ANDOR™
TECHNOLOGY

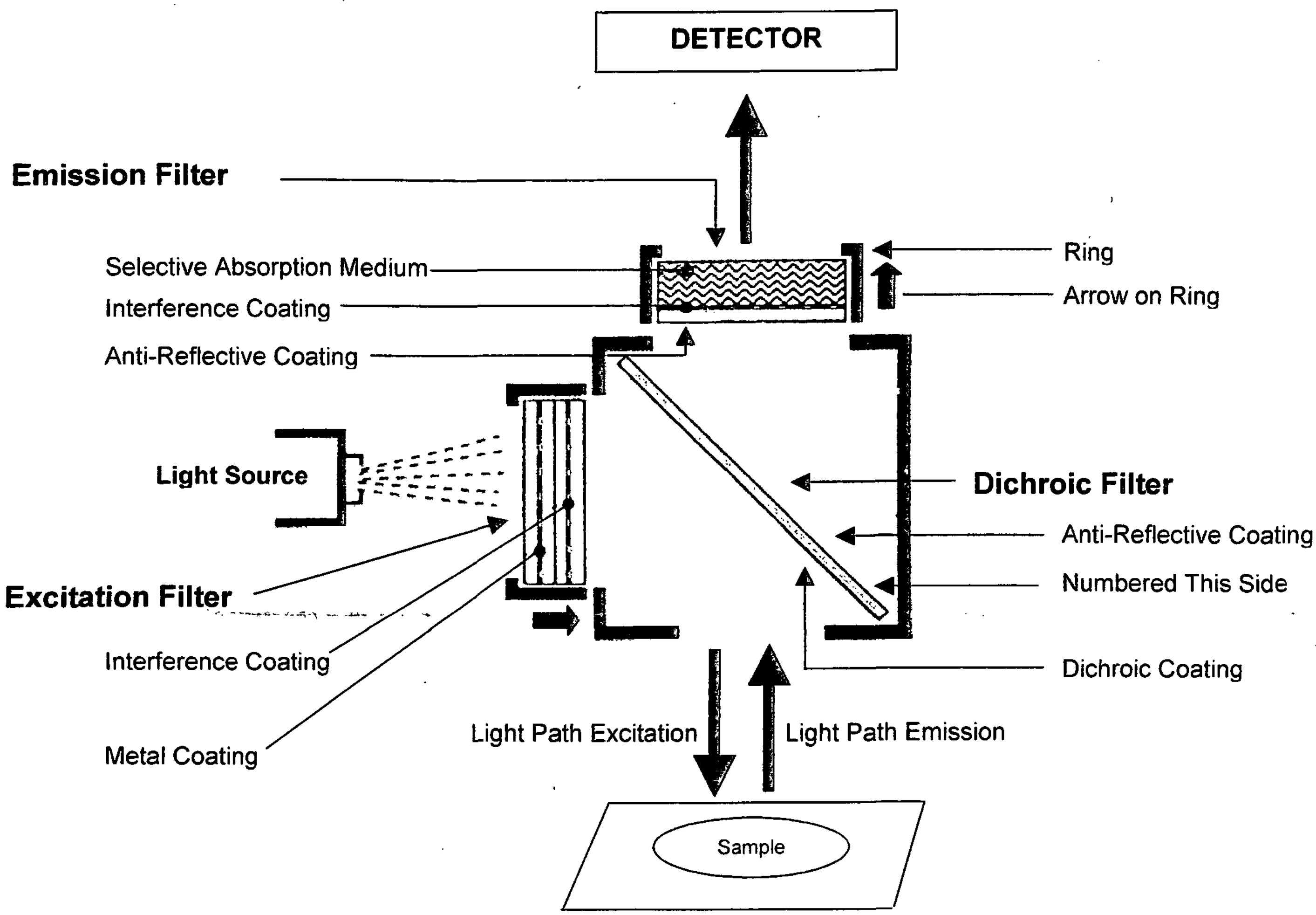
USERS GUIDE TO:

ANDOR™ TECHNOLOGY | iXon

www.andor.com

Version 3.5

Fluorescence Microscopy Filter Cube Schematic Diagram



Important Notes:

Orientation of Filters:

Omega exciter & emission filters have an arrow on ring pointing in the direction of the light path.

Care & Handling of Filters:

Precision optical components should be handled with reasonable care. Hold by edges only.

Cleaning of Optical Components:

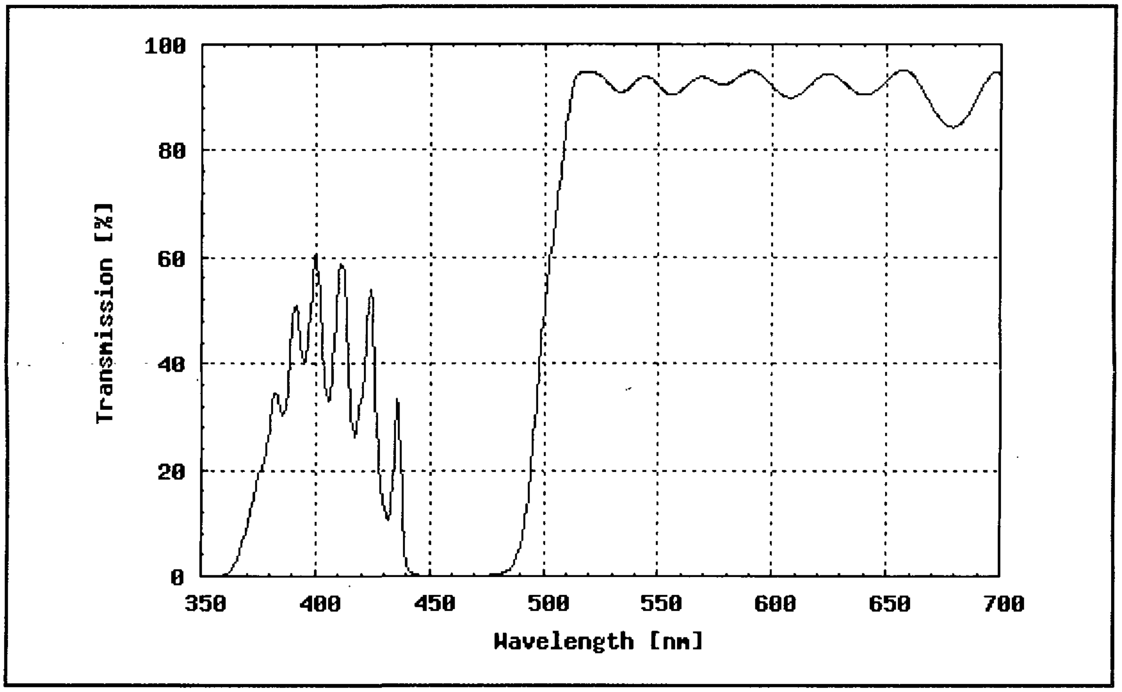
Remove any foreign particles with a puff of dry air. Wipe gently with a soft, lint-free cloth. A final wipe with a few drops of pure anhydrous alcohol will result in a clean, undamaged component.

OMEGA OPTICAL

Curv-o-matic

An interactive database of fluorophore absorption and emission spectra, along with filter set recommendations and accompanying filter spectral curves. For technical or sales questions e-mail sales@omegafilters.com.

Dichroic: XF2077 500DRLP



Performance & Tolerance Note

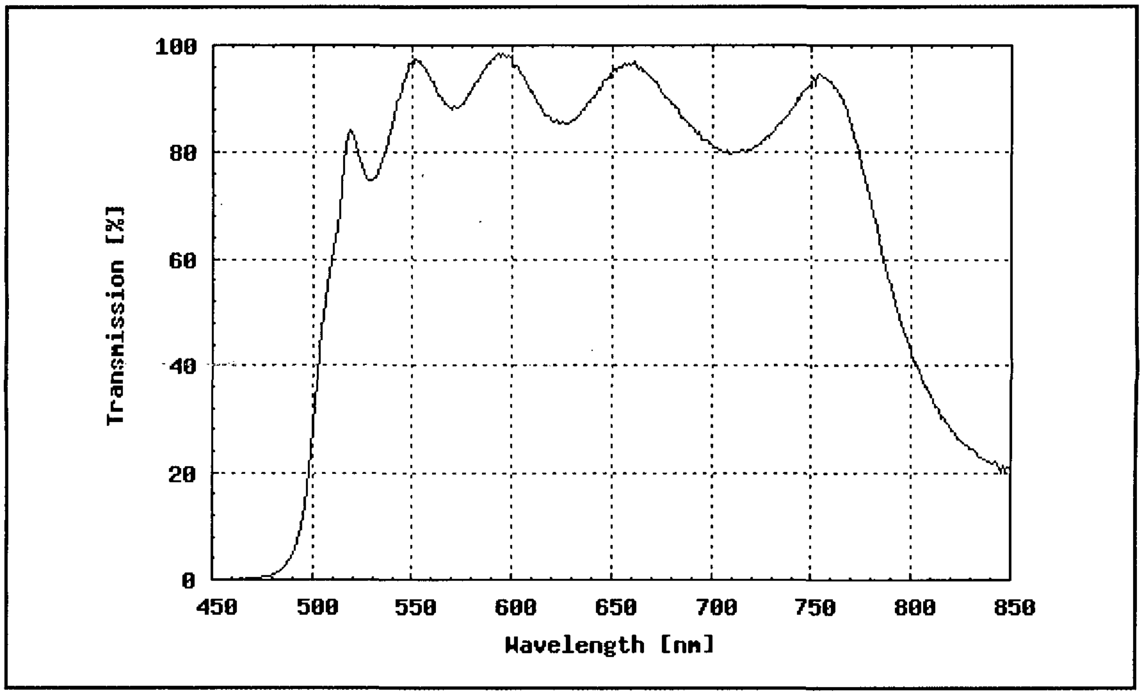
Standard microscopy filter sets and individual filters are designed as "functional" units for the visualization of specific fluorophores in a microscope. Curves and data presented in Curvomatic are measured from actual products which are designed to meet this definition. Spectral performance of individual sets and filters may vary from published data within normal manufacturing tolerances. Filters and sets in this program are not guaranteed for use in other applications. For applications and instrumentation requiring tighter tolerances, contact sales@omegafilters.com.

OMEGA OPTICAL

Curv-o-matic

An interactive database of fluorophore absorption and emission spectra, along with filter set recommendations and accompanying filter spectral curves. For technical or sales questions e-mail sales@omegafilters.com.

Dichroic: XF2026 495DRLP



Performance & Tolerance Note
Standard microscopy filter sets and individual filters are designed as "functional" units for the visualization of specific fluorophores in a microscope. Curves and data presented in Curvomatic are measured from actual products which are designed to meet this definition. Spectral performance of individual sets and filters may vary from published data within normal manufacturing tolerances. Filters and sets in this program are not guaranteed for use in other applications. For applications and instrumentation requiring tighter tolerances, contact sales@omegafilters.com.

TABLE OF CONTENTS

TABLE OF CONTENTS

PAGE

PAGE

PAGE

PAGE

SECTION 6 (continued)

TABLE OF CONTENTS

PAGE

PAGE

PAGE

TABLE OF CONTENTS

PAGE

SECTION 1 - ABOUT THE ANDOR iXon

Thank you for choosing the **Andor iXon** . 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. 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**.
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 directly, or your local representative or supplier, if you have any questions regarding your iXon system.

COMPONENTS

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 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. The main components of the Andor iXon CCD system are as follows:

- **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) & cable**

Optional Extras

The following items can also be added to the system as necessary:

- C-Mount Lens
- C-Mount Lens Adaptor
- F-Mount Lens
- F-Mount Lens Adaptor
- Mounting Posts

The detector head (**figure 1**) contains the following:

- **CCD Sensor with Pre-Amplifier**
- **16-bit analogue to digital converters that digitize data from the analogue controller boards. Under software control these data are transferred to the computer, where they are stored in computer memory.**
- **Temperature Sensor with Pre-Amplifier**
- **Thermoelectric Cooler & Cooling circuitry**
- **Input & output connectors**

The head can be attached to a **microscope** or other optical device for acquiring data.

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

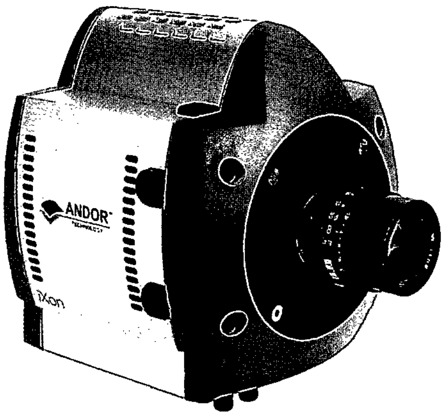


Figure 1: iXon Camera

Connectors

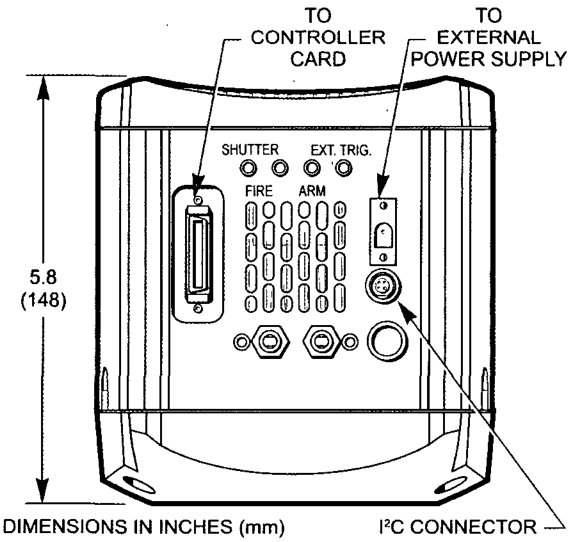


Figure 2: iXon CONNECTORS

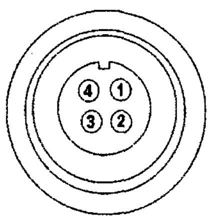
There are 4x industry-standard SMB (Sub Miniature B) connectors fitted to the rear of the detector. These are labelled from left to right as follows:

- **Fire**
- **Shutter**
- **Arm**
- **Ext Trig**

These are used to send or receive Triggering and Firing signals, which are described later in this manual. The SMB outputs (Fire & Shutter) are CMOS compatible & Series terminated at source (i.e. in the camera head) for 50Ω cable. **NOTE: The termination at the customer end should be high impedance (<u>not</u> 50Ω) as an incorrect impedance match could cause errors with timing and triggering. The SMB Ext Trig input is TTL Level & CMOS compatible and has 470Ω impedance.**

The other connectors are as follows:

- **Controller:** Connection for the 26 pin interface between the detector head and the PCI controller card.
- **Cooler Power:** Connection for the **P**ower **S**upply **B**lock (**PSB**) described on the next page.
- **I²C Connector:** Philips™ introduced the I²C™ bus 20 years ago and today it is the de facto standard for controlling and monitoring applications in computing, communications and industrial segments. The pin-outs used on the 4-way connector are as follows:



PIN	FUNCTION
1	I²C DATA
2	I²C CLOCK
3	+5V
4	GROUND

Figure 3: I²C connection (facing in) with pinouts

EMCCD Sensor

All of Andor's CCD detectors use our proprietary all-metal, glass and ceramic vacuum 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 If the sensor is cooled. Moisture and hydrocarbons cannot and will not build up in our detector head, even after years of use. The two water pipes allow water to be passed through the head to assist cooling as required.

Controller Card & Cable

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. 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

A 2.1 mm Jack connector, links the detector head to the **P**ower **S**upply **B**lock (**PSB**).

NOTE: Cooling is only available when the PSB is connected to the detector. For details of temperatures that can be achieved when using the PSB supplied, please refer to COOLING later in this section.

Mounting Posts

Mounting posts can be fitted on three sides of the camera that can be used to mount the camera if the C-Mount is not used, or to mount accessories. There are 3 pairs of holes for the mounting posts, each with 2.0" spacing.

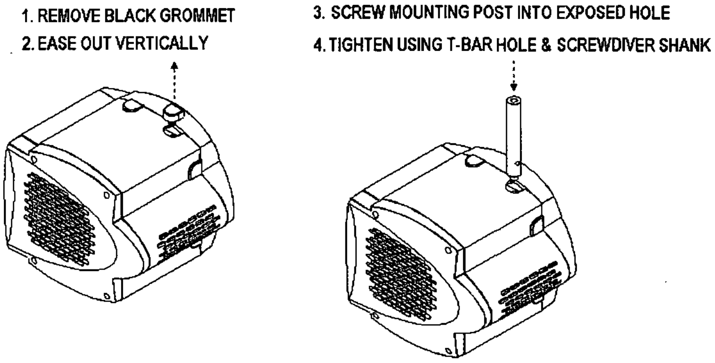


Figure 4: Mounting post installation

NOTE: A bag containing two Ø1/2" x 80mm long x1/4-20unc posts is included with all kits.

COOLING

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 heat sink). Therefore the minimum absolute operating temperature of the CCD depends on the temperature of the heat sink. Our vacuum design means that we can achieve a maximum temperature difference of over 110°C (DU models with optional PS-25), a performance unrivalled by other systems. The maximum temperature difference that a TE device can attain is dependent on the following factors:

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

The heat that builds up on the heat sink must be removed. This can be done in one of two ways:

1. **Air cooling:** a small built-in fan forces air over the heat sink.
2. **Water cooling:** external water is circulated through the heat sink using the water connectors on the 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 be dependant on or vary with track the heat sink temperature. Therefore a temperature sensor on the CCD, combined with a feedback circuit that controls the operating current of the cooler, allows stabilisation of the CCD to any desired temperature within the cooler operating range.

NOTE: In order to achieve the maximum cooling performance specified for your iXon CCD, you must use the PSB which has been supplied 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 (see note below), a heat sink 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.

NOTE: The fan does not operate until the heat sink temperature has reached between 20°C and 22°C. It is therefore quite normal for the fan not to operate when the system is first switched on. See also Setting Temperature for information on fan settings.

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

Table 1: Evacuated housing - High performance air cooling with power supply block

Air Temperature	External PSU box
20°C	-60°C
25°C	-58°C
30°C	-56°C

NOTES

1. **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.**
2. **Systems are specified in terms of the minimum Dark Current achievable, rather than Absolute Temperature. For Dark Current specifications, please refer to the specification sheet for your camera.**
3. **Cooling the CCD detector helps you reduce dark signal and its associated shot noise. Cooling will also affect the iXon CCD's Electron Multiplying Gain.**

Water Cooling Performance

A flow of water through the heat sink removes heat very efficiently, since the heat sink 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 **Table 2** below.

Water cooling, either chilled though a refrigeration process or re-circulated (which is water forced air cooled then pumped) 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 heat sink, the water taps and other metal parts of the head. This will quickly destroy the head and must never be allowed to happen. However this is not an issue when using a Recirculator which eliminates the dew point problem.

NOTE: Never use cooling water that is colder than the dew point of the air in the room. Damage caused in this way is not covered by the warranty.

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

Table 2: Evacuated housing - High performance water cooling with power supply block

Water Temperature	External PSU box
10°C	-75°C
15°C	-73°C
20°C	-71°C
25°C	-69°C

Dew Point

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

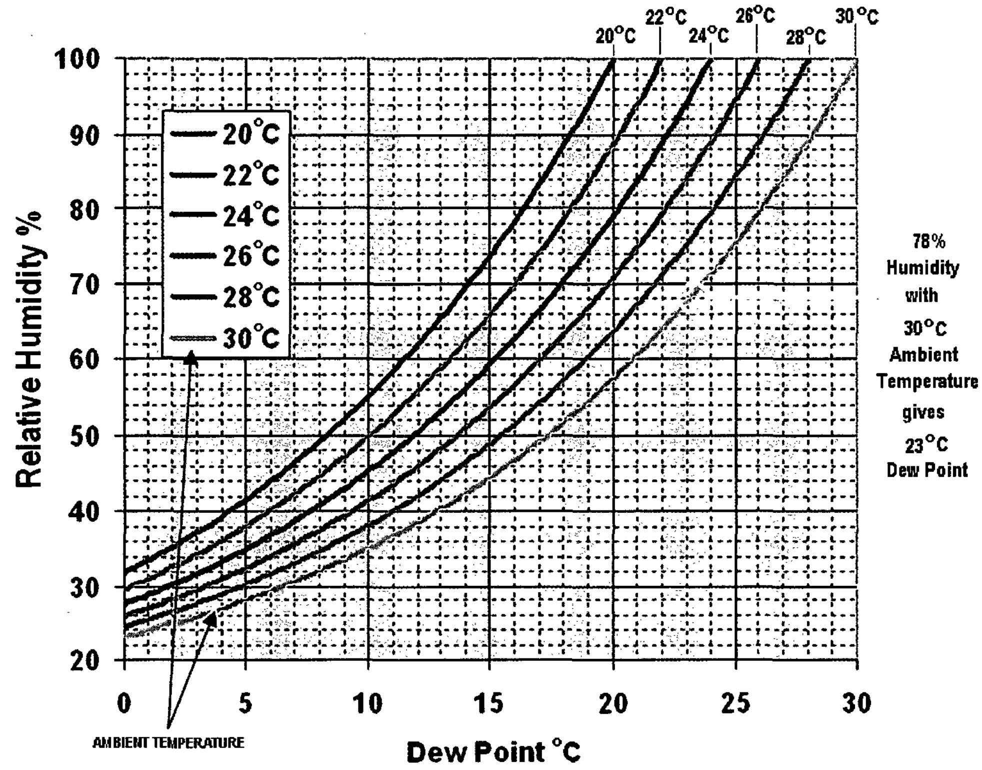


Figure 5: Dew point graph

For example, using a DU model & optional PS-25 you will need 10ºC cooling water to guarantee performance down to -100ºC. In the relatively dry atmosphere of an air-conditioned lab, cooling water at 10º should not present any problems. However, in humid conditions (such as exist in some parts of the world) condensation may occur, resulting in damage to the head. In such conditions you will have to use warmer water (20ºC or even higher if it is very humid). The minimum CCD temperature in this example would then be limited to between -90ºC to -95ºC.

Head Overheating

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

- The air vents on the sides of the head are accidentally blocked or there is insufficient or no water flow
- You are using air cooling with the optional PS-25 and have selected **Deep Cooling.** Air cooling may not be possible if the ambient air temperature is over 20°C.

To protect the detector from overheating, a thermal switch has been attached to the heat sink. If the temperature of the heat sink rises above 47°C, the current supply to the cooler will cut out and a buzzer will sound. The cut-out will automatically reset once the head has cooled. It is not recommended that you operate in conditions that would cause repeated cut-outs as the thermal switch has a limited number of operations.

NOTE: Never block the air vents on the detector head.

WORKING WITH THE USERS GUIDE

This User's Guide is your 'road-map' to the Andor iXon software and hardware.

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 and use standard Windows terminology to describe the features of the user interface.

If you are unfamiliar with Windows, the documentation supplied with your Windows installation will give you a more comprehensive overview of the Windows environment.

HELP

The software provides On-Line Help typical of Windows applications.

When the application is running, click the [?]. Button or press **F1** on the keyboard and the Andor MCD dialog box will open. Click on the area for which you require help and you will be provided with information relevant to the part of the application from which help was called.

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. To activate, with the cursor on or immediately after a reserved word, press **Ctrl + F1**.

So, whenever you're working with a particular window, you'll find a section in the User's Guide that sets that window in context, reminding you how the window is launched, letting you know what it can do, and telling you what other windows and operations are associated with it.

We hope you find use of our product rewarding. If you have any suggestions as to how our software, hardware and documentation might be improved, please let us know. You'll find the address of our nearest representative on the next page.

TECHNICAL SUPPORT

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

Europe

Andor Technology
7 Millennium Way
Springvale Business Park
Belfast
BT12 7AL
Northern Ireland
Tel. +44 (0) 28 9023 7126
Fax. +44 (0) 28 9031 0792
e-mail: sales@andor.com

USA

Andor Technology
425 Sullivan Avenue
Suite # 3
South Windsor
CT 06074
USA
Tel. (860) 290-9211-1085
Fax. (860) 290-9566
e-mail: sales@andor.com

Asia-Pacific

Andor Technology
7F Ichibancho Central Building
22-1 Ichiban-Cho
Chiyoda-Ku
Tokyo 102-0082
Japan
Tel. +81 3 3511 0659
Fax. +81 3 35110662
e-mail: sales@andor.com

WORLDWIDE DISTRIBUTORS

Country/Region	Contact	Phone Number	e-mail
Australia (I)	Mr Con Sapounas	**00 61 39416 9959**	con@scitech.com.au
Belgium, Luxembourg, Netherlands (S)	Mr Olaf Koschuetzke	**00 49 6151 88060**	koschuetzke@lot-oriel.de
China (S/I)	Mr Depei Wang	**00 86 10 649 71708**	dwang@bost-ltd.com
Denmark (S)	Mr Michael Lund	**00 45 3930 0403**	michael.lund@azpect.se
France	Mr Lionel Sudrie	**0033 1 69194949**	sudrie@lot-oriel.fr
Germany (I)	Mr Gerhard Haunert	**00 49 89 8901 3523**	gerhard.haunert@bfioptilas.avnet.com
Germany (S)	Mr Olaf Koschuetzke	**00 49 6151 88060**	koschuetzke@lot-oriel.de
Hong Kong	Ms Judy Chang	**00 852 2810 6090**	judy@teltec.biz
India (S/I)	Mrs Kaveri Mukherjee	**00 91 805534004**	specmco@vsnl.com
Israel (S/I)	Mr Moti Zvi	**00 972 3635 6650**	moti@newteh.co.il
Italy (S,I)	Mr Paolo Antonucci	**00 39 06 500 4204**	antonucci@lot-oriel.it
Poland	Mr Henryk Dluzewski	**0048 32 342 0666**	Dluzewski@lot-oriel.com
Portugal (S)	Mr Pedro Fernandes	**00 351 218 150760**	biodin@individual.eunet.pt
Singapore (S)	Mr Justin Kow	**00 65 6734 8619**	justinkow@teltecpacific.com
South Africa	Dr Maurice McDowell	**00 27 12 348 0569**	ceo@measureit.co.za
South Korea (S/I)	Mr Dong Hyun Kim	**00 82 31 765 0300**	optron@optron.co.kr
South Korea (S/I)	Mr Ji-Soung Park	**00 82 2 587 4369**	jspark@hkic.co.kr
Spain (S)	Ms Paloma Gonzalez	**00 34 91 806 4390**	info@iberlaser.com
Switzerland (S/I)	Mr Andre Galliker	**00 41 21 869 90 33**	galliker@lot-oriel.com
Taiwan (S/I)	Mr Richard Lin	**00 886 3 485 2036**	richard@mrnanotec.com
Turkey	Mr Akan Sahin	**00 90 312 472 5390**	aksahin@globalanalitik.com.tr

S = Spectroscopy Cameras

I = Imaging Cameras

(Details correct at time of going to print)

SECTION 2 - INSTALLATION

SAFETY PRECAUTIONS

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.

Care of the Detector Head

YOUR DETECTOR IS A PRECISION SCIENTIFIC INSTRUMENT CONTAINING FRAGILE COMPONENTS. ALWAYS HANDLE WITH THE CARE ACCORDED TO ANY SUCH INSTRUMENT.

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.

NOTE: Please refer to COOLING in Section 1 for further operating characteristics and safety features of your system.

INSTALLING THE HARDWARE

PC Requirements

The system requires a PCI-compatible computer. The PCI slot you use must have bus master capability. The minimum recommended specification is:

- 2.4 GHz Pentium Processor or better.
- 1GB of RAM
- Minimum 10,000 RPM Hard drive (RAID 15,000 RPM preferred for extended Kinetic series)
- 32 MB free Hard Disc space.
- Auxiliary internal power connector available

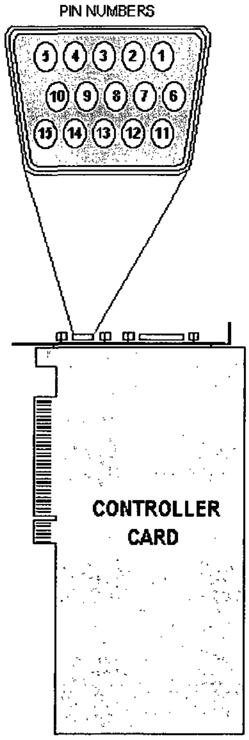
The operating system must Windows 2000 or XP.

