon CCD, you must use the Power Supply Block (PSB) with your system.

Air Cooling Performance

Air cooling is the most convenient method of cooling, but it will not achieve as low an operating temperature as water cooling (see below). Even with a fan, a heatsink typically needs to be 10°C hotter than the air (room) temperature to transfer heat efficiently to the surrounding air. Therefore the minimum CCD temperature that can be achieved will be dependent on the room temperature.

For detailed performance figures see **Performance Tables: Air Cooling** below.

Water Cooling Performance

A flow of water through the heatsink removes heat very efficiently, since the heatsink is never more than 1°C hotter than the water. With this type of cooling, the minimum temperature of the CCD will be dependent only on the water temperature, and NOT on the room temperature.

For detailed performance figures see **Performance Tables: Water Cooling** below.

Water cooling allows lower minimum operating temperatures than air cooling. However, there is a very important point relating to water cooling: if the water temperature is lower than the dew point of the room, condensation will occur on the heatsink, the water taps and other metal parts of the head. This will quickly destroy the head and must never be allowed to happen.

NEVER USE COOLING WATER THAT IS COLDER THAN THE DEW POINT OF THE AIR IN THE ROOM.

For example, with a typical Hermetic Vacuum Head you will need 10°C cooling water to guarantee -75° C performances. In the relatively dry atmosphere of an air-conditioned lab, cooling water at 10° 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 (20°C or even higher if it is very humid). The minimum CCD temperature in this example would then be limited to between -65°C to -70°C.

Head Overheating

Whichever cooling method you are using, make sure that the detector head does not overheat, as this can cause component failure. Overheating may occur if:

1. The air vents on the sides of the head are accidentally blocked.
2. You are using air cooling and have selected an operating temperature that is lower than the minimum achievable temperature. To check the minimum achievable temperature, refer to the Air Cooling Performance table supplied with your system specifications.

To protect the detector from overheating, a thermal switch has been attached to the heatsink. If the temperature of the heatsink rises above 40°C, the current supply to the cooler will cut out and a buzzer will sound. The cut-out will automatically reset itself 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.

NEVER BLOCK THE AIR VENTS ON THE DETECTOR HEAD

Performance Tables

Air Cooling

The table below is a guide to the minimum achievable operating temperatures for various room temperatures. Performance of individual systems will vary slightly.

Evacuated Housing-High Performance Cooling with Power Supply Block (PSB)	
Air Temp	External PSU Box
20°C	-60°C
25°C	-58°C
30°C	-56°C

Note that the relationship between the air temperature and the minimum CCD temperature in the table is not linear, this is because TE coolers become less efficient as they get colder.

The table below is a guide to the minimum CCD operating temperatures for various water temperatures. Performance of individual systems will vary slightly.

Water Cooling

Evacuated Housing-High Performance Cooling with Power Supply Block (PSB)	
Water Temp	External PSB
10°C	-75°C
15°C	-73°C
20°C	-71°C
25°C	-69°C

Never use cooling water that is colder than the dew point of the air in the room: **condensation causes serious damage to the head.** You may have to use warmer water and cool to a higher minimum temperature than you intended.

The **Window** Menu offers a number of options which help you manage the windows or icons that you have created while working with the system. The following options are available:

Window

Cascade arranges any open *Data Windows* or *Program Editor Windows* (see SECTION 6 **WORKING WITH PROGRAMS**) into a stack which runs diagonally across the *Main Window* and which allows you to see the title bars of each of the windows in the stack.

Cascade

Tile Horizontal arranges and resizes any open *Data Windows* or *Program Editor Windows* so that all run the full width of, and are fully visible within, the *Main Window*. Any icons that have been created within the system will be arranged into a row starting at the bottom left of the screen.

Tile Horizontal

Tile Vertical arranges and resizes any open *Data Windows* or *Program Editor Windows* so that all run the full height of, and are fully visible within, the *Main Window*. Any icons that have been created within the system will be arranged into a row starting at the bottom left of the screen.

Tile Vertical

Arrange Icons places in a row (starting at the bottom left of the screen) any icons that have been created within the system.

Arrange Icons

Close All removes all windows and icons from the *Main Window*. You will be prompted to save any unsaved data.

Close All

(For use only with *Data Windows*.) **Copy to Clipboard** copies the contents of the active *Data Window* into the Windows ‘clipboard’ - the store in which Windows normally holds data that has been cut or copied.

Copy to Clipboard

Data, which have been copied to the clipboard, can be pasted into other Windows applications, where it will be treated as a picture or ‘bitmap’.

You might, for example, want to include a data trace as an illustration in a word-processed document. Once you have copied your data to the clipboard, use your word-processing package to bring up the document in which you want to perform the paste. Insert the data as a bitmap using the word-processing package’s usual paste command.

In addition to the window-handling options described above, the *Window Menu* displays a list (using filenames where appropriate) of any *Data Windows* or *Program Editor Windows*, which are currently open or are currently iconized within the system. Click the name of a window or icon in the list to bring the corresponding window to the front of the *Main Window* (if the *Data Window* or *Program Editor Window* was iconized it will now be opened as well).

Document List

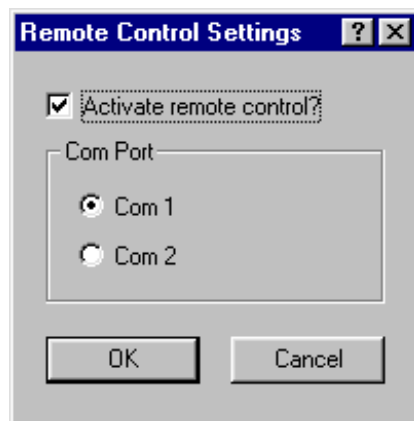
The **Remote Control** allows various commands of the iXon software to be modified using an infra-red signal sent to a **Receiver**. The range of operation of the remote control is up to a distance of 12m from the receiver along the line of sight.

- Connect the lead from the **Remote Control Receiver** to a serial RS232 Com. Port at the back of your computer. The receiver should be positioned in a slightly shadowed place, away from the glare of the room lights (underneath the computer monitor or on a shadowed shelf above the monitor, for example). This should be done as certain energy-saving fluorescent lighting may generate interference that may cause erratic windows pointer movement or erroneous commands to occur.
- Insert two AAA batteries (included) into the back of the remote control handset.

