# ANDOR™
TECHNOLOGY

## Software Guide

# Solis

Andor's camera control and analysis software platform



Solis
Data Acquisition and Analysis for Imaging and Spectroscopy

# iXon Ultra

## TABLE OF CONTENTS

## SECTION 1: USING THE iXon Ultra

## 1.1 - STARTING THE APPLICATION

On the desktop, click on the  icon which was installed during the installation process and the **Solis Splash Screen** appears briefly:



The **Main Window** then appears, e.g.:
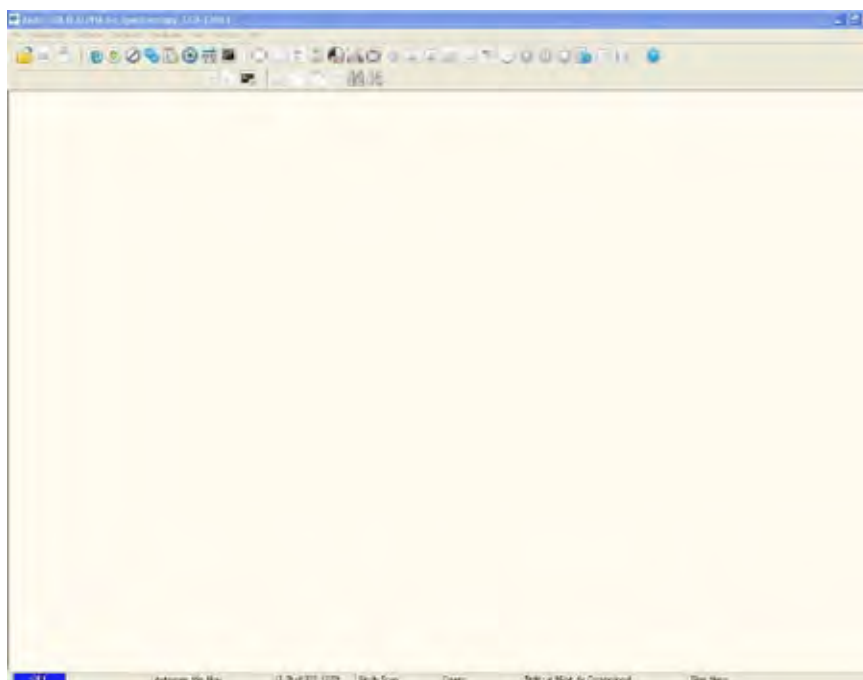
## 1.2 - MAIN WINDOW

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 the table below.

Table 1: Main Window buttons

| Icon | Title | Icon | Title | Icon | Title |
|---|---|---|---|---|---|
| | Open | | Command Line | | 3D display mode |
| | Print | | Help | | Image Display mode |
| | Save | | Select Autoscale area | | Change false colour pallette |
| | Video | | Reset | | Timestamp |
| | Take Signal | | Rescale | | Play |
| | Abort Acquisition | | Acquisition Autoscale ON | | Pause |
| | Setup Acquisition | | 99:1 Contrast Ratio | | Stop |
| | Run-Time Control | | Data Histogram | | Playback autoscale-off |
| | Shutter | | Analysis Region of Interest | | Periodic Table |
| | Post Process Photon Counting | | File Information | -80°C | Temperature control ON |
| | Spurious Noise Filter | | 2D with peak labels | OFF | Temperature control OFF |
| | Run Program | | 2D display mode | | Count Convert |

**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.:
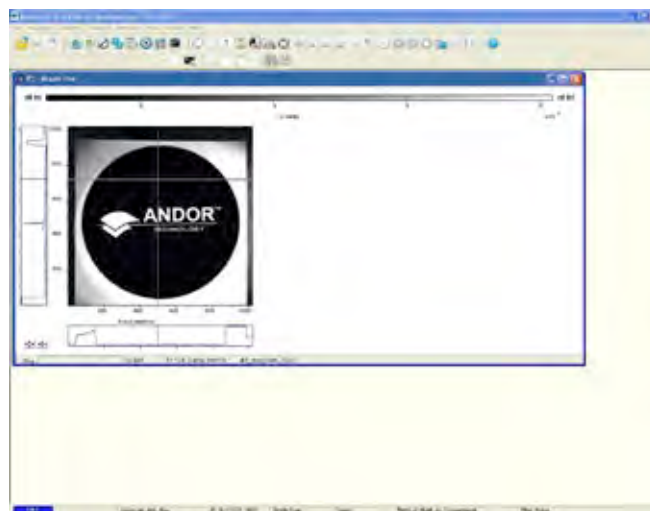


The Edit & Search menus and their associated buttons appear only when a Program Editor Window is active, e.g.:
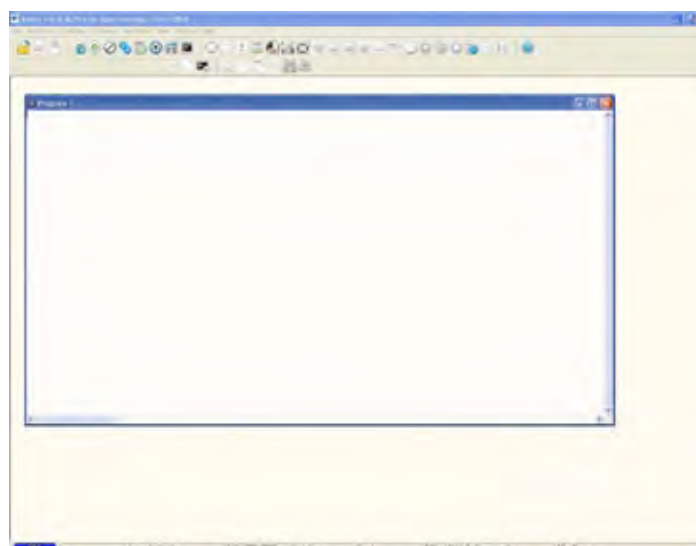


## 1.2.1 - Main Window Status Bar

The following information is displayed on the bottom line of the display:

- Current temperature status (see **pages 15 - 16** for further information)

- Autoscale acquisition status (see **page 26** for further information)

- Sub-Image (see **page 37** for further information)

- Acquisition mode (see **pages 27 - 29** for further information)

- Data type (see **pages 23 - 25** for further information)

- Readout speed, No of bits and amplifier in use (see **Hardware Manual** for further information)

## 1.3 - HOT KEYS

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

Table 2: Data Acquisition Hot Keys

| KEY STROKE(S) | DESCRIPTION |
|---|---|
| F5 | Take signal |
| F6 | Autoscale Acquisition |
| Ctrl + B | Take background |
| Ctrl + R | Take reference |
| Esc | Abort Acquisition |

Table 3: 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.<br>If maintain aspect ratio on, expand x-axis and y-axis | ✓ | ✓ | ✓ |
| Del | If maintain aspect ratio off, contract x-axis.<br>If maintain aspect ratio on, contract x-axis and y-axis | ✓ | ✓ | ✓ |
| / | On image, if maintain aspect ratio off, expand y-axis.<br>On image, if maintain aspect ratio on, expand x-axis and y-axis. | | | ✓ |
| Home | Move cursor furthest left | ✓ | ✓ | ✓ |
| End | Move cursor furthest right | ✓ | ✓ | ✓ |
| PgUp | Scroll up through track | ✓ | ✓ | ✓ |
| 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 | ✓ | ✓ | ✓ |
| F7 | Toggle Palette | ✓ | ✓ | ✓ |
| F8 | Reset | ✓ | ✓ | ✓ |
| F9 | Rescale | ✓ | ✓ | ✓ |
| Alt + F9 | Toggle Rescale Mode | ✓ | ✓ | ✓ |
| Ctrl + F9 | Scale to Active (see Section 4 - Displaying Data) section) | ✓ | ✓ | ✓ |
| F10 | File information | | | |

Table 4: Andor Basic Programming Language Hot Keys

| KEY STROKE(S) | DESCRIPTION |
|---|---|
| Ctrl + N | 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. |
| Ctrl + S | Save |
| Ctrl + P | Print |
| Ctrl + O | Open |

## SECTION 2 - PRE-ACQUISITION

## 2.1 - SETTING TEMPERATURE

For accurate readings, the EMCCD 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:
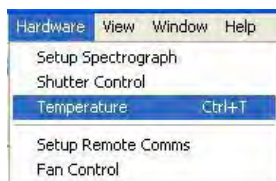


or click the [OFF] button in the bottom-left of the screen. This will open up the **Temperature** dialog box :
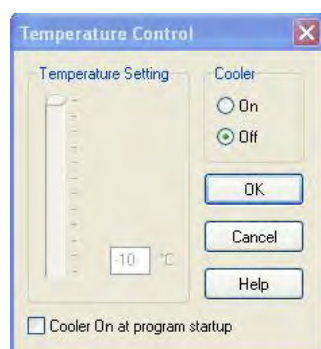


Select the **On** radio button in the **Cooler** area.

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.:
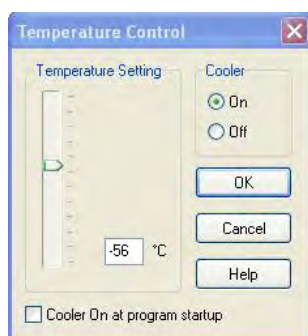


To adjust the temperature, either type in the new figure in the Degrees (C) box or move the slider bar down or up. Once the desired temperature has been selected, click OK. The dialog box will disappear and the Temperature Control button in the bottom-left of the screen will show the current temperature highlighted in red e.g.: [-45°C]

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 switched on as soon as you start the application. This is selectable in the bottom-left of the Temperature dialog box.

**PLEASE REFER TO THE iXon Ultra HARDWARE GUIDE FOR DETAILS OF MINIMUM ACHIEVABLE TEMPERATURES AND IMPORTANT ADVICE ON AVOIDING OVERHEATING.**

## 2.1.1 - 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 EMCCD).

**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.**

## 2.2 - SETUP ACQUISITION

To select the mode of acquisition prior to data capture, you can carry out one of the following actions:

- Click the 🔧 button

- Key in **Ctrl+A** from the keyboard

- Select **Setup Acquisition** from the **Acquisition** drop-down menu:



The Setup Acquisition dialog box appears, e.g.:
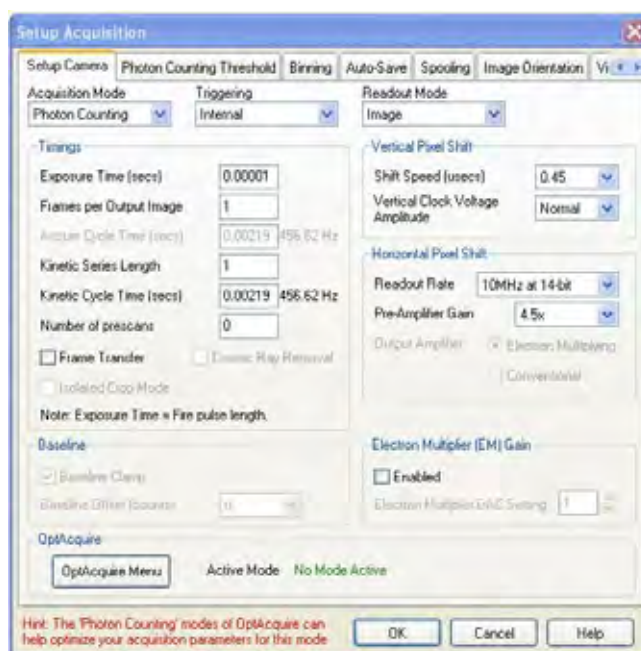


As you select an Acquisition Mode you will notice that you are able to enter additional exposure-related parameters in a column of text boxes. Appropriate text boxes become active as you select each Acquisition Mode. The value you enter in one text box may affect the value in another text box. The following 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 | ✓ | | ✓ | ✓ | ✓ |

## 2.2.1 Run time control

The Run time control provides the user with the ability to control the following parameters using slider controls:

- EM Gain range can be varied depending on the system
- Exposure time can be varied from the minimum exposure setting

The controls are activated by clicking the button on the Main Window. When selected, the Run time control appears, e.g.:
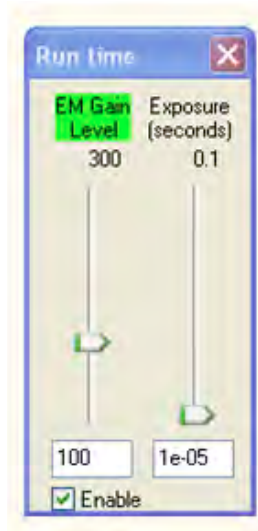
The gauge that is active has its name highlighted in green and the actual setting of each gauge is given in the text boxes below them.

**NOTE: The exposure time upper limit will auto-range as the setting is increased.**

## 2.3 - OptAcquire

OptAcquire is a unique control interface, whereby a user can conveniently choose from a pre-determined list of set-up configurations, each designed to optimize the camera for different experimental acquisition types, thus removing complexity from the extremely adaptable control architecture of the iXon Ultra. The control architecture of the iXon Ultra is extremely tuneable, meaning the camera can be adapted and optimized for a wide variety of quantitative experimental requirements, ranging from fast single photon counting through to slower scan, 16-bit dynamic range measurements. However, successfully optimizing EMCCD technology is not a trivial exercise, with various set-up parameters directly influencing different camera performance characteristics.

OptAcquire, has been designed as a unique interface, whereby a user can choose from a pre-determined list of nine camera set-up configurations. A variety of set-up parameters are balanced behind the scenes through the OptAquire menu. Furthermore, advanced users may wish to create their own additional OptAcquire.

When the [OptAcquire Menu] button on the Set-up Acquisition dialog is selected, the following dialog appears.



The user can then select between modes of operation which are described on the next 2 pages.