PCI Controller Card

The **PCI Controller Card** is installed as you would other slot-in cards such as graphics cards.

NOTE: Please consult the manual supplied with your personal computer to ensure correct installation of the Controller Card for your particular PC.

CONTROLLER CARD: AUXILIARY CONNECTOR DESCRIPTION



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

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

Perform the installation as follows:

1. Exit the computer's operating system and switch off the computer and its accessories (see above).
2. Unplug the computer and its accessories from the wall outlet(s).
3. Unplug all cables from the rear of the computer.
4. Unscrew the cover mounting screws on the computer and set them aside safely.
5. Gently remove the cover of the computer.
6. 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, as you will need it later in the installation procedure. You are now ready to install the Controller Card.
7. While observing appropriate static control procedures 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.
8. 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.
9. Replace the cover of the computer and secure it with the mounting screws.
10. Reconnect any accessories you were using previously.

CONNECTING THE SYSTEM

Connect the elements of your system as follows:

1. Plug your PC into the mains outlet to ensure grounding, but keep the power switched off.
2. 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.
3. Your system has been supplied with a **P**ower **S**upply **B**lock 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.
4. There is only one socket on the detector head that the PSB can be connected to.
5. For best performance the PSB should be plugged into the same power source as the computer.

INSTALLING THE SOFTWARE

Windows 2000/XP

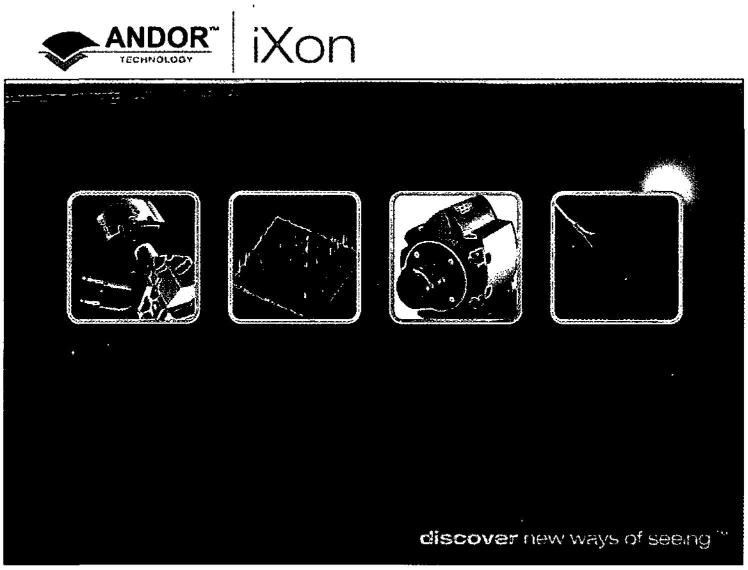
During the start up sequence the operating system will detect the Andor plug-in card and a dialogue box will prompt you for the location of the device driver.

- Insert the **Andor CD** and navigate from the dialogue 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 desktop select **Start...Programs...Andor\iXon**. **...Andor iXon**. .

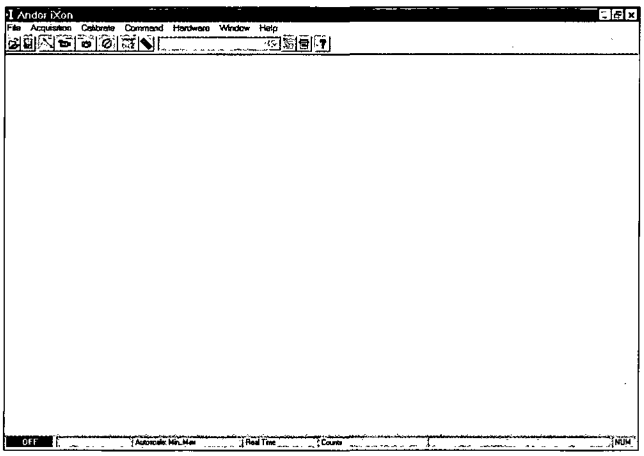
SECTION 3 – USING THE iXon

STARTING THE APPLICATION

On the desktop, click on the icon and the **iXon Splash Screen** appears briefly:



The **Main Window** then appears, e.g.:



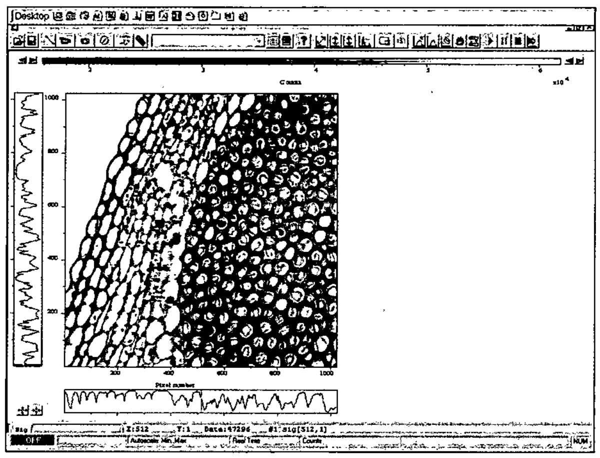


Figure 6: Example of data window displaying data (image)

MAIN WINDOW

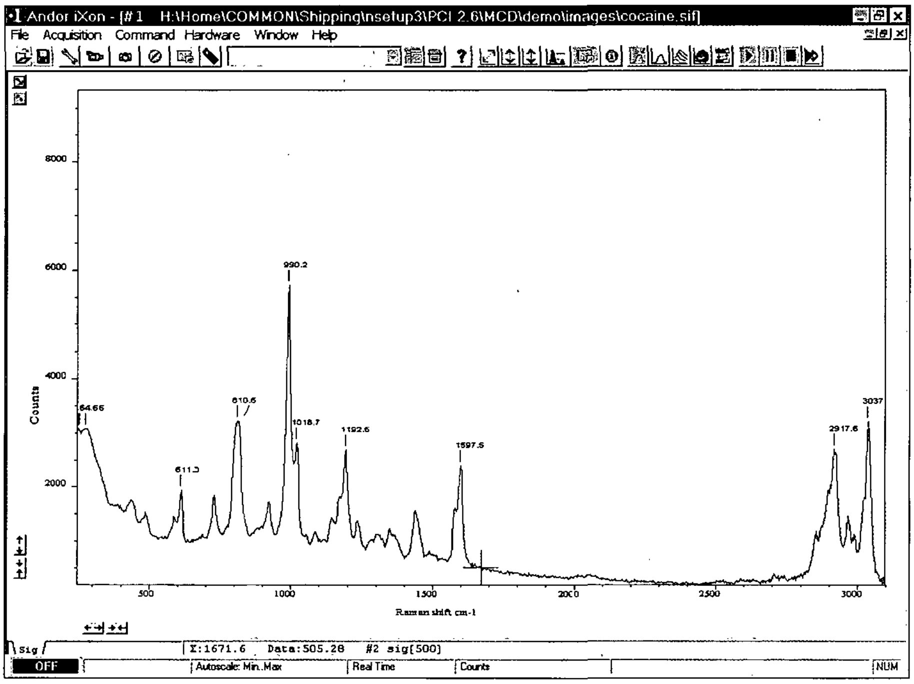
The **Main Window** is your "entry point' to the system. The menu options that you select from either execute functions directly, or launch further windows/dialog boxes that let you select the functionality you require. Some menu options on the Main Window are also represented as easy-to-use radio buttons, as shown in **Table 1** below.

Table 3: Main Window buttons

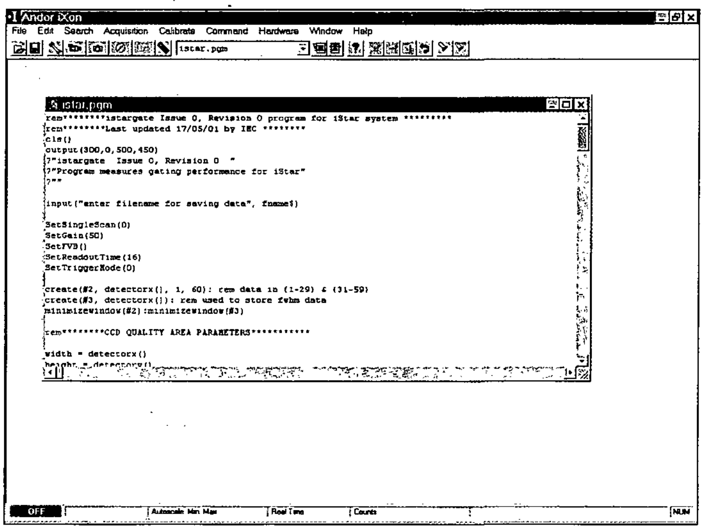
ICON	TITLE	ICON	TITLE	ICON	TITLE
	OPEN		CONTENTS		2D DISPLAY
	PRINT		SELECT SUBIMAGE AREA (NOT USED)		3D DISPLAY
	SAVE		SELECT AUTOSCALE AREA (NOT USED)		IMAGE DISPLAY
	REAL TIME		RESET		CHANGE FALSE COLOR PALETTE
	TAKE SIGNAL		RESCALE		TIME STAMP
	ABORT ACQUISITION		ACQUISITION AUTOSCALE #0		PLAY
	SETUP ACQUISITION		DATA HISTOGRAM		PAUSE
	RUN-TIME (NOT USED)		REGION OF INTEREST		STOP
	RUN PROGRAM		FILE INFORMATION		PLAYBACK AUTOSCALE OFF
	COMMAND LINE		2D DISPLAY WITH PEAK LABELS	OFF or -45°C	TEMPERATURE CONTROL

NOTE: Some menu titles and buttons appear on the Main Window only under certain circumstances as shown on the next page.

- The **Display** menu and its associated buttons will not appear until you open a **Data Window**, e.g.:



- The **Edit** & **Search menus** and their associated buttons appear only when a **Program Editor Window** is active, e.g.:



HOT KEYS

Hot keys (or shortcuts) as shown in **Tables 2, 3 & 4** enable you to work with the system directly from the keyboard, rather than via the mouse.

Table 4: Data Acquisition Hot Keys

KEY STROKE	DESCRIPTION
F5	Take signal
F6	Autoscale Acquisition
Ctrl + B	Take background
Ctrl + R	Take reference
Esc	Abort Acquisition

Table 5: Data Window Hot Keys

KEY STROKES	DESCRIPTION	DISPLAY MODE 2D	3D	IMAGE
+	Expand ('Stretch') data-axis	✓	✓	✓
-	Contract ('Shrink') data-axis	✓	✓	✓
Ins	If maintain aspect ratio off, expand x-axis. If maintain aspect ratio on, expand x-axis and y-axis	✓	✓	✓
Del	If maintain aspect ratio off, contract x-axis. If maintain aspect ratio on, contract x-axis and y-	✓	✓	✓
/	On image, if maintain aspect ratio off, expand y-axis.			✓
Home	Move cursor furthest left	✓	✓	✓
End	Move cursor furthest right	✓	✓	✓
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	✓	✓	✓
Ctrl + Left Arrow	Peak search left	✓		
Ctrl + Right Arrow	Peak search right	✓		

Table 5: Data Window Hot Keys (continued)

KEY STROKES	DESCRIPTION	DISPLAY MODE 2D	3D	IMAGE
Left Arrow	Move cursor left	✓	✓	✓
Right Arrow	Move cursor right	✓	✓	✓
Up Arrow	Scroll trace up	✓	✓	✓
Down Arrow	Scroll trace down	✓	✓	✓
Shift + Left Arrow	Scroll trace/image left	✓	✓	✓
Shift + Right Arrow	Scroll trace/image right	✓	✓	✓
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 section)	✓		
F10	File information	✓	✓	✓

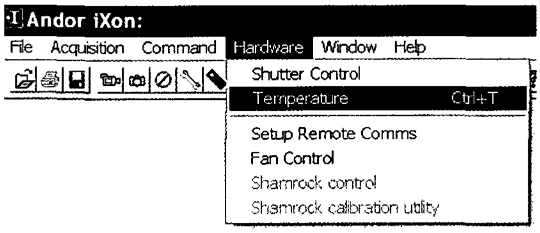
Table 6: Andor Basic Programming Language Hot Keys

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 **Program Editor Window**.

SECTION 4 – PRE-ACQUISITION

SETTING TEMPERATURE

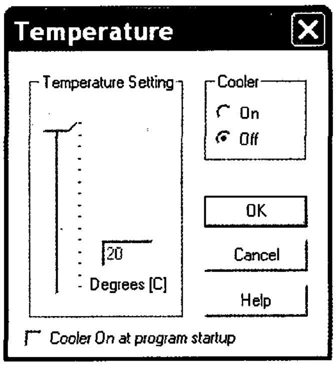
For accurate readings, the CCD should first be cooled, as this will help reduce dark signal and associated shot noise. To do this, either select the **Temperature** option from the **Hardware** drop-down menu on the Main Window:



or click the 

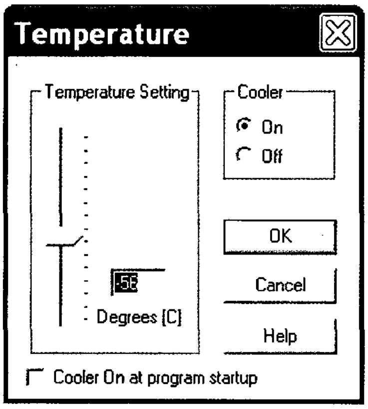
button in the bottom-left of the screen.

This will open up the **Temperature** Control dialog box :



Select **On** in the Cooler check box.

The **Degrees (C)** field in the **Temperature Setting** section will now be highlighted in blue and the Cooler will be indicated as **On,** e.g.:



To adjust the temperature, either type in the new figure in the **Degrees (C)** box or slide the arrowed bar down or up.

Once the desired temperature has been selected, click **OK**. The dialog box will disappear and the **Temperature Control** button in the bottom-left of the screen will show the current temperature highlighted in red e.g.:

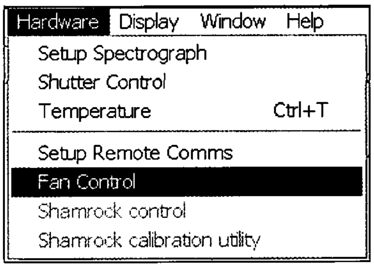
This figure will change as the head cools. Once the head has reached the desired temperature, the highlighted area changes to blue.

You can also select the option to have the Cooler is on as soon as you start the application. This is selectable in the bottom-left of the Temperature dialog box.

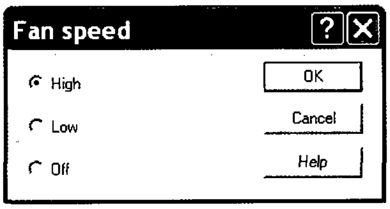
PLEASE REFER TO <u>COOLING</u> IN SECTION 1 FOR DETAILS OF MINIMUM ACHIEVABLE TEMPERATURES, AND IMPORTANT ADVICE ON AVOIDING OVERHEATING.

Fan Control

The speed of the cooling fan can also be controlled. Select Fan Control from the Hardware drop-down menu as shown:



The Fan speed dialog box will appear:



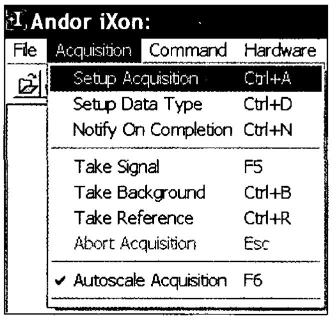
Select the speed you require as necessary (this may affect the cooling ability of the CCD).

NOTE: After changing from High to Low, it may be necessary for the camera temp to stabilize before acquiring data. However for optimum performance it is recommended to leave the fan setting at HIGH.

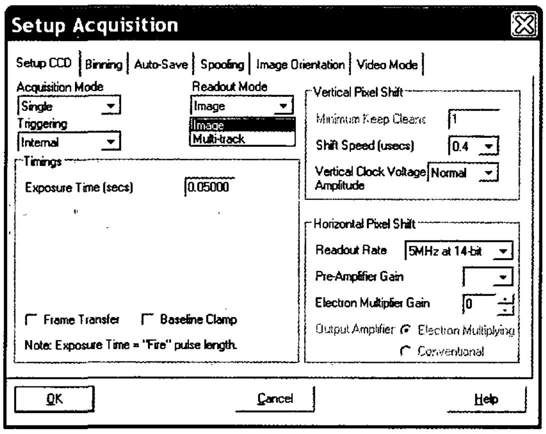
SETUP ACQUISITION

To select the mode of acquisition prior to data capture, you can choose one of the following options:

- Click the button,
- Type in **Ctrl+A** from the keyboard
- Select **Setup Acquisition** from the **Acquisition** drop-down menu:



The **Setup Acquisition** Dialog box appears, e.g.:



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:

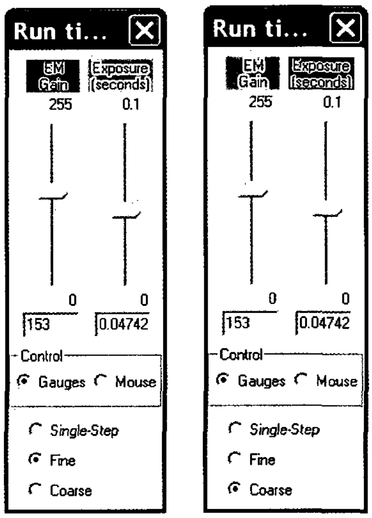
Mode	Exposure Time	Accumulate Cycle Time	No. of Accumulations	Kinetic Cycle Time	No. in Kinetic Series
Single Scan	✓				
Accumulate	✓	✓	✓		
Kinetic	✓			✓	✓

Run Time Control

The **Run Time Control** provides the user with the ability to control the following parameters using slider controls:

- EM Gain
- Exposure time of the CCD

The controls are activated by clicking on the  button on the Main Window. When selected, the dialog box appears, e.g.:



Each dialog box has sliders that can be moved up and down to control the required parameters and there are three levels of control:

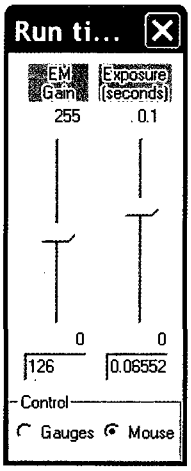
- **Single-Step**
- **Fine**
- **Coarse**

The controls can also be accessed either by using the relevant buttons on the **Remote Control** (the buttons are explained later in this section) or by moving the windows pointer with the mouse or the remote control unit (if attached). 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: The exposure gauge upper limit will auto-range as the setting is increased.

When the **Control** section is selected for **Mouse**, the Single-Step, Fine & Coarse selectors are removed and the slider titles are no longer highlighted in green, e.g.:



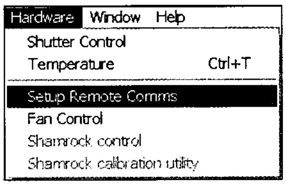
While the remote is being used to access the Run Time Control dialog box, the Trigger on the underside of the Remote Control is used to make or cancel an acquisition. It has the same function as pressing the green Take Signal button or the red Cancel Take Signal on the toolbar of the main window.

Remote Control

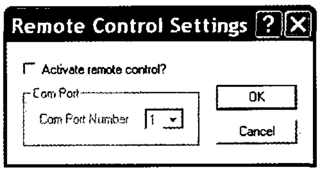
The **Remote Control** (**figure 5, page 47**) allows various commands of the iXon software to be modified using an infra-red signal sent to a **Receiver** (**figure 6, page 47**). The range of operation of the remote control is up to a distance of 12m from the receiver along the line of sight. Connect 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.

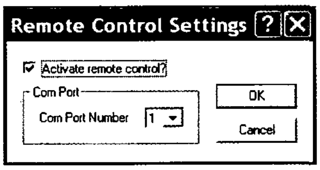
To enable the infra-red Remote Control to be activated, go to the **Hardware** Menu on the main window of the software and select **Setup Remote Comms** as shown:



The **Remote Control Settings** dialog box appears as shown:



Click in the **Activate remote control?** box and the dialog box changes as shown:



Select the tick box to activate the remote control. Then from the Com Port Number drop-down menu select the Port that the receiver is connected to.

NOTE: Com Port Number 1 is the default.

The remote control has two modes of operation.

The first mode provides direct control over the **Run Time Control** dialog box (see Run-Time Control), where the following parameters may be varied:

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

Note Gain and Exposure time may also be modified using the Setup Acquisition Dialog Box

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.

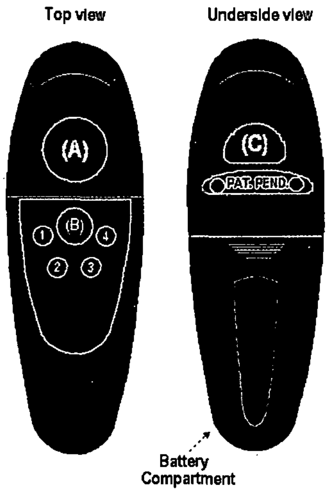


Figure 5: Remote Control

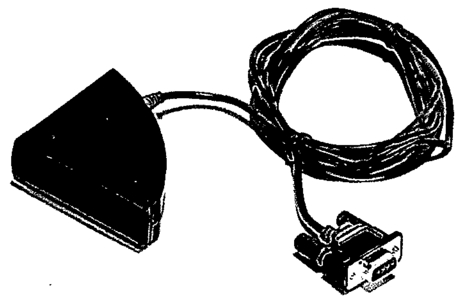


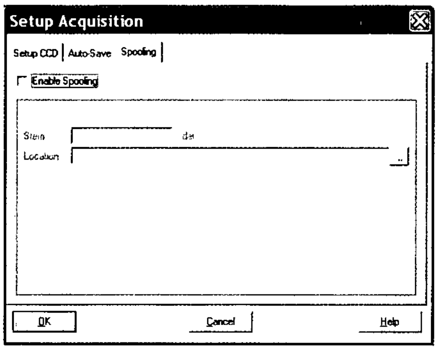
Figure 6: IR Receiver

BUTTON	RUN TIME FUNCTIONS	WINDOWS FUNCTIONS
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 control over the gauges between single-step, fine and coarse	**INACTIVE**
3	Switches the Autoscale function on or off	
4	As button 1	**INACTIVE**

SPOOLING

The Andor MCD software allows you to spool acquisition data direct to the hard disk of your PC. This is particularly useful when acquiring a series of many images. The amount of data generated by a Kinetic Series of, for example 1000 acquisitions, is huge and more than most PC RAM can handle.

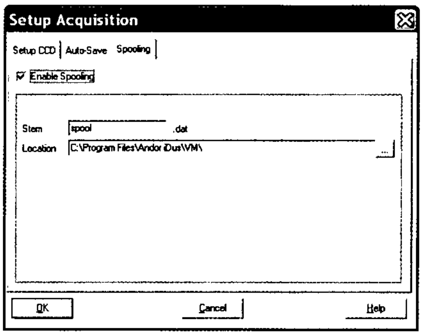
To select click on the **Spooling** tab and the Spooling dialog box appears e.g.:



With the spooling function enabled, data is written directly to the hard disk of you PC, as it is being acquired.

To enable the spooling function on your software, tick Enable Spooling on the Spooling dialog Box.

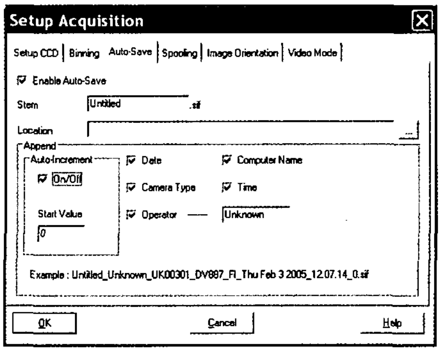
You must also enter a stem name, and also select a location for the for this spooled data file, e.g.:



NOTE: Spooling large amounts of data straight to hard disk for later retrieval requires a hard disk of sufficient read-write speed. Andor recommends only very high-speed hard disk drives be used for this type of operation.

AUTO-SAVE

Auto-Save allows you to set parameters and controls for the auto saving of acquisition files thus removing the worry of lost data and files. To select, click on the Auto-Save tab on the Setup Acquisition dialog box The Auto-save dialog box appears, e.g.:



Tick the **Enable Auto-Save** 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.

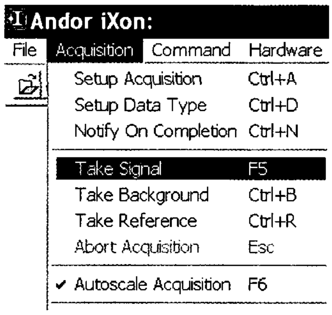
NOTE: This function will only auto-save single scan, kinetic series, fast kinetics or accumulated images, <u>not</u> data acquired in video mode.

SECTION 5 – ACQUIRING DATA

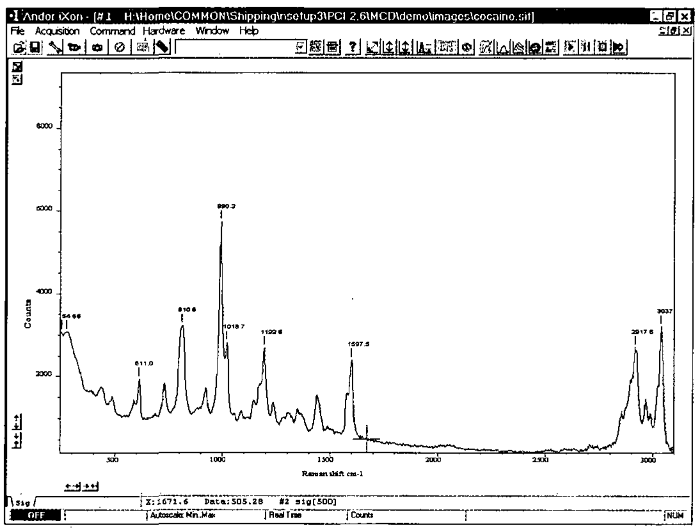
INITIAL ACQUISITION

To start an initial data acquisition you can either:

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



The **Data Window** opens (labeled **#0 Acquisition)** and displays the acquired data, according to the parameters selected on the **Setup Acquisition** Dialog box. e.g.:



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.

#n uniquely identifies the data set while the data set is being displayed and is temporary. It ceases to be associated with the data set once you close all data windows bearing the same #n. It is often referred to as an **Acquisition Window.**

NOTE: Each Data Window has the same name and #n (which identify the data set), but a unique number, following the data set name, to identify the window itself. 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.

If you have selected **Accumulate** or **Kinetic** as the Acquisition Mode, new data will continue to be acquired and displayed until you carry out one of the following actions:

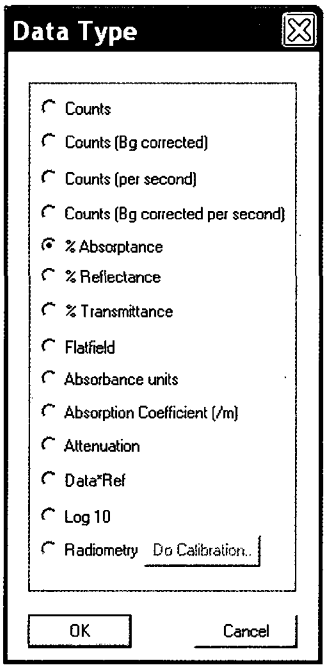
- Select **Abort Acquisition** from the Acquisition drop-down menu.
- Click the Abort Acquisition button
- Press the **<ESC>** key.

This stops any data capture process that may be under way.

Information on how to capture & view more detailed data is contained in the pages that follow.