Connecting the Remote Control Receiver

To enable the infra-red Remote Control to be activated, go to the **Hardware Menu** on the *main window* of the software and select **Setup Remote Comms**. A **Remote Control Settings** dialog box will open. Select the tick box to activate the remote control. Then select the relevant **Com. Port** radio button (the default is Com. Port 1) to indicate which port the receiver has been connected to if your computer has more than one.

Activating the Remote Control



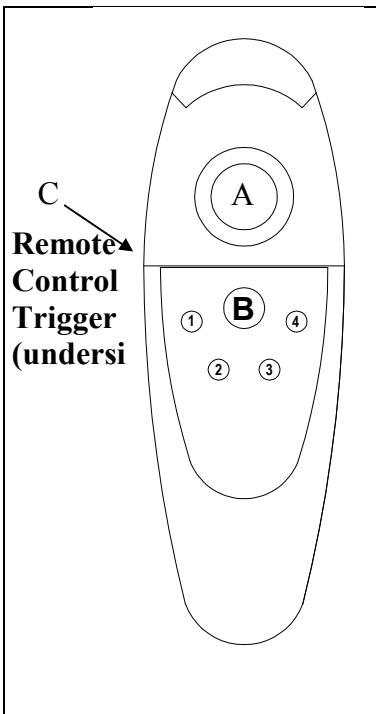
Remote Control Settings Dialog Box

The remote control has two modes of operation. The first provides direct control over the **Run Time Control dialog box** (see next page), where the following parameters may be varied:

- **Run/Abort acquisition**
- **EM Gain**
- **Exposure Time**
- **Autoscale ON/OFF**

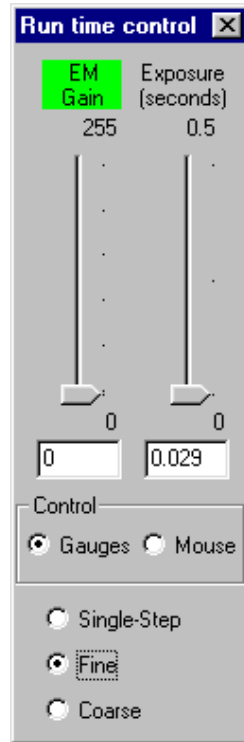
Note that the Gain and the exposure time may also be modified using the **Setup Acquisition Dialog Box** (see SECTION 4.4).

The second mode of operation allows the remote to be used as a general tool for moving the Windows pointer around and accessing all the various menus and dialog boxes of both the iXon software and the general Windows interface.

	Button	Accessing Run Time Control dialog box	Windows pointer control
	A	Moves the gauges of the dialog box	Moves the Windows pointer
	B	Switches between control of the Run Time Control dialog box and the Windows pointer	
	C	Run/Abort acquisition	Activates the relevant menu selection made by the Windows pointer
	1	Selects between the two Run Time control gauges	Inactive
	2	Selects between single-step, fine and coarse control over the gauges	Inactive
	3	Switches the Autoscale function on and off	
	4	As button 1	Inactive

To open the **Run Time Control dialog box**, press the Run Time Control button on the toolbar of the main software window. The default settings of the dialog box are shown below.

Operating The Run Time Control Dialog Box



The dialog box has two gauges that can be moved up and down to control the EM Gain and the Exposure of the CCD. The gauges have three degrees of control: Single-Step, Fine and Coarse. This dialog box may be accessed either by using the relevant buttons on the remote control or by moving the windows pointer with the mouse or the remote.

Button A (the large grey button) is used to move the active gauge of the Run Time Control dialog box up or down. The gauge that is active has its name highlighted in green and the actual setting of each gauge is given in the text boxes below them. The **EM Gain** gauge can be varied from a setting of 0 to 255. The **Exposure** gauge can be varied from the minimum exposure setting. Note that the exposure gauge upper limit will auto-range as the setting is increased.

Button B (the unmarked black button on the main panel of the remote) switches between the two modes of operation of the Remote Control. Press the button to either control the Run Time Control dialog box exclusively or to operate the windows pointer.

While the remote is being used to access the Run Time Control dialog box, the **Trigger** on the underside of the Remote Control is used to make or cancel an acquisition. It has the same function as pressing the green **Take Signal** button or the red **Cancel Take Signal** on the toolbar of the main window.

Button 1 is used to toggle between the two gauges of the Run Time Control dialog box. Each sliding bar is activated in turn.

Button 4 operates as for button 1.

Button 2 is used to toggle between the three degrees of control over the gauges i.e. Single Step, Fine and Course. The **Single Step** control allows the active gauge to move in discrete steps of one. **Fine** control enables the gauges to be moved at a faster speed than single step control and the **Coarse** control enables the gauges to be moved even faster.

Button 3 is used to switch the 'Autoscaling' of the data in the active data window on and off (see SECTION 4.7 **Displaying Data**).

Introduction	5.1
Editing Programs	5.2
Running Programs	5.3

Working with Andor Basic Programs

The **Andor Basic** programming language allows you to create programs for customized control of data acquisition and customized manipulation of data. See the **Programmer's Guide to Andor Basic**.

The system provides facilities to let you **Edit, Save** and **Run** your programs.

The Program Editor Window

Opening a program file, or selecting **New Program** from the **File** Menu (see SECTION 2 **INSTALLATION**) launches a *Program Editor Window* where you can enter unformatted text.

Context Sensitive Help

While you are working in the *Program Editor Window*, context sensitive help is available on the 'reserved words' of the programming language: with the cursor on or immediately after a reserved word, press **Ctrl + F1**.

Accessing the Edit Functions

Edit facilities are available either as edit buttons on the *Main Window* or as options on the **Edit** and **Save** Menus. Some options (**Cut, Copy**) become available only when you have highlighted a segment of text; others are available only when preceded by another operation (**Paste** must be preceded by **Cut** or **Copy**).

The following pages provide details of how to work with Andor Basic.

Edit

Cut or **Copy** text that you have highlighted. Paste the text into a new position.

Cut  Copy 

Paste inserts cut or copied text into the position following the cursor, or replaces text that you have highlighted.

Paste 

Undo causes the text to revert to its state before the last change was made.