## 2.3.1 - OptAcquire pre-set modes



iXon Ultra control parameters include:

- **EM Gain** – This parameter has a direct bearing on both sensitivity and dynamic range

- **Vertical clock speed** – Flexibility in this parameter is critical to optimizing the camera for lowest noise, fastest speed, minimal frame transfer smear or maximum pixel well depth

- **Vertical Clock Amplitude** – Can be employed to help 'over-clock' the sensor to achieve faster frame rates and can also be used to reduce charge leakage into the image area when there is saturated signal in the frame transfer storage area (e.g. when combining very short exposure with a slow readout speed)

- **Horizontal readout speed** – Ranging between 10MHz and 1MHz. 10MHz for faster frame rates, 1MHz for best dynamic range

- **Pre-amplifier gain** – Trading off reduced digitization noise versus accessing full pixel well depth

- **EM / Conventional amplifier** – To choose between ultrasensitive EMCCD operation or traditional high dynamic range EMCCD operation, the latter recommended for relatively 'brighter' signals or when it is possible to apply long exposures to overcome read noise floor

- **Frame Transfer (overlap)** – Overlapped readout is used to achieve 100% duty cycle, ideal for fastest frame rate measurements without switching exposure time between frames. This mode should be deselected for time-lapse experiments

## 2.3.2 - OptAcquire create user-defined modes



Pre-defined OptAcquire modes include:

- **Sensitivity & Speed (EM Amplifier)** – Optimized for capturing weak signal at fast frame rates, with single photon sensitivity. Suited to the majority of EMCCD applications

- **Dynamic Range & Speed (EM Amplifier)** – Configured to deliver optimal dynamic range at fast frame rates. Moderate EM gain applied

- **Fastest Frame Rate (EM amplifier)** – For when it's all about speed! Optimized for absolute fastest frame rates of the camera. Especially effective when combined with sub-array/binning selections

- **Time Lapse (EM Amplifier)** – Configured to capture low light images with time intervals between exposures. Overlap ('frame transfer') readout is deactivated

- **Time Lapse & Short Exposures (EM Amplifier)** – Configured to minimize vertical smear when using exposure < 3ms

- **EMCCD Highest Dynamic Range (EM amplifier)** – Combines EMCCD low light detection with the absolute highest dynamic range that the camera can deliver. Since this requires slower readout, frame rate is sacrificed

- **EMCCD Highest Dynamic Range (Conventional Amplifier)** – Optimized for slow scan EMCCD detection with highest available dynamic range. Recommended for brighter signals OR when it is possible to apply long exposures to overcome noise floor

- **Photon Counting** – Configuration recommended for photon counting with individual exposures < 10sec

- **Photon Counting with Long Exposures** – Configuration recommended for photon counting with individual exposures > 10sec

## SECTION 3 - ACQUIRING DATA

## 3.1 - INITIAL ACQUISITION

To start an initial data acquisition you can either:

- Click the [image] button on the Main Window

- Press F5 on the keyboard

- Select the Take Signal option from the Acquisition drop-down menu, e.g.:
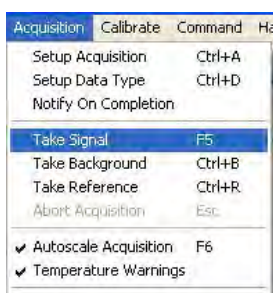


The Data Window opens (labeled #0 Acquisition) and displays the acquired data, according to the parameters selected on the Setup Acquisition Dialog box. e.g.:
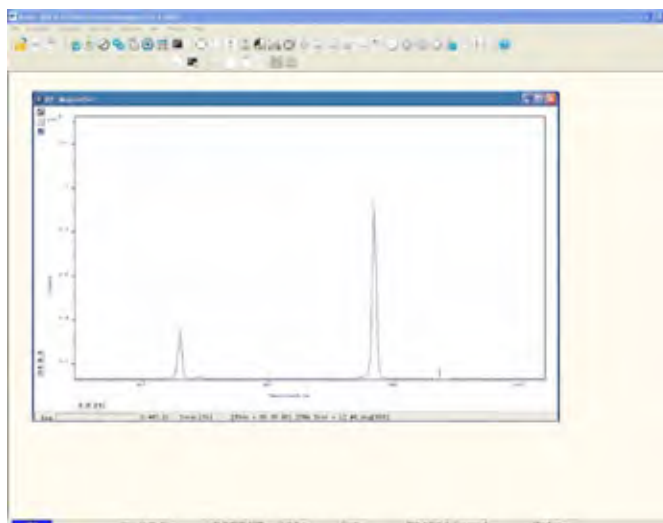


When you acquire data, by reading out a scan or a series of scans the data are stored together in a "Data Set", which exists in your computer's **R**andom **A**ccess **M**emory **(RAM)** or on its Hard Disk.

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

- Select Abort Acquisition from the Acquisition drop-down menu

- Click the [image] button

- Press the **<ESC>** key

## 3.2 - Data Type Selection

You can select the type of information that you want the system to compute and display whenever you perform the **Take Signal** function. When the **Setup Data Type** option of the **Acquisition** drop-down menu is selected, the **Data Type** dialog box opens, e.g.:
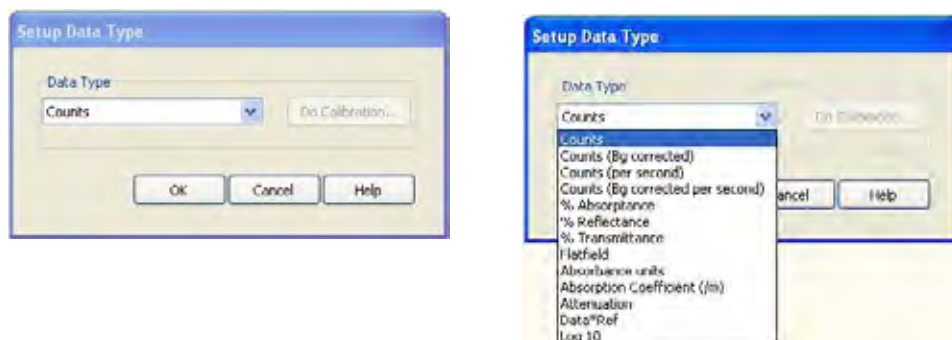


The descriptions of the data types are shown in **Table 5** which follows on the next page. The acquired data are presented under the Sig tab of an Acquired Data Window.

The data type selected will also 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 on **page 27**).

## 3.2.1 - Definitions of data types

- **Signal:** Data in uncorrected Counts

- **Background:** Data in uncorrected Counts, acquired in darkness

- **Reference:** Background corrected data. Reference data are normally acquired from the light source, without the light having been reflected from or having passed though the material being studied

| OPTION | FUNCTION |
|---|---|
| Counts | Counts represent raw, digitized data (i.e. no calculations have been performed on the data) from the EMCCD detector's analog to digital (A/D) converter. Please refer to the detailed performance sheet accompanying your particular EMCCD detector for the number of electrons that correspond to 1 count |
| Counts (Bg corrected) | Counts (Background Corrected) is digitized Data from the EMCCD detector's analog to digital (A/D) converter, where Background (or dark signal) has been removed.<br>Counts (Bg. Corrected) = Signal - Background |
| Counts (per second) | Counts ÷ Exposure Time |
| Count (Bg corrected per second) | Counts (Bg corrected) ÷ Exposure Time. |
| %Absorptance | Represents the light absorbed by an object.<br>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:<br>% Absorptance = 100 x (1 - (Signal - Background) / Reference) |
| %Reflectance | Represents the light reflected by an object.<br>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:<br>% Reflectance = 100 x (Signal - Background) / Reference |
| %Transmittance | Represents the light transmitted by an object.<br>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:<br>% Transmittance = 100 x (Signal - Background) / Reference |
| Flatfield | Flatfield is used to remove any pixel-to-pixel variations that are inherent in the EMCCD sensor. If Reference is the background corrected incident intensity, the Signal is divided by the Reference so:<br>Flatfield = M x Signal / Reference<br>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.<br>Absorbance Units are defined as $Log_{10}$ (1 / Transmission).<br>Therefore: Absorbance Units = $Log_{10}$ (Reference / (Signal - Background)) |
| Absorption Coefficient (/m) | Indicates the internal absorptance of a material per unit distance (m).<br>It is calculated as $-log_e t$, where t is the unit transmission of the material and $log_e$ is the natural logarithm.<br>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:<br>Transmission = (Signal - Background) / Reference<br>and:<br>Absorption Coefficient = $-log_e$ ((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.<br>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:<br>Attenuation = 10 x $log_{10}$ ((Signal - Background) / Reference) |
| Data*Ref | Allows you to 'custom modify' the background corrected signal:<br>Data x Ref = (Signal - Background) x Reference Store Value<br>See the Andor Basic Programming Manual for similar operations |
| Log 10 | Calculates the logarithm to the base 10 of the background corrected signal counts.<br>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.° |

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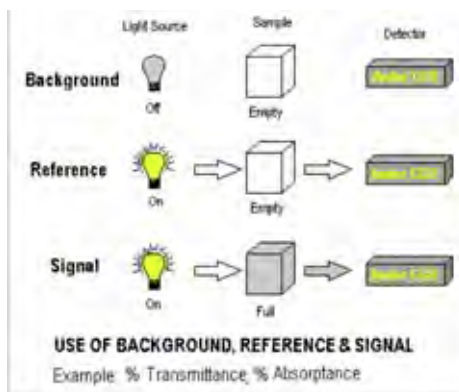
The default data type (used when you capture data and have not explicitly made a selection from the Data Type dialog box) is **Counts**.

If you select 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**: Uncorrected raw data acquired via Take Signal. "Signal", as used in the definitions of the calculations, refers to "raw" data from the EMCCD and should not be confused with the possibly "processed" data to be found under the Sig tab of the Data Window

- **Background:** Data in uncorrected Counts, acquired in darkness, via Take Background

- R**eference:** Background corrected data, acquired via Take Reference

## 3.3 - 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.

## 3.3.1 - Autoscale Acquisition

Prior to the Take Signal function being activated, Autoscale Acquisition can be selected from the Acquisition drop-down menu as shown (alternatively, 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)

- 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:
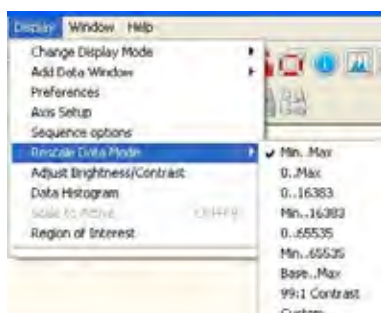


You can choose to display values between the following parameters:

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

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

- Zero & 16383 **(0..16383)**

- Minimum & 16383 **(Min..16383)**

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

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

- Custom setting as required

For further information on Rescale, please refer to **page 79.**.

## 3.3.2 - 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**

**NEW background data are required.**

## 3.3.3 - 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.**

## 3.3.4 - Acquisition errors

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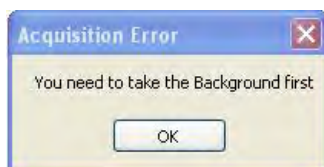


## 3.4 - ACQUISITION MODES & TIMINGS

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 a single readout of data from the EMCCD-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.

## 3.4.1 - Single scan

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



**NOTE: Should you attempt to enter too low a value, the system will default to a minimum exposure time.**

## 3.4.2 - Video

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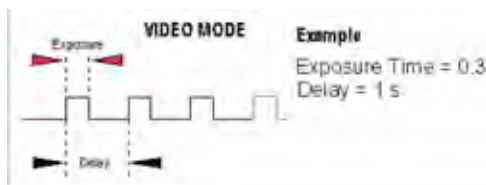


**NOTE: This is a useful mode for focusing your iXon Ultra and for watching experimental events happening in real time. However, this mode will not allow you to save any of the acquired images or data, except for the last frame of the sequence.**

## 3.4.3- 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.:
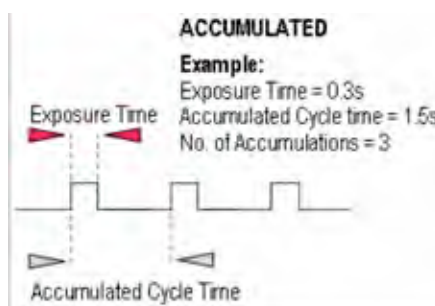


You can select the following parameters in the Setup Acquisition dialog box:

- Exposure Time
- Accumulated Cycle Time: the period in seconds between each scan. This parameter is only available if you have selected Internal triggering (please refer to Triggering Modes on **page 35**).
- No. of Accumulations: the number of scans you want to add together

**NOTE: This mode is used to improve the Signal to Noise ratio.**

## 3.4.4 - Kinetic Series

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

- Exposure Time

- Kinetic Cycle Time: the time between the start and finish of each kinetic scan

- Number in Kinetic Series: the number of scans taken in the kinetic series
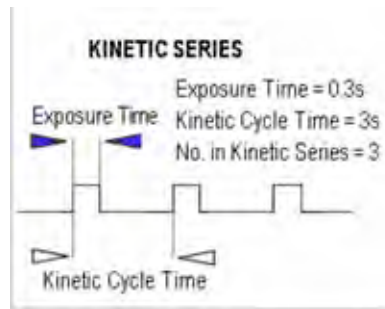


**NOTE: This mode is particularly well suited to recording the temporal evolution of a process.**

## 3.4.4.1 - 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.

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

## 3.5 - COUNT CONVERT

One of the distinctive features of the iXon Ultra is the capability to quantitatively capture and present data in units of electrons or photons, the conversion applied either in real time or as a post-conversion step. Photons that are incident on pixels of an array detector are captured and converted to electrons. During a given exposure time, the signal in electrons that is collected in each pixel is proportional to the signal intensity. In EMCCDs, the signal in electrons is further multiplied in the EM gain register. The average multiplication factor is selected in software from the RealGain™scale. It can be desirable to directly quantify signal intensity, either in terms of electrons per pixel or in terms of incident photons per pixel. However, during the readout process, array detectors must first convert the signal in electrons (the multiplied signal in the case of EMCCDs), into a voltage which is then digitized by an Analogue to Digital Converter (ADC). Each Analogue to Digital Unit (ADU) is presented as a 'count' in the signal intensity scale, with each count corresponding to an exact number of electrons. Furthermore, the signal value in counts will sit on top of an electronic bias offset value. In the iXon Ultra this 'baseline' can be clamped at 100 counts.