DATA TYPE SELECTION

When the **Setup Data Type** option of the Acquisition drop-down menu is selected, the **Data Type** dialog box opens:



From the dialog box you can select the type of information (e.g. **%Absorptance**) that you want the system to compute and display whenever you perform Take Signal. The acquired data are presented under the **Sig** tab of an Acquired Data Window. 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. These options are described in more detail later in this section.

The descriptions of the data types are shown in **Table 7** which follows on the next 2 pages.

Table 7: Data Types

OPTION	FUNCTION
Flatfield	Flatfield is used to remove any pixel-to-pixel variations that are inherent in the CCD sensor. If **Reference** is the background corrected incident intensity, the **Signal** is divided by the **Reference** so: **Flatfield = M x Signal / Reference** where **M** is the **Mean of Reference**.
Absorbance units	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 **Transmission = (Signal - Background) / Reference**. Absorbance Units are defined as **Log_{10} (1 / Transmission)**. Therefore: Absorbance Units = **Log_{10} (Reference / (Signal - Background))**.
Absorption Coefficient (/m)	Indicates the internal absorptance of a material per unit distance (**m**). It is calculated as **-loge t**, where **t** is the unit transmission of the material and loge is the natural logarithm. If **b** 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: **Transmission = (Signal - Background) / Reference** and: **Absorption Coefficient = -loge ((Signal - Background) / Reference**)
Attenuation	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 = 10 x log_{10} ((Signal - Background) / Reference)**
Data*Ref	Allows you to 'custom modify' the background corrected signal: **Data x Ref = (Signal - Background) x Reference Store Value** See the Andor Basic Programming Manual for similar operations.
Log 10	Calculates the logarithm to the base 10 of the background corrected signal counts. **Log Base 10 = log_{10} (Signal - Background)**
Radiometry (OPTIONAL EXTRA)	Allows you to calculate values for radiance or irradiance. The system requires that you supply calibration details. This option must be ordered separately.

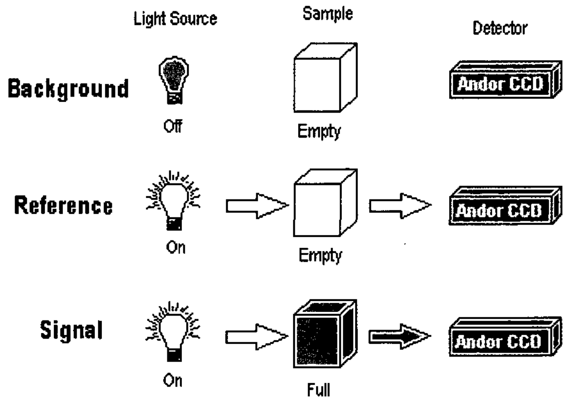
Table 7: Data Types (continued)

OPTION	FUNCTION
Counts	Counts represent raw, digitized data (i.e. no calculations have been performed on the data) from the CCD detector's analog to digital (A/D) converter. Please refer to the detailed performance sheet accompanying your particular CCD detector for the number of electrons that correspond to 1 count.
Counts (Bg corrected)	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) = **Signal - Background**
Counts (per second)	Counts divided by Exposure Time.
Count (Bg corrected per second)	Counts (Bg corrected) divided by Exposure Time.
%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 = 100 x (1 - (Signal - Background) / Reference)**
%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 = 100 x (Signal - Background) / Reference**
%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 = 100 x (Signal - Background) / Reference**

As an example, the system will compute % Absorptance as:

100 x (1 - (Signal - Background) / Reference).

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



USE OF BACKGROUND, REFERENCE & SIGNAL
Example: % Transmittance, % Absorptance

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.**

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.

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 though the material being studied.

If you require raw or background corrected data pertaining to the light source itself, Signal will be data acquired directly from the source.

If you intend to compute the reflection, transmission or absorption characteristics of a material, Signal will be data acquired from light that has passed through or has been reflected from the material being studied.

NOTES:

1. **'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.**
2. **Functionality for displaying and manipulating data is only available if a Data File has been opened, 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.**

ACQUISITION TYPES

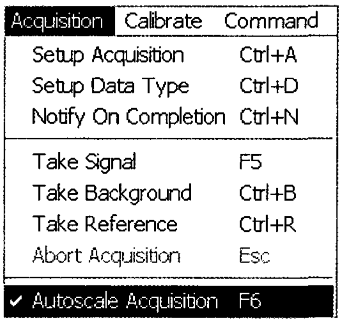
From the **Acquisition** drop-down menu on the Main Window, you can make the following data acquisition selections:

- **Take Signal**
- **Take Background**
- **Take Reference**

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.

Take Signal (Autoscale Acquisition OFF)

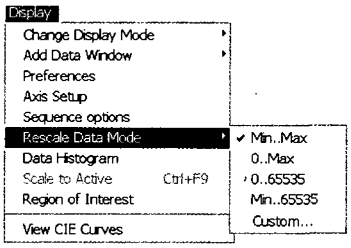
Autoscale Acquisition can be selected from the Acquisition drop-down menu as shown (or press F6 on the keyboard):



With Autoscale Acquisition deselected, the display will remain the same size regardless of brightness settings, etc. When selected off, the button appears (click this button to switch back on).

Take Signal (Autoscale Acquisition ON)

With Autoscale Acquisition selected, 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 button appears when selected on. The data are displayed in accordance with the selection made on the **Rescale Data Mode** on the **Display** Menu:



You can choose to display values between the following parameters:

Minimum & maximum **(Min..Max)**

Zero & maximum **(0..Max)**

Zero & 65535 **(0..65535)**

Minimum & 65535 **(Min..65535)**

Custom setting as required.

For further information on **Rescale**, please refer to **page 105**.

Take Background

The **Take Background** option of the Acquisition drop-down menu instructs the system to acquire raw background data.

These are as counts of the Acquisition Window. No calculations are performed on these data.

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: 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 <u>new</u> background data are required.

Take Reference

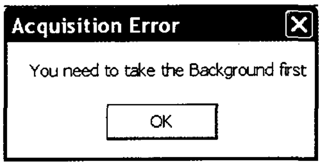
The **Take Reference** option of the Acquisition drop-down menu instructs the system to acquire background corrected data that will be used subsequently in calculations that require a reference value. Before executing this function you must therefore perform a **Take Background**.

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

NOTE: 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.

Acquisition Errors

If you perform an operation '**out of sequence**', the system will prompt you by launching an **Acquisition Error** message, e.g.



ORGANIZING YOUR WINDOWS

The **Window** menu offers a number of options which help you manage the data windows or icons that you have created while working with the system. **Table 8** below explains the available options.

Table 8: Window Options

OPTION	DESCRIPTION
CASCADE	Arranges any open Data Windows or Program Editor Windows) into a stack which runs diagonally across the Main Window and allows you to see the title bars of each of the windows in the stack.
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 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.
ARRANGE ICONS	Places in a row (starting at the bottom left of the screen) any icons that have been created within the system.
CLOSE ALL	Removes all windows and icons from the Main Window. You will be prompted to save any unsaved data.
COPY TO CLIPBOARD	For use only with Data Windows - Copies the contents of the active Data Window into the Windows 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'.

In addition to the window-handling options described, the Window Menu displays a list below the Copy to Clipboard option of any open Data Windows or Program Editor Windows or are currently iconized within the system. When the name of the window or icon in the list is selected, the corresponding window or will be brought to the front of the Main Window (if the Data Window or Program Editor Window was iconized it will now be opened as well).

ACQUISITION MODES & TIMINGS

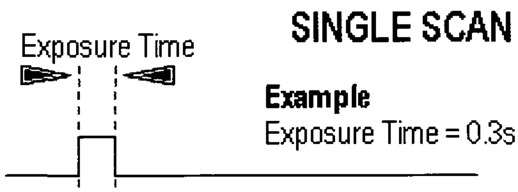
An acquisition is taken to be the complete data capture process that is executed whenever you select Take Signal, Take Background, or Take Reference from the Acquisition Menu or whenever you click the Take Signal button.

By contrast, a scan (an **'Acquired Scan'** in the definitions that follow) is **1x readout** of data from the CCD-chip. Several scans may be involved in a complete data acquisition.

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

Single

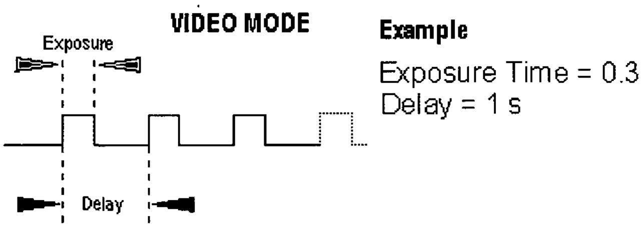
Single scan is the simplest acquisition mode, in which the system performs one scan of the CCD.



NOTE: Should you attempt to enter too low a value, the system will default to a minimum Exposure Time.

Video

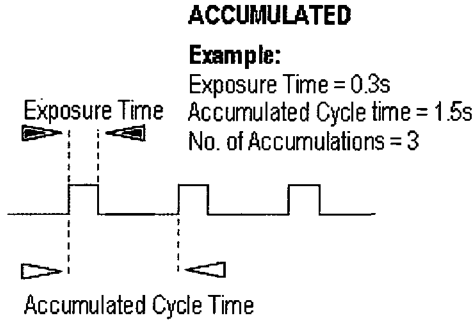
If you click the button, the system repeatedly performs a single scan and updates the data display.



NOTE: This is a useful 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.

Accumulate

Accumulate mode allows you to add together in computer memory the data from a number of scans to create an '**Accumulated Scan'** e.g.:



You can select the following parameters in the Setup Acquisition dialog box:

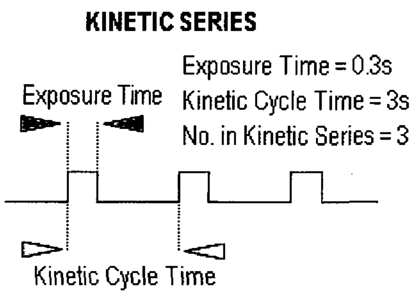
- **Exposure Time**
- **Accumulated Cycle Time** - i.e. the period in seconds between each scan. This parameter is only available if you have selected **Internal triggering** (please refer to **Triggering Modes** later in this section.)
- **No. of Accumulations** - i.e. the number of scans you want to add together.

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

Kinetic Series

In the Setup Acquisition dialog box you can key in the following parameters:

- **Exposure Time** (should you attempt to enter too low a value, the system will default to a minimum Exposure Time).
- **Kinetic Cycle Time** - i.e. the time between the start and finish of each kinetic scan.
- **Number in Kinetic Series** - i.e. the number of scans taken in the kinetic series.



NOTE: This mode is particularly well suited to recording the temporal evolution of a process.

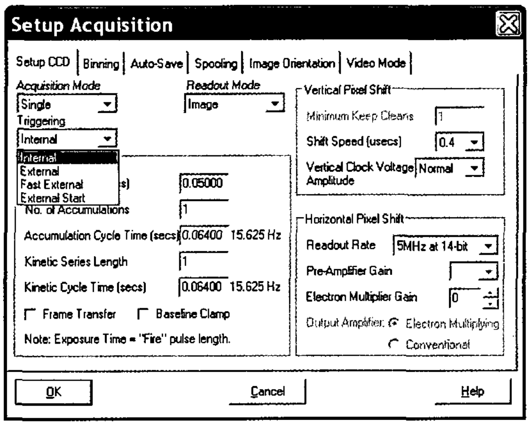
Frame Rates

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.

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

TRIGGERING MODES

The **Triggering** modes are selected from a drop-down list on the Setup Acquisition dialog box:



Internal

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

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

Internal Trigger Mode is also used with '**C**ontinuous **W**ave' (**CW**) sources (e.g. an ordinary room light) where incoming data, for the purposes of your observation, are steady and unbroken. This means that acquisitions can be taken at will.

External

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

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

Fast External

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

NOTE: If you need Maximum Repetition Rate, have a shutter connected and are using Fast External triggering, you must ensure that the shutter is open before the optical signal you want to measure occurs.

External Start

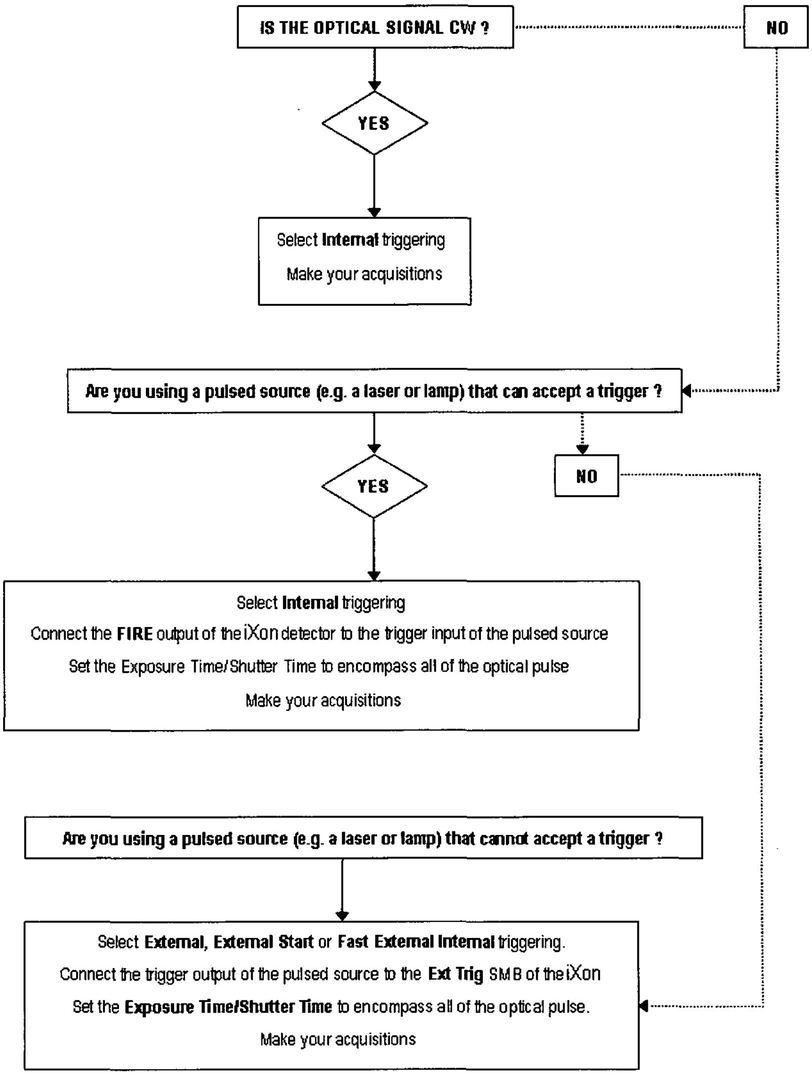
With **External Start** triggering, once you issue a data acquisition command, data will not be acquired until your system has received an external trigger signal generated by an external device. The system will then continue to acquire data based on user options set within the Acquisition Dialog.

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

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.

TRIGGERING TYPE SELECTION

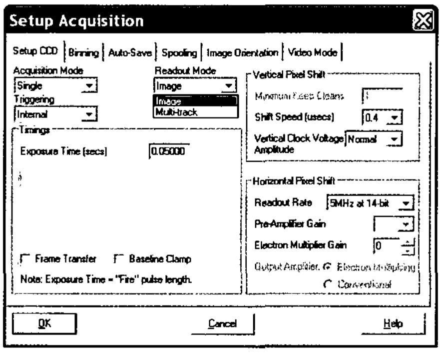
The following flowchart will help you decide whether you should use **Internal**, **External**, **External Start** or **Fast External** triggering.



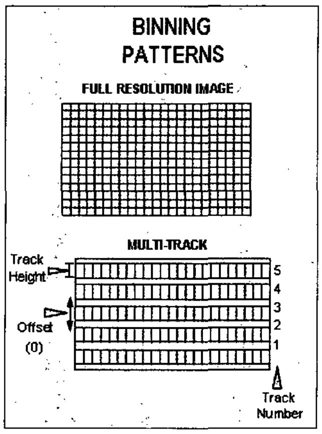
READOUT MODES

The **Readout Modes** available from the Setup Acquisition dialog box let you use the CCD chip at the heart of the detector to collect/readout data. The options available are as follows:

- Image
- Multi-track



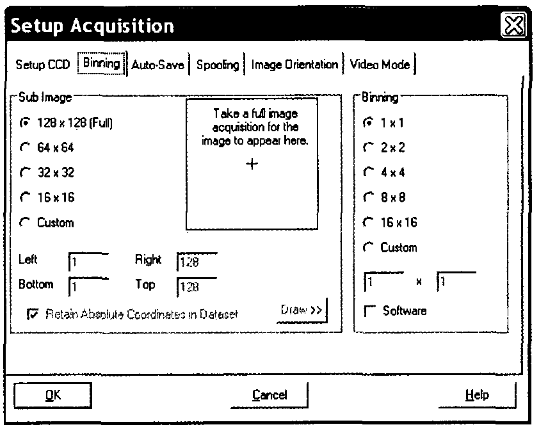
The **Binning** patterns used in each readout mode are as follows:



Binning is a process that allows charge from two or more pixels to be combined on the CCD-chip prior to readout. For a full explanation of binning please see **pages 171 - 173**.

NOTE: The examples given in this manual to illustrate the use of binning patterns are based on a 30-11 chip with 1024 x 256 pixels, each pixel measuring 26 μm^2.

When the **Setup Image** tab is clicked, the **Setup Image** dialog box appears, e.g.:



The user can then to set and control various **Binning Patterns** and define **Sub Images** of the iXon CCD.

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, e.g.:

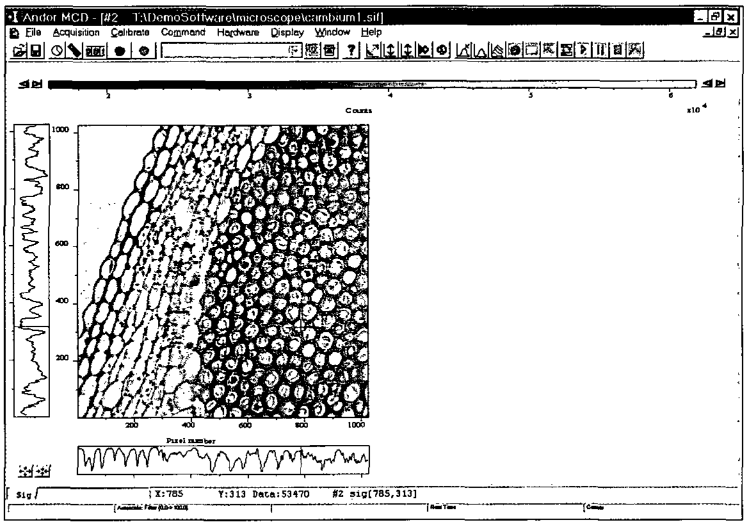
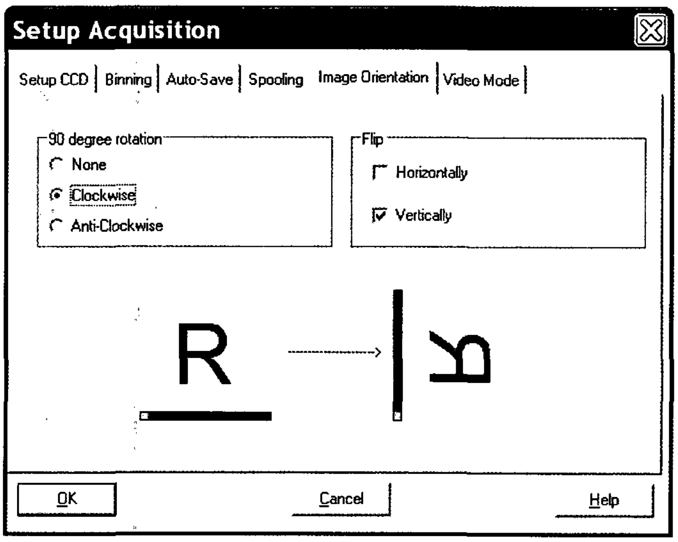


Image Orientation

In **Image** mode, the data can also be orientated as they are acquired. This is particularly useful if the CCD-chip has a readout register along its short, vertical edge. Without rotation, images would by default appear sideways on screen.

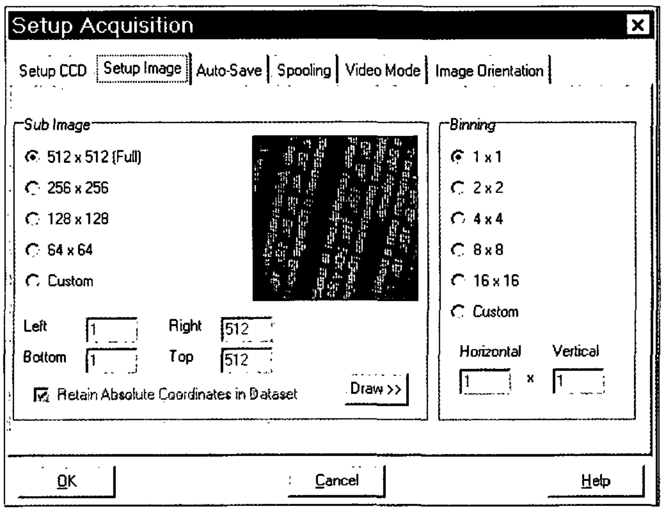
To orientate the image data, click the **ImageSpectral Orientation** tab on the Setup Acquisition dialog box, then select the required parameters with the appropriate check buttons, e.g.:



Sub Image

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. To select Sub Image mode, click on the button 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, e.g.:



The software offers a choice of three defined sub images:

- 256 x 256 pixels
- 128 x 128 pixels
- 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 tick the **Custom** button, then use the co-ordinate entry dialogue boxes to select the size and location of your sub image.

Draw

In addition to the previous 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.

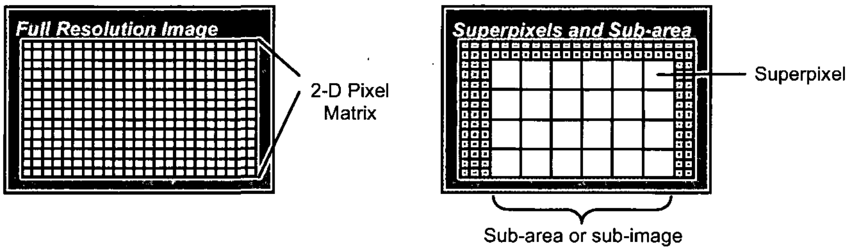
Click on the Draw >> button then use the Draw tool to select the size and position of your Sub Image by dragging rulers form the X and Y-axis. Alternatively, a Sub Image can be drawn on the template by positioning your cursor on the image, and dragging out the shape of the Sub Image area you require.

Superpixels

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.

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:

- 1 x 1 pixels
- 2 x 2 pixels
- 4 x 4 pixels
- 8 x 8 pixels
- 16 x 16 pixels.

For example, if you enter 4 x 4 binning the CCD-chip is notionally divided into a matrix of superpixels which each measure 4 x 4 pixels and provide a signal for readout.

There is also an option for you to define a **Custom Binning Pattern.** Tick the **Custom** radio button and enter the dimensions of the superpixels (as a number of pixels vertically and horizontally) in the text boxes.

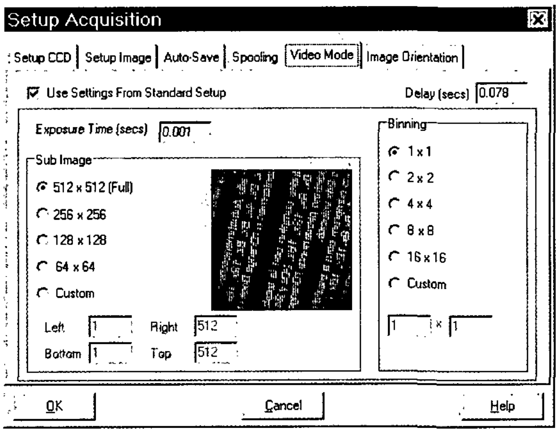
Working with Superpixels

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 4 x 4.

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).

Video Mode

When the **Video Mode** tab on the Setup Acquisition dialog box is clicked, the Video Mode dialog box opens, e.g.:



When the **Use Settings From Standard Setup** option is selected, the following parameters can be adjusted :

- **Exposure Time**
- **Delay (i.e. the time you require between scans).** If you attempt to enter too low a value, the system will default to a minimum delay.
- **Resolution (Sub-image area)**
- **Binning pattern**

The system will acquire data only as quickly as the data can be displayed. If you perform Take Background or Take Reference in video Mode, the system will perform one scan only rather than repeatedly performing a scan at the delay indicated. New data will continue to be acquired and displayed until you either :

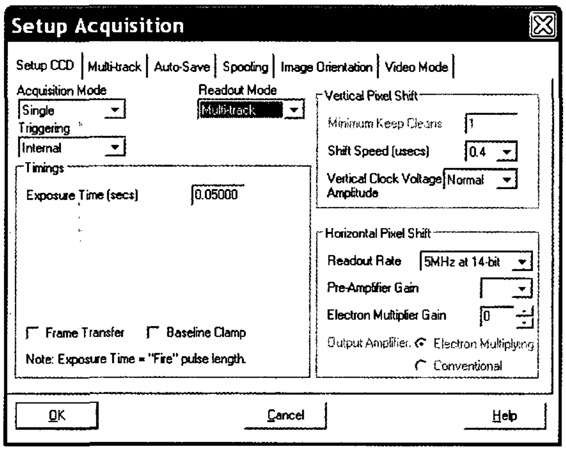
- Select **Abort Acquisition** from the **Acquisition** Menu.
- Click the ⊘ button
- Press the **<ESC>** key.

This stops any data capture process that may be under way.

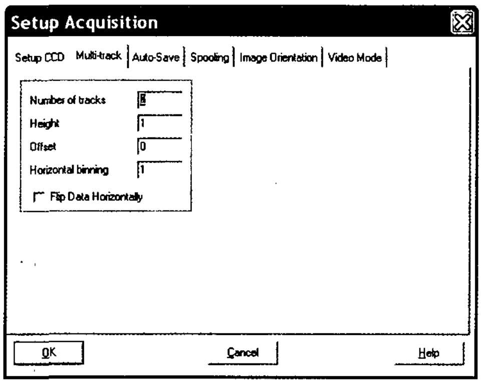
Multi-Track

Multi-Track mode allows you to create one or more tracks. You can define (in rows) the height of each track, and the offset, which in effect 'raises' or 'lowers' on the CCD-chip the pattern of tracks from which you will readout charge. In this way you can adjust the position of the tracks to match a light pattern produced on the CCD-chip by a fiber bundle, for example.

To define multiple tracks on the CCD-chip, select **Multi-track** from the Readout Modes drop-down menu in the Setup Acquisition dialog box. The **Setup Multi-track** dialog box appears e.g.:



Click the **Multi-track** tab and the Multi-track dialog box opens, e.g.:

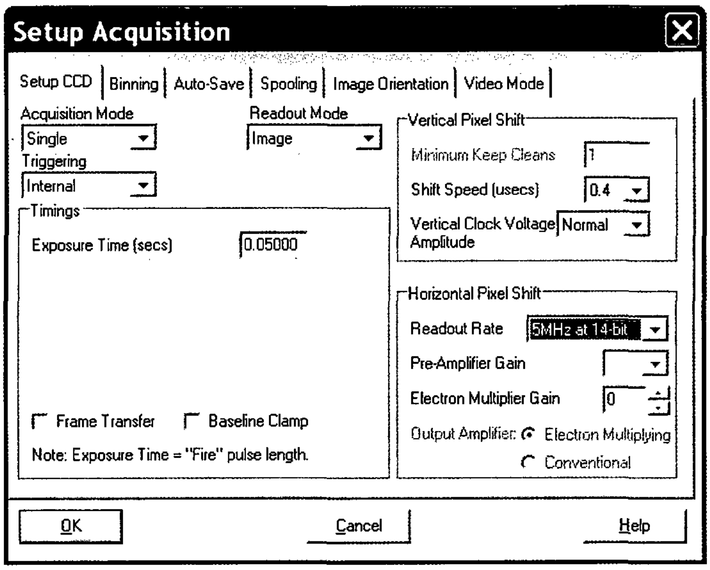


Type in the required parameters then click **OK.**

Readout Rate

The Horizontal Pixel Shift Readout Rate defines the rate at which pixels are read from the shift register. The faster the Horizontal Readout Rate the higher the frame rate that can be achieved. Slower readout rates will generate less noise in the data as it is read out.