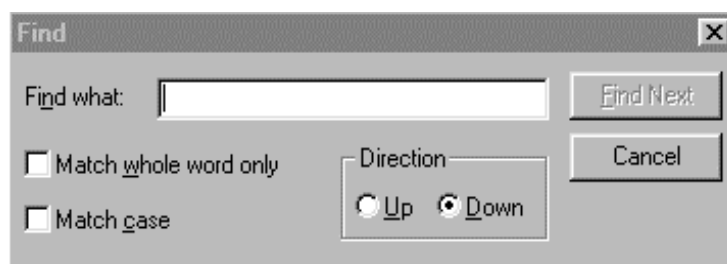
Undo 

Search

- In the **Find what** text box, type the word or phrase (the 'search string') that you want to find in the *Program Editor Window*.
- Click the appropriate check box to look for a complete word (**Match whole word only**), or for the same combination of capital and/or small letters as occur in the search string (**Match case**).
- Click the radio buttons to determine the **Direction** of the search.
- To activate the search, click on **Find Next**.

Find 

Find Next 

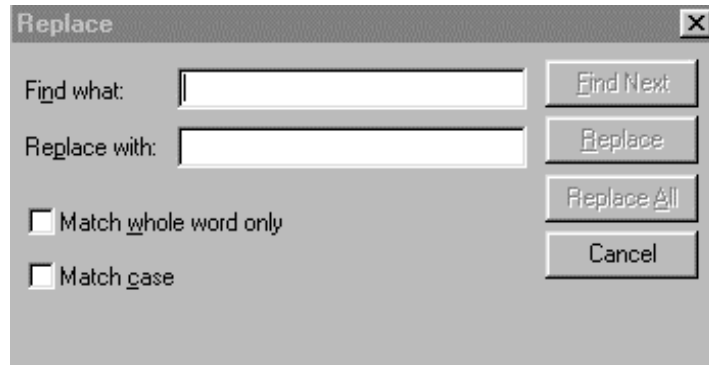


Find Dialog Box

- In the text box marked **Find what**, type the search string. In the text box marked **Replace with**, type the word or phrase that you want to use instead.
- Click the **Find Next** button to highlight the next occurrence of the search string.
- Click the **Replace** button to change the next occurrence of the search string (or the highlighted search string if you have just used Find Next).
- Click **Replace all** to replace the search string wherever it occurs after the current cursor position.
- Check boxes let you **Match whole word only** or **Match case**.

Replace

Find Next 



Replace Dialog Box

To run a program, make sure you have opened the appropriate .pgm file and ensure that the file name appears in the drop-down list box next the **Run Program** button; then click the Run Program button.

Run Program



To change the name of the file you want to run: either open the drop-down list and click the name of the file; or select an open *Program Editor Window*; or open the .pgm file from the **File** menu.

You may also run a program by means of the **Run Program by Filename** option on the **File** Menu.

Run Program by Filename

Clicking Run Program by Filename launches an *'Open' Dialog Box*, from which you may select the file whose contents you want to run. The file containing the program appears on screen as an iconized *Program Editor Window* and the program begins to execute immediately.

Entering Program Input

Any text-based input required by the program (i.e. in **Andor Basic** you have indicated that the user must manually enter a value at a particular point in the program's execution) is entered via an *Input Dialog Box*.

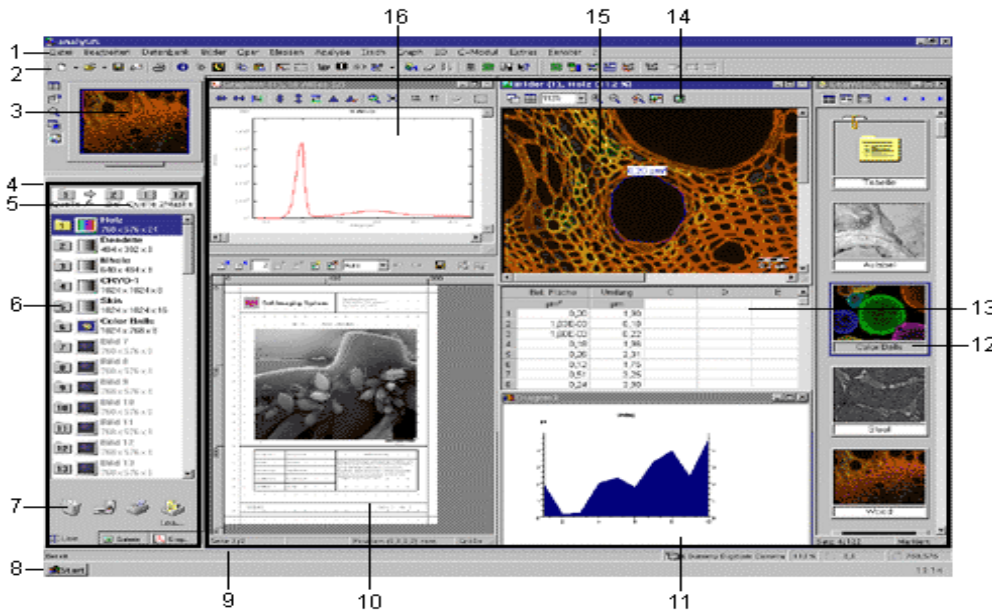
Viewing Program Output

Any text-based output from the program (regardless of how execution of the program was initiated) will appear in an *Output Dialog Box* which you may edit and save.

Soft Imaging System	6.1
Other S/W Supported	6.1

Soft Imaging System

Andor's preferred imaging partner is SiS, **Soft Imaging System**, the imaging software specialists. We are working together to provide you with imaging software for all your imaging requirements. AnalySIS software, a flexible software package has been designed to work in conjunction with your Andor camera with minimum effort.



1 Menu Bar	7 Icon Area	13 Measurement Sheet
2 Button Bar	8 Status Bar	14 Image Button Bar
3 Viewport Manager	9 Documents Area	15 Image Document
4 Image Manager	10 Report Generator	16 Graph Document
5 Operands Box	11 Diagram	
6 Image Buffer Box	12 Database Document	

SiS Analysis User Interface

We also provide drivers for the following software packages: -

AQM 2002 from Kinetic Imaging
 Image Pro+ and Image Pro Express from Media Cybernetics
 V++ from Digital Optics
 LabVIEW from National Instruments
 GRAMS from Galactic

**Other Software
Supported**

- Tutorial 1:** Measuring Gain Factor at a Given Temperature Setting Using a Weak LED Light Source 7.1
- Tutorial 2:** Extending Dynamic Range in ‘Slow Scan’ Measurements By Accumulating Signal 7.2
- Tutorial 3:** Performing Basic Low-Light Microscopy Functions Using the iXon 7.3

Measuring Gain Factor at a Given Temperature Setting Using a Weak LED Light Source

Additional Requirements

Stable LED light source, Compatible camera lens, Darkened ambient conditions, Optional - Neutral density filters

The object of this experiment is to make an approximate measurement of the true gain factor applied at a series of working empirical gain settings.