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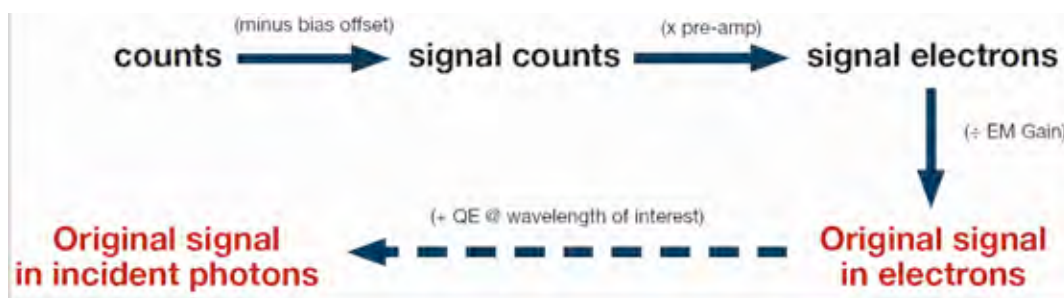


Figure 1:
Count Convert calculation path

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

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

## 3.5.1 - Count Convert dialogs

When the  button is clicked, the **Count Convert** dialog box appears, e.g.
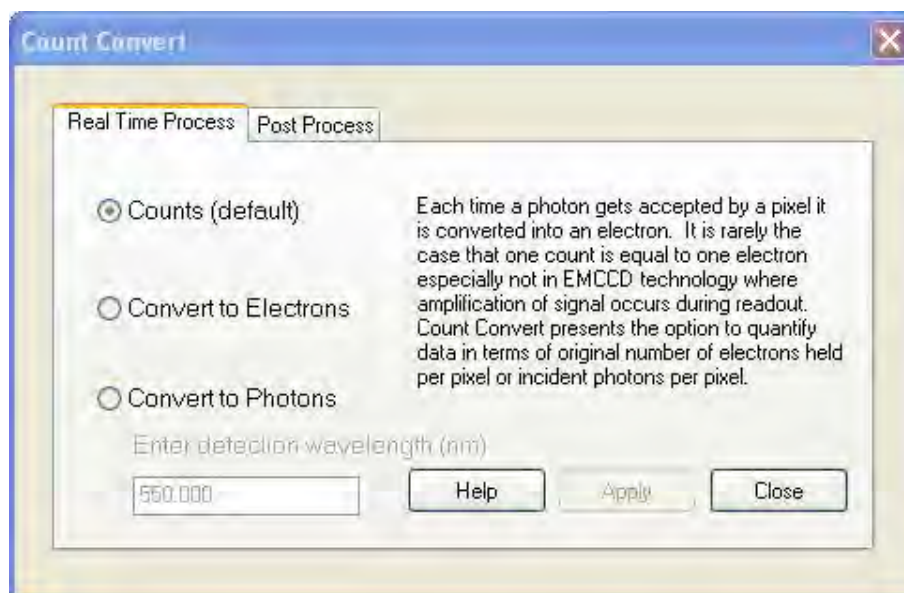


Count Convert dialogs allow the user to select between the following parameters:

- Data (in counts)
- Data in electrons
- Data in photons

When displaying data in photons, the user must input the detection wavelength in nm, e.g.:
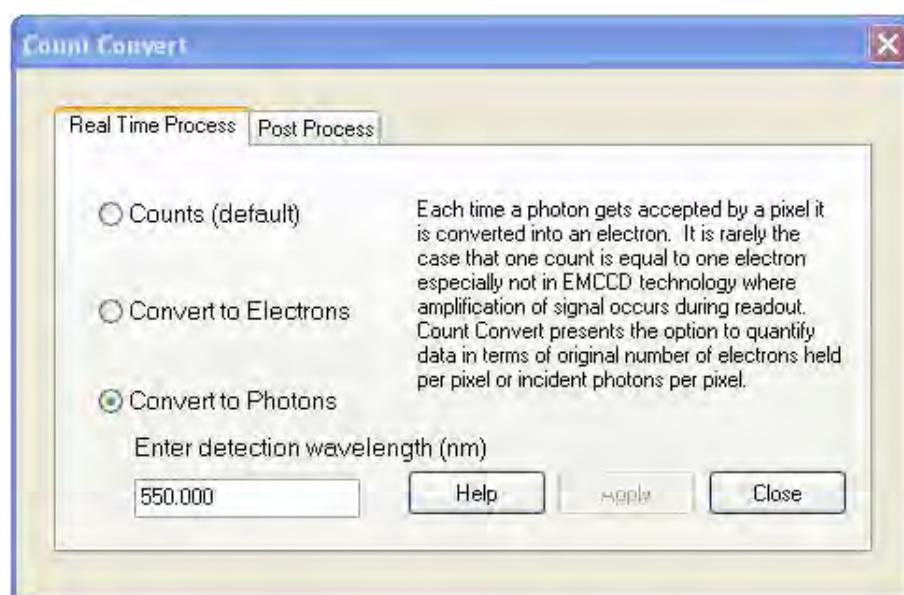


**NOTE: The dialog shown above can remain on-screen at all times and users can select between the counts / convert options during real time.**

Count Convert can also be applied to data as a post-processing step and the options are as shown below:
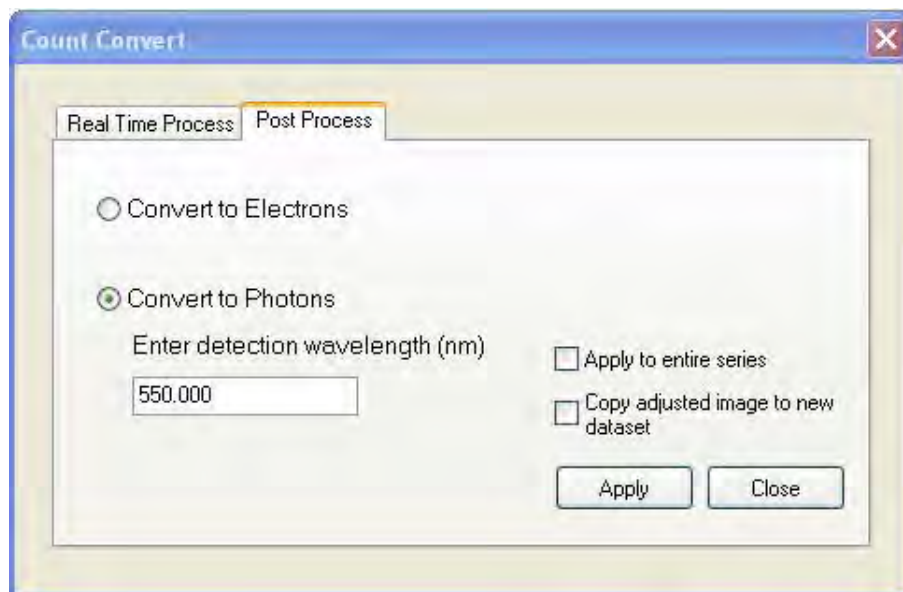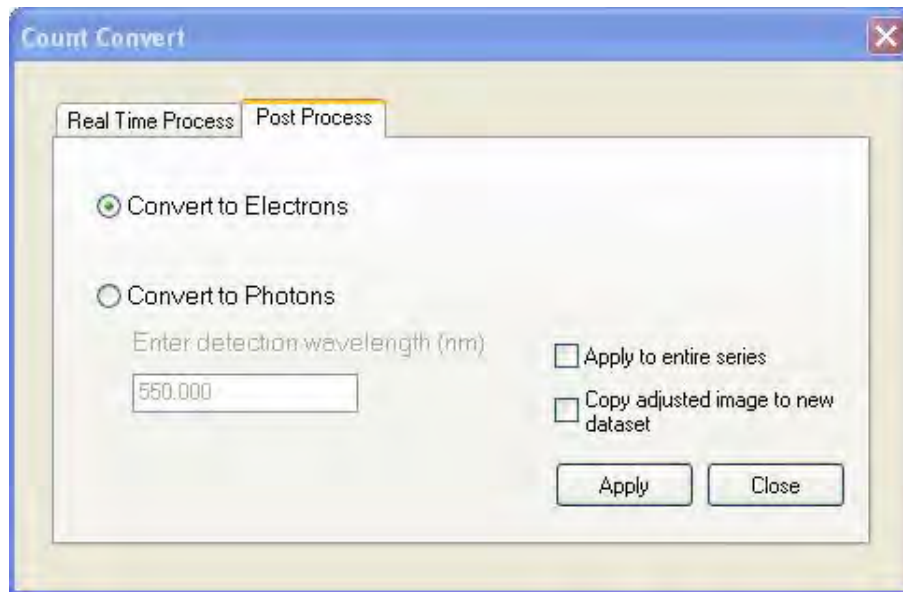
## 3.6 - SPURIOUS NOISE FILTER

It can be desirable to optionally filter spurious EM-amplified background events to give as 'black' a background as possible, eradicating any remaining 'salt and pepper' noise. It is important to utilize noise selection and filter algorithms that are intelligent enough to accomplish this task without impacting the integrity of the signal itself. This is realized through the new Spurious Noise Filter (SNF) functionality of iXon Ultra, which offers the user a choice of advanced algorithms to try. SNF can be applied either in real time or as a post-processing step. The options available to the user for using Spurious Noise filter are as follows:

- Median (available in Real Time & Post-processing)

- Level Above (available in Real Time & Post-processing)

- Noise Threshold (available in Post-processing)

- Interquartile Range (available in Post-processing)

These can be selected from the **Real Time** or **Post Process** options, e.g.
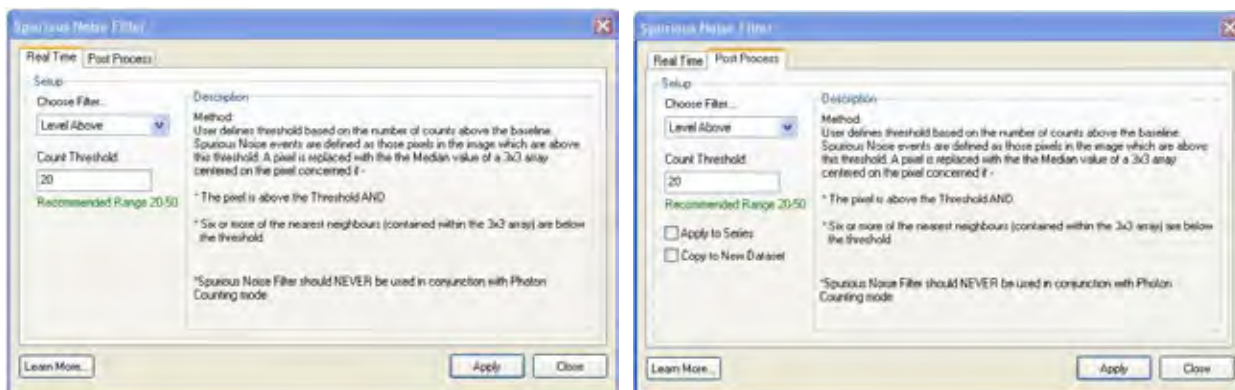


NOTE: Andor spurious noise filters' options make use of advanced algorithms that offer excellent discrimination of spurious noise events with minimal effect on signal integrity. As such, some 'slow down' of display rates may be noticed on some lower specification PC's when using these filters in real time. In such circumstances, post-process application is recommended.

## 3.7 - CROPPED SENSOR MODE

If an experiment demands fast temporal resolution, but cannot be constrained by the maximum storage size of the sensor (as is the case for 'Fast Kinetics Mode' of readout), then it is possible to readout the iXon Ultra in 'Cropped Sensor Mode', e.g.:
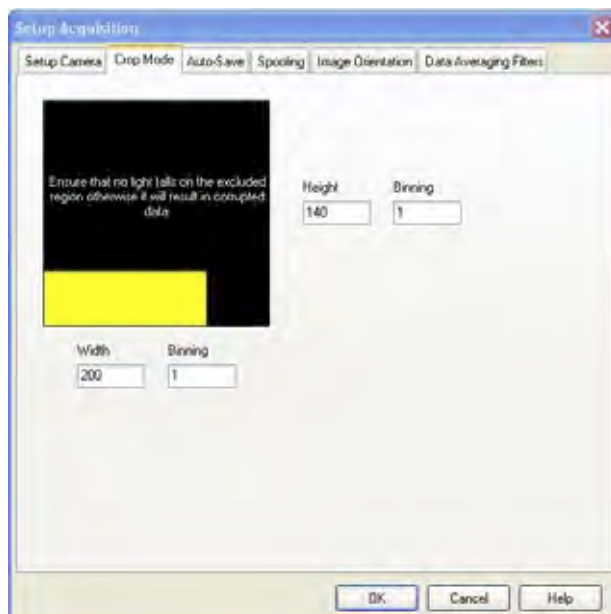


In this mode, the user defines a 'sub-array' size from within the full image sensor area, such that it encompasses the region of the image where change is rapidly occurring (e.g. a 'calcium spark' within a cell). The sensor subsequently "imagines" that it is of this smaller defined array size, achieved through software executing special readout patterns, and reads out at a proportionally faster frame rate. The smaller the defined array size, the faster the frame rate achievable. The cropped area must be positioned in the bottom-left of the sensor, thus the subject of study should be first positioned in this area rather than centrally located.

# 3.8 - TRIGGERING MODES

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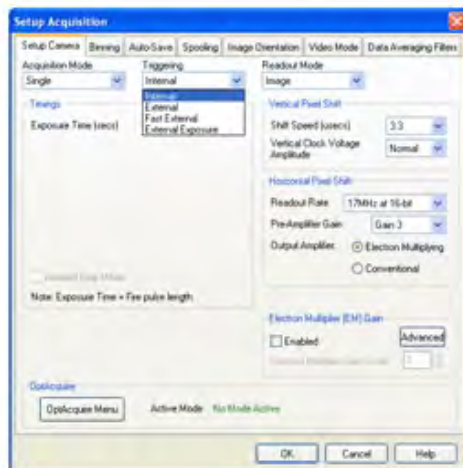


Depending on the system and other acquisition parameters selected, the modes you can select are as follows:

- Internal
- External
- Fast External
- External Start (Accumulate & Kinetic Series modes only)
- External Exposure (only available on the 885 model)

For further information on Triggering, please refer to the Hardware Manual supplied with your iXon Ultra

# 3.9 - READOUT MODES

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

- Image (including sub-array and binning)
- Multi-Track
- Full Vertical Binning (FVB).

## 3.9.1 - Image mode

When Image mode is selected and the Binning tab is clicked, a window similar to the following opens:
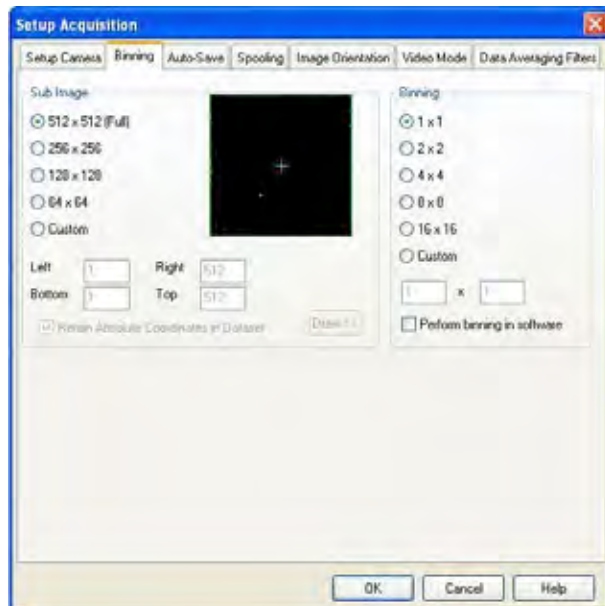


The user can then set various Binning Patterns and define Sub Images of the EMCCD.