The rate can be selected from a drop-down list on the Setup Acquisition dialog box:



TIMING PARAMETER

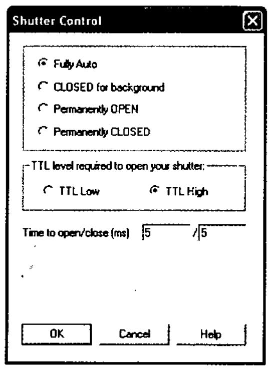
Depending on which combination of Acquisition, Readout & Triggering modes is selected, various timing parameters are available as follow:

- **Exposure Time (secs)**
- **No. of Accumulations**
- **Accumulation Cycle Time (secs)**
- **Cosmic Ray Removal**
- **Kinetic Series Length**
- **Kinetic Cycle Time (secs)**

SHUTTER

A shutter can be used to take a reference or background if Full Vertical Binning is selected. For either Multi-Track or Image mode, the shutter is required to avoid unnecessary signals/light falling on the CCD during the readout process; otherwise the image will be smeared.

When the **Shutter Control** option is selected from the **Hardware** drop-down-menu, the **Shutter Control** dialog box opens e.g.:



You can use this to indicate when and how a hardware shutter should be used. With a CCD, the shutter is used for background shuttering.

Certain settings (e.g. **Permanently OPEN** & **Permanently CLOSED)** take effect as soon as you close the Shutter Control dialog box. Other settings will be applied whenever you acquire data.

- **Fully Auto** is the simplest shutter mode, as it leaves all shuttering decisions to the system. When you perform **Take Signal** the shutter opens for the duration of the **Exposure Time** you have entered in the Setup Acquisition dialog box.

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.

- If **CLOSED for background** mode is selected, any shutter driven from the Shutter output will be closed as you perform Take Background. If you want the shutter to be open so that the Take Background function records genuine optical background data, deselect the option.

NOTE: Usually a background scan is used to subtract the dark signal and the Fixed Pattern Noise (FPN) of the sensor. For this reason the background scan is usually performed in darkness. A shutter may be used to stop light entering the spectrograph or other imaging system. Strictly speaking though, the background acquisition may be regarded as comprising all light with the single exception of the source. Thus, when you are working with a pulsed or independently shuttered source, it may be appropriate to have the mode deselected.

- In **Permanently OPEN** mode, the shutter will be open before, during and after any data acquisition.
- **Permanently CLOSED mode** can be useful if you want 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.

The **TTL** (Transistor-Transistor Logic) buttons, **TTL Low** & **TTL High**, let you instruct the system as to how it should control the opening and closing of the shutter.

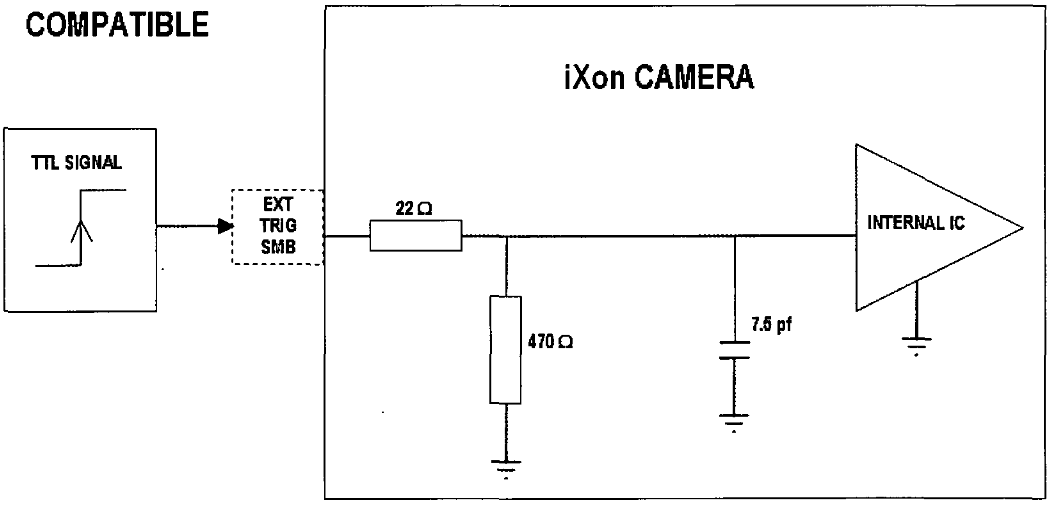
- If you select **TTL Low,** the system will cause the output voltage from the iXon to go '**low**' to open the shutter.
- If you select TTL **High**, the system will cause the output voltage from the iXon to go '**high**' to open the shutter.

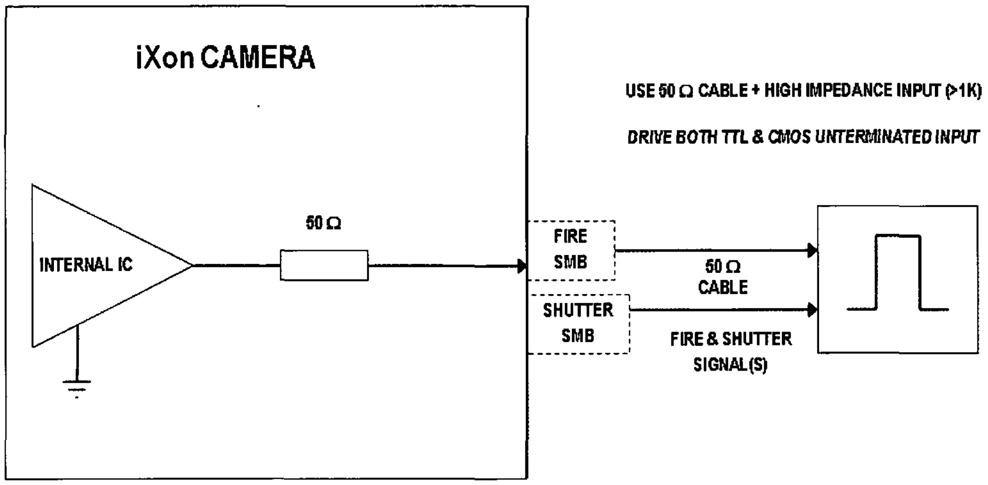
The documentation supplied by the shutter manufacturer will show whether your shutter opens at a high or a low TTL level.

NOTES:

1. **The iXon contains a Frame Transfer CCD device, so 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 Background is required then close the shutter using Permanently Closed, take Background and reopen.**
2. **The shutter pulse is not capable of driving a shutter. It is only a 5V pulse designed to trigger TTL & CMOS compatible shutter drivers. Also there is no shutter pulse during the Take Signal and Take Reference data acquisitions.**

TTL & CMOS COMPATIBLE





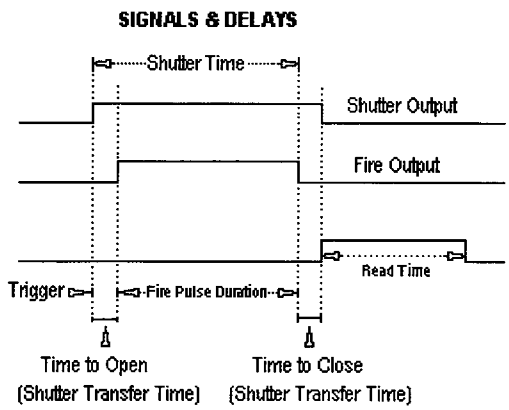
NOTE: The shutter pulse is fed from the Shutter out pin on the back of the camera.

Time to Open or Close

Shutters take a finite time to open or close and this is sometimes called the **S**hutter **T**ransfer **T**ime (**STT**). The documentation supplied by the shutter manufacturer should indicate the STT you can expect from your particular shutter. In the case of a CCD detector, the STT gives enough time for the shutter to open before acquisition starts and enough time to close after acquisition finishes and before readout commences.

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 shutter output is sent before the FIRE output signal by an amount equal to the STT. 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 as shown in the following example diagram:



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.

Accumulate Cycle Time & No. of Accumulations

If you have selected **Accumulate** or **Kinetic** as the acquisition mode, with **Internal** triggering, you can also select the **Accumulation Cycle Time** and **No. of Accumulations**.

The Accumulation Cycle Time is the period in seconds between each of a number of scans, whose data are to be added together in computer memory to form an **Accumulated Scan**.

The Number of Accumulations indicates the number of scans you want to add together.

Kinetic Series Length & Kinetic Cycle Time

When **Kinetic** is selected as the acquisition mode, with **Internal** triggering you can also select the **Kinetic Series Length** and **Kinetic Cycle Length (secs).**

The Kinetic Series Length is the number of scans you require in your series.

The Kinetic Cycle Length is the interval (in seconds) at which each scan (or accumulated scan) in your series begins.

Fast Kinetics

Fast Kinetics allows exposure times on a microsecond timescale. Use Fast Kinetics when you need an exposure time that is smaller than the minimum Kinetic Cycle Time in a standard Kinetic Series.

In Fast Kinetics the image to be recorded is imaged across a certain section of the CCD. The un-illuminated part of the CCD is used for storage of image before readout. You must ensure that light does not fall on this storage part of the CCD by using, for example, an imaging spectrograph.

By way of explanation, take the example of an illuminated sub-area of height 8 rows. A CW spectrum is imaged along this sub-area and the resulting image is then shifted down 8 rows into the un-illuminated area, thus sampling the image in time.

In this way the image is temporarily stored on the CCD itself, rather than in the computer. The process is repeated until the frame of the CCD is filled with time-sampled images or until the number of images you have specified for your series has been acquired.

Next each image is transferred into the shift register in turn, and read out in the normal way.

With a CCD-chip of height 512 pixels and a sub-area height of 8 rows, 63 discrete images can be stored on the CCD (not 64 images, since the top 8 rows of the CCD constitute the illuminated sub-area, which cannot be used for storage.)

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

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

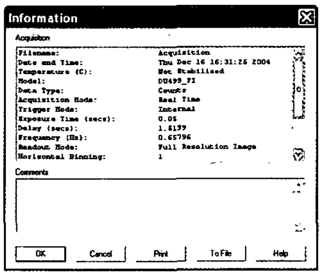
The data from each of the spectra in the Fast Kinetics series is stored as an image. Select Image from the Readout Mode drop down list on the Setup Acquisition Window. Fast kinetics is not available in multi-track mode.

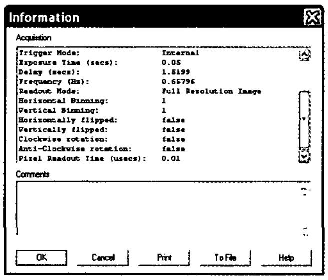
With Fast Kinetics you may use the following Trigger Modes: Internal, External, Fast External and External Start.

- In Internal Trigger Mode, the system determines when the acquisition begins, and then uses the acquisition settings defined by the user. This mode is equivalent to the internal triggering mode for Single Scan etc.
- In External Trigger Mode, a trigger pulse is required to start each scan in the series. The rising edge of the trigger starts the exposure time. After the exposure time has elapsed, the number of rows (specified by the user0 are vertically shifted. The system then waits for the next trigger to start the next scan. As there is no keep clean cycle running while waiting for the external trigger the "real "exposure time is the time between each trigger. A consequence of this is that if your experiment has a constant background signal but your trigger period is not fixed you may see different background levels in your signal.
- In External Start Trigger mode, data will not be acquired until the system receives an initial external trigger signal from an external device, like a laser. From that point on, the system alone determines when data are acquired based on the user settings, as in the case of Internal Trigger. As the system changes from an external trigger mode to internal trigger mode on receipt of the initial trigger signal the exposure time of the first scan in the series will not be the same as the subsequent scans. The exposure time is defined as the time between vertical shifts.

FILE INFORMATION

Details of the Acquisition selection can be viewed by clicking the File Information button on the Main Window which opens the **Information** dialog box (you can enter your own notes in the Comments box):





The table below details the type of information contained in the dialog box.

Table 9: Information dialog box

Filename	The filename associated with the active Data Window. If the data has not yet been saved this will default to Acquisition
Date and time	The date & time at which the acquisition was made.
Temperature (C)	The temperature to which the detector had been cooled.
Model	The model number of the detector.
Data Type	Data Type – Counts, % Transmittance, etc.
Acquisition Mode	Single, Accumulate or Kinetic
Trigger Mode	Internal, External or Fast External
Exposure Time	"Fire" pulse length
Delay (secs)	Value in microseconds
Horizontal Binning	**Always = 1**
Vertical Binning	Minimum = 1, Maximum = 256
Horizontally	True or False
Vertically flipped	True or False
Clockwise rotation	True or False
Anti-clockwise	True or False
Pixel Readout	Value in microseconds

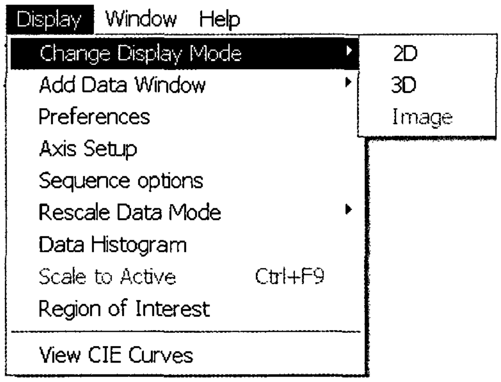
SECTION 6 – DISPLAYING DATA

DISPLAY MODES

Once the parameters for the data acquisition have been set and data has been successfully acquired, there are 3 main options available to display the data, which are as follow:

- Image
- 2D
- 3D

The **Display** drop-down menu also offers various options to change the various formats of the display as shown :



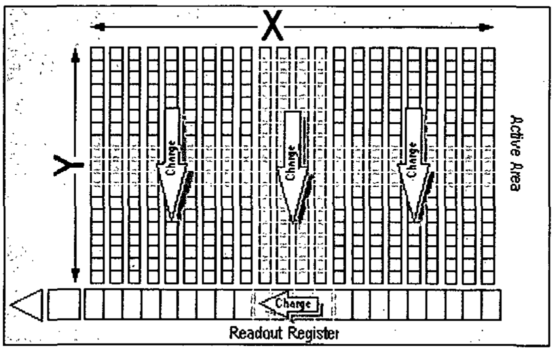
Some of the options are also available via icons on the Main Window and these are shown later in this section.

NOTE: 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. This is explained in more detail in Overlay later in this section.

AXIS DEFINITIONS

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

- The **x-** and **y-**axes define a pixel's position on the two-dimensional CCD-chip, e.g.:



- The **x-axis** (or horizontal display axis) is parallel to the readout register and may be calibrated in pixels or in a unit of your choice.
- The **y-axis** (or vertical display axis) is perpendicular to the readout register and may be calibrated in pixels or in a unit of your choice.
- The **data-axis** is calibrated in the unit of your choice and has data values plotted against it. In **2D** and **3D** display modes, the data-axis is the vertical display axis. In **Image** Display Mode, data are represented by a color or a grayscale tone.

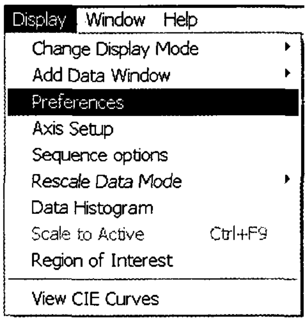
For further information on **Calibration**, please refer to **section 9**.

In all display modes, x- and data-values are displayed on the status bar along the bottom edge of the Data Window.

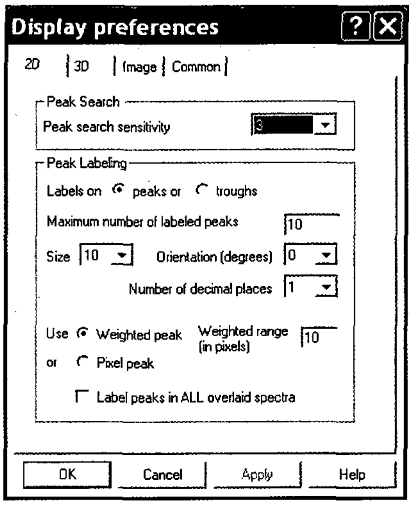
When data has been acquired in a mode other than Full Vertical Binning, y-values are also displayed in the status bar.

DISPLAY PREFERENCES

The way data is displayed in the various modes can also be changed. From the **Display** menu drop-down options, select the **Preferences** option as shown:



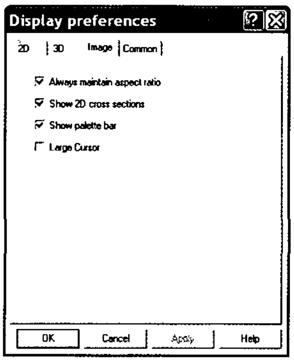
The **Display Preferences** dialog box appears, e.g.:



By clicking on the appropriate tab, you can select or deselect certain features associated with the data window for the mode of your choice.

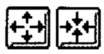
IMAGE DISPLAY MODE PREFERENCES

When the **Image** tab on the Display Preferences dialog box is selected, the following options are made available:



Always Maintain Aspect Ratio (ON)

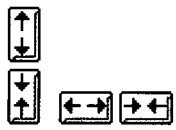
When the **Always Maintain Aspect Ratio** option is ticked, 2 special buttons appear on the data window as shown:



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 (OFF)

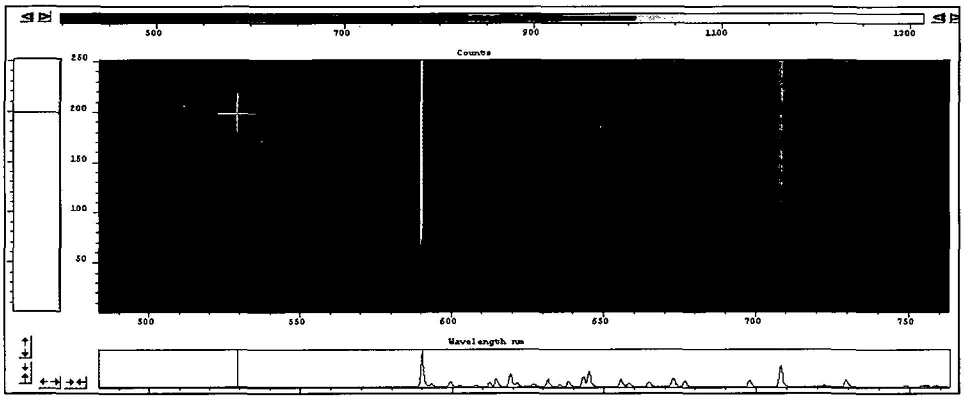
When the **Always Maintain Aspect Ratio** option is deselected, the following buttons appear instead of those shown previously:



Clicking these buttons allows you to stretch or shrink the scale on **either** the x- or y-axis.
This creates the effect of Zooming In or Zooming Out in either the vertical or horizontal dimension.

Show 2D Cross Sections

When **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, e.g.:

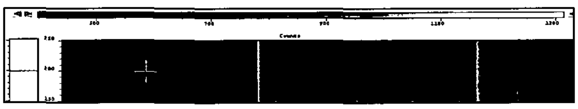


The long edge of each Side Trace is calibrated in the same units as the corresponding edge of the display. Plotted against the unmarked 'short edge' of the vertical and horizontal Side Traces are the data values taken (respectively) along a vertical or horizontal line running through the cross-hair.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

Show Palette Bar

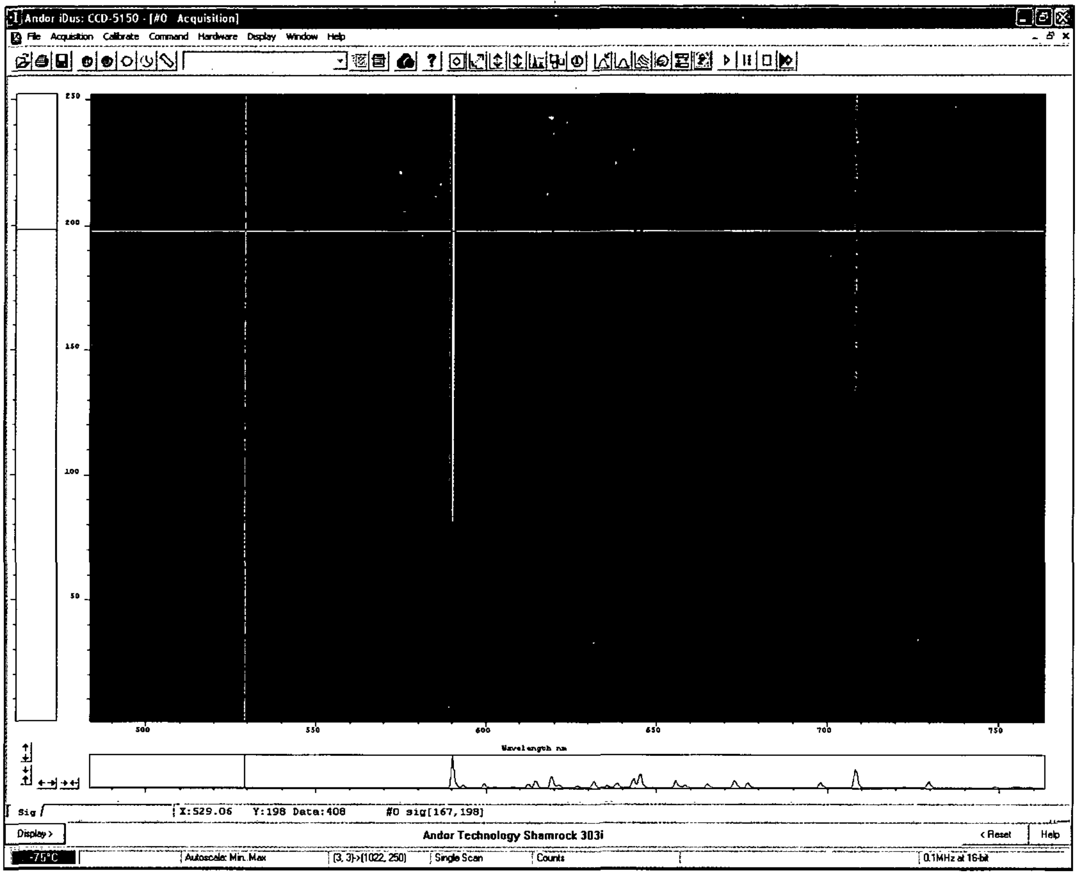
Show Palette Bar causes the black and white (or false color) palette and it's control to be displayed or removed e.g.:



The arrow buttons at either end of the palette bar can be used to adjust the manner in which values are mapped against colors, and so change the brightness and contrast of the image.

Large Cursor

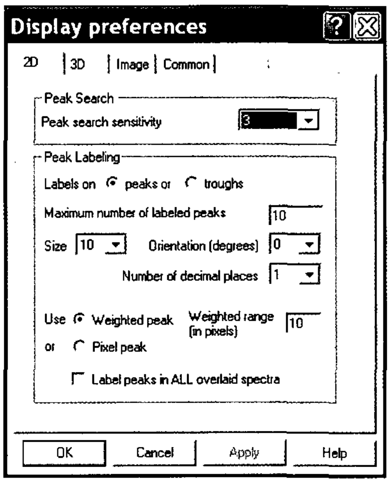
When the **Large Cursor** option is selected, the cross-hairs run the full height and width of the display, e.g.:



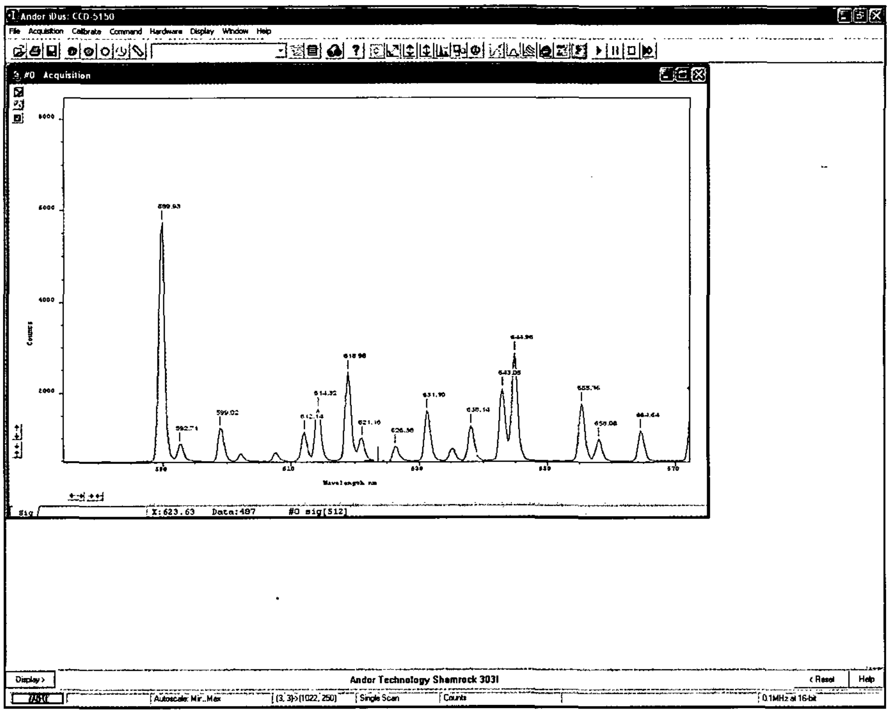
This makes it easier to identify the corresponding points on the Side Traces.

2D DISPLAY PREFERENCES

The following options are available to view peaks when in **2D Display** mode:



The display is similar to the following:



Peak Search

Peak Search Sensitivity

The Peak Search Sensitivity option Determines the manner in which the cursor moves between peaks/troughs when you key in **Ctrl + Right Arrow** or **Ctrl + Left Arrow.**

A low sensitivity (e.g. 1) means the system will find the most prominent peaks or troughs.

A high sensitivity (e.g. 5) means less obvious peaks or troughs will be found.

NOTE: This parameter relates only to peak search, not to Peak Labeling.

Peak Labeling

Labels on Peaks or Troughs

Lets you choose whether to mark the highest points (peaks) or lowest points (troughs) on the trace.

Maximum Number of Labeled Peaks

Causes only the highest peaks or lowest troughs, up to the total number of peaks/troughs indicated, to be labeled automatically.

Format Labels

Size, Orientation & Number of Decimal Places lets you format the peak labels. For **Orientation,** 0° is horizontal; 90° vertical. You can have up to 4 decimal places in the label.

Weighted Peak

Weighted Peak in combination with a **Weighted Range** (centered on the highest/lowest positioned pixel) lets the system calculate and label a weighted mean to represent the peak or trough.

NOTE: The Weighted Peak feature works best on peaks or troughs which are symmetrical about the highest/lowest point.

Pixel Peak

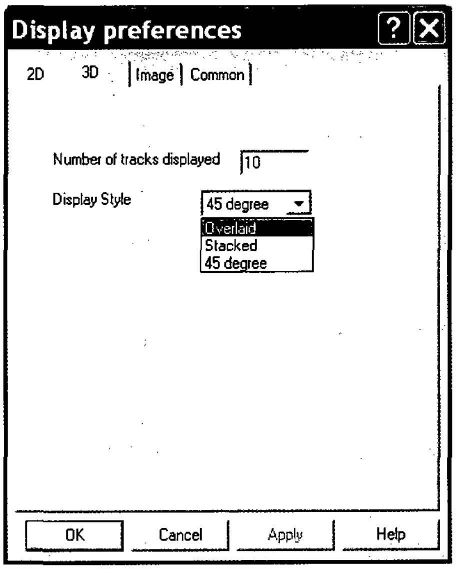
The system can label the **Pixel Peak**, the highest/lowest positioned pixel.