Aim

Attach the camera to the lens, either through C or F-mount adaptor as appropriate. Spacers can be inserted in order to optimise the working focal distances. Ideally the camera should be mounted on a retort stand, pointing downwards. The experiment can however be conducted with the camera lying flat on a bench, provided the light source can be suspended within the field of view.

Description

Bring the camera to its operational temperature, at which gain values need to be determined. Ensure that the temperature has stabilized before commencing, as is the case if the temperature indicator at the bottom right of the *Main Window* turns blue. The temperature can be accessed through the command line within the Andor software – from the **Command** menu on the *Main Window*, select the option **Command Line**. Type in the command - `?gettemperature()` – and click on **Execute**.

Focus on the light source - from the **Acquisition** menu on the *Main Window*, select **Setup Acquisition**. In the **Setup CCD** tab, ensure gain is set to zero and set exposure time to approximately 200 ms. In the **Video Mode** tab, ensure that **Use Settings From Standard Setup** is selected. In the **Setup Image** tab, ensure that **1 x 1 Binning** and **Full Area** is selected. Click the **Video Mode Run** button and focus on the light source. Click the cursor onto some of the brightest pixels of the LED image – if the intensity readings for these pixels appear so high that application of gain is likely to result in the CCD becoming saturated (i.e. amplification of signal beyond the full well capacity - a value of 16384 counts), the exposure time may be reduced (this is easily done through the **Exposure Slide Control Bar**).

Alternatively, neutral density filters may be inserted in front of the light source, or the aperture on the lens may be lessened in order to reduce the intensity of light reaching the sensor. If the signal appears too low, relative to the surrounding noise floor, the exposure time may be increased accordingly.

Staying in video mode, increase the gain to the maximum empirical value that is being used within the experiment and measure the values from same pixels within the brightest region of the image – this can readily be done using the **Gain Slide Control Bar**. If at this setting, the amplified signal in these brightest pixels saturates the CCD, the light intensity will need to be reduced by either inserting neutral density filters in front of the LED (in the absence of such filters, thin paper or filter paper may be used to reduce the intensity) or adjusting the lens aperture. Once the signal level has been reduced sufficiently, the signal at 0 gain needs to be re-checked in order to ensure that it can still be confidently observed above the noise floor.

If this cannot be satisfied, measurements can be taken at varying exposure times for different gains, providing that this is corrected for when calculating the gain factor at each setting, e.g. if the exposure has to be halved at the maximum selected gain setting relative to the exposure used at 0 gain, the measured value at maximum gain will have to be doubled in order to determine the expected value had it been possible to maintain that longer exposure time. However, in order to ensure that the minimum exposure time employed in these measurements remains significantly larger than the readout time (therefore aiding accuracy), it is recommended not to use exposures less than 100ms.

You should now be ready to make measurements. Measurements must be recorded with background subtraction *for each gain setting* – from the **Acquisition** menu on the *Main Window*, select **Setup Data Type**, then click on **Counts (Bg corrected)**. Then from the **Acquisition** menu, select **Setup Acquisition**. In the **Setup CCD** tab, set gain to zero and the exposure time to optimal value determined for this gain setting. Select **Single Acquisition Mode**. Turn off the light source, and from the

Acquisition menu select **Take Background**. Background data is displayed on-screen under the **Bg** tab of an *Acquisition Window*. Turn the light source on again.

Now acquire the signal image for this gain setting – from the **Acquisition** menu on the *Main Window*, select **Take Signal** (or alternatively click the **Take Signal** button). The image should automatically be background corrected.

The signal intensity from this image may be measured now or the image may be saved for measurement later. Assuming the latter, repeat the previous two steps for the gain settings of interest, using the pre-determined optimal exposure times for each setting (should this parameter need to be varied at all), saving each image using easily recognisable file names.

Signal values may be determined for each image as follows – from the **Display** menu on the *Main Window*, select **ROI** (Region of Interest). This opens the ROI display window, which lists parameters such as mean, standard deviation, variance etc. for the ROI and also displays a list of ROI manipulation buttons along the bottom. Click the **Add ROI** button (bottom left), and a ROI will appear on screen. Pan the ROI centrally onto the image of the LED, then resize until it closely contains the image. Click the **Hot Spot Approximation** button and select 50%. Make a note of the mean value (this is the mean of the top 50 % of pixel values within the ROI – i.e. will not take into account pixels from outside the round image of the light source). Open another image and click **Add ROI**, the box should have the same dimensions as that defined for the first frame, and must simply be panned on top of the LED image. The mean value appears automatically and again must be noted.

The gain factor for each gain setting can now be determined - divide the value obtained for the 0% gain setting into the values obtained at each other gain setting. If shorter exposure times had to be employed at higher gain settings in order not to saturate the detector, this must be corrected for as outlined earlier. If sufficient gain settings are measured a calibration curve

can be plotted and extrapolated, from which gain factors at any gain settings can readily be estimated at this system temperature.

Extending Dynamic Range in 'Slow Scan' Measurements By Accumulating Signal

Although the iXon camera, due to its high-speed operation and ultimate EMCCD sensitivity performance, is very well suited to dynamic low light measurements, it can just as readily be applied to 'slow scan' non-dynamic quantitative measurements such as microtitre plate, gel or biochip reading. Operation under conditions of applied gain can enable very weak signals to be amplified above the camera read noise (this can be particularly pertinent in chemiluminescence / bioluminescence measurements), using lower exposure times than would otherwise need to be employed.

However, such formats can typically be demanding on dynamic range, requiring extremely weak signals to be imaged in the same frame as significantly stronger signals. Whilst the iXon can deliver true 14 bit dynamic range at 5 MHz readout, due to an extended full well capacity in the gain register (enabling a degree of gain to be applied before saturation), this may sometimes not be enough.