By default, taking an Acquisition supplies you with a count from each pixel on the EMCCD, 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 and it can be viewed in grayscale or false color ,e.g.:
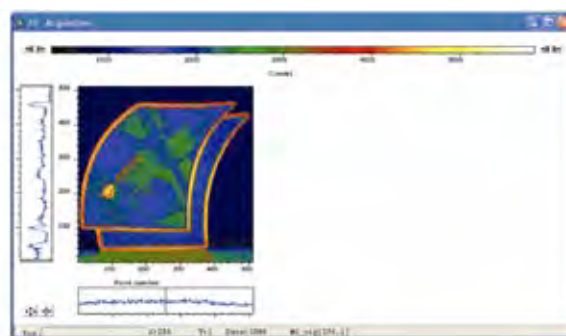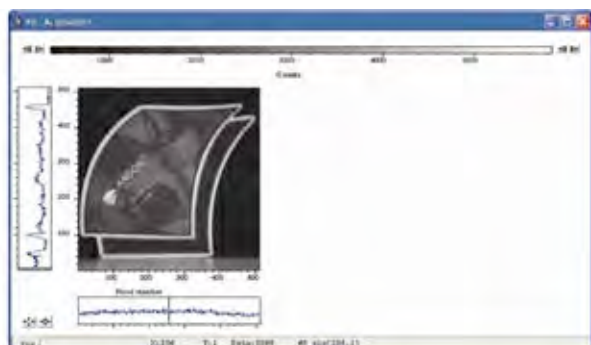
## 3.9.1.1 - Sub Image

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. When the camera is running in Sub Image mode, only data from the selected pixels will be read out, data from the remaining pixels will be discarded. To read out data from a selected area (or Sub Image) of the EMCCD, use the radio buttons to select the resolution which you require, e.g.:
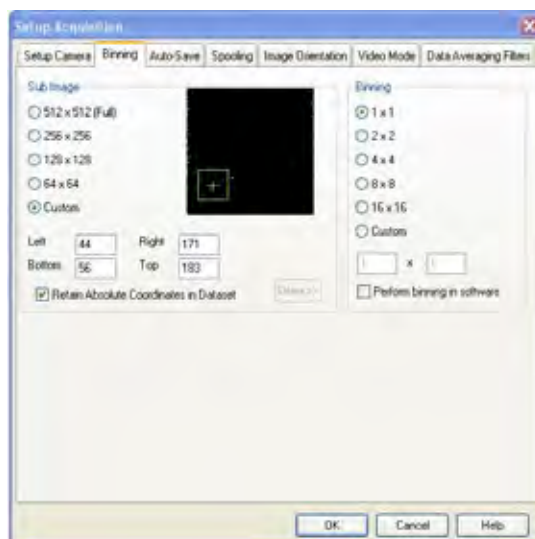


Depending on the type of camera being used, the software offers a choice of defined sub images, e.g.:

- 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 EMCCD chip. To define a Custom Sub Image tick the Custom radio button then use the co-ordinate entry dialogue boxes to select the size and location of your sub image.

## 3.9.1.1.1 - Draw

In addition to the previous methods of defining a Sub Image on the EMCCD, 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.

## 3.9.1.1.2 - Binning

As well as selecting a Sub Image of the EMCCD, 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 EMCCD, or your selected sub-area, then becomes a matrix of superpixels. For further information on Binning, please refer to the Hardware manual.

## 3.9.2 - Video mode

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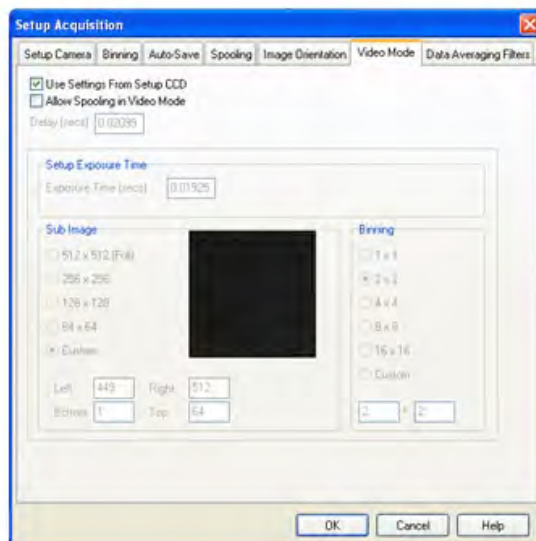


The following parameters can then be changed:

- **Exposure Time**

- **Delay:** The interval required between scans. NOTE: If you attempt to enter too low a value, the system will default to a minimum delay

- Res**olution (Sub-image area):** see previous page

- **Binning pattern:** Please refer to the Hardware manual supplied with your camera

- **Moving Calculation:** when a figure >1 is selected, the displayed signal is either a running average or summation of multiple scans. This improves the look of the displayed image by effectively boosting the Signal to Noise ratio

**NOTE: When the Use Settings From Standard Setup option is selected, these parameters cannot be altered.**

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 carry out one of the following actions:

- 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.

### 3.9.3 - Image orientation

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

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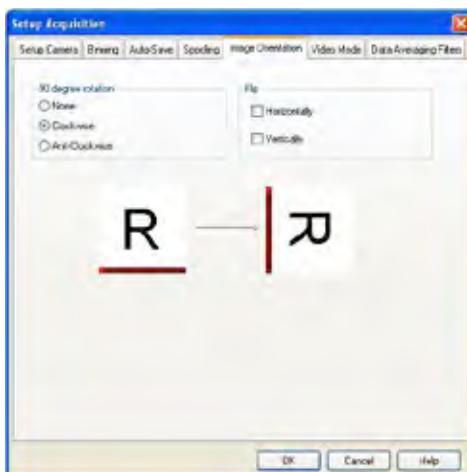


### 3.9.4 - Vertical Pixel Shift

### 3.9.4.1 - Shift Speed

Shift Speed (μsecs) specifies the time taken to shift charge from one row on the EMCCD sensor to the next. Speeds which appear un-bracketed in the drop-down list are guaranteed to meet all the system specifications. In some instances, using a slightly slower vertical shift speed may result in a slight increase in the single well capacity for imaging applications. However it may also increase the Clock Induced Charge (CIC) of the system and will reduce the maximum frame/spectral rates achievable.

The iXon Ultra has the capability to "over-clock" the vertical shift transfer speed. To this end the use of the faster (bracketed) shift speeds can have the benefits of (a.) reduced CIC and (b.) faster frame rates (particularly in combination with sub-array and binning). Note that using the bracketed shift speeds may result in reduced single pixel well depth.
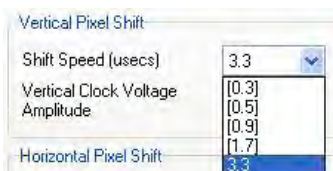
## 3.9.4.2 - Vertical Clock Amplitude Voltage

The Vertical Clock Voltage Amplitude can be used to increase the amplitude of the clock pulses used to perform row shifts on the EMCCD. The Normal setting is the default amplitude which has been set at the factory during the optimization and testing of the camera. The other settings (if available) specify the voltage increase to be applied to this clock amplitude. In some imaging applications, increasing this voltage can provide a slightly higher single pixel well depth and improve charge transfer efficiency, at the expense of slightly higher CIC.

Application of higher voltage may be required in combination with the fastest of the bracketed vertical pixel shift speeds in order to overcome image distortion effects that result from reduced charge transfer efficiency. Thus, higher voltage settings in combination with the fastest vertical shift speeds enables the frame rate to be pushed to the maximum, especially apparent when using sub-arrays and binning. Best practice is to select the fastest vertical shift speed, then step the vertical clock voltage 1 unit at a time until distortive effects disappear from the image.
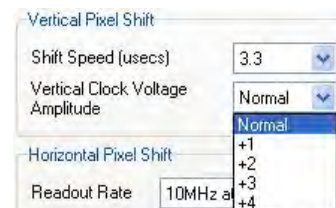
## 3.9.5 - Horizontal Pixel Shift

## 3.9.5.1 - Readout Rate

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 available rates are system dependant and can be selected from a drop-down list on the Setup Acquisition dialog box, of which an example is shown below:
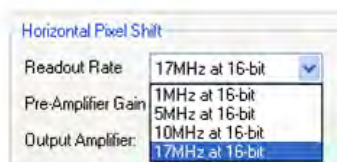
## 3.9.5.2 - Pre-Amplifier Gain

Pre-Amplifier gain determines the amount of gain applied to the video signal emerging from the EMCCD and allows the user to control the sensitivity of the camera system. Depending on the system there are up to three options available. They are again selected from a drop-down menu, e.g.:
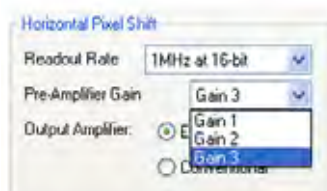
These normalized gain settings will correspond to system sensitivities specified on the performance sheets (in terms of electrons per A/D count) which accompany the system.

Selecting higher pre-amplifier gain values (i.e. x2 or x4) will increase the sensitivity of the camera (i.e. fewer electrons will be required to produce one A/D count) and provide the lowest readout noise performance. However this may result in the A/D converter saturating before the single pixel / register capacity of the EMCCD sensor is reached.

### 3.9.5.3 - Output Amplifier Selection

For the 888 & 897 models, the output amplifier radio button allows the user to select which of the on-chip amplifiers (Electron Multiplying: EM) or Conventional (non-EM) the data will be outputted, e.g.:

The various options available (if any) will vary depending on the EMCCD sensor type incorporated in the camera system.

### 3.9.5.4 - EM Gain Amplifier

If the Electron Multiplying (EM) Gain amplifier has been selected, the user can set the amount of electron multiplying gain required. This gain level is a "Real Gain" level as opposed to an arbitrary value. The maximum value is set to 300, unless the Advanced button is clicked, and the following message appears:

Using gains greater than 300 is not generally recommended for most applications as this will reduce the lifespan of the EMCCD. For further information on the correct use of EM Gain, please refer to the Hardware Manual.

## 3.9.6 - Multi-track mode

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 EMCCD-chip, the pattern of tracks from which you will read out charge.

In this way you can adjust the position of the tracks to match a light pattern produced on the EMCCD-chip by, for example, a fiber bundle. To define multiple tracks on the EMCCD-chip, select **Multi-track** from the **Readout Mode** drop-down menu in the **Setup Acquisition** dialog box:



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



There are two modes of operation, i.e. **Standard** or **Custom**.

- In **Standard** mode, the user defines the **Number of tracks**, the **Height** & **Offset**. The software automatically calculates the position of the tracks by distributing the evenly across the sensor.

- In **Custom** mode, the user has the ability to position the tracks as required, e.g.:
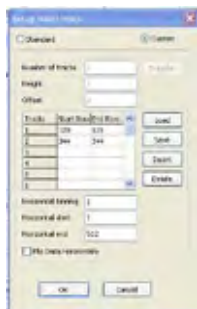


The user can also save the position of the individual tracks to a file that can be reloaded later. The **Load** and **Save** buttons are used to achieve this. The user can also utilize the **Insert** and **Delete** buttons to define or remove tracks. If the Advanced button is clicked, the **Advanced Multi-track** options dialog box appears:



This allows the user to define the Horizontal binning and position of all the tracks. The **Transfer** button can be used to quickly load the automatically calculated tracks from the Standard mode setup into Custom mode.

## 3.9.7 - Full Vertical Binning

**FVB** allows you to use the EMCCD-chip as a **L**inear **I**mage **S**ensor (or **LIS** - a photodiode array), typically for spectroscopy applications. The charge from each column of pixels (each column being the chip height) is combined, or binned, on the chip to give a single value per column. To define the binning in **FVB** mode, click on the **FVB** tab in the Setup Acquisition dialog box:
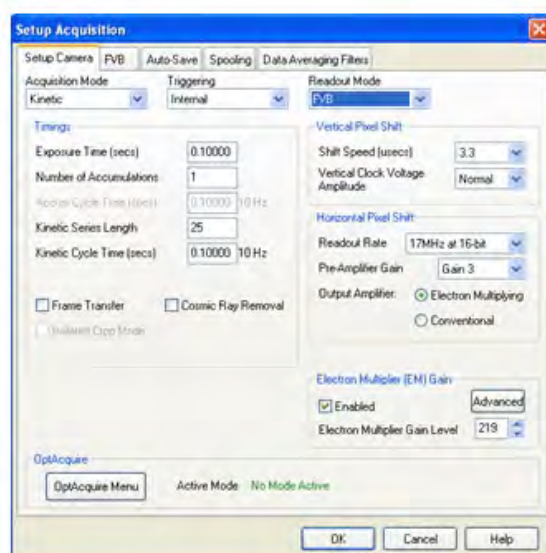


For some spectrographs, it may be necessary to change the direction in which the data is displayed on screen. click on the **FVB** tab in the Setup Acquisition dialog box and the following dialog box appears:



Simply click the **Horizontally** tick box in the **Flip** section as shown below:



An example of flipped spectra is shown on the next page.
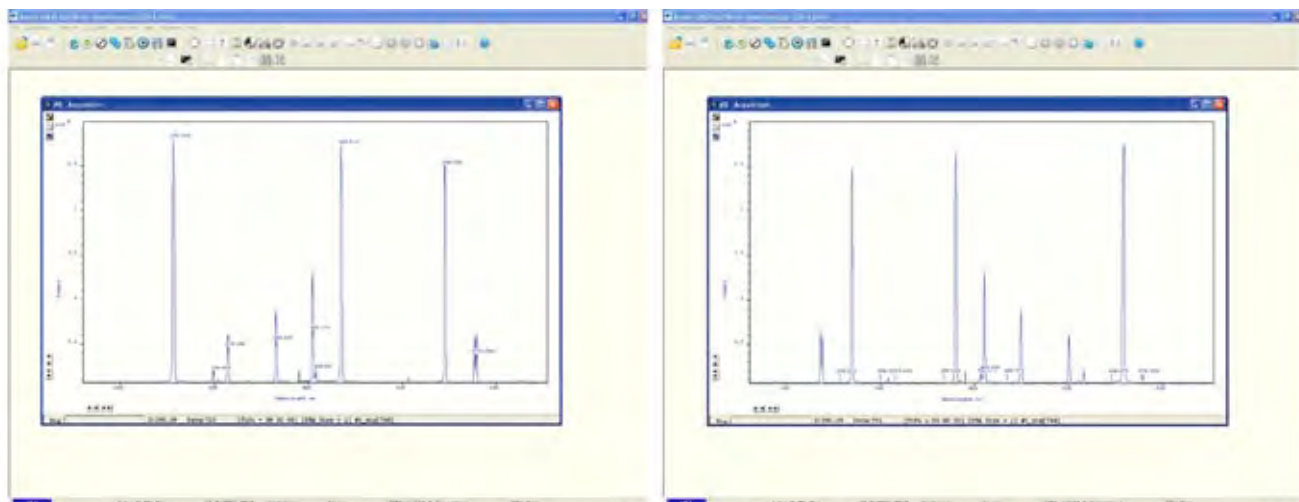
In some instances spectral rate can be more important than spatial/spectral resolution. For such applications horizontal binning can be applied by setting the number in the Horizontal binning section to a value greater than one.

For example, using the iXon Ultra 888 model in FVB with horizontal binning = 1 will produce a spectrum 1024 pixels wide, with each pixel containing the summation of the charge in one column. Changing the horizontal binning to 4 will produce a spectrum 256 pixels wide, however now each pixel will contain the summation of the charge in four columns.