Label Peaks in all Overlaid Spectra

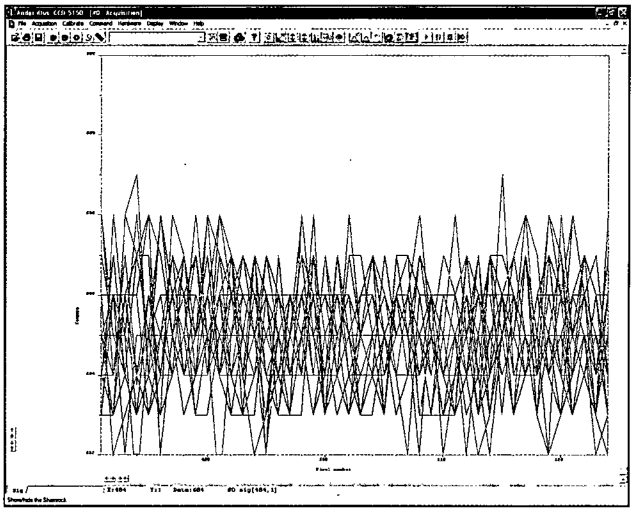
You can also choose whether to **Label peaks in all overlaid spectra** or to have peaks labeled only on the active trace. See the following subsection on **Overlaying Data**.

3D DISPLAY PREFERENCES

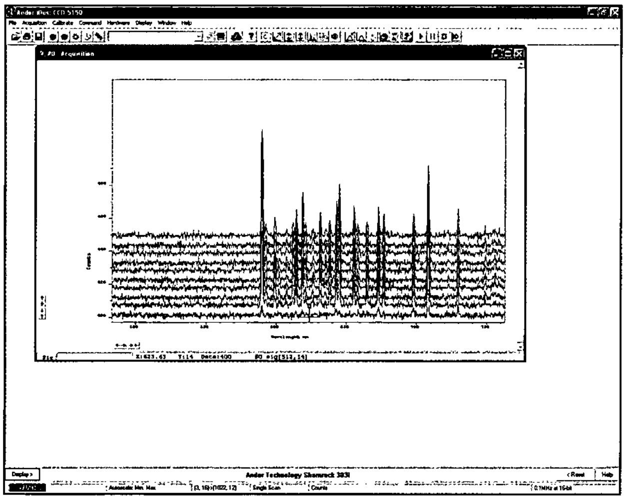
When 3D display mode is selected, the following options are available from the **Display preferences** dialog box:



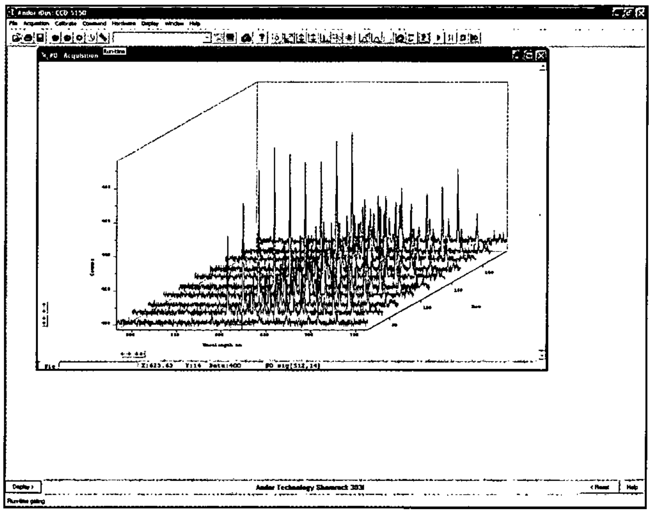
You can display multiple tracks and either **Overlaid** on one another:



or **Stacked**:

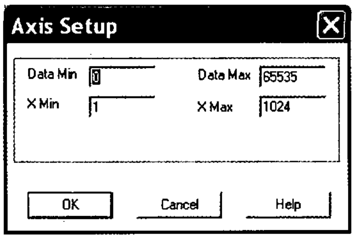


or at **45 degrees**:



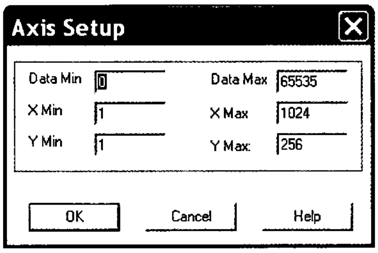
AXIS SETUP

When you are in **2D** or **3D** display mode and the **Axis Setup** option on the **Display** menu is selected, the **Axis Setup** dialog box opens as shown:



The minimum & maximum values you wish to appear on the x- and data-axes (the horizontal and vertical display axes respectively) of your data window can be entered in the text boxes.

If you select **Axis Setup** while you are viewing data in **Image** display Mode, the **Axis Setup** dialog box opens as shown:



You can now also enter, in the text boxes, minimum & maximum 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 now enter minimum & maximum **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** in **Preferences,** 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.

ZOOM BOX

In **2D** & **Image** modes, you can also **zoom** into an area by drawing a **Zoom Box.** In both instances, hold down the primary mouse button and pull the cursor in a diagonal across the screen around the area that you are interested in.

- In **2D** mode, 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 zoom width is 30 pixels.

Note: You may wish to perform a Rescale (see page 105) 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.

- In **Image** mode, release the mouse button to perform the zoom operation. The image will zoom to show the selected area in greater detail. The minimum zoom 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 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.

ZOOMING & SCROLLING

The following functions are available in data windows whilst in 2D & 3D Display modes:

- Zoom in
- Zoom out
- Scroll

Zoom In & Zoom Out

On a data window in **2D** or **3D d**isplay 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.

Scrolling

If you have stretched a scale by zooming, you can slide the scale to cover a different range and the display will scroll in synchronization 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 through the display.

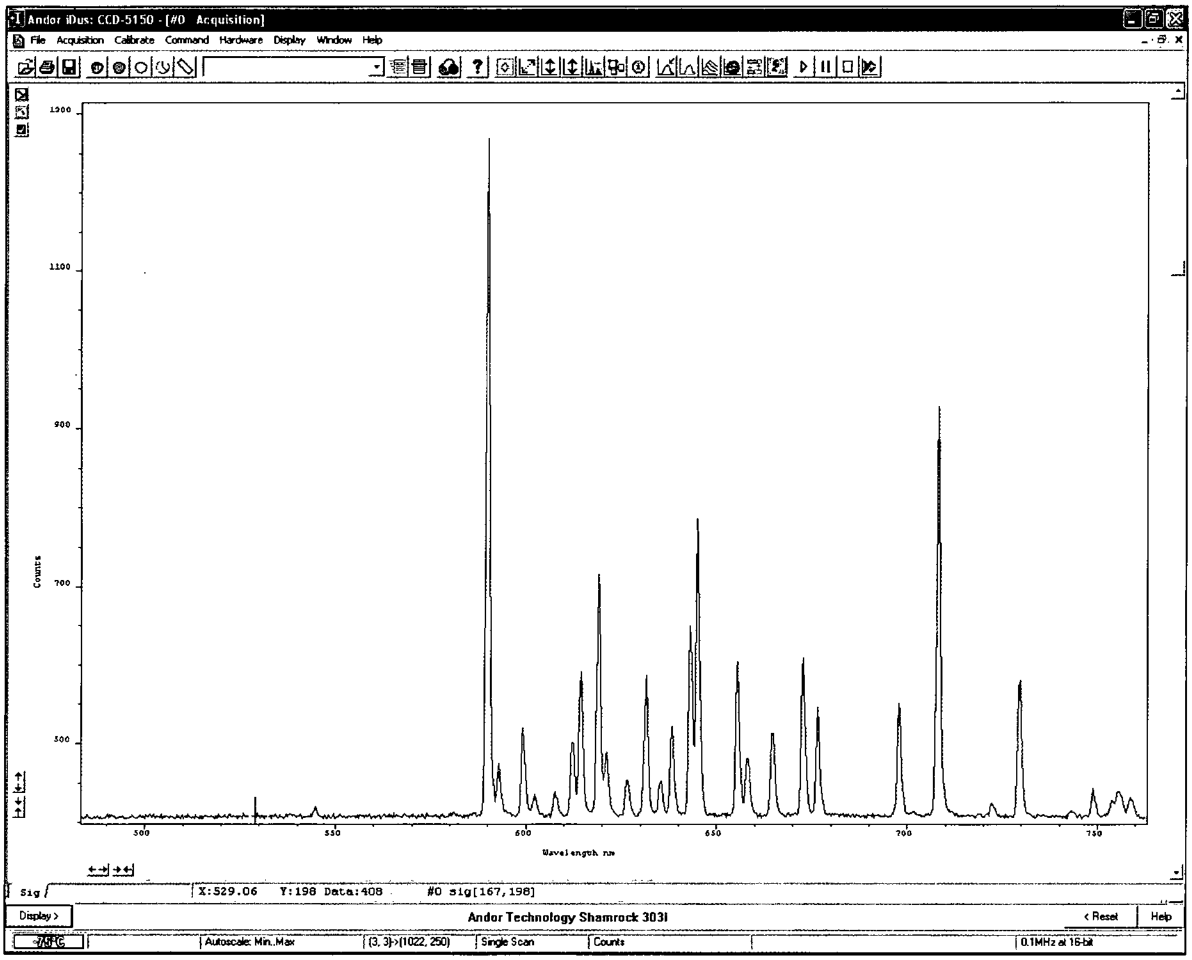
If you place the finger cursor at either end of an axis you will notice that a single arrow appears beside it, indicating the direction in which the scale will slide automatically when you depress the primary mouse button: the display "fast scrolls" accordingly.

Reset

Clicking the button when a Data Window is open, returns the displayed data to its original configuration, thus undoing any adjustment to scale that you may have performed in accordance with the descriptions given in Zooming In, Zooming Out, Scrolling and **Rescale** (see **page 105**). **Reset** is available for all display modes.

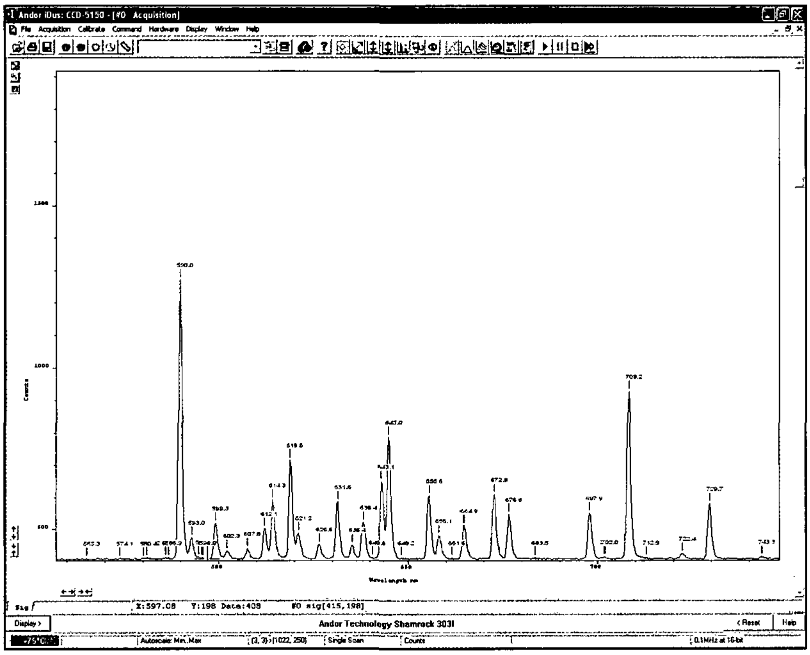
2D

To view data in 2D, either select **2D** from the drop-down menu or click on the  button. Data is then displayed as an unlabeled trace, e.g.:



2D with Peak Labeling

To label peaks automatically, either select **2D** from the drop-down menu or click the button. The data window display will change e.g. :



When labeling is selected, you can label a peak manually by double clicking it. To remove a peak label, double click it again. If you switch off peak labeling, by clicking the button, your manual labeling will be lost.

NOTE: To manually label peaks accurately it is best to zoom in on the trace as described previously.

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, etc.). The data-axis will by default be calibrated in counts. **For details of how to change the calibration on the x-axis, please refer to Section 9.**

If you have acquired data in an imaging mode 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.

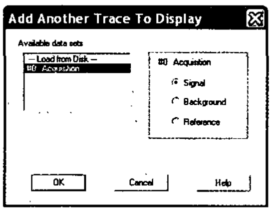
To read off a data value, click on the trace to position the cursor on the point of interest (you may need to use the left and right arrow keys on your keyboard to position the cursor precisely). The numeric display on the status bar along the bottom edge of the data window will indicate the corresponding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

Overlay

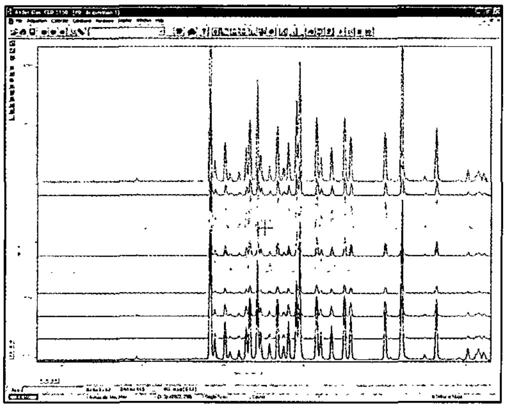
The ability to **Overlay** data traces is useful if you wish to compare several traces on the same axes. You can display up to nine 2D traces simultaneously in the same data window on the same set of axes. The data window in which you intend to display the overlaid traces must be in 2D display mode. Only the data which were 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 window from which the 2D overlays are taken can be in any display mode.

To add an overlay, click the button on the Data Window. The **Add Another Trace To Display** dialog box appears:



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 (the **Load from Disk** option). Buttons also let you specify **Signal**, **Background** or **Reference** data (if these are contained in the data set you have selected for overlay). When you have made your selection and clicked **OK**, the display changes, e.g.:



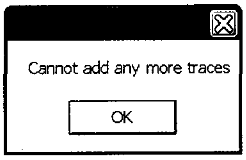
NOTE: You can add up to a maximum of 8 overlays to your original data trace.

Active Trace

The original data trace is always displayed in blue. Each new overlay appears in a unique identifying color and an **Active Trace** button is displayed on the left in the same color. To manipulate the trace you want, click on the Active Trace button corresponding to the color of the trace you wish to work with.

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 the same manner as any **2D** Display.

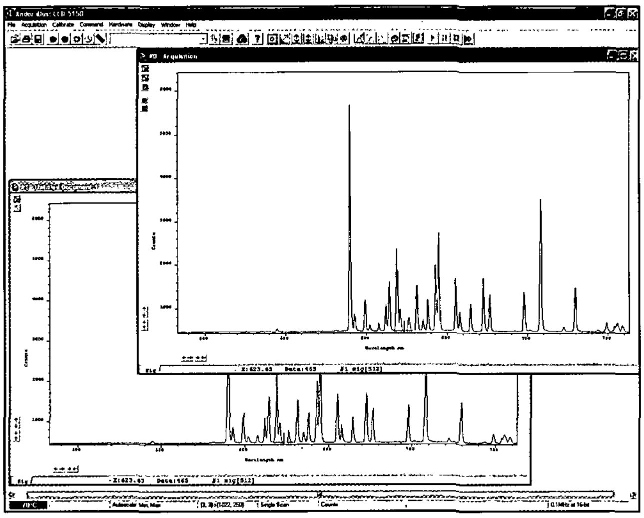
If you try to add too many traces, you will be prompted with the following message:



Overlay & Keep

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 the button.

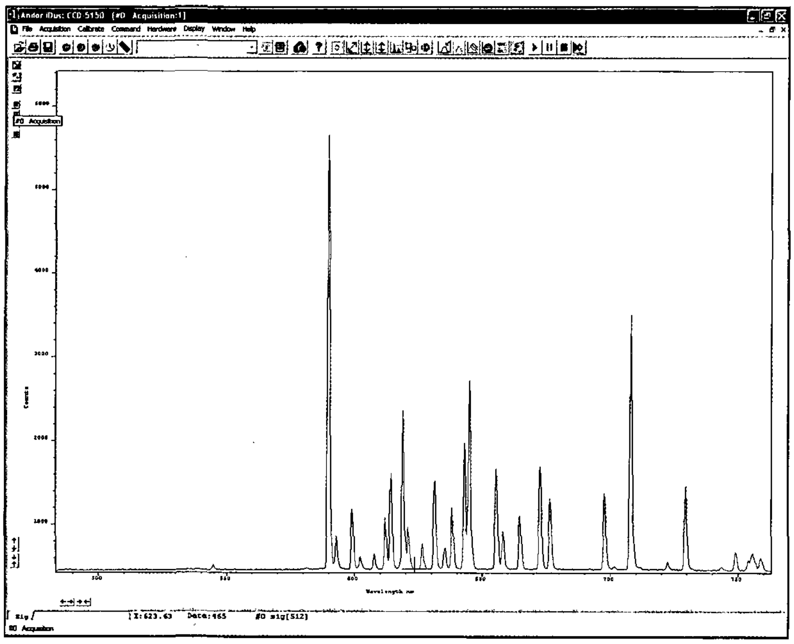
In the Acquisition Window you will see the 'live' trace in blue along with an overlaid copy. In addition, you will see a new data window containing the selected data. The new data window is minimized at the bottom of the screen, e.g.:



At a later stage you may choose to save the contents of this new data window to file.

Scale to Active

If you have overlaid a number of traces, the **Scale to Active** option becomes available on the **Display** Menu. When Scale to Active is selected, all the data traces in your data window will be plotted against the scales of the active trace, e.g. :



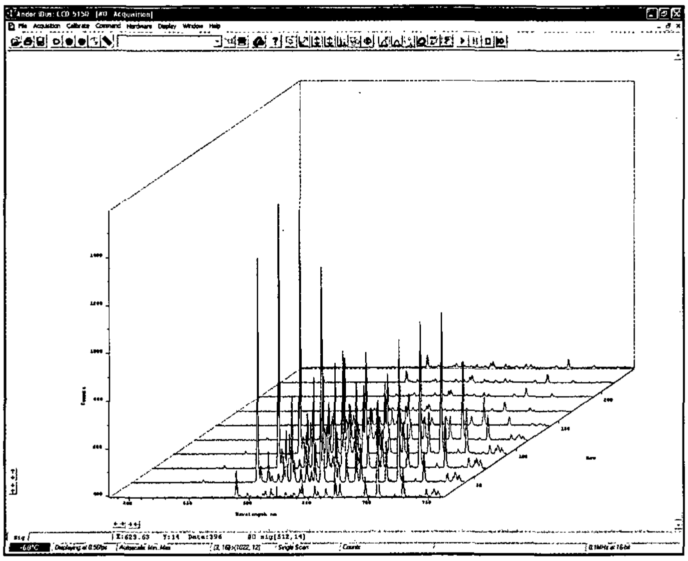
Vertical axes will be rescaled even if the units do not match those of the active trace.

Remove Overlay

If you want to remove an overlay, first make sure that it is the Active Trace, then click 
If necessary use the Active Trace buttons to select a new active trace.

3D

If you have acquired data in **Imaging** mode or as a **Kinetic** series you can view the traces taken from all the rows or tracks on one set of axes in a data window. Select the **3D** option from the **Change Display Mode option** on the **Display** menu, or click the button. A data window will appear e.g.:



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 3rd (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.

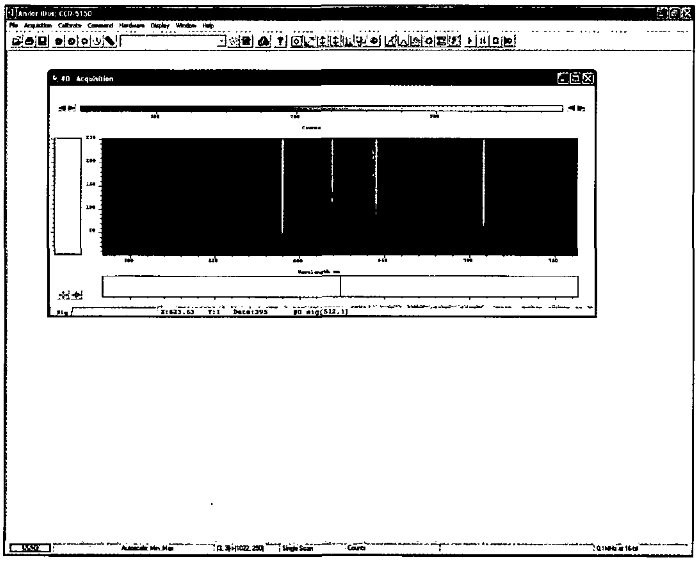
If your data set contains a series of images (each of which may represent data acquired in Kinetics Mode) you will notice that the data window has two scroll bars, placed end to end. The upper scroll bar allows you to move between the members of the series, while the lower scroll bar allows you to view the traces that make up the particular member of the series.

NOTE: The Zooming, Scrolling and Reset functions are the same as for 2D mode.

IMAGE

Data acquired in **Imaging** mode can be viewed as an image in a data window. 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 another color mode) and adjust the color or brightness using the controls associated with the **Palette Bar** (see below).

Select the **Image** option from the **Display** drop-down menu or click the  button and an image will appear, e.g.:



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.

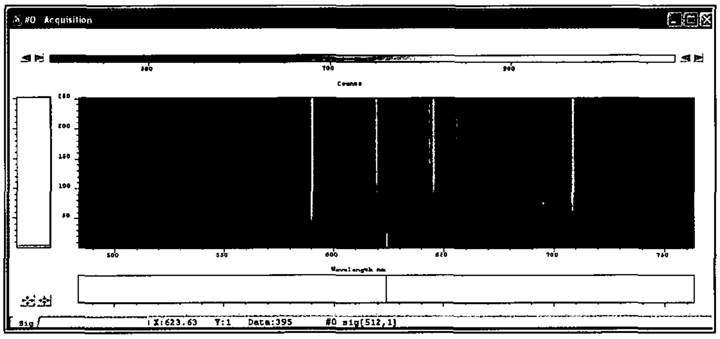
The data value for the point, along with the pixel numbers on the x- and y-axes that identify the point, is displayed on the bottom edge of the data window.

NOTE: 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.

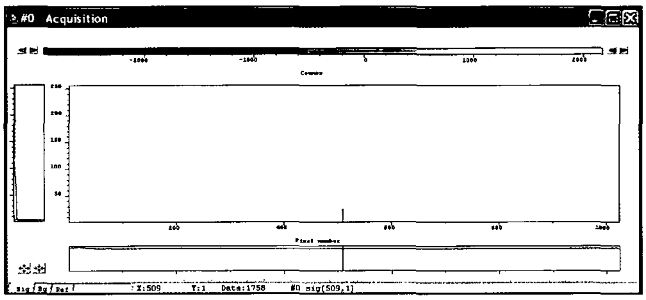
Palette Bar

The **Palette Bar:**, which runs across the top of the data 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 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), e.g.:

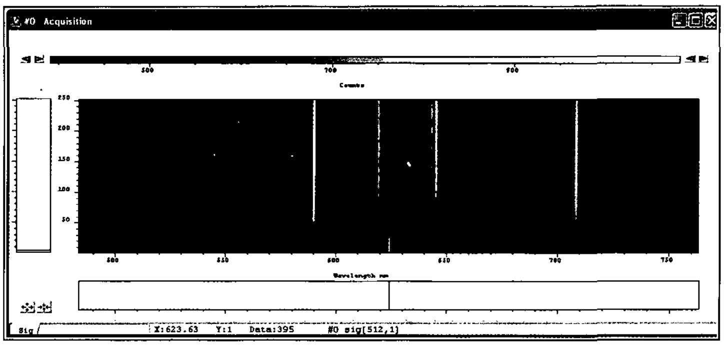


The arrows to the right of the Palette Bar on the Data Window allow you to adjust the maximum distinguishable data value (i.e. max, the value at which the Palette Bar becomes white)., e.g.:



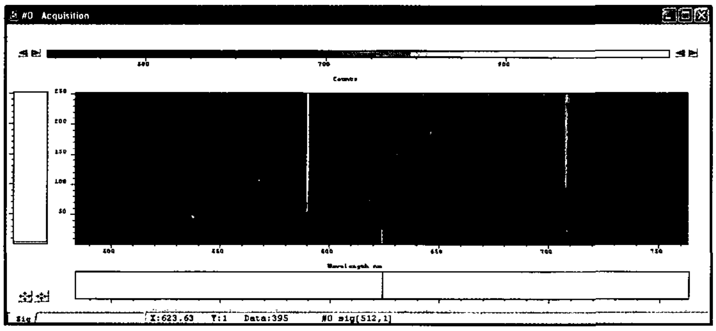
The scale on the Palette Bar is calibrated in your chosen data units. By default, the full range of colors/grays is correlated with the full range of data in your data set. This is known as '**Rescaling**' (see **Rescale** on page **105**). **Adjusting the Brightness & Increasing and Decreasing Contrast** later in this section explain how you can use these controls to change the brightness and contrast of the display.

The display appears initially in grayscale, e.g. :

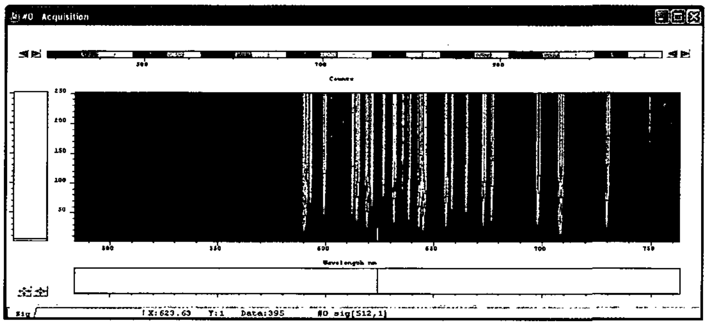


Clicking on the  button causes the data window to cycle through the following 2 modes:

- **Color**, e.g.



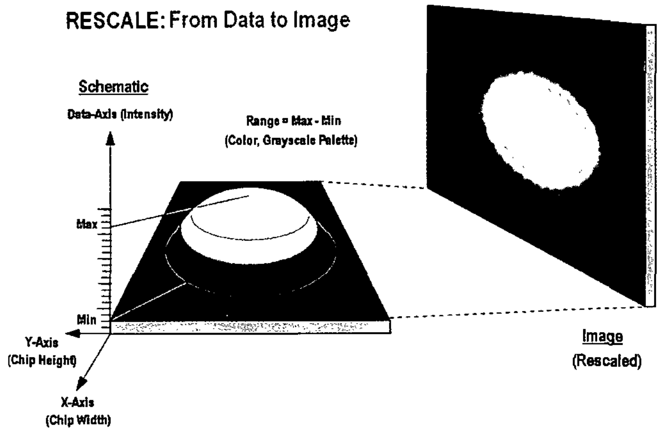
- **Iterated Grayscale** (a small sequence of grays is repeated at intervals to cover the same range of data as grayscale), e.g.:



Rescale

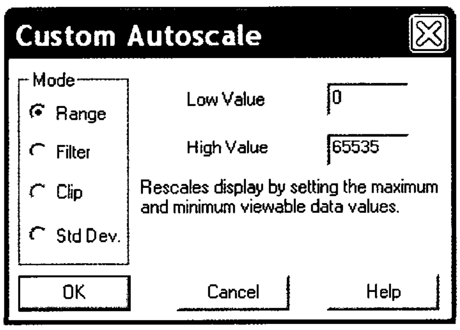
When a data window is open and you click on the button on the button bar of the Main Window, the system displays against an appropriate data scale all data that falls within the range selected on the x-axis.

In **Image** display Mode, it will also be displayed in appropriate colors or grayscale tones.