The object of this tutorial, is to outline the method by which both very weak signals, and much stronger signals, may be quantitatively determined in an image, without saturating the pixels of the CCD with the stronger signal. .

The first stage is to define the maximum exposure time (E1) at a given gain setting, at which the strongest signal may be quantitatively measured before the pixels become saturated. This can be accomplished by recording an image at a given exposure time and measuring from one or two pixels within the most intense signal. From the **Acquisition** menu on the *Main Window*, select **Setup Acquisition**. In the **Setup CCD** tab, set exposure time for approximately 1000 ms (or set appropriately for the experiment).

In the **Setup Image** tab, ensure gain is set to the level at which the measurement is to be performed and that **1 x 1 Binning** and **Full Area** is selected. Click the cursor on some pixels within the strongest signals to

Background

Aim

Description

determine approximately how close the most intense pixels are to the saturation level. If this signal level is less than the saturation limit of 16384 counts, the exposure time may be proportioned up accordingly to approach this maximum value. If the signal is already saturating the CCD, the exposure time will have to be reduced and the above procedure repeated. Carry out this measurement with the calculated optimum exposure time in order to ensure that it does not saturate.

You must now calculate the signal to background (S:B) ratio of the lowest intensity signal at this exposure time, as defined by the individual user or assay criteria. *Background relates to the combined contribution from sources of 'undesirable' signal and noise, such as readout noise (remember that the main benefit of EMCCD technology is to amplify the weakest of signals above the readout noise 'detection limit'), clock induced charge, darkcurrent and 'leaked' background light level (the latter three are amplified by EMCCD gain).* A suggested way to measure S:B ratio is to integrate the signal, from say 20 pixels, from within the signal area (S) and from a neighbouring background region (B). This can be accomplished within the iXon software as follows:

From the **Display** menu on the *Main Window*, select **ROI** (Region of Interest). This opens the ROI display window, which lists parameters such as mean, standard deviation etc. for the ROI and also displays a list of ROI manipulation buttons along the bottom. Click the **Add ROI** button (bottom left), and a ROI will appear on screen. Pan the ROI centrally onto the image of the LED, then resize until it falls within the signal area (or define it numerically to measure say 5x5 pixels, then drag it onto the signal). *Take an average reading from within this ROI.* Then drag the ROI to a neighbouring background area and repeat this measurement. The S:B ratio of the weakest signal can now readily be calculated.

Next, the exposure time (E2) must be determined at which the S:B ratio from the weakest signal exceeds the assay S:B cut-off limit. Set the exposure time to a greater value within the **Setup CCD** sub-window. The intensity of the highest concentration signal will now saturate the detector

(for sensors without anti-blooming, the weakest signal should be sufficiently spaced within the image from the stronger signals, such that charge does not ‘spill over’ into a measured region). Measure the S:B ratio of the weakest signal using ROI, as in the above step, and repeat this procedure until an optimal exposure time (E2) has been determined which meets the S:B requirements of the assay.

Division of E2 by E1 (and round upwards) should determine approximately the number of accumulations needed, using exposure time E1, to approach the S:B threshold required for the weakest signal to be considered quantitatively valid. In practice, more accumulations may be necessary due to extra spurious noise sources such as clock induced charge (CIC), which will accumulate with each readout and contribute to the overall background. Simple trial and error above this calculated number should determine the optimal number of accumulations required. Importantly, irrespective of the accumulation number used, if E1 is used as the exposure time, the strongest signal should not result in saturation of any of the pixels within the image.

This procedure can be carried out at a variety of gain settings in order to find the setting which affords the required S:B level for the lowest intensity signal, at the minimum overall accumulation time. Primarily, EMCCD gain should be applied in order to overcome the readout noise of the detector, which in a well optimised assay (in which the detector performance defines the detection limit and not extraneous background sources), defines the assay detection limit, i.e. the ‘concentration’ yielding a signal equal to the readout noise. The EMCCD can effectively reduce the readout noise to sub-1 electron rms, therefore significantly reducing the detection limit at a given exposure time. Alternatively gain may be employed to significantly reduce exposure times, therefore increasing the throughput rate of the assay procedure.

Bear in mind also that gain performance varies with temperature (see tutorial one), so ensure that temperature remains stable, as is the case if the temperature indicator at the bottom right of the *Main Window* turns blue. The temperature can be accessed through the command line within the

Andor software – from the **Command** menu on the *Main Window*, select the option **Command Line**. Type in the command - `?gettemperature()` – and click on **Execute**. Furthermore, with electron multiplying technology, operation at a lower temperature reduces darkcurrent, which would otherwise be amplified in the gain register and appear as spurious noise spikes (the extent of which depends on exposure time). The extensive cooling performance of the iXon can essentially render this noise source negligible.

Rather than carrying out this procedure with the sample to be scanned within the actual experiment, an alternative is to perform a serial dilution within microtitre wells of chemiluminescent material (e.g. Horse Radish Peroxidase/luminol/peroxide) spanning a concentration range close to that expected from the experiment. The maximum exposure time determined from the strongest signal (E1) can be used with the exposure time (E2) needed to exceed the cut-off S:B threshold in order to work out the minimum number of accumulations required at E1 exposure and the gain level applied. A neighbouring blank well (without chemiluminescent material) can be used to represent background levels. Furthermore, this simple test platform could also be used to determine actual gain levels, as an alternative to the stable light source recommended in tutorial 1.

Performing Basic Low-Light Microscopy Functions Using the iXon

Additional Requirements

Epifluorescence Microscope and camera adaptor. Optical filter set matched to fluorescence beads. Fluorescent microspheres, e.g. Fluorescein containing micron scale polystyrene FluoSpheres® from Molecular Probes. Darkened ambient conditions.

The object of this experiment is to perform some basic low light microscopy functions with the iXon, including recording kinetic series and determination of optimal gain, utilising as a simple example, micron scale fluorescent microspheres in solution.

Aim

Attach the camera to the microscope via the appropriate mounting adaptor. Ensure the camera is stabilized at the chosen temperature (a lower temperature will essentially negate darkcurrent noise spikes). The temperature indicator at the bottom left of the *Main Window* turns blue, when the system has reached the requested temperature.

Description

Prepare a solution of microspheres - e.g. a 1:100 dilution of the stock solution. Deposit a small amount onto a microscope slide and place a cover slip on top. Focus onto the beads using a reasonably high magnification, e.g. x100 oil immersion objective. While the beads are still in solution, they can be seen to translate, both laterally and also in and out of the focal plane in the z-direction.