## 3.10 - TIMING PARAMETERS

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

- Exposure Time (secs)

- No. of Accumulations

- Accumulation Cycle Time (secs)

- Cosmic Ray Removal

- Kinetic Series Length

- Kinetic Cycle Time (secs)

## 3.11 - PHOTON COUNTING

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

The industry-leading Darkcurrent and Clock induced charge (CIC) specification of the Andor's back-illuminated iXon Ultra 897 & 888 models render them uniquely suited to imaging by Photon Counting.

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

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

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

To successfully photon count with EMCCDs, there has to be a significantly higher probability of seeing a 'photon spike' than seeing a darkcurrent/CIC 'noise spike'. The lower the contribution of this 'spurious' noise source to a single exposure within the accumulated series, the lower the detection limit of photon counting and the cleaner the overall image will be, as demonstrated in **Figure 2** on the next page.

The iXon Ultra has the most effective combined cooling/CIC minimization on the market, lower than other competing EMCCDs utilizing the same 512 x 512 sensor. As such, the detection limit for photon counting is markedly lower. The iXon Ultra intuitively offers photon counting modes, either as a real time acquisition or as a post-processing step.
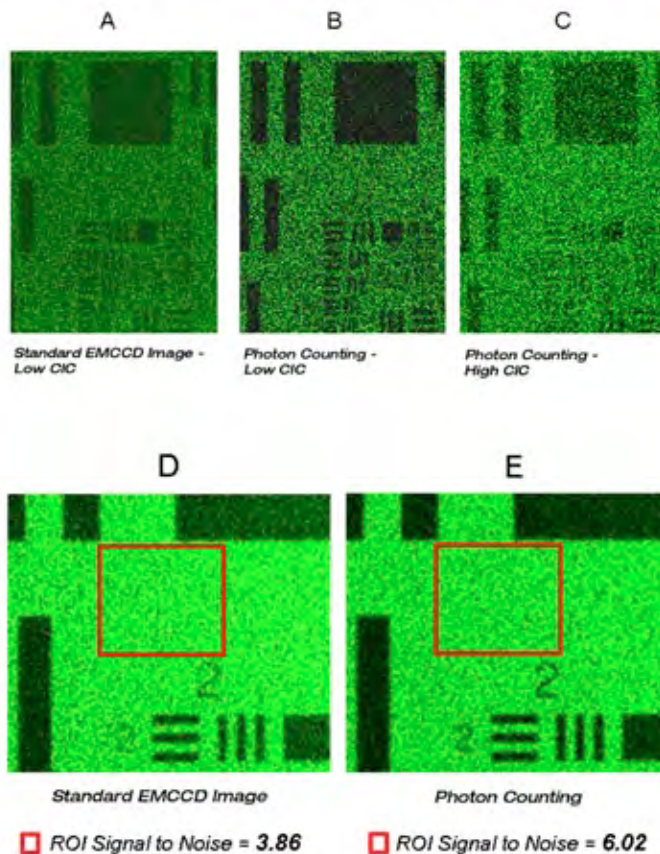


Figure 2: 'Photon Counting' vs. 'Standard EM-on' Imaging for very weak signals. Images A, B and C were recorded under identical illumination conditions, identical exposure times and each with EM Gain set at x1000. The benefits of photon counting under conditions of low clock induced charge (CIC) are evident. Images D and E are derived from a larger number of accumulated images, to yield a greater measurable signal to noise ratio. An identically positioned Region of Interest on each image was used to determine S/N of 3.86 and 6.02 for standard and photon counted images respectively. This factor improvement is in accord with the theory of Photon Counting circumventing the influence of multiplicative noise (noise factor) in EMCCD signals.

**NOTE: OptAcquire can be used to first optimize the camera for photon counting acquisition.**

## 3.11.1 - Photon Counting in real time

As the EMCCD is continually scanned, the signal builds up in memory and can be viewed live on the screen.

In order to use Photon Counting, select **Photon Counting** from the **Acquisition Mode** drop-down menu of the dialog box, e.g.:



Tick the **Frame Transfer** box, then input the **Exposure Time** per frame, number of **Frames per Output Image** and the **Kinetic Series Length** required. Click on the **Photon Counting Threshold** and the following dialog box appears:



Input an appropriate value for **Min.Threshold**; any spikes appearing above this figure will be counted as a single event. Click **OK** and the acquisition can then be started as normal.

## 3.11.2 - Photon Counting by post-processing

The **Post Process Photon Counting** dialog box is opened by clicking the ⬚ button.
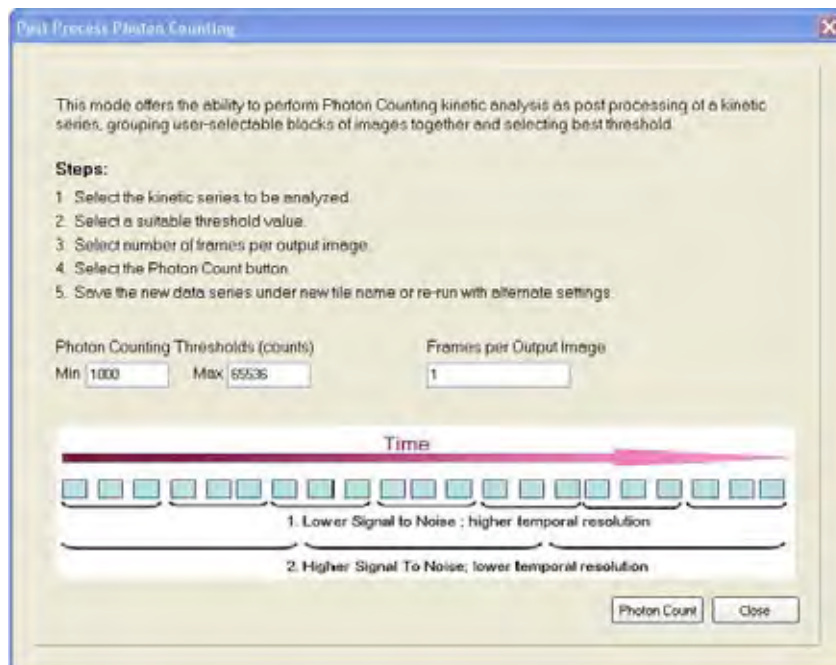


As a post-processing analysis, the user holds the flexibility to 'trial and error' photon count a pre-recorded kinetic series, trading-off temporal resolution vs SNR by choosing how many images should contribute to each photon counted accumulated image.

For example, a series of 1000 images could be broken down into groups of 20 photon counted images, yielding 50 time points. If it transpires that better SNR is required, the original dataset could be re-treated using groups of 50 photon counted images, yielding 20 time points.
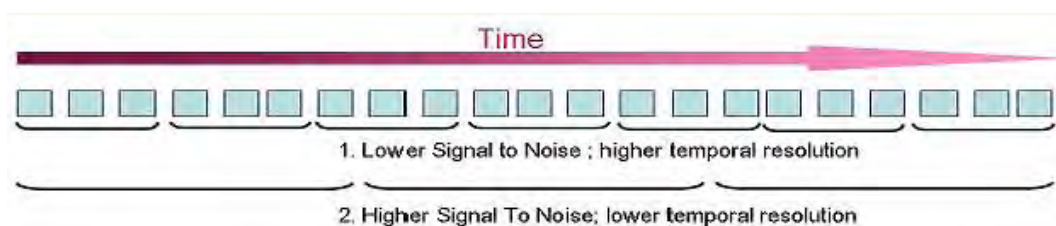


Figure 3: Schematic illustration of how photon counting can be applied to a kinetic series as a post processing step, affording increased flexibility in 'trail and error' trading SNR vs temporal resolution.

## 3.12 - 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 may be required to avoid unnecessary signals/light falling on the EMCCD during the readout process; otherwise the image will be smeared.

When the Shutter Control option is selected from the Hardware drop-down-menu, or the [image] button is clicked, the Shutter Control dialog box opens, e.g.:
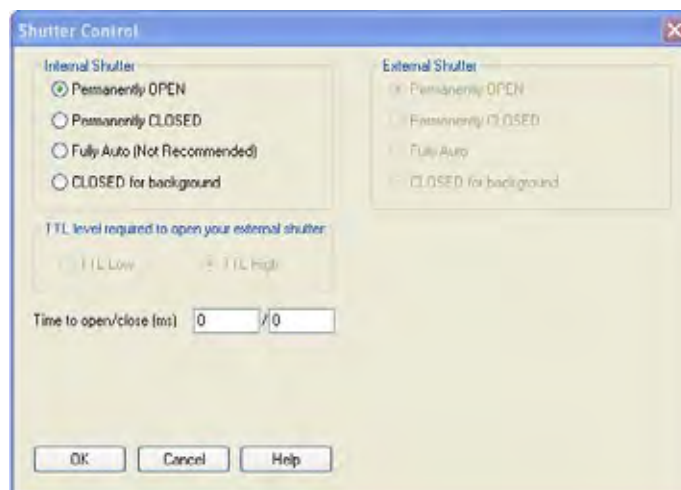


You can use this to indicate when and how a hardware shutter should be used. With a EMCCD, the shutter is used for background shuttering. Certain settings (e.g. Permanently OPEN & Permanently CLOSED) take effect as soon as you close the Shutter Control dialog box. Other settings will be applied whenever you acquire data.

**NOTE: The iXon Ultra shutter is a slow background type and the repetition rate is only a few Hertz. Thus for rapid Kinetic Series measurements it is recommended the shutter be left Permanently OPEN.**

- Fully Auto is the simplest shutter mode, as it leaves all shuttering decisions to the system. When you perform a Take Signal operation, the shutter opens for the duration of the Exposure Time you have entered in the Setup Acquisition dialog box. NOTE: This option is not recommended for the iXon Ultra as it is a Frame Transfer device and utilizes a slow shutter.

- 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. NOTE: This is the preferred option for the iXon Ultra. However if the camera is to be left unattended for long periods, it is recommended that the shutter is closed.

- 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. NOTE: If the camera has an internal shutter, you must select TTL High. If the camera does not have an internal shutter, you can select either mode. 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 Ultra contains a Frame Transfer EMCCD 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 the 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.**

## 3.12.1 - Shutter Transfer Time

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 an EMCCD 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:
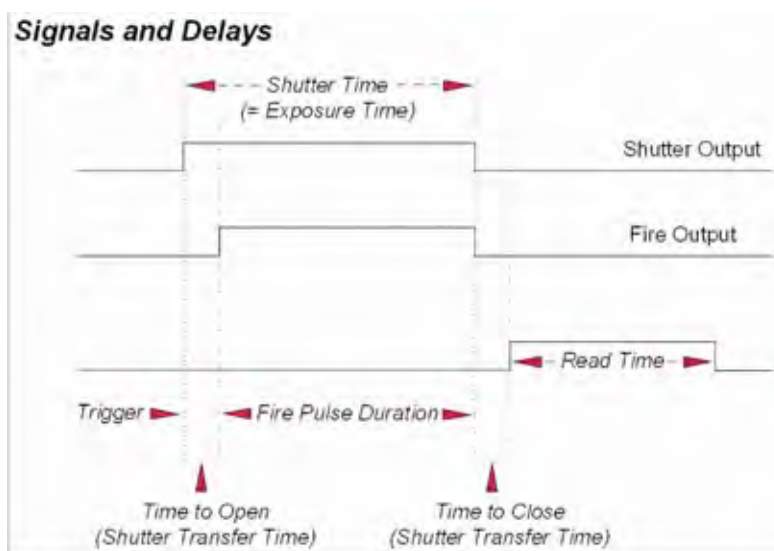


If you do not have a shutter connected, set the Time to open or close to 0. Setting the Time to open or close to any other value will insert extra delays into cycle time calculations.

## 3.12.2 - Accumulate Cycle Time & No. of Accumulations

If you have selected Accumulate as the acquisition mode you can select the Accumulation Cycle Time and **No. of Accumulations**.

- 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

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

## 3.12.3 - Kinetic Series & Kinetic Cycle Lengths

When Kinetic is selected as the acquisition mode, you can 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

To improve the Signal to Noise ratio, the number of accumulations per final image in the series can also be set.

## 3.12.4 - 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 EMCCD. The non-illuminated part of the EMCCD is used for storage of image before readout. You must ensure that light does not fall on this storage part of the EMCCD by using, for example, an imaging spectrograph or the OptoMask (see Andor website for details). 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 non-illuminated area, thus sampling the image in time. In this way the image is temporarily stored on the sensor itself, rather than in the computer. The process is repeated until the frame of the EMCCD 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 an EMCCD-chip of height 512 pixels and a sub-area height of 8 rows, 128 discrete images can be stored on the EMCCD. From the Setup Acquisition dialog box, you can change the following parameters:

- • **Exposure Time:** the Exposure Time also represents the cycle time of the Fast Kinetics series. There is no separate parameter for a Fast Kinetics cycle time

- • **Sub Area Height** in rows

- • **Number in Series:** the number of time-sampled images you want to acquire

- • **Binning** of the images

- • **Offset** of the active area from the bottom of the detector can also be specified

## 3.12.4.1 - Readout Mode & Fast Kinetics

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. **NOTE: 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 (as specified by the user) are vertically shifted. The system then waits for the next trigger to start the next scan. As there is no keep clean cycle running while waiting for the external trigger the "real" exposure time is the time between each trigger. A consequence of this is that if your experiment has a constant background signal but your trigger period is not fixed, you may see different background levels in your signal

- In External Start Trigger mode, data will not be acquired until the system receives an initial external trigger signal from an external device, e.g. 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.

## 3.13 - FILE INFORMATION

Details of the acquisition selection can be viewed by clicking the 🛈 button on the Main Window which opens the Information screen (you can enter your own notes in the Comments box):



The table below details the type of information contained in the 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 |
| **Software Version** | The version of Solis software in use |
| **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, Kinetic, etc. |
| **Trigger Mode** | Internal, External, Fast External, etc. |
| **Exposure Time (secs)** | "Fire" pulse length |
| **Readout Mode** | Image, FVB, etc. |
| **Horizontal Binning** | Minimum = 1, Maximum = System dependant |
| **Vertical Binning** | Minimum = 1, Maximum = System dependant |
| **Horizontally flipped** | True or False |
| **Vertically flipped** | True or False |
| **Clockwise rotation** | True or False |
| **Anti-clockwise rotation** | True or False |
| **Baseline Clamp** | OFF or ON |
| **EM Gain Level** | As per factor set in Electron Multiplier (EM) Gain area |
| **Baseline offset** | As set in the Acquisition dialog |
| **Vertical Shift Speed (μsecs)** | System dependant |
| **Clock Amplitude** | Normal, +1, +2, +3 or +4 |
| **Output Amplifier** | Electron Multiplying or Conventional |
| **Pre-Amplifier Gain** | As per Multiplication factor set in Horizontal Pixel Shift area |
| **Serial Number** | Serial number of the camera in use |
| **Pixel Readout Rate (MHz)** | Value in MHz |

## 3.14 - SPOOLING

The Andor Solis software has an extensive range of options that allow 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 the Enable Spooling box. You must also enter a stem name, and also select a location for this spooled data file, e.g.:
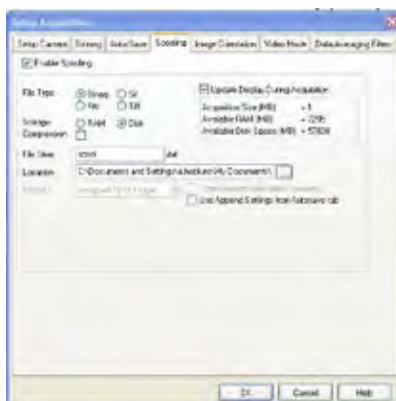


NOTE: Spooling large amounts of data straight to hard disk for later retrieval requires a hard disk of sufficient read-write speed. Andor recommends only very high-speed hard disk drives be used for this type of operation and these need to be dedicated for Spooling.