From the **Rescale Data Mode** menu of the **Display** drop-down menu, you can also select the maximum number of counts that can be recorded for a single pixel, your rescaled data distinguishes values between the following parameters:

- Minimum and Maximum **(Min..Max)**
- Zero and Maximum **(0..Max)**
- Zero and 65535 **(0..65535)**
- Minimum and 65535 **(Min..65535)**
- Custom Setting. When **Custom..** is selected, the **Custom Autoscale** dialog box appears, e.g.:



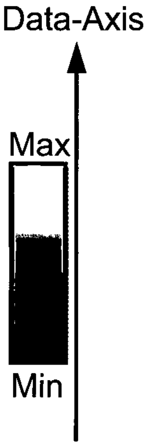
Autoscale Acquisition performs a similar function for the display of data as they are being acquired. It can be selected from the Acquisition drop-down menu, or by clicking the buttons:

 **= ON**

 **= OFF**

or by pressing **F6** on the keyboard.

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.)



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 defaults to Minimum **(Min)** and Maximum **(Max)** and scales the available number of colors to the range.

Alternatively, depending on the rescale submenu item you have selected, the range may be between **0 and Max**, or between **0 and 65535**.

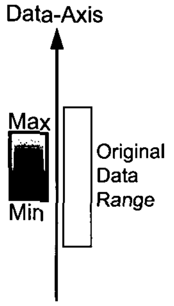
Rescale

For various reasons (e.g., 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 & Decreasing Contrast** and **Adjusting the Brightness** that follow.

Assume your original data are rescaled.

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

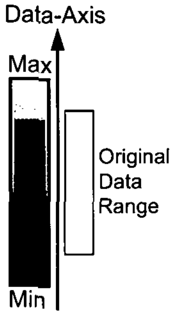


High Contrast

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.



Low Contrast

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.

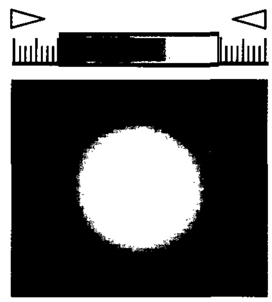
Increasing & Decreasing Contrast

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. You can also use the **+** and **–** keys to do the same function.

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

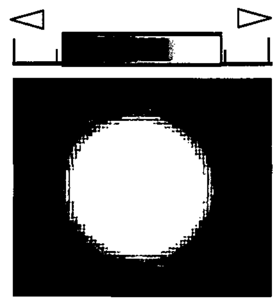
Decreasing the displayed data range ('stretching' the scale).

Increase Displayed Data Range



Decrease Contrast

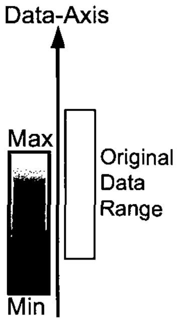
Decrease Displayed Data Range



Increase Contrast

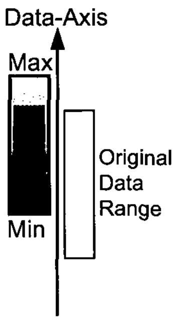
By holding down the left-right arrows at either end of the Palette Bar you can alter the minimum and maximum values in the displayed data range independently of each other, and so adjust the contrast to the level you require. As you hold down the arrows (place the cursor over an arrow button and hold 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.



Brighter

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.



Darker

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.

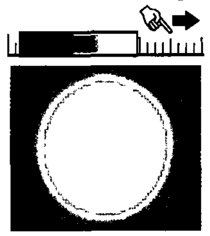
NOTE: If you wish to undo your changes, use Rescale.

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 changes to a finger) and, holding down the primary mouse button, 'slide' the scale on the palette to the left or right.

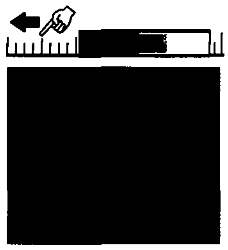
Slide Data Scale Right



Brighten Image

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.

Slide Data Scale Left



Darken Image

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.

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.

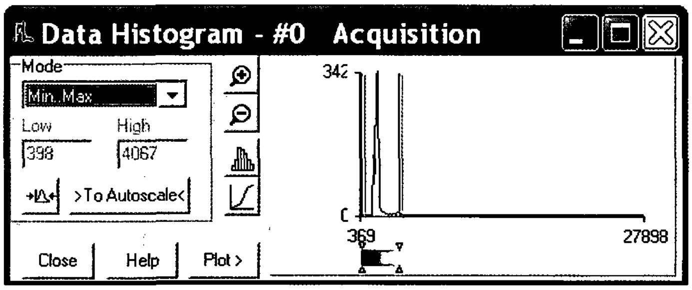
IMAGES & DISPLAYED DATA RANGES SUMMARY

In summary, the general rules that follow apply:

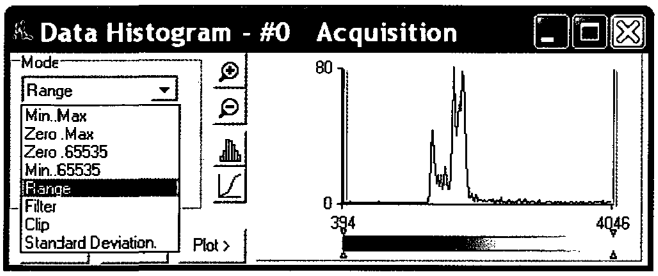
- **Larger displayed data range = LESS CONTRAST**
- **Smaller displayed data range = GREATER CONTRAST**
- **Displayed data range covers higher values = DARKER DISPLAY**
- **Displayed data range covers lower values = BRIGHTER DISPLAY**

DATA HISTOGRAM

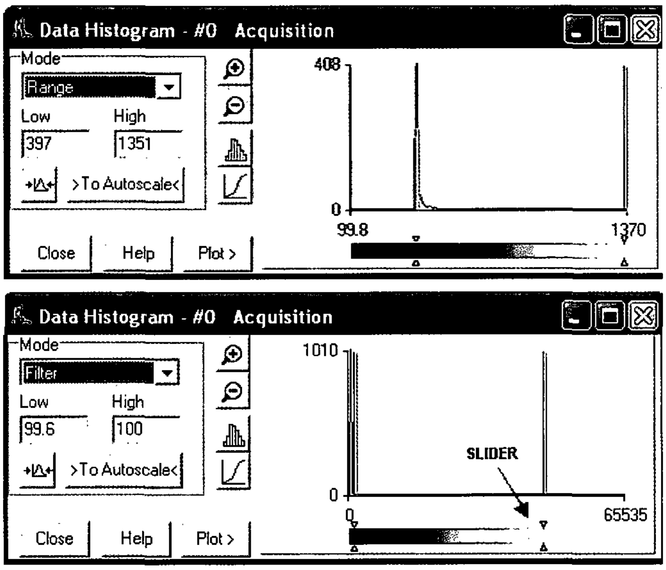
The **Data Histogram** dialog Box is launched either by clicking on the icon on the Main Window, or selecting **Data Histogram** from the Display drop-down menu. This tool allows you to plot a histogram, or graph, between the maximum and minimum data points in the displayed range. It also contains a filter drop down menu, which allows for more accurate analysis and presentation of data values.



By clicking on an open data window, its histogram will be displayed on the Data Histogram dialog. When plotting the histogram, the focus is transferred to the new data window and the displayed histogram is that of the plotted histogram. The data from which the histogram is displayed is indicated on the title bar of the Data Histogram dialog. To return the focus on the original data, click on the original data window. Modes to view specific areas of spectra can be selected through the drop-down menu:



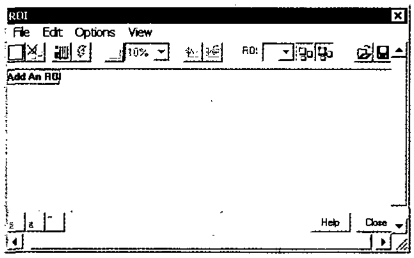
Values can be modified either by typing in the new values in the **Low** and **High** text boxes or by dragging the red arrows and bars below the histogram, e.g.:



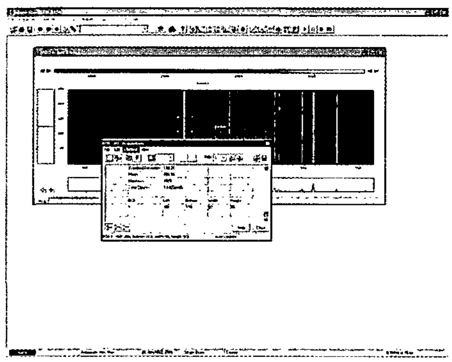
- **Update**: Any change on the mode and/or the Low/High values is updated when the Update button is clicked.
- **Autoscale**: After clicking the >To Autoscale< button, acquisitions that follow will use these scaling settings as default
- **Expand to bounds**: clicking on the button zooms in on the histogram
- **Zoom Out**: clicking on the button zooms out the histogram
- **Bar Chart:** clicking on the button toggles between x-y and bar chart histogram display
- **Cumulative**: clicking on the button toggles between cumulative (integral) and non-cumulative histogram display
- **Plot:** clicking on the Plot > button plots the histogram into a data window

REGION OF INTEREST

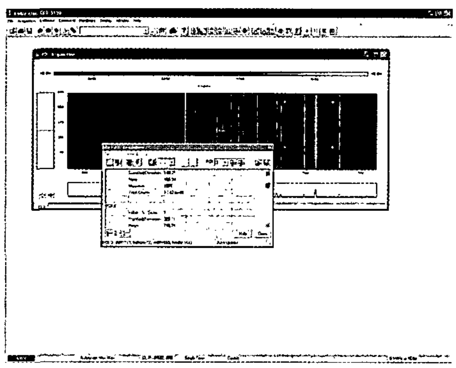
Region of Interest (ROI) is an important post-acquisition tool, used for quantitative analysis and it can be selected either by clicking the button or selecting **Region of Interest** from the Display drop-down menu. When ROI is selected, the following dialog box opens:



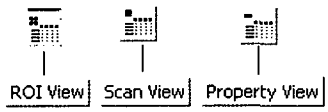
An ROI can be drawn on the image by positioning your cursor on the red ROI, and dragging out the using the corner handles.



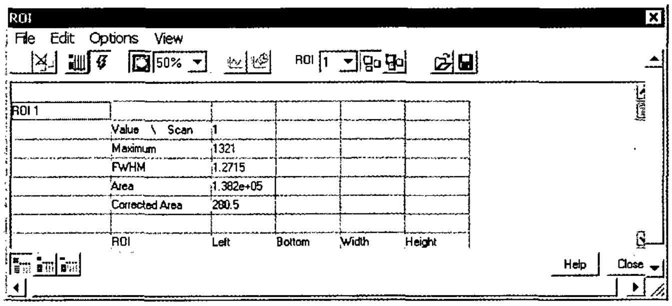
You can use this tool to select and draw multiple ROI's onto your image, then use the ROI data set to compare the values obtained, e.g.:



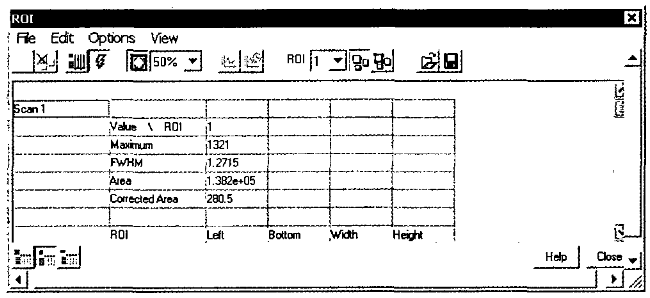
There are 3x buttons in the bottom-left of the ROI dialog box:



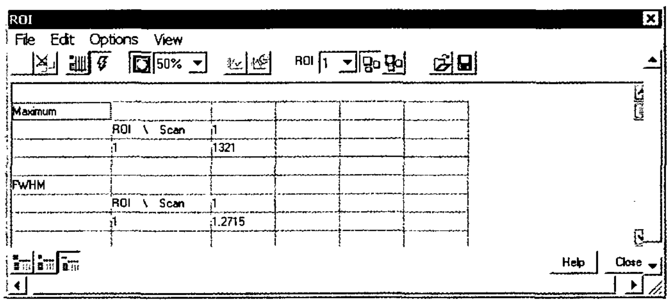
Clicking the **View** button will present and group your ROI data, according to each individual ROI region selected on the image. It will also display the pixel co-ordinates of all the ROI's for that scan, e.g.:



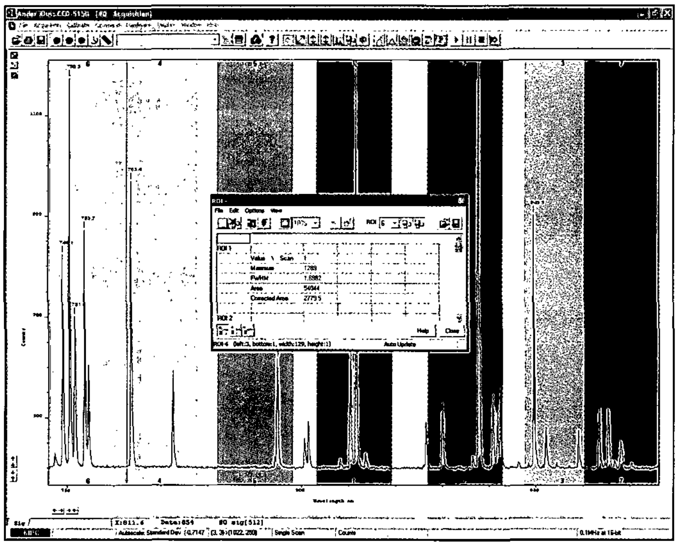
Clicking the **Scan** button will present and group your ROI data, according to **individual data scans**. It will also display the pixel co-ordinates for all the ROI's for that scan, e.g.:



Clicking the **Property View** button will present and group your ROI data, according to value regions, i.e. **Mean, Range** and **Standard Deviation**. It will also display the pixel co-ordinates for all the ROI's for that scan, e.g.:



The ROI can be switched on and off by clicking the (**Show ROI**) button. When **Show ROI** is selected on, the selected ROI will be displayed and outlined by red boxes, e.g. for Full Vertical Binning.:



When Show ROI is selected OFF, the red ROI boxes are hidden.

NOTE: When Show ROI is selected On, it is not possible to position the Cross Hair cursor on the image to perform Zoom In or Zoom Out functions.

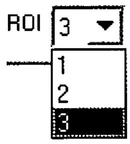
The **Edit ROI** function is only available when **Show ROI** is selected **On.** To activate, click on the (**Edit ROI**) button. You can then change the size and location of the ROI's.

NOTE: When the Edit ROI tool is selected OFF, the red ROI boxes are locked and cannot be altered. This is useful tool to prevent accidental interference with ROI's.

The current ROI (including the ROI data set) can be deleted by clicking on the (**Delete current ROI**) button.

ROI Counter

The **ROI Counter**:



identifies the current active ROI. It can also be used to select and isolate a particular ROI, which can be a useful tool, e.g. if two ROI's are overlapping or are layered on top of each other. By clicking the down arrow, you can also see how many ROI's are currently defined.

Hot Spot Approximation

Hot Spot Approximation can be used to take a selected percentage of the highest data values within a given ROI.

To select, click on the button, then select the % required from the drop-down menu. For example selecting 50% will give you the mean value for the top 50% of pixel values within the ROI.

Recalculate

To recalculate the values in the ROI window, click on the button.

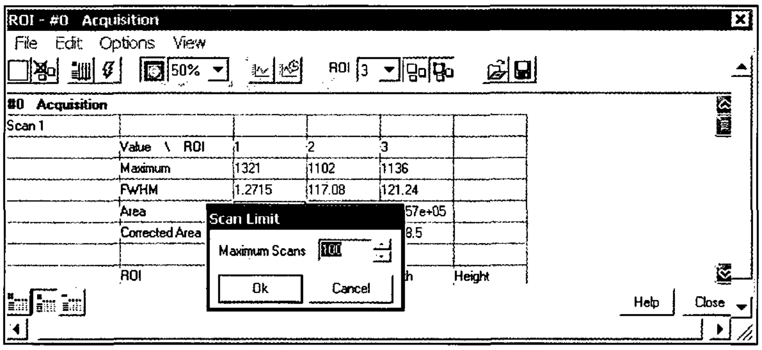
Live Update

You can receive and calculate ROI data, while the system is acquiring a **Kinetic Series** or running in **Real Time** Mode.

To select, click on the button. The software is then able to acquire data and at the same time calculate and tabulate ROI data.

Maximum Scans

When the Maximum Scans is selected from Options drop-down menu of the ROI dialog box, the Maximum Scans dialog appears, e.g.:

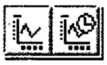


You can then enter the length of the history buffer you require (i.e. the number of previous values stored when acquiring in **Real Time** Mode with the **Live Update** feature enabled).

NOTE: This defaults to 100 and can be modified for longer series.

Plot Series

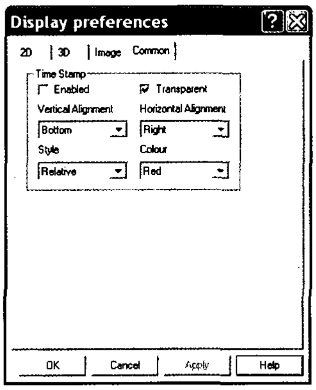
Select any data value for a particular ROI and series position and the following buttons become available:



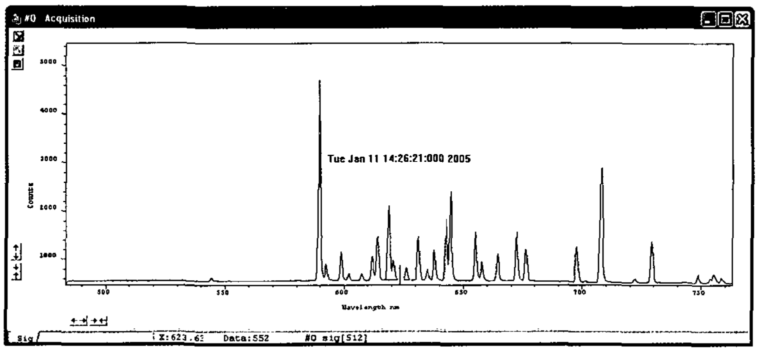
Clicking on these buttons will create a new dataset window displaying the currently selected property values plotted against series position or time.

TIME STAMP

When the (**Time Stamp**) button is clicked, the Display Preferences dialog box opens, e.g.:



The Time Stamp feature allows you to add to the display the time at which the acquisition, or each scan in a kinetic series, occurred., e.g.:



Time & date information, or time relative to the start of the acquisition can be displayed by selecting **Enabled**, then selecting the appropriate option in the **Style** drop down list.

- The position of the time stamp within the display can be set by adjusting the **Vertical** & **Horizontal Alignment** controls.
- The **Color** of the text can also be changed
- The time can be made to print on a solid background by de-selecting **Transparent.**

To remove the Time Stamp from the display de-select **Enabled**.

PLAYBACK

After a Kinetic series acquisition has been has been taken, it can be played back again for analysis.

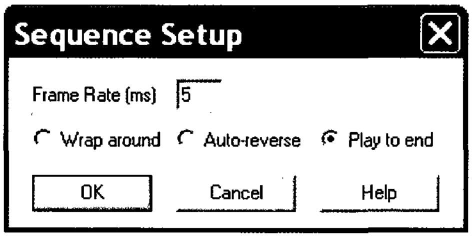
To replay, click the button and the acquisition will display again as taken.

To pause, click the button.

To stop click the button.

Playback autoscale performs a similar function to Autoscale acquisition and is selected from the button on the top of the main window (**=ON, = OFF).**

The sequence can also be viewed with different parameters set.
Select **Sequence options** from the Display drop-down menu and the **Sequence Setup** dialog box appears, e.g.:



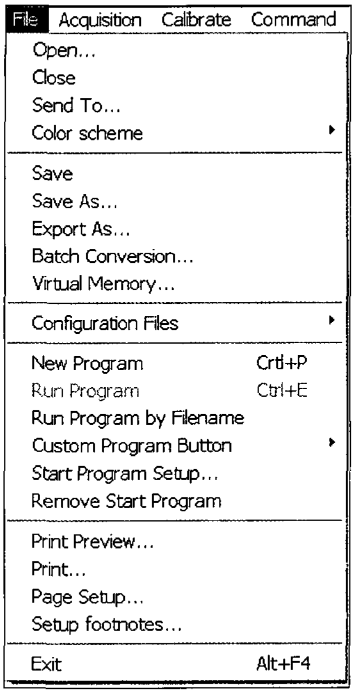
Select the parameters as required, click OK, then playback the sequence as normal.

NOTE: This can be exported as an MPEG or other similar file for use in presentations, etc. Please refer to Section 7 for further details of handling files.

SECTION 7 – HANDLING FILES

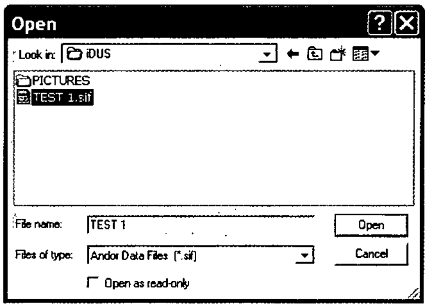
MENU OPTIONS

The **File** drop-down menu on the Main Window has the following options:

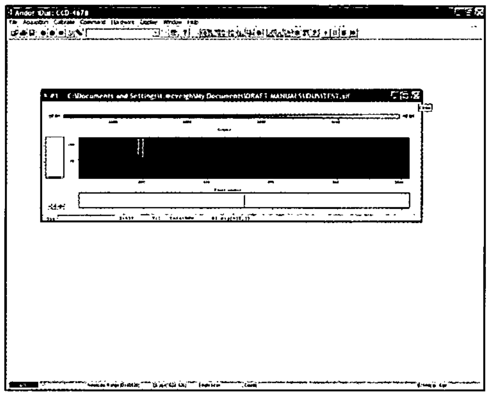


Some of the options available are typical **"Windows facilities"** to **OPEN, SAVE, PRINT** files, etc but some are specific to Andor's Software to let you create or run programs.

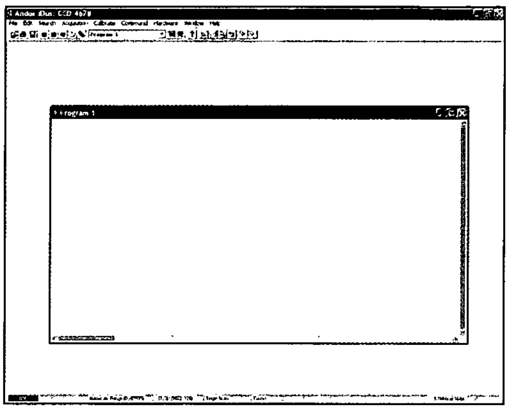
Open.. or  opens a standard dialog box, e.g.:



If you select a **Data** file **(.sif),** the system launches a data window with the appropriate file displayed, e.g.:



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 (please refer to **EDITING PROGRAMS** in **section 9**), e.g.:



CLOSE

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

SAVE

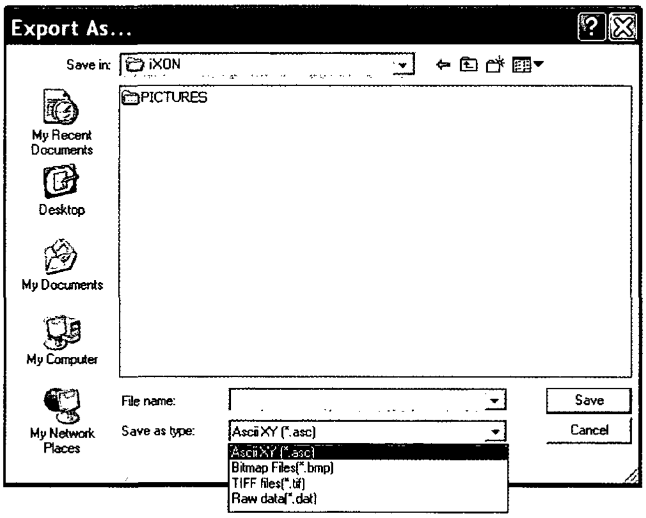
Save or stores the contents of an active and previously saved Data Window or Program Editor Window under the current filename.

SAVE AS

Save as... launches the **Save As** dialog box, which lets you save the contents of an active Data Window or Program Editor Window under a filename and in a directory of your choice.

NOTE: If data is saved as a .sif file, the information contained within File Information is saved. If the data is exported to another file type, the information within File Information is lost

Export As... opens the **Export As** dialog box, e.g.:



Depending on the **Display & Readout modes** selected, the file can be saved in one or more of the following formats:

1. **ASCII (.asc)**
2. **BITMAP (.bmp)**
3. **GRAMS (.spc)**
4. **RAW DATA (.dat)**
5. **TIFF (.tif)**

Table 10 shows a matrix of the actual combinations available.

Table 10: File Export combinations

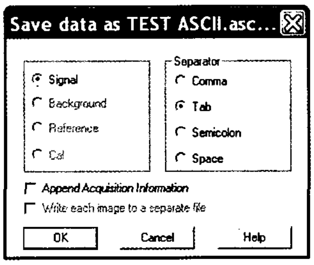
READOUT MODE	DISPLAY MODE	ASCII	BMP	GRAMS	RAW DATA	TIFF
Image	IMAGE	✓	✓		✓	✓

FILE EXTENSION = .asc

ASCII (**A**merican **S**tandard **C**ode for **I**nformation **I**nterchange) is the most common format for text files in computers and on the Internet. In an ASCII file, each alphabetic, numeric, or special character is represented with a 7-bit binary number. 128 possible characters are defined.

Exporting Data as ASCII text means you can subsequently import your data into other applications (such as spreadsheets) that use the ASCII format.

After you have selected **ASCII XY** (*.asc) from the **Save as type** drop-down menu, allocated a filename and clicked **Save**, the **Save data as** dialog box appears, e.g.:



The radio buttons on the left allow you to choose whether you want to save the **Signal, Background** or **Reference** data from the live data set.

The radio buttons on the right allow you to choose a character that will serve as a **Separato**r 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 an application such as Microsoft 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 Single track or Full Vertical Binning 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 represent the height (and/or the member of the kinetic series) and the rows the width of the CCD-chip.

BITMAP

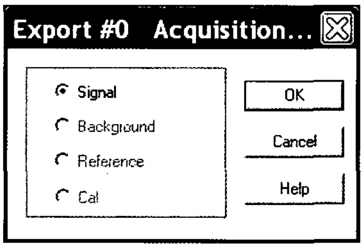
FILE EXTENSION = .bmp

BMP exports data in Microsoft Windows bitmap format that can be embedded into documents created in word processing and presentation packages, etc. If you adjust the image, it is the adjusted image that will be exported. After you have selected **Bitmap Files (*.bmp)** from the **Save as type** drop-down menu, allocated a filename and clicked **Save**, the file is saved.

GRAMS

FILE EXTENSION = .spc

GRAMS (Graphic **R**elational **A**rray **M**anagement **S**ystem), is a software package that supports advanced data visualization and management. It is produced by Galactic Industries Corporation of Salem, New Hampshire.
After you have selected **GRAMS Files (*.spc)** from the Save as type drop-down menu, allocated a filename and clicked Save, the **Export #** dialog box appears, e.g. :

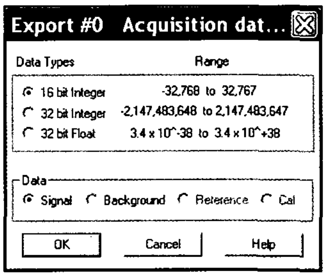


Provided data has been acquired in each format, you can select **Signal, Background**, **Reference** or **Calibration** data from the active data set.

FILE EXTENSION = .dat

The .dat file comprises data only and has no header information of any kind. The original data set remains unchanged.

After you have selected **Raw data (*.dat)** from the **Save as type** drop-down menu, allocated a filename and clicked **Save**, the **Export #** dialog box appears, e.g.



This allows the user to save a data set (currently in memory) to a file located on disk. A **data set** refers to a collection of data comprising one or more of the following subsets:

- **Signal**
- **Background**
- **Reference**
- **Cal**

A .dat file, however, will contain only one of the above data subsets.

Note: 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 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 types range is shown in **table 11** on the next page.