Obtain an Image on CCD - Switch the microscope from eyepiece to camera observation. From the **Acquisition** menu on the *Main Window*, select **Setup Acquisition**. In the **Setup CCD** tab, ensure gain is set to zero and set exposure time to approximately 10-20 ms. In the **Video Mode** tab, ensure that **Use Settings From Standard Setup** is selected. In the **Setup Image** tab, ensure that **1 x 1 Binning** and **Full Area** is selected. Click the **Video Mode Run** button – an image of the beads should appear on screen at the frame rate accessed through the selected settings.

Record a Kinetic Series - From the **Acquisition** menu on the *Main Window*, select **Setup Acquisition**. In **Acquisition Mode** select **Kinetic Series**, and type in the number of frames to be recorded on the run, e.g. 50. In the **Setup CCD** tab, ensure gain is set to zero and set exposure time to approximately 10-20 ms (the **Kinetic Cycle Time** will update as exposure time is changed, as will the indicated **Frame Rate** in Hz). Now acquire the signal image – from the **Acquisition** menu on the *Main Window*, select **Take Signal** (or alternatively click the **Take Signal** button).

Playback Kinetic Series - Playback of the kinetic series can easily be done through the **Play**, **Pause** and **Stop** icons at the RHS of the top row of icons. Furthermore, **Playback Autoscale** may be selected or deselected as required. The dynamics of the fluorescent beads moving across the field of view and in and out of the focal plane should be readily observable.

Use of ROI and Kinetic Plots – Using the scroll bar at the RHS of the *Main Window*, select a frame for processing. From the **Display** menu on the *Main Window*, select **ROI** (Region of Interest). This opens the **ROI display window**, which lists parameters such as mean, standard deviation etc. for the ROI and also displays a list of ROI manipulation buttons along the bottom. Click the **Add ROI** button (bottom left), and a ROI will appear on screen. Pan the ROI centrally onto the image of a fluorescent bead, then resize until it falls within the signal area (or define it numerically to measure say 5x5 pixels, then drag it onto the signal).

Click the **Add ROI** button again and a second ROI will appear of the same resized dimensions as the first. Drag this over the position of another fluorescent bead. More ROIs can be added in this fashion. The mean value of pixels within each ROI will appear for each frame, and can be accessed for that particular frame by sliding across the scroll bar within the ROI display window. The mean of the top 10%, 20%, 30% etc. pixels can be calculated by clicking on the **Hot Spot** Approximation icon in the ROI display window. This enables spot intensities to be compared without taking the immediately surrounding background pixel values into account.

The row of mean values can be highlighted for any ROI then plotted vs. either frame number or time by clicking the appropriate icon in the ROI display window. The kinetic plot will appear in a new window. In the present example, the fluctuations in the plot over time represent fluorescent beads passing through or coming into focus within the field of view. Furthermore, a smaller number of frames may be selected and plotted in order to concentrate more closely on the interesting sections of the kinetic sequence.

Use of Gain to Enhance S:N of Weak Signal - from the **Acquisition** menu on the *Main Window*, select **Setup Acquisition**. In the **Setup CCD** tab, ensure gain is set to zero and set exposure time to approximately 10 ms. In the **Video Mode** tab, ensure that **Use Settings From Standard Setup** is selected. Click the **Video Mode Run**, the images of dynamic fluorescent beads should appear in real time. From the **Acquisition** menu on the *Main Window*, toggle **Autoscale Acquisition** (or alternatively press F6).

Reduce the aperture of the excitation light, insert neutral density filters and lower the exposure time (this is easily done through the **Exposure Slide Control Bar**), such that the intensity of fluorescence coming from the beads will be significantly reduced, representing a low light signal (using a mismatched emission filter can also markedly reduce the signal intensity). Ideally, the intensity of emission detected from the beads will approach, or become lost within the readout noise of the camera under this condition of zero gain setting. (Operating under subdued ambient light conditions will increase the probability that the background detection limit is indeed defined by the camera readout noise).

Then increase gain using the **Gain Slide Control Bar**, visually monitoring the markedly enhancing effect this has on the S:N ratio. Estimate the optimum approximate gain setting required to amplify the signal from the fluorescence beads significantly above the readout noise floor.

If the quantitative level of the noise floor can be seen to increase as gain is

increased (this may be more easily visualised by clicking the cursor in a background region of the image, then selecting the **2D** icon), it is likely that background light is being leaked into the system. This can markedly reduce the effectiveness of this technology, since leaked background or unwanted background fluorescence is primarily contributing to the detection limit at higher gain settings, rather than the camera readout noise. This principle applies to all low-light microscopy techniques and efforts should be made to eliminate as far as possible any sources of background light.

N.B. At higher gain settings, another source of spurious noise may be observed, in the form of occasional and random *single pixel* spikes on top of the readout noise floor which are typically of lower intensity than the true signal (furthermore, the signal in techniques such as Single Molecule Microscopy is normally dispersed over a few pixels). This is called **Clock Induced Charge** and is due to the electrons being generated electronically during the charge shifting process, then amplified within the gain register. Care must be taken also since single photon amounts of background light may also appear as single pixel spikes above the readout noise floor, upon application of gain.