## 3.14.1 - Virtual Memory

In addition to the Spooling function, it can also be useful to have the Virtual Memory (VM) function enabled. This will speed up the retrieval of large data sets and allow larger data sets to be acquired. This works by buffering data in the Hard Drive of the PC. To switch on, select the **Virtual Memory**… option from the File menu, e.g.:
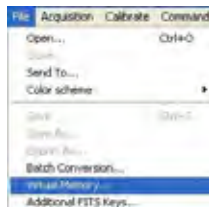


This will open the **Virtual Memory** dialog box,e.g.:



Tick the **Enable** box and select the required **Threshold**. The data is normally saved to the default directory shown in the **Location** field. Alternatively, you can click on the ⬚ button and choose a different area to save the data. Click **OK** and the data will be saved during acquisition.

**NOTE: It is recommended to have the option activated for images > 50Mb.**

## 3.15 - 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.:
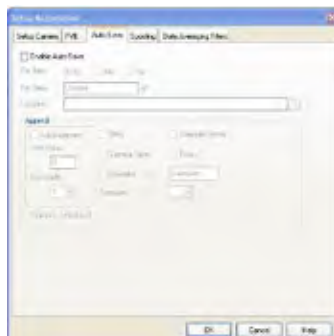


Tick the **Enable Auto-Save** box. If selected, acquisitions will be saved automatically when each one is completed. Each subsequent auto-saved file will over-write the previously auto-saved one.

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, e.g.:

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

Any combination of these may be selected by activating the relevant tick box, e.g.:
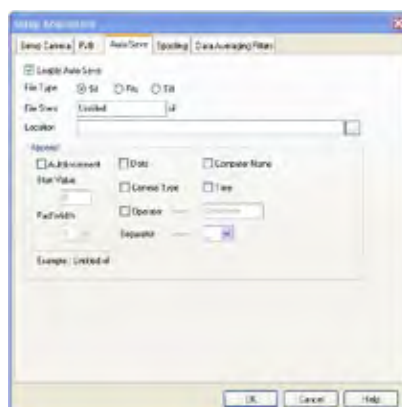


**NOTE: This function will only Auto-save Single Scan, Kinetic Series or Accumulated images.**

## SECTION 4 - DISPLAYING DATA

## 4.1 - 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 follows:

- 2D
- 3D
- Image

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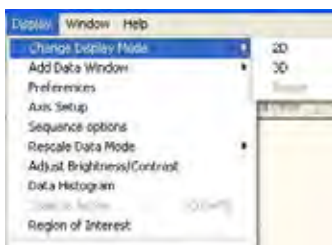


Some of the options are also available via icons on the Main Window and these are shown later in this section.

**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 on pages 65-69.**
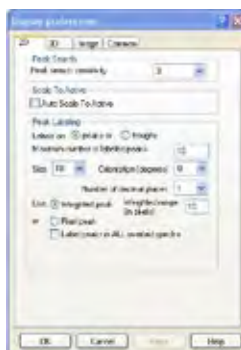
## 4.2 - DISPLAY PREFERENCES

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:
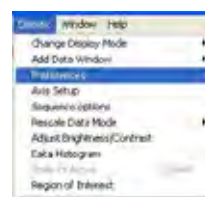


The **Display Preferences** dialog box appears, e.g.:



By clicking on the appropriate tab, you can select or deselect certain features associated with the data window for the mode of your choice. **See pages 62-63** for the main features available in each display mode.

## 4.3 - 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 EMCCD-chip, e.g.:
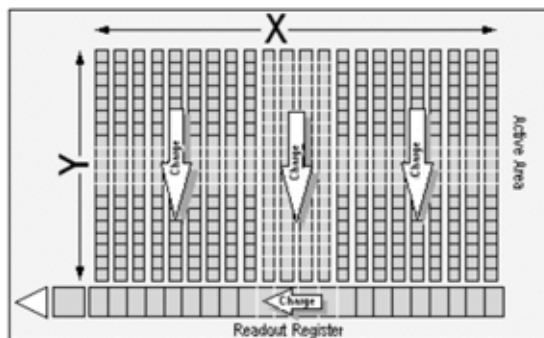


- The x-axis (or horizontal display axis) is parallel to the readout register and may be calibrated in pixels or in a unit of your choice

- 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 information on Calibration, please refer to Section 6

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.

## 4.4 - 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, e.g.:



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 EMCCD-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 less of the available window space, but the aspect ratio is maintained.

## 4.5 - 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 79) 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 the EMCCD-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.**

## 4.6 - ZOOMING & SCROLLING

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

- Zoom in

- Zoom out

- Scroll

## 4.6.1 - Zoom In & Zoom Out

On a data window in 2D or 3D display mode, pairs of Zoom In; ⬚ and Zoom Out; ⬚ buttons are provided on both the x- and data-axes of the trace. These 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.

## 4.6.2 - 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 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.

## 4.6.3 - Reset

Clicking the [icon] 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 79** for more information on rescaling). Reset is available for all display modes.
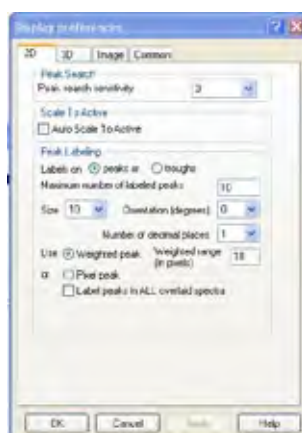
## 4.7 - 2D DISPLAY MODE

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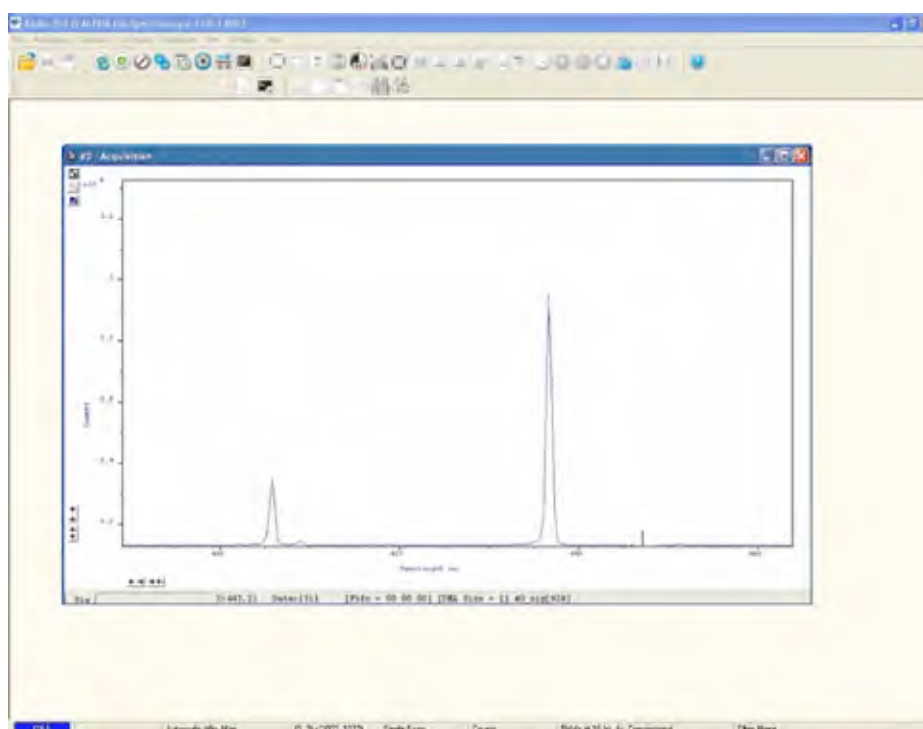


## 4.7.1 - 2D display mode preferences

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

## 4.7.1.1 - Peak Search

## 4.7.1.1.1 - 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 Labelling.**

## 4.7.1.2 - Peak Labelling

## 4.7.1.2.1 - Labels on Peaks or Troughs

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

## 4.7.1.2.2 - 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.

## 4.7.1.2.3 - 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.

## 4.7.1.2.4 - 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: This feature works best on peaks or troughs which are symmetrical about the highest/lowest point.**

## 4.7.1.3 - Pixel Peak

The system can label the Pixel Peak (the highest/lowest positioned pixel).
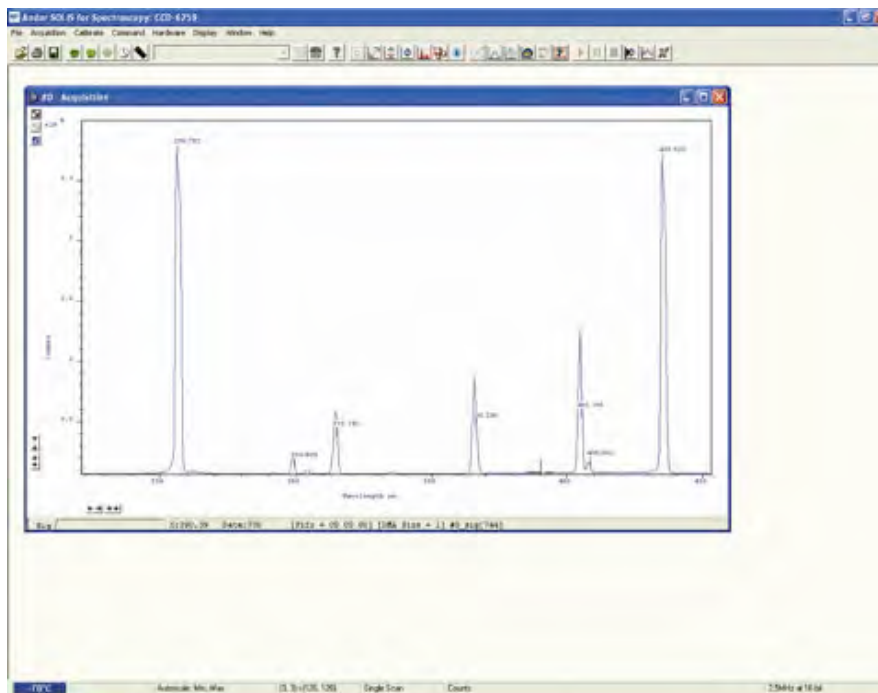
## 4.7.1.3.1 - Label Peaks in all Overlaid Spectra

You can also choose whether to Label peaks in all overlaid spectra or to have peaks labeled only on the active trace. **See Overlay on pages 65-69.**

## 4.7.2 - 2D with Peak Labelling

To label peaks automatically, either select 2D from the drop-down menu or click the ▥ button. A data window similar to the following will appear :



When labelling 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 labelling (by clicking the ▥ button) your manual labelling 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 EMCCD-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 6**.

- If you have acquired data in an imaging mode you will be able to view the data from each track on the EMCCD-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.

## 4.7.3 - 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. 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. **NOTE: The examples shown for Overlay functions are taken from an Andor Newton camera setup, but the principle is exactly the same**
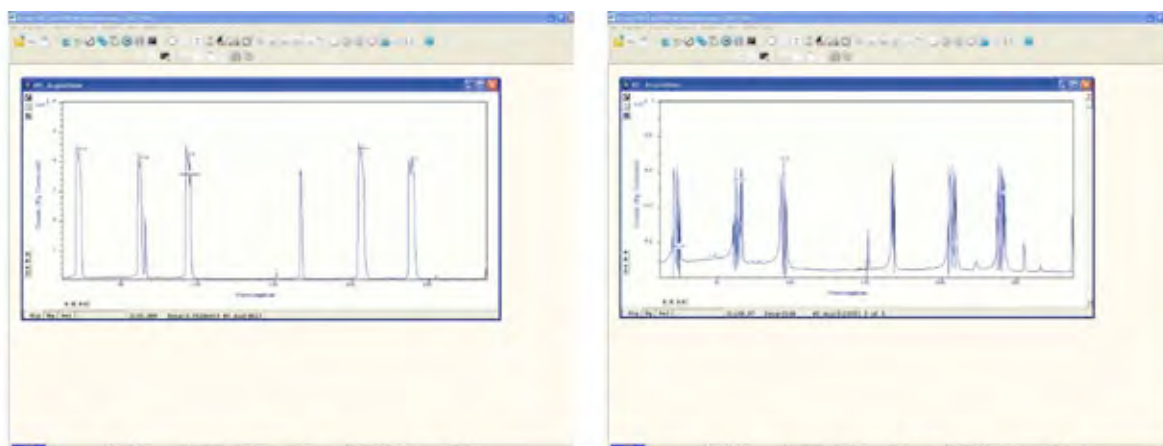
- To add an overlay, first obtain a live signal, e.g.:
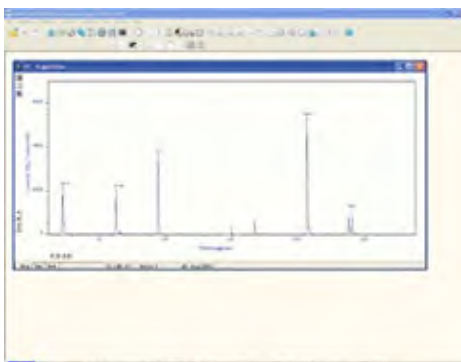


- Then save the image as a **SIF** file, e.g.:
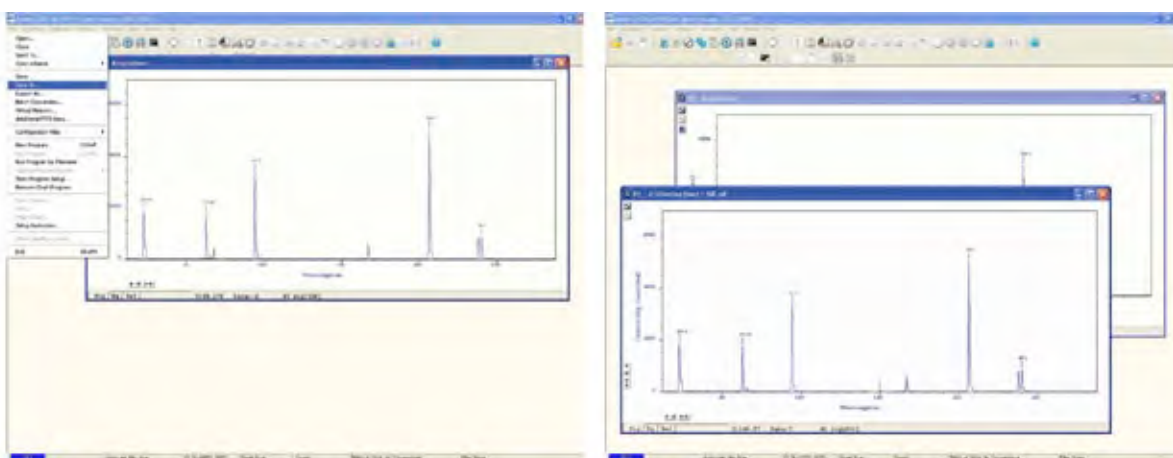


Take some further acquisitions (this is normally done with different parameters set) and save these as SIF files again. The examples below show the same source as above but acquired in different modes:

When you have taken all the reference acquisitions required, keep the last live acquisition window active and open the previously saved SIF files.