Table 11: .dat types

	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 $3.4 \times 10^{+38}$

(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 units then the value is truncated to 32,767 units).

(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.

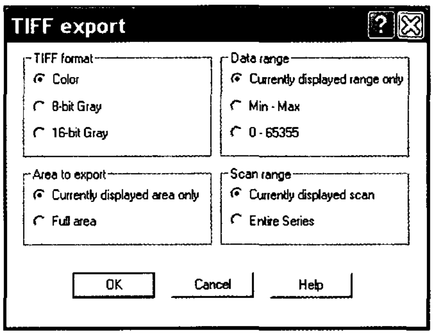
(3) Saves a data set to a 32 bit floating point .dat file.

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.

FILE EXTENSION = .tif

TIFF (**T**agged or Tag **I**mage **F**ile **F**ormat) is used for storing bitmapped images and is widely supported by commercial publishing packages.

After you have selected from the Save as type drop-down menu and allocated a filename, the **TIFF export** dialog box appears, e.g.:



You can then choose the parameters you require to be displayed.

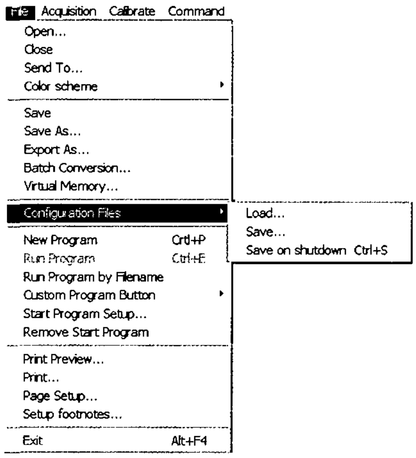
FILE EXTENSION = .cfg

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 .cfg is loaded automatically.

The files are accessed from the **Configuration Files** menu on the **File** drop-down menu of the Main Window as shown:



- **Load...** selects the configuration file you currently want to use. The system will immediately use the settings in the newly loaded file.
- **Save...** stores your current settings under a filename and in a directory of your choosing. You can, if you wish, overwrite an existing configuration file.
- **Save on shutdown** stores your current settings under a filename when the computer is shutdown

Program Selection

The menus for working with **Programs** are selected from the **File** drop-down menu of the Main Window as shown:

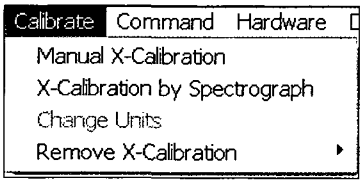
New Program	Crtl+P
Run Program	Ctrl+E
Run Program by Filename	
Custom Program Button	▸
Start Program Setup...	
Remove Start Program	

Working with Programs is explained in more detail in **section 9.**

SECTION 8 - CALIBRATION

CALIBRATION OPTIONS

The following calibration options for data displays are available from the **Calibration** Menu on the Main Window:



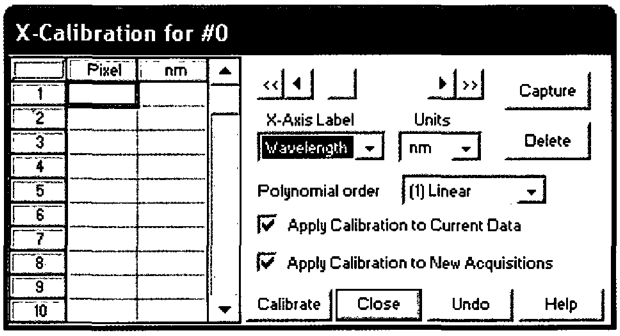
- **Manual X-Calibration** lets you calibrate the x-axis of data displays by manually setting values (time, pixel number, wavelength, Raman shift or spatial position) against recognizable features of a particular 2D data trace.
- **X-Calibration by Spectrograph** lets you calibrate the x-axis of your data displays with reference to your spectrograph's specifications. Calibration may be in wavelength or Raman shift.
- **Change Units** lets you change the units on the x-axis of a data display **(e.g. nm to µm, cm to pixel number, etc.)**

In addition, the option **Remove X-Calibration** has 2 further options:

1. **Current Data** returns the data in the active Data Window to its default pixel x-calibration.
2. **New Acquisitions** causes future data to be acquired with the default pixel x-calibration.

MANUAL X-CALIBRATION

Using newly acquired or previously stored data, select **Manual X-Calibration** from the **Calibrate** menu. The **Manual X-Calibration** dialog box appears, e.g.:



The number (**#n**) of the data window (or **#0** in the case of an Acquisition Window) appears on the title bar of the dialog box.

To manually calibrate a data window, first use the drop-down lists on the Manual X-Calibration dialog box to choose the **label** and the **units** you wish to use for the x-axis. The available labels and units are shown in **table 12** below.

Your chosen unit will appear on the top of the right-hand column of the two columns to the left of the Manual X-Calibration dialog box.

Table 12: Labels & Units

X-AXIS LABEL	UNITS
Wavelength	nm, µm, cm^{-1}, eV
Raman Shift	cm^{-1}
Position	µm, mm, cm, µi(nches), in(ches)
Time	ms, secs
Pixel	pixels

Another drop-down list allows you to choose a **polynomial order** for your calibration. The following polynomial options are available:

POLYNOMIAL ORDER	DESCRIPTION
Linear	The linear fit is best for situations where only 2 or 3 spectral features can be identified.
Quadratic	A quadratic polynomial produces the best calibration fit if the known spectral features are located near the center of the CCD sensor.
Cubic	A cubic polynomial produces the best calibration fit if the known spectral features are evenly distributed across the CCD sensor.

To perform the calibration:

1. Place the cross-hairs on a feature which you know to have a particular x-axis value and click **Capture**. If you wish to remove a point that you had previously selected, click **Delete**.
2. The pixel number will appear in the left-hand column. Against it, in the right-hand column, enter a value.
3. Repeat 1. & 2. for at least one other point. However, to achieve a good quality calibration you should choose a polynomial order fit commensurate with the spread of known spectral features across your sensor.

Rather than position the cross-hair by clicking a point, you may choose to move the cross-hair left or right using the horizontal scroll bar on the Manual X-Calibration dialog box.

Click the Single Scroll Arrows at either end of the scroll bar to move the cross-hair by one pixel at a time.

Click the Double Scroll Arrows to move the cursor from peak to peak (i.e. conduct a Peak Search) in accordance with the sensitivity level you have set using the Display Preferences dialog box.

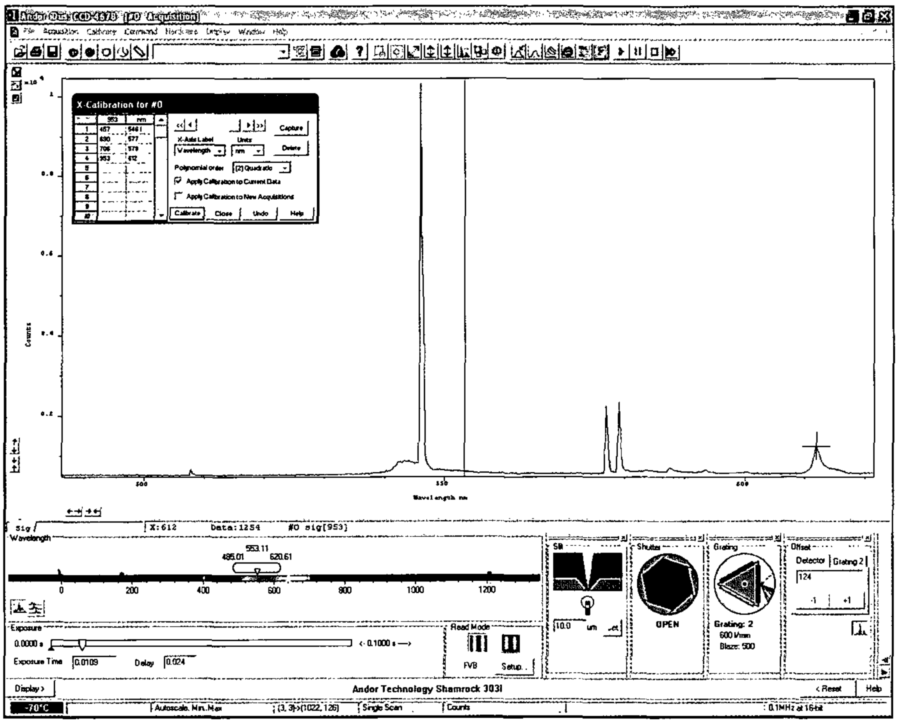


FIGURE 7 – EXAMPLE OF CALIBRATION USING ANDOR SHAMROCK

NOTE: You can also perform a peak search via the keyboard by pressing Ctrl ← or Ctrl → (2D display mode).

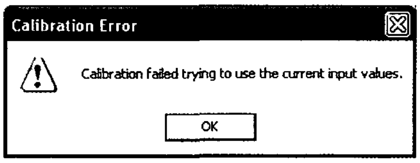
Applying Calibration

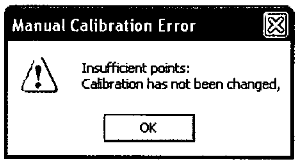
Calibrate

Depending on the selections made using the check boxes on the Manual X-Calibration dialog box, clicking the **Calibrate** button will apply calibration to the active data window and/or to future data acquisitions.

In the event that **Manual X-Calibration** fails, it typically does so for one of two reasons:

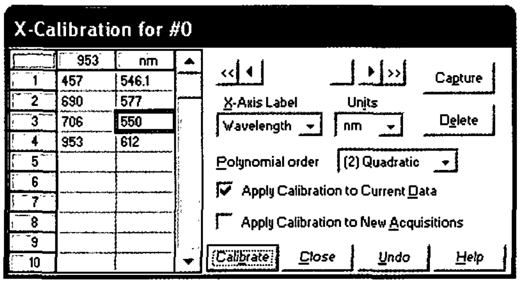
- Data that you are attempting to calibrate are **non-monotonic** (for example, a wavelength that should correspond to a single pixel value has several pixel values)
- You have identified **too few points** (i.e. 0 or 1) for the system to perform a calibration. The system displays one or other of the following **Error** dialog boxes:





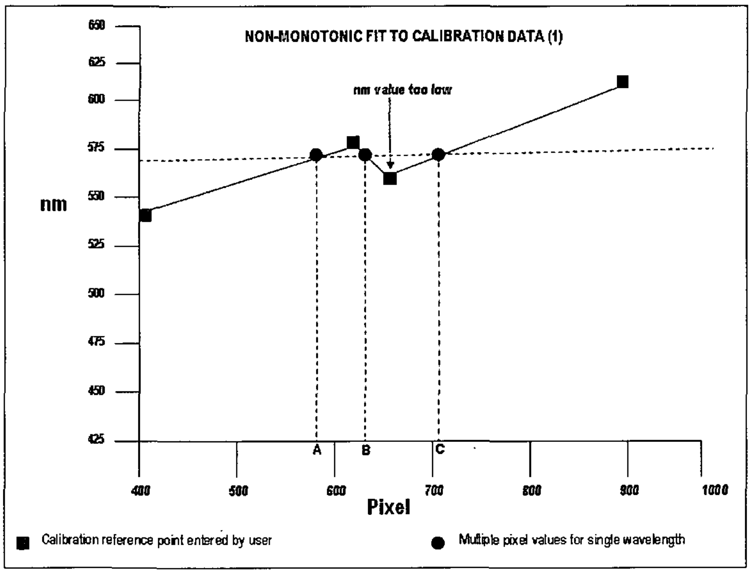
Data are Non-Monotonic

Your data may be **Non-Monotonic** if you have entered an incorrect value for one or more points on your data trace. An instance of grossly inaccurate manual calibration is shown here:



From Pixel 706 to Pixel 953 the user has indicated a fall in wavelength, despite the preceding rise in pixel 690. In such a case, an illegal non-monotonic calibration (as shown on the graph below) results.

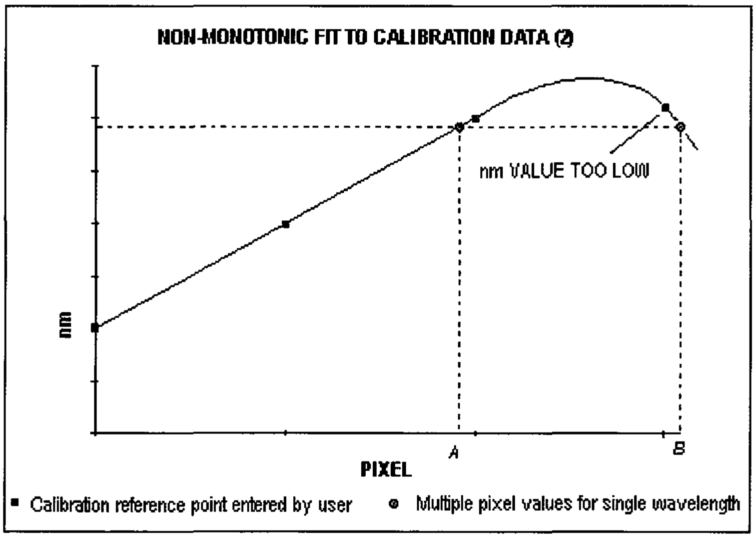
The squares indicate points entered by a user, pixel values *A*, *B* and *C* illustrate the non-monotonicity of a sample wavelength of around 570 nm (i.e. three different pixel values for a single wavelength).



However, a non-monotonic calibration may come about even in cases where your data are not as grossly inaccurate as those shown in the example above. A non-monotonic calibration sometimes results if you attempt to calibrate points that are very close together on your trace, even if, for example, you are entering rising wavelength values against rising pixel values.

In its background processing, the system models the calibration data (the user-supplied reference points) as a cubic polynomial. Inaccurate values mapped to pixels that are close together may cause the system to model the calibration data as shown in the following graph.

Again certain (wavelength) values are non-monotonic relative to pixel value (see pixel values *A* and *B*, for example). The squares on the graph indicate points entered by the user, the rightmost point being at slightly too low a wavelength value.



Too Few Points

A calibration error will also occur if you have entered no data points, or only one data point, in the Manual X-Calibration dialog box. As general rule to obtain a good quality calibration, use more than five reference points, at regular intervals, across the full width of the CCD sensor.

Undo

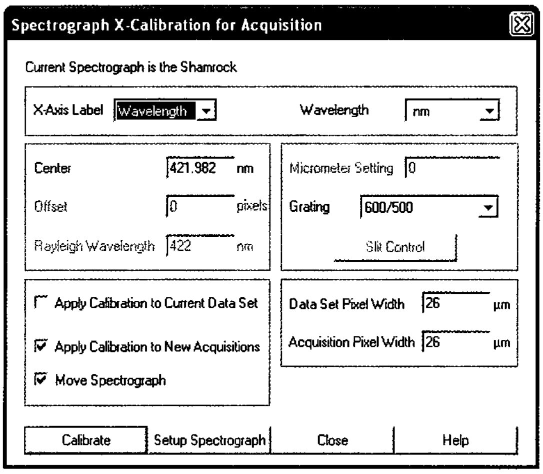
Click the **Undo** button to exit the Manual X-Calibration dialog box and to undo any calibrations that you have performed since entering the dialog box.

Close

Click the **Close** button to exit the Manual X-Calibration dialog box.

X-CALIBRATION BY SPECTROGRAPH

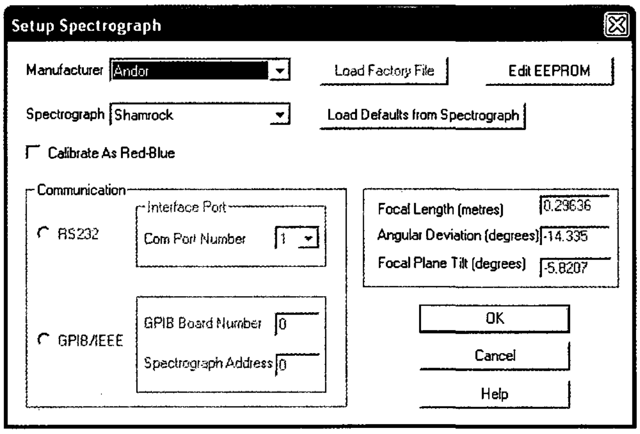
To calibrate data using the spectrograph, select the **X-Calibration by Spectrograph** option from the **Calibration** Menu. The **Spectrograph X-Calibration** dialog box will appear e.g.:



NOTE: The dialog box can also be opened by selecting the Setup Spectrograph option from the Hardware menu on the Main Window.

Setup Spectrograph

Before you can perform a calibration using the spectrograph, you must ensure that the system knows which spectrograph you are using. To select the type of spectrograph to be used, click the **Setup Spectrograph** button on the Spectrograph X-Calibration dialog box and the **Setup Spectograph** dialog box appears, e.g.:



From the **Manufacturer** drop-down lit, select the appropriate company name and from the **Spectrograph** drop-down list, select the model type being used, then click OK. The Spectrograph X-Calibration will appear again, with the details of the selected spectrograph.

With the exception of User Defined, selecting one of these options will cause the system to select and display (in grayed - i.e. non-writable - text boxes) your spectrograph's **Focal Length**, **Angular Deviation** and **Focal Plane Tilt**.

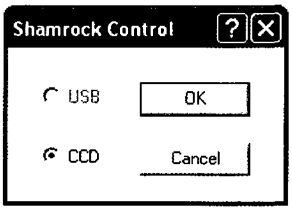
If you select the **User Defined** option from the drop-down list, you should consult the manufacturer's handbook for details, then key in the values for the spectrograph.
If you are using a motorized spectrograph, the system may be able to load spectrograph attributes (number of gratings, lines/mm, etc.) directly. The **Load Defaults from Spectrograph** button is enabled if an appropriate motorized spectrograph is chosen from the Spectrograph drop-down list.

Depending on the type of spectrograph being used, you can also select the type of interface needed from the **Communications** section of the dialog box.

Communications

The radio buttons in the Communications section of the Spectrograph Setup Dialog box can be used to establish an interface between your computer and the spectrograph.

If you are using an Andor Shamrock, you can also choose between USB or I^2C control links. Select **Shamrock Control** from the Hardware drop-down menu and the **Shamrock Control** dialog box appears e.g.:



Select **USB** for USB control or for **I^2C** select **CCD**, then click **OK.**

NOTE: When a Shamrock is connected, the Shamrock icon appears in the menu bar of the Main Window.

In the event of an error in communications occurring, you will be prompted by a message, e.g.:

Other Spectrographs

If for example you have selected the **Oriel MS257** spectrograph, you can now **Load Factory File**. This file supplies the system with important configuration details of your particular spectrograph. It must be loaded for the system to control the spectrograph correctly. The Factory File will have been supplied on diskette with your MS257 spectrograph. Performing Load Factory File lets you save the file contents to the **.ini** file included with Andor's software. The Factory File need not be loaded again.

When you click the **Load Factory File** button you will see a typical Windows **'Open File'** dialog box. Select the directory and the filename of the file you wish to load, and click **OK**. The Factory File is now loaded.

Reverse Spectrum

Some spectrographs produce somewhat 'atypical' spectra, where longer wavelengths (red) are to the left (as viewed from the detector), and shorter wavelengths (blue) are to the right. If you select the **MS127** or the **FICS** spectrograph from the drop-down list for example, you will notice a tick in a **Reverse Spectrum** check box. Because both these spectrographs are of this type, the Reverse Spectrum check box is ticked by default when these spectrographs are selected.

The system then uses software to 'reverse' the output of the detector when it generates a data window, presenting the display in the more usual orientation.

You may, if you wish, disable the Reverse Spectrum function by clicking the check box.

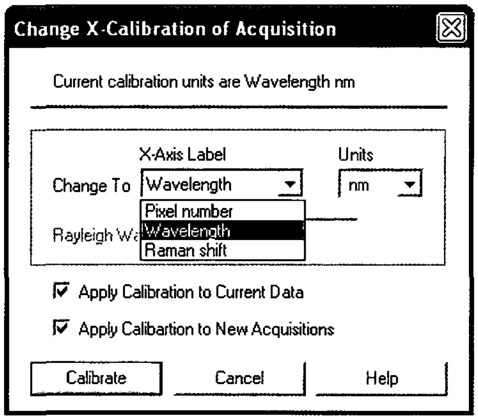
X-Axis Label & Units

The Spectrograph X-Calibration Dialog box allows you to select, from scrollable drop-down list boxes, an **X-Axis Label** for your data window and an appropriate **Unit** of measurement.

The following label & unit combinations are available:

X-Axis Label	Units
Wavelength	nm, µm, cm^{-1}, eV
Raman Shift	cm^{-1}

To change the x-axis units of an active data window which you have previously calibrated, select the Change Units option on the Calibrate Menu. The **Change X-Calibration of Acquisition** dialog box will appear on your screen, e.g.:



From the **X-Axis Label** drop-down list choose whether you want the x-axis to represent **Wavelength, Pixel Number** or **Raman Shift.**

From the **Units** drop-down list, choose the units that you want to use for your recalibration.

The available combinations depend on how the data were previously calibrated. If for instance, the data were previously calibrated in Wavelength and Nanometers the available combinations for recalibration are as follows:

Wavelength	nm, μm, cm^{-1}, eV
Raman Shift	cm^{-1}
Pixel Number	-

Table 13 on the next page shows the available combinations for all modes.

Table 13: Label & Unit changes

	X-Axis Label	Units	Can Change to
0	User Type	-	
1	*Pixel Number (see NOTE below)	-	
2	Wavelength	nm, µm, cm^{-1}, eV	**1, 2, 3**
3	Raman Shift	cm^{-1}	**1, 2**
4	Position	µm, mm, cm, µi(nches), in(ches)	**1, 4**
5	Time	ms, s	**1, 5**
6	Sample	-	**1**

NOTES:

1. **Changing from Pixel Number actually constitutes a new calibration and can only be performed by using Manual X-Calibration** or **X-Calibration by Spectrograph**.
2. **If you choose to recalibrate a data window in pixels, you will not be able to perform any further recalibrations on that window using the Change X-Calibration of Acquisition dialog box. If you save (under its previous filename) data that has been recalibrated in pixels, you will lose any previously saved calibration.**

Center Wavelength / Center of Raman Shift

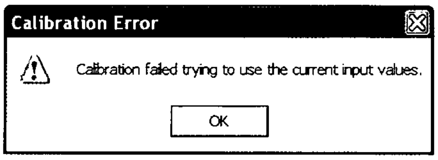
The system allows you to adjust your spectrograph so that light of a chosen **Wavelength** or a chosen **Raman Shift** falls on the center of the CCD-chip.

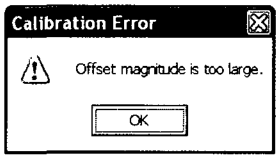
These are referred to as the **Center Wavelength** and the **Center of Raman Shift** respectively.

If you have chosen **Wavelength** as your X-Axis Label, enter the **Center Wavelength** in the text box provided on the Spectrograph X-Calibration dialog box. If you have chosen **Raman Shift** as your X-Axis Label enter the **Center of Raman Shift** in the text box.

In each instance, the value is expressed in the units you selected previously above.

If you enter too large or too small a value an **Error** dialog box appears, e.g.:





Note on Raman Shift

The **Raman Shift** is calculated as follows:

If ***scatter*** is the wavelength of the Raman scattered light in nanometers and ***laser*** is the wavelength of the incident laser light in nanometers, then the Raman Shift in cm^{-1} (i.e. ***rs***) is calculated as follows: ***rs*** = 10^7 x [(***scatter*** - ***laser***) / (***scatter*** x ***laser***)].

Thus, if ***scatter*** < ***laser***, a negative Raman Shift (**anti-Stokes transition**) will result.

If ***scatter*** > ***laser***, a positive Raman Shift (**Stokes transition**) will result.

Positive and negative values for Raman Shift may thus appear on the x-axis of a data window that is calibrated for Raman Shift, and may be used in the calibration process itself.

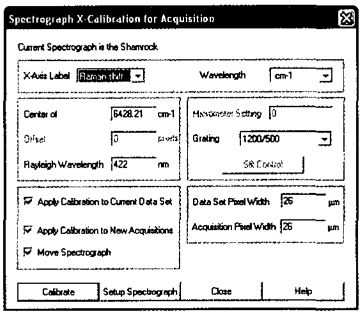
Offset

By entering a value in the **Offset** text box of the Spectrograph X-Calibration dialog box you can compensate for small misalignments of the detector or the wavelength drive in your spectrograph. A positive value will cause the x-axis of the data window to move to the right (relative to the trace) by the corresponding number of pixels. A negative value will cause the x-axis to move to the left.

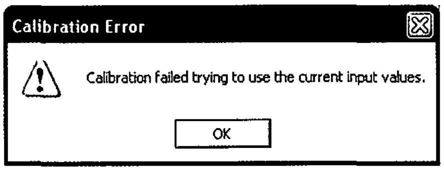
NOTE: To assess the accuracy of any calibration you have performed, you will need a calibration spectral line source, such as a helium neon laser or a mercury vapor lamp. Ideally, set the spectrograph to one of the prominent spectral lines, take a scan, and use the cursor on the data window to determine any offset (in pixels) of the line from its true wavelength.

Rayleigh Wavelength

If you have selected **Raman Shift** as your X-Axis Label, you must enter a value in nanometers for the **Rayleigh Wavelength**. In Raman Spectroscopy the Rayleigh Wavelength is that element of a spectrum line (in scattered radiation) whose wavelength is equal to that of the incident radiation (i.e. the laser wavelength) and is a product of ordinary or Rayleigh scattering.



An Error Message will appear if you attempt to perform a calibration without having entered a valid Rayleigh Wavelength, e.g.:



Micrometer Setting

For certain non-motorized spectrographs, the system will calculate a Micrometer Setting that corresponds to the Grating and the Center Wavelength / Center of Raman Shift you have chosen. The Micrometer Setting allows you to manually adjust the angle of the diffraction grating (by means of the micrometer on the spectrograph housing), so that light of the wavelength / Raman shift of your choice falls on the centre of the CCD-chip.

The Micrometer Setting appears in a text box on the Spectrograph X-Calibration dialog box. You should use this value to manually set the micrometer on the spectrograph.

NOTE: If you choose to enter a Micrometer Setting, the system will calculate a value for the Center Wavelength / Center of Raman Shift, and vice versa. You need enter only one of the two values.

Because Raman shift does not correlate linearly with wavelength (or pixel positions), the center column of pixels on the CCD-chip (e.g. column 512 on a chip of 1024 pixels) is likely to be represented off-center on the x-axis of a data window linearly calibrated for Raman shift. Column and where appropriate, row number, are expressed in the form [x,y] on the status bar along the bottom edge of the data window.

NOTES:

1. **If you choose Raman Shift as the X-Axis Label, the Center Wavelength text box is relabeled Center of Raman Shift.**
2. **In the case of motorized spectrographs, the wavelength drive is under direct software control.**

Grating

From the scrollable drop-down list select the specification of the diffraction grating you are currently using. **Grating** specifications are shown as a line density followed by (where applicable) a blaze wavelength.

Close

To exit the Spectrograph X-Calibration dialog box, click **Close**.

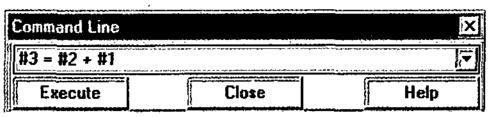
NOTE: The details you supply regarding your spectrograph, including any retrieved data will appear by default whenever you open the Spectrograph X-Calibration dialog box subsequently. You can change them whenever you choose.

Processing Data via the Command Line

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. Several command lines can be entered and they are separated by ":".

The open the **Command Line** dialog box, either click the button or select the **Command Line** option from the **Command** drop-down menu. The dialog box opens as per the following example:



To run a command, click **Execute.** For further details of how to use the **Andor Basic** programming language, please refer to the **User's Guide to Andor Basic.**

Calculations & Configure Calculations

The **Calculations** option lets display the output of colorimetry calculations in a **CIE Calculations Window**.