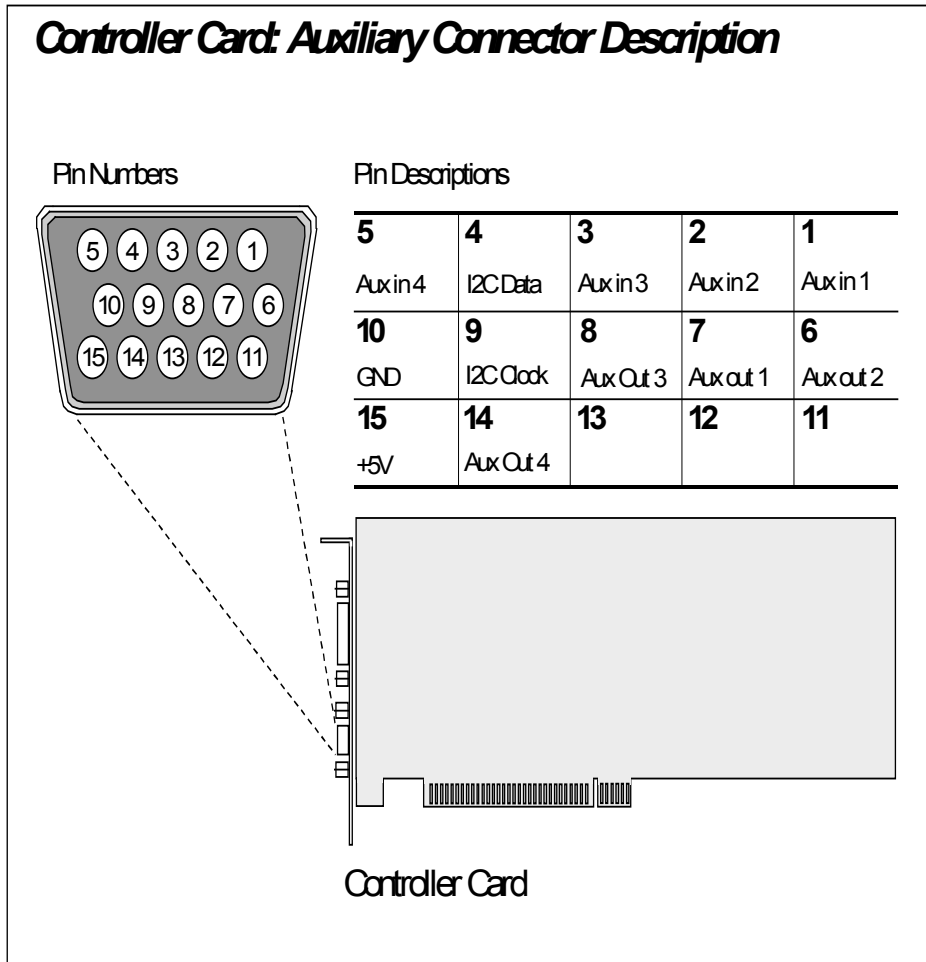
Record Immobilised Beads and Use ROI - Leave the slide to dry for a while, in order to immobilise the beads. Running the camera in **Video Mode** as described above, at a suitable exposure time and gain setting for detection, focus on one or more immobilised beads within the camera field of view. Record a **Kinetic Series** of images as described above, whilst performing an action which will result in the intensity of the fluorescence fluctuating periodically during the total acquisition time (such as cycling of the excitation aperture, insertion/extraction of ND filter, sliding gain setting up and down, tune in and out of focal plane etc.).

An alternative approach to recording a kinetic series of full images, is to record only an image sub-area. This has the effect of both increasing the frame rate (by decreasing the readout time required for each frame) and decreasing the file size of each image. To do this, it is necessary to first define the minimum dimensions of the sub-area. This can readily be accomplished by clicking the cursor onto four pixels on the full image

(such as was recorded in video mode) in order to determine the position of the sides of a sub area (square or rectangle) encompassing the fluorescent beads of interest. In the **Setup Image** tab of the **Setup Acquisition** Dialog box, select **Custom Sub-Image** and enter the sub-area dimensions into the left, right, top and bottom boxes. In the **Setup CCD** tab, the new frame rate created under these conditions may be viewed.

Then, place ROI areas over the immobilised spots and create kinetic plots of the mean values vs. time as described above. The plots should readily represent the fluctuations in intensity over time as the attenuating action was performed. This observation may be considered as representative of the fluctuations in intensity that is commonly observed for single fluorescing molecules.

The Controller Card	8.1
Windows Glossary	8.2
Imaging Glossary	8.3
Terms and Conditions	8.4



The Andor PCI Controller Card provides a 15 pin Auxiliary Connector should you wish to connect an external device.

The assignment of the pins is shown in the diagram above. There are several type of connection possible:

- Power – Pins 10 and 15 provide GROUND and +5 V DC power respectively.
- I²C – Pins 4 and 9 provide the DATA and CLOCK channels to control additional I²C devices you may have. I²C commands can be generated using the *Andor Basic* language.
- AUX IN – Pins 1, 2, 3 and 5 provide up to 4 auxiliary input channels. The inputs are standard 74LS TTL input driver that can be read via the *Andor Basic* language.

· AUX OUT – Pins 6, 7, 8 and 14 provide up to 4 auxiliary output channels. The outputs are SN74128 50 V TTL output driver that can be controlled via the *Andor Basic* language.

WARNING: Pins 11, 12 and 13 are RESERVED, these pins are not available for auxiliary use. Do not make electrical connection to these pin locations when attaching external devices via the Controller Card Auxiliary Connector port or damage may occur to the Controller Card, the Detector Head, or your external device.

As far as possible the **User's Guide** employs standard Windows terminology to describe the features of the user interface. If you are unfamiliar with Windows, the definitions that follow cover some of the most significant terms used in this User's Guide. The documentation supplied with your Windows installation will give you a more comprehensive overview of the Windows environment.

The window which you are currently working with is known as the **Active Window**. It is always positioned in the foreground of your screen. By the bolder color of its **title bar**, the active window is distinguished from other **windows** you may have open.

Active Window

A **Check Box** is included on a **dialog box** to let you select optional functionality. When you click the box, a \surd appears in it, indicating that the particular option has been chosen. Several check boxes may appear together on a dialog box. You may select whatever combination of options you believe to be appropriate for your particular application (by contrast, **radio buttons** allow you to choose only one of a number of options).

Check Box

A **Dialog Box** is a specialized window that asks you to supply information. The dialog box may request that you enter text or choose between a number of options.

Dialog Box

A **Drop-Down List Box** is a one-line, rectangular display area that shows an item (a name or number, for instance) that has been selected from a list. By clicking the downward pointing arrow at the side of the **list box** you can see the **Drop-Down List** itself and select a new item.

**Drop Down List;
Drop Down List
Box**

To **Highlight** text (when, for example, you are using a text editor and wish to mark text to be copied) click the text at the point where you want the highlighting to begin and keeping the left mouse button depressed, draw the cursor over the text you wish to highlight. Release the mouse button. The text is highlighted.

Highlight

A **Menu** is a list of commands that you can select by clicking. The menu titles are arranged in a row, or **Menu Bar**, across the top of a window. Clicking the menu title causes the menu itself to ‘drop down’, allowing you to select the option you require. Menus often provide an alternative to an on-screen button as a means of invoking commands.