Click the ⬛ button on the live #0 Acquisition data window and the **Add Another Trace To Display** dialog box appears:
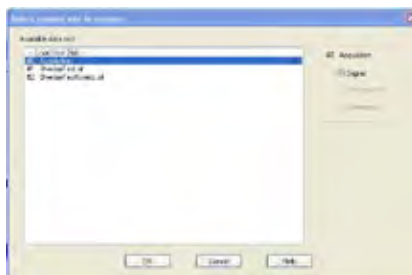


The selection list displays the names of data sets that are already being displayed in a data window. Alternatively you can select files previously stored by selecting the Load from Disk option and choosing the appropriate file or files, e.g.:
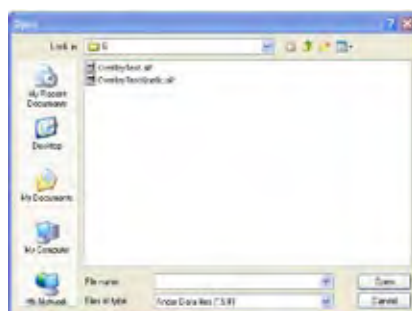


Buttons also let you specify Signal, Background or Reference data (if these are contained in the data set you have selected for overlay).

Once you have selected the required file(s) and clicked OK, the display will change, e.g.:
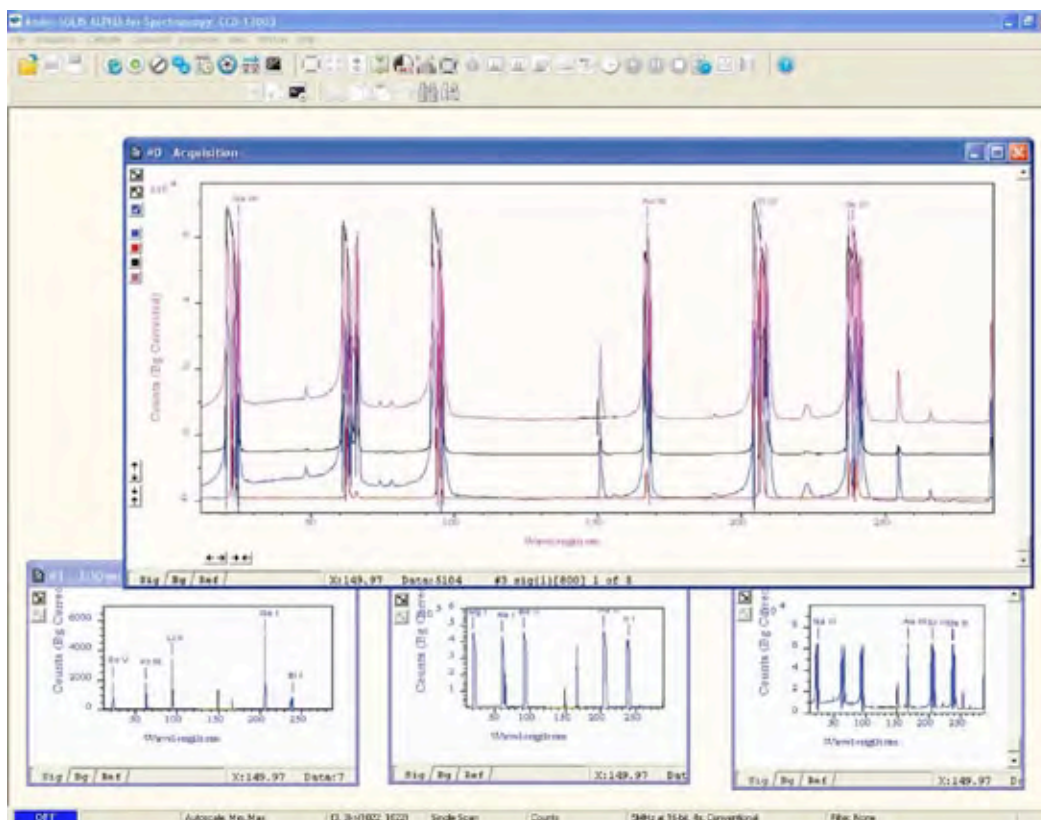


**NOTE: You can add up to a maximum of 8 overlays to your original data trace.**

The original data trace is always displayed in blue. Each new overlay appears in a unique identifying color and the 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:
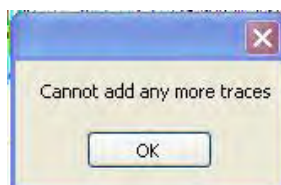
## 4.7.3.1 - Overlay and Keep

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 #0 Acquisition window, the live trace will appear in blue but overlaid with a copy in red and the new data window containing the selected data will be shown minimized at the bottom of the screen, e.g.:
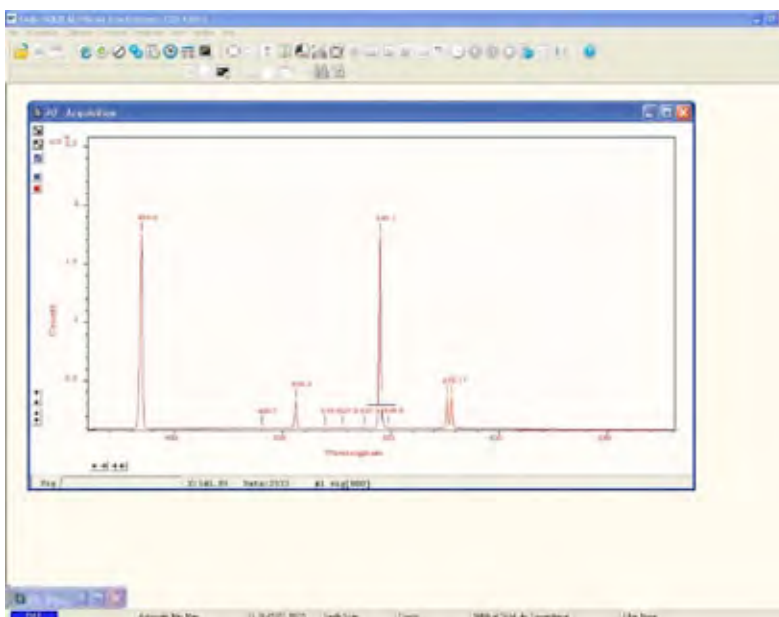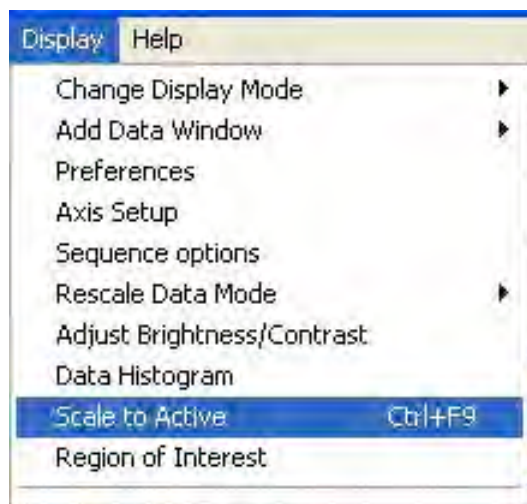


You can display the #1 data window again by clicking on the minimized window, e.g.:
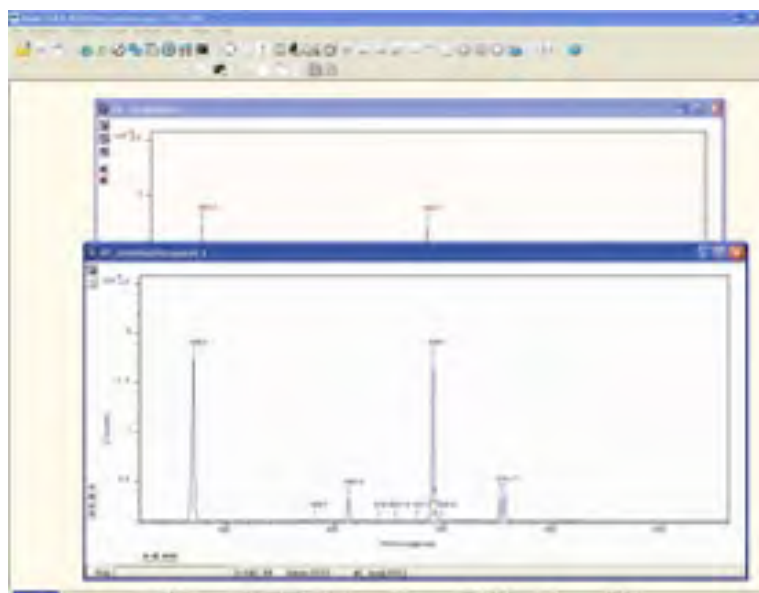


If required, you can then save this new data window to file for subsequent analysis.

## 4.7.3.2 - Scale to Active



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. for the red trace:
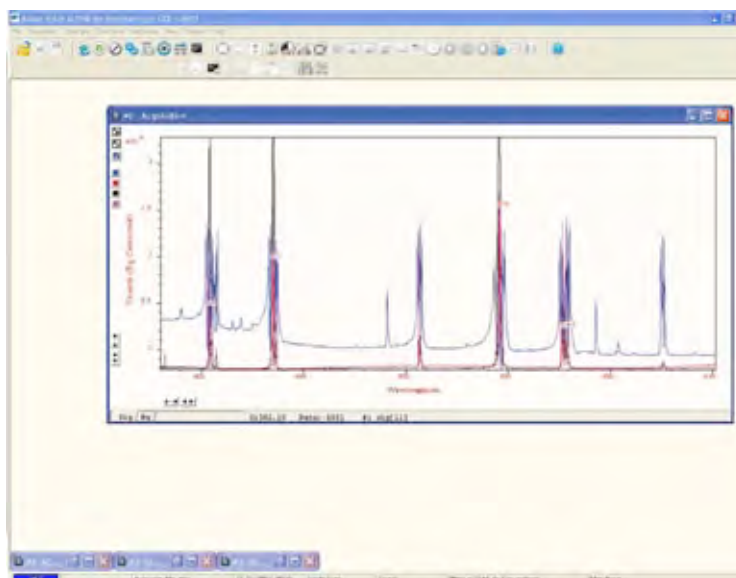


## 4.7.3.3 - Remove Overlay

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

## 4.7.4 - 2D with Chemical Labels

If an Andor Shamrock spectrograph is connected to the iXon Ultra, the following buttons become active:

- Chemical Labels: 
- Periodic Table: 

When the  button is pressed, the Periodic Table of Elements dialog box opens, e.g.:



When the required labels have been selected and the  button is button is then clicked, the 2D display will show the labels, e.g.

## 4.7.5 - Baseline Correction
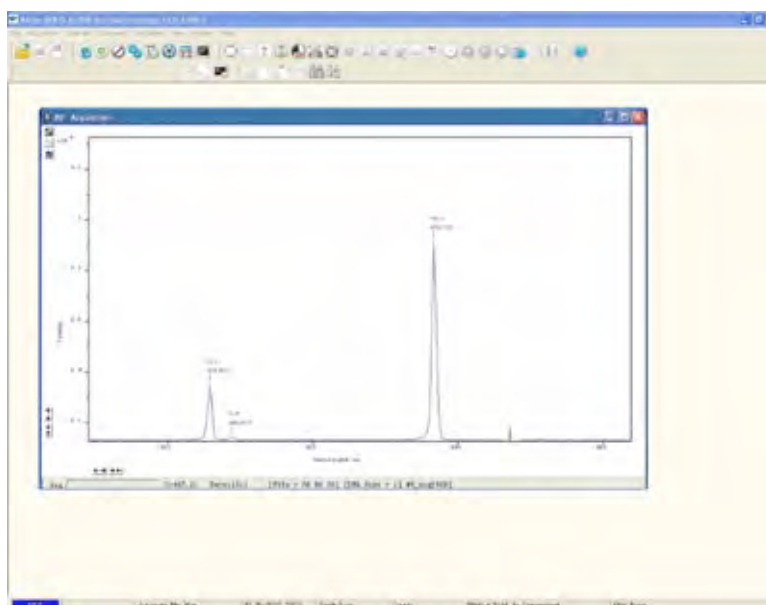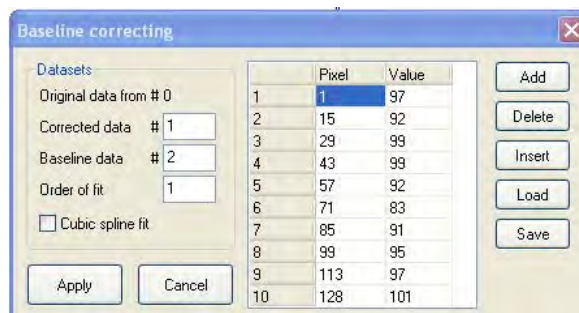
With Baseline Correction, a series of points on a signal are selected through which a smooth, continuous reference is obtained. This reference is subsequently subtracted from the original signal and the resultant spectrum saved to a new data set.

To select the function, click the 📈 button and the Baseline correcting dialog box appears, e.g.:



The user can now enter and define customized baselines. The values to be subtracted are entered against individual pixel numbers in the dialog box. The user can select whether to fit the data using polynomial or cubic spline. The user can also select where the Corrected and Baseline data are stored. When the necessary parameters have been set and the **Apply** button is clicked, other data windows will be generated as defined by the user, e.g.:
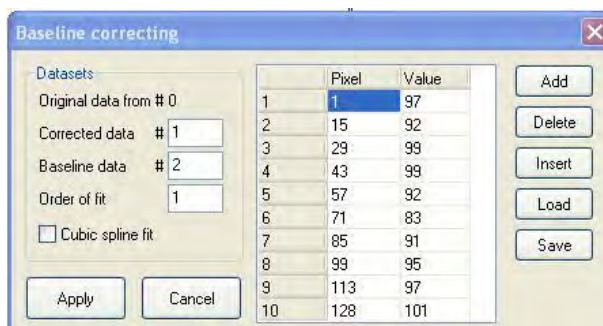


Window **#0** will show the raw data as acquired during the last acquisition, by default window **#2** will show the profile of the customized baseline to be subtracted off the raw data and window **#1** will show the baseline corrected data i.e. **#0** - **#2**). The window number labels of the customized baseline profile and corrected data can be adjusted by changing the numbers detailed in the Baseline correcting dialog box.