The **Configure Calculations** option lets you choose which colorimetry calculations you are going to perform and which parameters you are going to use.

For further information, please refer to **Colorimetry Calculations** in the **Andor Radiometry Guide**.

SECTION 9 – WORKING WITH PROGRAMS

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. Please refer to the **Programmer's Guide to Andor Basic**.

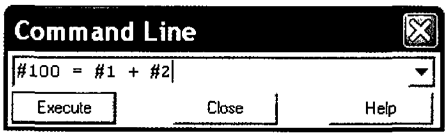
The system provides facilities to let you **Edit**, **Save** and **Run** your programs.

Command Line

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.

To open the Command Line, click the button.

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:



Program Editor Window

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

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.**

Cut, Copy, Paste, Undo

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

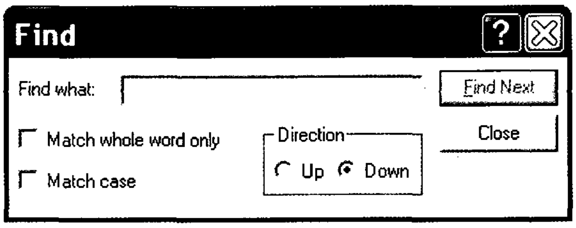
Paste inserts cut or copied text into the position following the cursor, or replaces text that you have highlighted.

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

Search

To search for items, either click on the **Find** button on the Program Window or select **Find...** from the **Search** drop-down menu:

The **Find** dialog box appears:



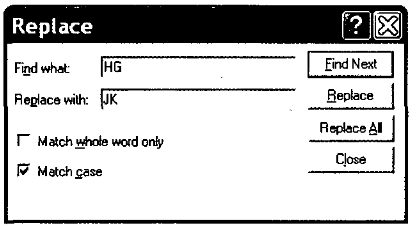
In the **Find what** text box, type the word or phrase (the **'search string'**) that you want to find.
Select **Match whole word only** to look for a complete word or for the same combination of capital and/or small letters as occur in the search string, select **Match case**.
Select **Direction** to determine in which way the search will be carried out search

To activate the search, click on **Find Next** or click the button.

Replace

To Replace items, select **Replace...** from the **Search** drop-down menu:

In the **Find what** text box, type in the search string and in the **Replace with** text box, type the word or phrase that you want to use instead, e.g.:



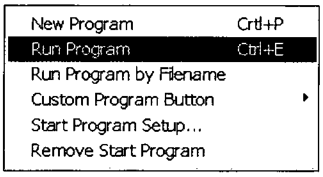
- Click the **Replace** button to change the next occurrence of the search string (or the highlighted search string if you have just used Find Next).
- Click **Replace all** to replace the search string wherever it occurs after the current cursor position.

Check boxes let you **Match whole word only** or **Match case**.

Run Program

To run a program, first ensure sure you have opened the appropriate **.pgm** file and ensure that the filename appears in the drop-down list box.

Secondly either click the  button or select **Run Program** from the **File** drop-down menu:



The program will now start.

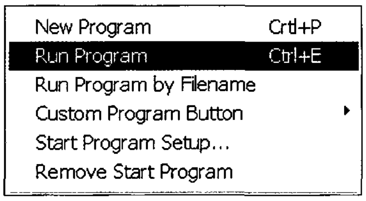
To change the name of the file you want to run carry out **one** of the following actions:

- Open the drop-down list and click the name of the file
- Select an open Program Editor Window
- Open the **.pgm** file from the **File** menu.

Run Program by Filename

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

Select **Run Program by Filename** from the File drop-down menu:



A standard **Open** dialog box appears, 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

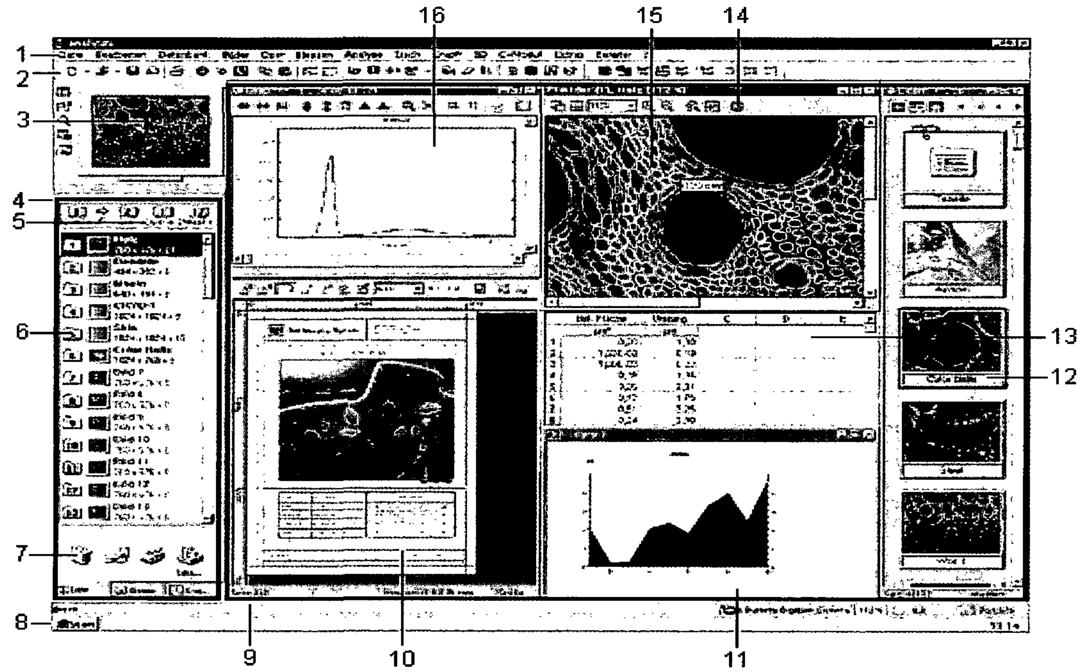
Any text-based input required by the program (i.e. in **Andor Basic** you have indicated that the user must manually enter a value at a particular point in the program's execution) is entered via an Input dialog box.

SECTION 10 – THIRD PARTY SOFTWARE

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. The layout of the **SiS** display is shown below:



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		

Other Software Supported

We also provide drivers for the following software packages: -

- Image Pro+ and Image Pro Express from Media Cybernetics
- V++ from Digital Optics
- LabVIEW from National Instruments
- GRAMS from Galactic

SECTION 11 - TUTORIALS

TUTORIAL 1 - 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

Aim

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.

Description

1. 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.
2. 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**.
3. 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**.
4. 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.
5. 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.

6. 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.
7. 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.
8. 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.
9. 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.
10. 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.

TUTORIAL 2 - EXTENDING DYNAMIC RANGE IN 'SLOW SCAN' MEASUREMENTS BY ACCUMULATING SIGNAL

Background

Although the iXon camera, due to it's 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.

Aim

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.

Description

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

TUTORIAL 3 - PERFORMING BASIC LOW-LIGHT MICROSCOPY FUNCTIONS USING THE IXON

Aim

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.

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.

Description

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.

- **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 10-20ms. 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.

1. **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).
2. 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.
3. 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.
4. **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).

5. 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).
6. 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.
7. 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. **NOTE: 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.**

8. **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.).
9. 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** dialogue 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.
10. 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.

APPENDIX

GLOSSARY

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

EMCCD Structure

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 (EMCCD)**, a revolutionary technology, capable of single photon detection.

An **EMCCD** is a silicon-based semiconductor chip bearing a two-dimensional matrix of photo-sensors, or **pixels**. This matrix is usually referred to as the image area. The pixels are often described as being arranged in rows and columns - the rows running horizontally and the columns vertically.

The EMCCD in the iXon detector is identical in structure to a conventional **CCD** but with the **shift register** extended to include an additional section, the **Multiplication (or Gain) Register** as shown below:

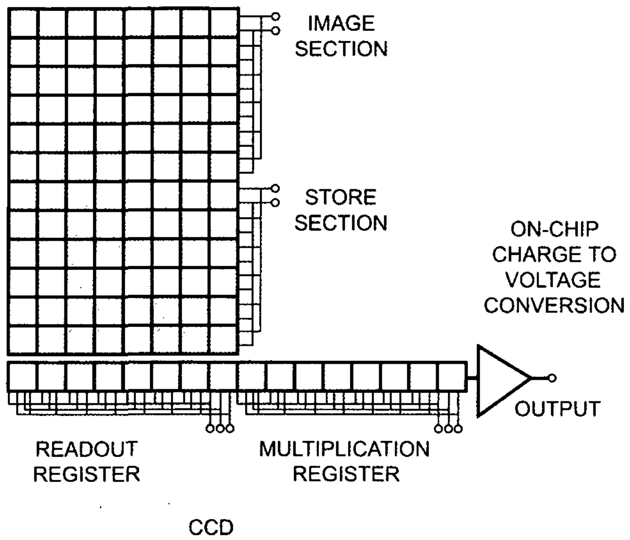


Figure 8: Typical EMCCD structure

Impact Ionisation

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 8)** the signal may be increased above the readout noise, hence effectively reducing the readout noise.

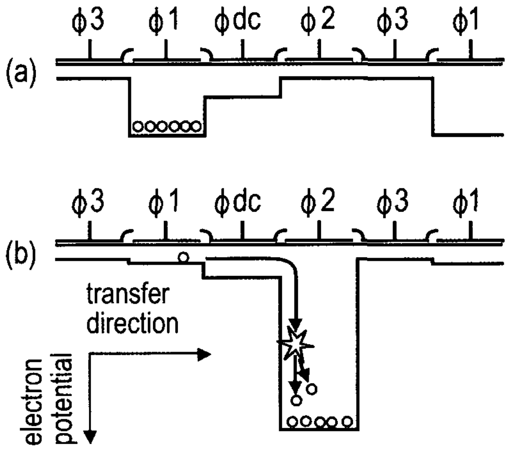


Figure 9: Gain register operation

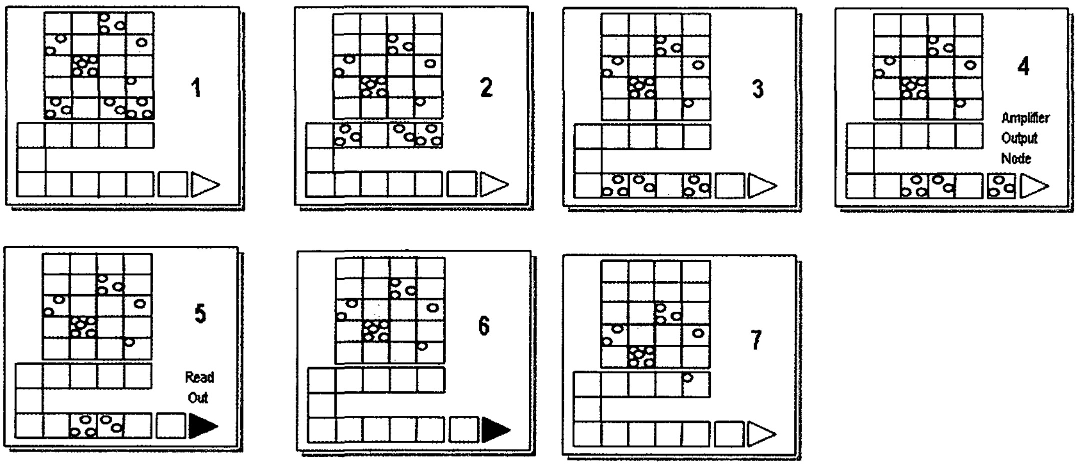
The **shift register** runs below and parallel to the light collecting rows. It has the same number of pixels as a light-collecting row, but is masked, to prevent light from falling on it. The gain register is also masked. When light falls on an element, electrons (photoelectrons) are produced and, in 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 **A**nalog-to-**D**igital **(A/D)** converter which in turn converts each charge packet into a 16-bit binary number. The A/D converter on the Newton 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 EMCCD-chip. Other binning patterns are achieved by summing charge in the shift register and/or the output node prior to readout. See **Vertical & Horizontal Binning** later in this section.

Readout sequence of an EMCCD (Only subset of pixels shown)



1 Exposure to light causes a pattern of charge (an electronic image) to build up on the frame (or Image Area) of the EMCCD-chip.

2 Charge in the frame is shifted vertically by one row, so that the bottom row of charge moves into the shift register.

3 Charge in the shift register is moved horizontally by one pixel, so that charge on the endmost pixel of the shift register is moved into the Gain register.

4 Charge is shifted into the output node of the amplifier.

5 The charge in the output node of the amplifier is passed to the analog-to-digital converter and is read out.

6 Steps 3 and 4 are repeated until the shift register is emptied of charge.

7 The frame is shifted vertically again, so that the next row of charge moves down into the shift register. The process is repeated from Step 3 until the whole frame is read out.

Accumulation

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

Acquisition

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

A/D Conversion

Charge from the CCD 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.

Background

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

Binning

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

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

There are two main variants of the binning process:

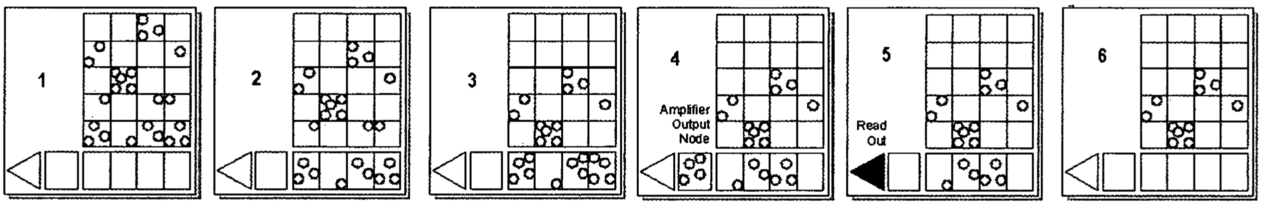
- **Vertical binning**
- **Horizontal binning**

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

Vertical Binning

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

Vertical Binning of Two Rows (Only subset of pixels shown)



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 down into the shift register.
3 Charge in the frame is shifted vertically by a further row, so that the next row of charge moves down into the shift register, which now contains charge from two rows - i.e. the charge is vertically binned.
4 Charge in the shift register is moved horizontally by one pixel, so that charge on the endmost pixel of the shift register is moved into the Gain Register
5 Charge is shifted into the output node of the amplifier
6 The charge in the output node of the amplifier is passed to the analog-to-digital converter and is read out
7 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 (Creating Superpixels)

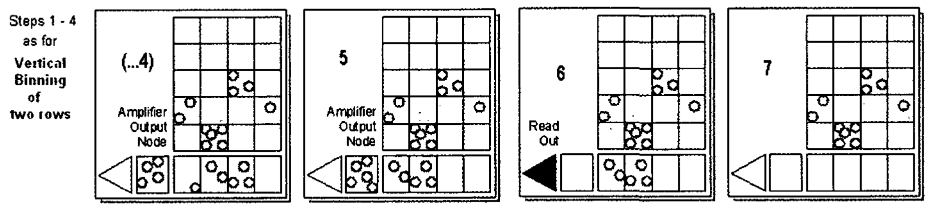
Shifting the charge horizontally from several pixels at a time into the output node is known as **Horizontal Binning.** Horizontal binning *in combination with* **Vertical binning** *allows you to define so-called* superpixels that in **Image** Display Mode represent as a single picture element charge 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,

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

In the following example, where each superpixel is of dimensions 2 x 2 pixels, charge from two rows is first binned vertically into the shift register; then charge from two pixels of the shift register is binned horizontally into the output node of the amplifier. The effect of the combined binning processes is a summed charge equating to a 2 x 2 "**superpixel**".

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

Vertical & Horizontal Binning (2x2 Superpixels)
(Only subset of pixels shown)



...4 Charge from two rows has already been vertically binned into the shift register (see **Vertical Binning of Two Rows** on the previous page*)*. 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 re see ad 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** on the previous page*)* until the whole frame is read out.

Counts

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

Dark Signal

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

The dark signal adds to your measured signal level, and increases the amount of noise in the measured signal. Since the dark signal varies with temperature, it can cause background values to increase over time. It also sets a limit on the useful exposure time.

Reducing the temperature of the CCD reduces dark signal (typically, for every 7°C that temperature falls, dark signal halves). CCD readout noise is low, and so as not to compromise this by shot noise from the dark signal, it is important to cool the detector to reduce the dark signal. If you are using an exposure time of less than a few seconds, cooling the detector below 0°C will generally remove most of the shot noise caused by dark signal.

Detection Limit

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

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

If the signal is small, we can ignore its shot noise.

Furthermore, if a suitably low operating temperature and short exposure time can be achieved, the lowest detection limit will equal the readout noise.

Exposure Time

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

Frame Transfer

Frame transfer is a special acquisition mode that is only available if your system contains a Frame Transfer CCD, (FT CCD). An FT CCD differs from a standard CCD in 2 ways:

1. Firstly, a FT CCD contains 2 areas, of approximately equal size, see diagram. 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.
2. 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 will the next image is being acquired. It also allows a FT CCD to be used in imaging mode without a shutter. **Note: This is only applicable when the camera is running in Accumulate or Kinetic mode.**

Gain

Gain & Dynamic Range

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. Therefore at 1MHz readout rates the Dynamic Range will be maintained at over 20,000:1 for gains up to about 20 times, while at 62.5kHz it will be maintained at over 43,000:1 for gains up to 6 times.

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 & Noise

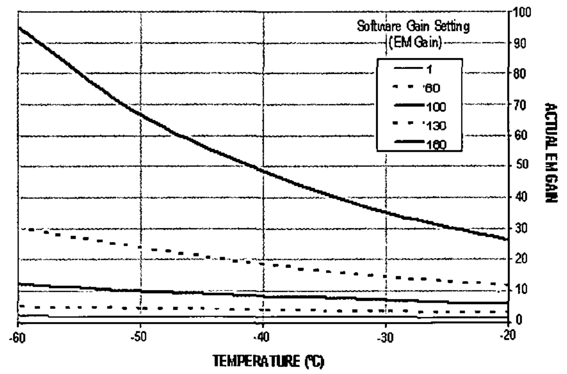
The output from the gain register is fed into a conventional CCD output amplifier. This amplifier, even in a scientific CCD, will have a readout noise of a few electrons rms and around 10 or 20 electrons rms at MHz readout rates. However this noise will effectively be reduced by the multiplication factor 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 an s readout noise of a few to tens of electrons, depending on read out speed.

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) 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.

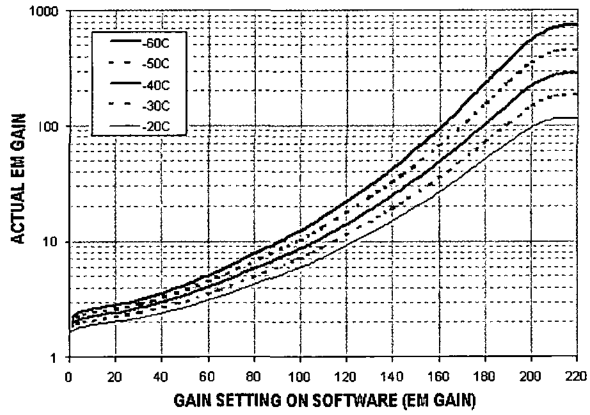
Conversely, if the signal is being lost in the readout noise then increasing the gain is the only way to detect it. If the gain is set high enough, then detection of single electron events will be possible. These events will appear on an image as a spike several hundred counts high. In Andor EMCCD cameras the gain is limited to a maximum of 255 times at –50°C for standard systems. This is comparable to high end ICCDs.

Gain Temperature Dependence

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.



The following 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.



Gain & Dynamic Range

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. Therefore at 1MHz readout rates the Dynamic Range will be maintained at over 20,000:1 for gains up to about 20 times, while at 62.5kHz it will be maintained at over 43,000:1 for gains up to 6 times.

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.

Keep Cleans

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.

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

Noise

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**
- **Fixed Pattern Noise**

These two categories are described in the following 2 pages.

Pixel Noise

Let us first attempt to define pixel noise. Assume that a light signal is falling on a pixel of the CCD. If the charge on the pixel is read, and the read process is repeated many times, the noise may be taken as the variation in the values read. The **R**oot **M**ean **S**quare (**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
- 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.

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.

Readout Noise

Readout noise (which in our detectors is, in any case, low) is due to the amplifier and electronics: it is independent of dark signal and signal levels; 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 falls to zero, their respective shot noise also falls to zero.

The total pixel noise is not, however, simply the sum of the three main noise components (readout noise, shot noise from the dark signal, and shot noise from the signal). Rather, the Root Sum Square (r.s.s.) gives a reasonable approximation - thus:

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

where:

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

Quantum Efficiency/Spectral Response

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

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

Readout

Readout is the process by which data are taken from the pixels of the CCD and stored in computer memory. The pixels, which are arranged in a single row, are read out individually in sequence.

Readout involves amplifying the charge on each pixel into a voltage, performing an A/D conversion, and storing the data in computer memory. The time taken to perform this operation is known as the "read time".

Saturation

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

Scan Types: Keep Clean & Acquired

The CCD 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 CCD (i.e. it is a keep-clean scan), the charge from the CCD 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 previously). In this User's Guide 'scan' generally refers to an acquired scan - unless the context specifically indicates otherwise.

Shift Register

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

Shot Noise

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. Most simply defined:

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

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

Signal to Noise Ratio

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

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.

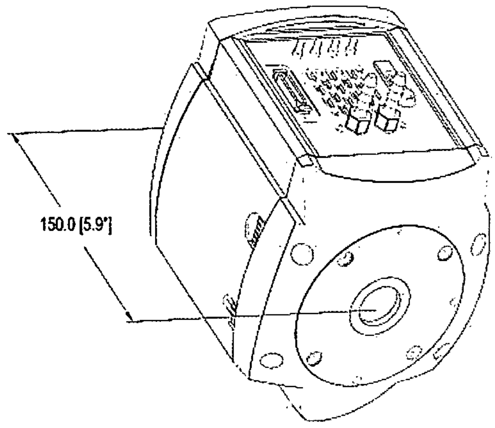
ELECTRICAL RATINGS & ENVIRONMENTAL CONDITIONS

- 5Vdc with 15 Watts
- 7.5Vdc with 30 Watts (PS-25 only)
- ±15Vdc with 3Watts0
- Indoor use only
- Altitudes up to 2000m
- Temperature 5°C to 40°C
- Maximum Relative Humidity 80% for temperatures up to 31°C, decreasing linearly to 50% relative humidity at 40°C
- Other voltage fluctuations as stated by the manufacturer
- Overvoltage category 1
- Pollution Degree 2

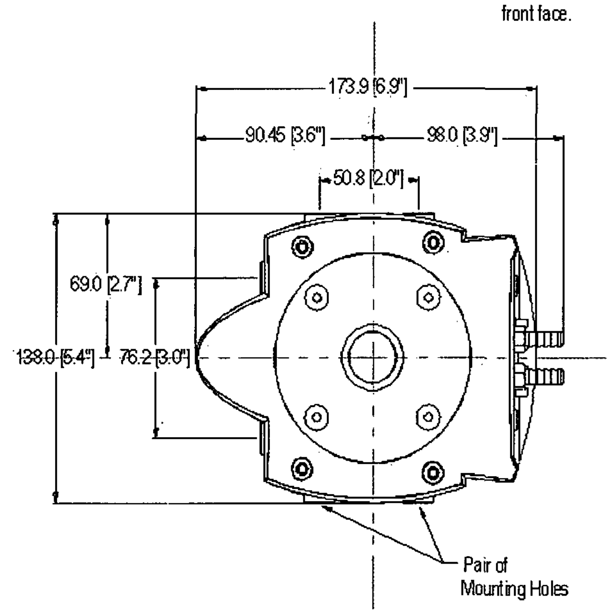
ADDITIONAL STATEMENT REGARDING EQUIPMENT OPERATION

IF THE EQUIPMENT IS USED IN A MANNER NOT SPECIFIED BY ANDOR TECHNOLOGY plc, THE PROTECTION PROVIDED BY THE EQUIPMENT MAY BE IMPAIRED.

MECHANICAL DIMENSIONS



Note: There are mounting holes (1/4-20UNC) located on three sides of the camera. They are positioned centrally at a distance of 40mm from the front of the front face.



TERMS & CONDITIONS

1. In these Conditions:

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

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

   'SELLER' means Andor Technology 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.

WARRANTIES & LIABILITY

1. Subject to these Conditions set out below, the Seller warrants that the Goods will correspond with their specification at the time of delivery and will be free from defects in material and workmanship for a period of 12 months from the date of delivery.

2. The above warranty is given by the Seller subject to the following conditions:

2.1 the Seller shall be under no liability in respect of any defect in the Goods arising from any drawing, design or specifications supplied by the Buyer;

2.2 the Seller shall be under no liability in respect of any defect arising from fair wear and tear, wilful damage, negligence, abnormal working conditions, failure to follow the Seller's instructions (whether oral or in writing), misuse or alteration or repair of the Goods without the Seller's approval;

2.3 the Seller shall be under no liability under the above warranty (or other warranty, condition or guarantee) if the total price for the Goods has not been paid by the due date for payment;

2.4 the above warranty does not extend to parts, material or equipment not manufactured by the Seller, in respect of which the Buyer shall only be entitled to the benefit of any such warranty or guarantee as is given by the manufacturer to the Seller.

3. Subject as expressly provided in these conditions, and except where the Goods are sold to a person dealing as a consumer (within the meaning of the Unfair Contract Terms Act 1977), all warranties, conditions or other terms implied by statute or common law are excluded to the fullest extent permitted by law.

4. Any claim by the Buyer which is based on any defect in the quality or condition of the Goods or their failure to correspond with specification shall (whether or not delivery is refused by the Buyer) be notified in Writing to the Seller within 7 days from the date of delivery or (where the defect or failure was not apparent on reasonable inspection) discovery of the defect or failure. If delivery is not refused, and the Buyer does not notify the Seller accordingly, the Buyer shall not be entitled to reject the Goods and the Seller shall have no liability for such defect or failure, and the Buyer shall be bound to pay the price as if the Goods had been delivered in accordance with the Contract. In no event shall the Buyer be entitled to reject the Goods on the basis of any defect or failure which is so slight that it would be unreasonable for him to reject them.

5. Where any valid claim in respect of the Goods which is based on any defect in the quality or condition of the Goods or their failure to meet specification is notified to the Seller in accordance with these Conditions, the Seller shall be entitled to replace the Goods (or the part in question) free of charge or, at the Seller's sole discretion, refund to the Buyer the price of the Goods (or a proportionate part of the price), but the Seller shall have no further liability to the Buyer.

6. Except in respect of death or personal injury caused by the Seller's negligence, the Seller shall not be liable to the Buyer by reason of any representation (unless fraudulent), or any implied warranty, condition or other term, or any duty at common law, or under the express terms of the Contract, for any indirect, special or consequential loss or damage (whether for loss of profit or otherwise), costs, expenses or other claims for compensation whatsoever (whether caused by the negligence of the Seller, its employees or against otherwise) which arise out of or in connection with the supply of the Goods, or their use or resale by the Buyer and the entire liability of the Seller under or in connection with the Contract shall not exceed the price of the goods, except as expressly provided in these Conditions.

7. The Seller shall not be liable to the Buyer or be deemed to be in breach of the Contract by reason of any delay in performing, or any failure to perform, any of the Seller's obligations in relation to the goods, if the delay or failure was due to any cause beyond the Seller's reasonable control. Without prejudice to the generality of the foregoing, the following shall be regarded as causes beyond the Seller's reasonable control:

7.1 Act of God, explosion, flood, tempest, fire or accident;

7.2 war or threat of war, sabotage, insurrection, civil disturbance or requisition;

7.3 acts, restrictions, regulations, bye-laws, prohibitions or measures of any kind on the part of any governmental, parliamentary or local authority;

7.4 import or export regulations or embargoes;

7.5 strikes, lock-outs or other industrial actions or trade disputes (whether involving employees of the Seller or of third party);

7.6 difficulties in obtaining raw materials, labour, fuel, parts or machinery;

7.7 power failure or breakdown in machinery.