**Menu;
Menu Bar**

The **Primary Mouse Button** is normally the left mouse button for right-handed users and the right mouse button for left-handed users.

**Primary Mouse
Button**

A **Scroll Bar**, usually positioned below and/or to the right of a window or a drop-down list allows you to see parts of the display that may initially be hidden from view. By moving the ‘slider’ on the scroll bar you can navigate quickly through the display. The scroll bar also has **Scroll Arrows** at each end: by clicking these you can move through the display or list more slowly. A window or list equipped with these facilities is described as **Scrollable**.

**Scroll Bar;
Scrollable;
Scroll Arrow**

A **Radio Button** is one of a number of on-screen buttons, only one of which may be selected at a time. (Compare with **check box**, which lets you select a number of options.)

Radio Button

A **Text Box** is usually a one-line rectangular area where you may enter characters.

Text Box

A **Title Bar** is a strip along the top edge of a **window** or **dialog box** and bearing the name of the **window** or **dialog box**.

Title Bar

A **Window** is a work area, which you can usually move, resize, or close.

Window

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**.

Accumulation

An **Acquisition** is taken to be the complete data capture process that is executed whenever you click **Take Signal**, **Take Background**, or **Take Reference** on the **Acquisition** Menu or whenever you click the **Take Signal** button (see SECTION 4.4 DATA ACQUISITION).

Acquisition

Charge from the **EMCCD** is initially read as an analog signal, ranging from zero to the saturation value. **A/D conversion** changes the analog signal to a binary number, which can then be manipulated by the computer.

A/D Conversion

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

Background

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

Binning

Summing charge on the **EMCCD** and doing a single readout results in 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 and horizontal binning. In addition there are several **binning patterns** that tailor the main binning variants to typical application usage.

The system uses the two main **binning** variants, vertical and horizontal binning.

Binning Patterns

Imaging: The default imaging mode is Full Resolution Image, which causes a value to be read out for each pixel on the CCD-chip. However, the *Image Dialog Box*, accessible from the *Setup Acquisition Dialog Box*, lets you define *superpixels* which in effect represent the charge binned (by means of a combination of vertical and horizontal binning) from groups of pixels (each superpixel being a rectangle of a given height and width in pixels). Viewed in Image Display Mode, data acquired in this way will appear either as a ‘high’ definition image (where data have been read out from individual pixels or from small superpixels) or as a more coarsely defined image, where data have been read out from larger superpixels. The advantage of defining superpixels is increased sensitivity and smaller data files. The *Image Dialog Box* also lets you define a sub-area of the chip from which to acquire data.

Counts refer to the digitization by the A/D 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 10photoelectrons on a **pixel** of the EMCCD.

Counts

Dark signal, a charge usually expressed as a number of electrons, is produced by the flow of **dark current** during the **exposure time**. All EMCCDs produce a dark current, an actual current that is measurable in (typically tenths of) milliamps per pixel.

Dark Signal

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 EMCCD reduces dark signal: typically, for every 7 degrees Celsius that temperature falls, dark signal halves. EMCCD 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 0 degrees Celsius will

generally remove most of the shot noise caused by dark signal.

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.

Detection Limit

Sources of noise are shot noise of the signal itself, shot noise of any dark signal, and 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.

The **Exposure Time** is the period during which the **EMCCD** collects light prior to **readout**. In the Andor system it is marked by a pulse on the Fire output.

Exposure Time

An electron multiplying charge coupled device. 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. A typical EMCCD-chip may comprise 512 rows and 512 columns, or 576 rows and 288 columns.

EMCCD

Noise is a complex topic, the full exploration of which is beyond the scope of this glossary. Noise may, however, be broken down into two broad categories: *pixel noise*; and *fixed pattern noise*. These two categories are described below.

Noise

Pixel Noise

Let us first attempt to define *pixel noise*. Assume that a light signal is falling on a **pixel** of the **EMCCD**. 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 (r.m.s.) of these variations is often used to express a value for noise. As a rule of thumb, the r.m.s. is four to six times 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; and *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.

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; it is only very slightly dependent on temperature; and 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 fall 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 Root Sum Square (r.s.s.) gives a reasonable approximation - thus:

$$total = \sqrt{readnoise^2 + darkshot^2 + sigshot^2}$$

where *total* is the pixel noise, *readnoise* is the readout noise, *darkshot* is the shot noise of the dark signal, and *sigshot* is the shot noise of the signal.

Fixed Pattern Noise

Fixed pattern noise (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.

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 **quantum efficiency (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 an **EMCCD**, but the most significant factor is the absorption coefficient of the silicon that serves as the bulk material of the device.

Quantum Efficiency;
Spectral Response

Readout is the process by which data are taken from the pixels of the **EMCCD** 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 **A/D conversion**, and storing the data in computer memory. The time taken to perform this operation is known as the ‘read time’.

Readout

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

Saturation

Each **pixel** is ultimately limited in the amount of charge it can hold - its ‘well depth’, in other words, is finite. This maximum charge, or well depth, is generally quoted in electrons or coulombs (where 1 coulomb = 1.6×10^{-19} times the number of electrons).

The **EMCCD** is continually being ‘scanned’ to prevent its becoming saturated with dark current (see **dark signal**). If the **Scan** is being used simply to ‘clean’ the EMCCD (i.e. it is a **keep-clean scan**), the charge from the EMCCD is discarded. In an **acquired scan**, however, the charge undergoes A/D conversion and is acquired into computer memory so that it can be used for subsequent processing and display: it is ‘read out’ (see **readout**). In the **User’s Guide** ‘scan’ generally refers to an acquired scan - unless the context specifically indicates otherwise.

Scan;
Acquired Scan;
Keep Clean Scan

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 **EMCCD**-chip. The shift register is protected from light by an aluminum mask. The elements in the shift register have a greater capacity to store charge (a greater ‘well depth’) than the other pixels on the EMCCD-chip.

Shift Register

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

Shot Noise

Most simply defined: if the signal or **dark signal** is 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 **EMCCD** at suitably low temperatures, the dark signal, and hence the noise from the dark signal, can be reduced.

The **Signal to Noise Ratio (S/N)** 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 Signal to Noise Ratio usually increases (improves) as the signal increases.

Signal to Noise Ratio

The maximum Signal to Noise Ratio 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.

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.

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.

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