## 4.8 - 3D DISPLAY MODE

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 [icon] button and a data window will appear e.g.:
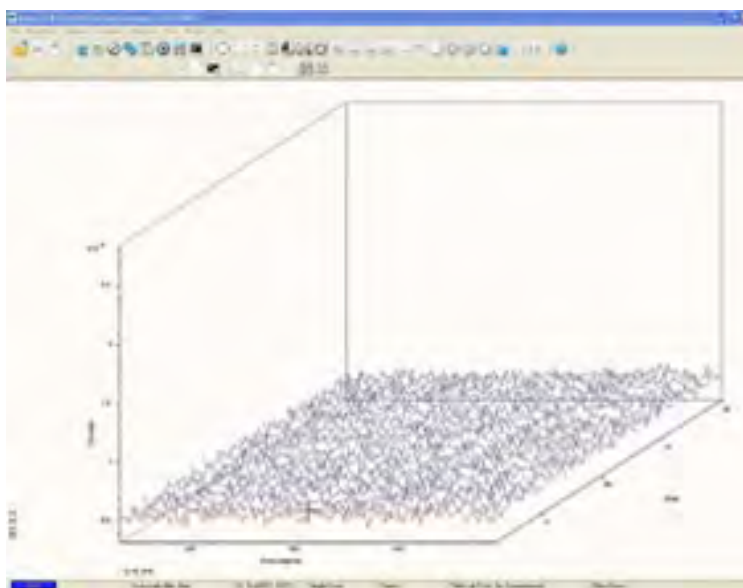


Along with the x-axis (calibrated by default to represent pixels across the EMCCD-chip) and the data-axis (calibrated by default in counts), you now see a 3rd (or y-axis) calibrated in rows or tracks, depending on the acquisition mode you have selected.

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.
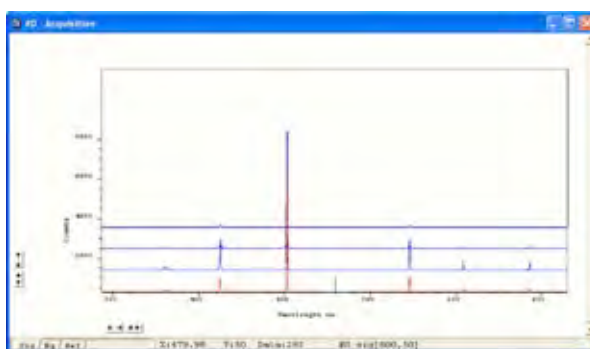
**NOTE: The Zooming, Scrolling and Reset functions are the same as 2D mode.**
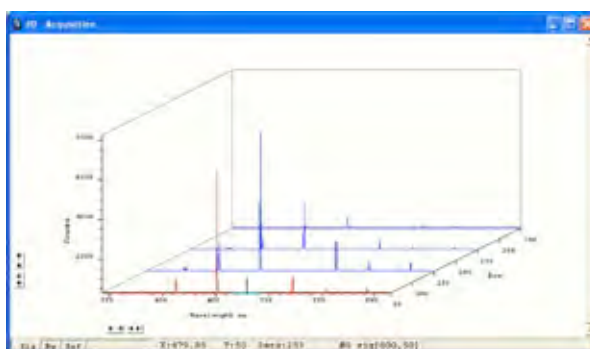
## 4.8.1 - 3D display mode preferences

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



You can display multiple tracks either Overlaid:



or Stacked:



or at 45 degrees:
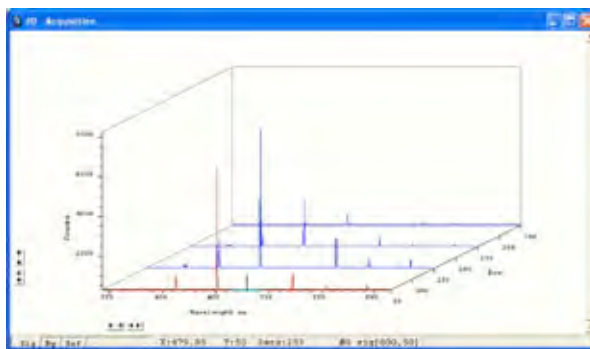
## 4.9 - IMAGE DISPLAY MODE

Data acquired in Imaging mode can be viewed as an image in a data window. Select the Image option from the Display drop-down menu or click the button and an image will appear, e.g.:
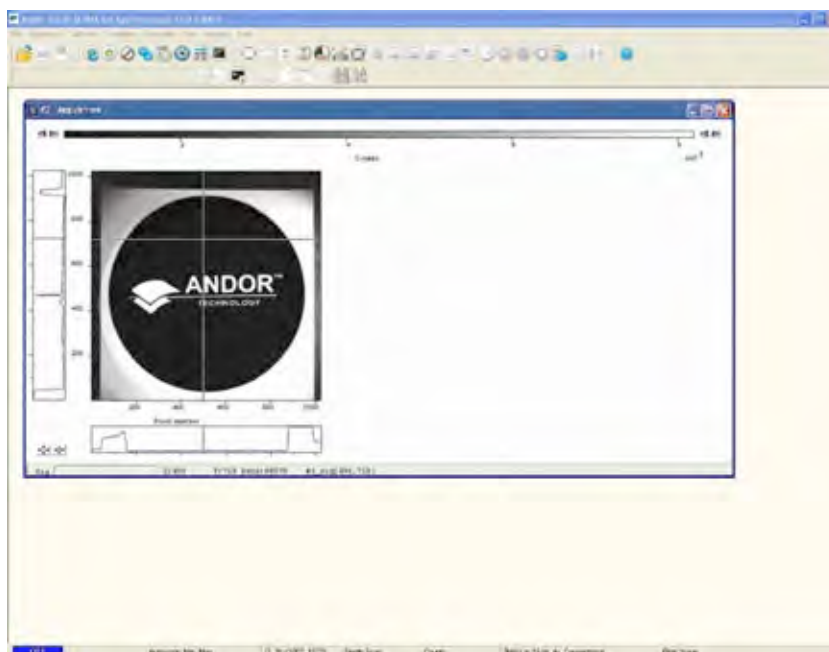


Clicking on any point on the image causes the cross-hair to move to that point. The data value for the point is displayed on the bottom edge of the data window, along with the x and y co-ordinates.

**NOTE: If your data set contains a kinetic series, 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.**

## 4.9.1 - Image display mode preferences

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

## 4.9.1.1 - Always maintain aspect ratio

- 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.

- 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.

## 4.9.1.2 - 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. Also see **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.

## 4.9.1.3 - Show palette bar

Show Palette Bar causes the palette and it's control to be displayed or removed e.g.:
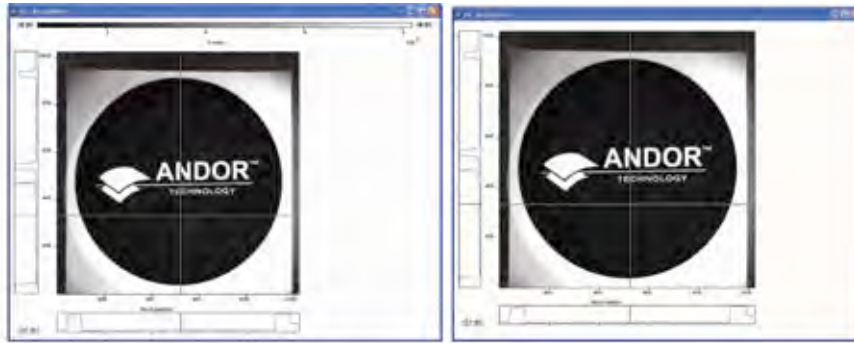


The arrow buttons at either end of the palette bar can be used to adjust the manner in which values are mapped against colors, and so change the brightness and contrast of the image. See next page for further details of the Pallete Bar

## 4.9.1.4 - Large Cursor

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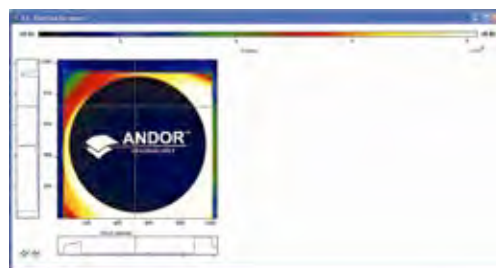


This makes it easier to identify the corresponding points on the Side Traces.

## 4.9.1.5 - Palette bar

The Palette bar: Runs across the top of the data window. This 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.:
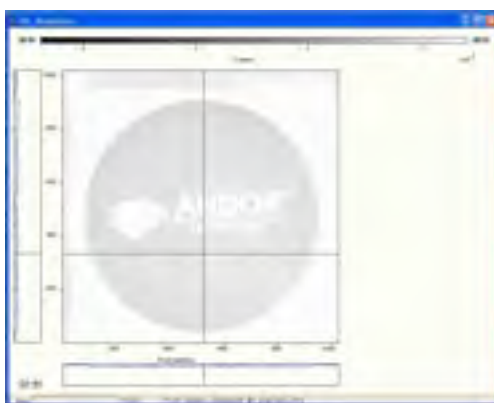


The scale on the Palette bar is calibrated in your chosen data units. By default, the full range of colors/grays is correlated with the full range of data in your data set. This is known as "Rescaling" (see **Rescale** on **page 79**). Adjusting the Brightness & Increasing and Decreasing Contrast later in this section explain how you can use these controls to change the brightness and contrast of the display.

## 4.9.1.6 - Selecting color/grayscale

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



Clicking on the [button] button causes the data window to cycle through the following 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.:

## 4.9.2 - Rescale
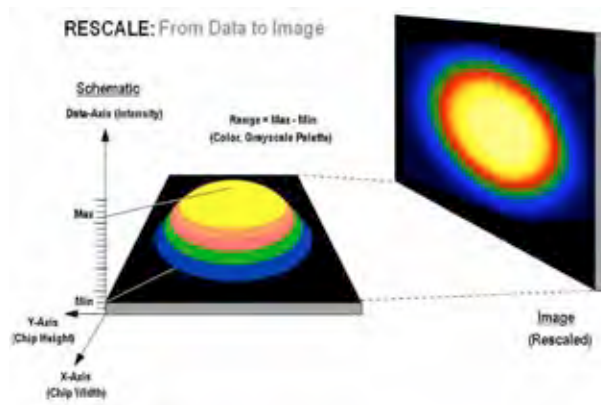


When a data window is open and you click on the 🌸 button of the Main Window, the system rescales the data axes such that all data within the currently selected region of interest is displayed. From the Rescale  Data Mode menu of the Display drop-down menu, you can also select the maximum number of counts that can be recorded for a single pixel, your rescaled data distinguishes values between the following parameters:

- Minimum & maximum (Min..Max)

- Zero & maximum (0..Max)

- Zero & 16383 (0..16383)

- Minimum & 16383 (Min..16383)

- Zero & 65535 (0..65535)

- Minimum & 65535 (Min..65535)

- Custom… setting as required. When 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 on the buttons:

[icon] = ON

[icon] = 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.)
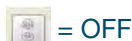
The computer rescales your display by default. The full available palette of colors/grays is correlated with the full range of data you have acquired.

When it rescales in Image display mode, the computer defaults to Minimum (**Min**) and Maximum (**Ma**x) 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**.

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.

## 4.9.3 - High & Low Contrast Overview
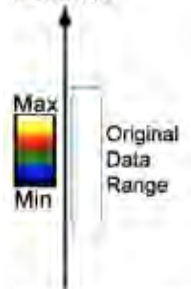
Assume your original data are rescaled.

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

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.
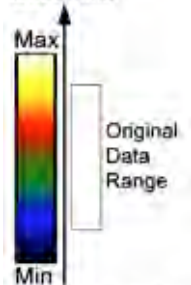
**Decreasing the displayed data range produces high contrast.**

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.**

## 4.9.3.1 - 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.

The diagrams to the right illustrate the following:

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

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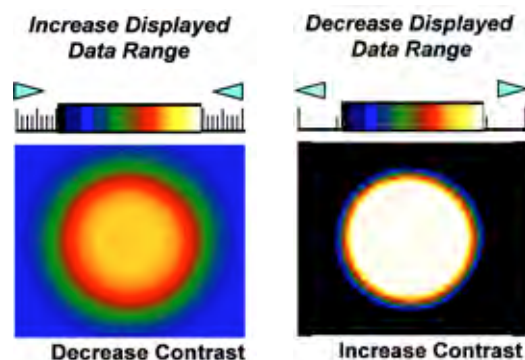


By holding down the left-right arrows at either end of the Palette Bar you can alter the minimum and maximum values in the displayed data range independently of each other, and so adjust the contrast to the level you require. As you hold down the arrows (place the cursor over an arrow button and hold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

## 4.9.4 - Brightness Overview

To make the data shown in Image display mode brighter, the computer uses the same number of colors as it used for rescaling to scale lower data values.

Thus any data originally represented by darker colors are now represented by brighter colors.
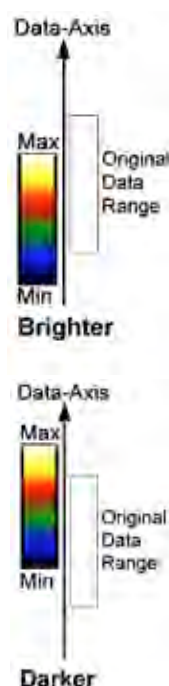


To make the data shown in Image Display Mode darker, the computer uses the same number of colors as it used for rescaling to scale higher data values.

Thus any data originally represented by brighter colors are now represented by darker colors.

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

# 4.9.4.1 - 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.

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

The display then becomes brighter as shown on the right.
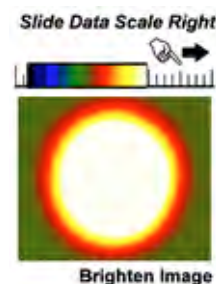
Sliding the scale to the left means that the displayed data range covers higher data values (the numbers on the scale increase).

The display then becomes darker as shown on the right.
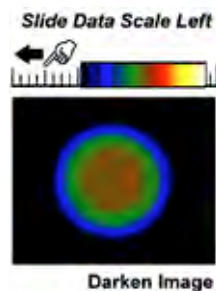
**NOTE: When you slide the scale, you will see the image flash periodically as the system performs the brightness adjustment. These flashes occur as the system refreshes the color palette to cover your new displayed data range. Continue to slide the scale until you reach your required brightness level.**

## 4.10 - DATA HISTOGRAM

The Data Histogram dialog Box is launched either by clicking on the ![icon] 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.
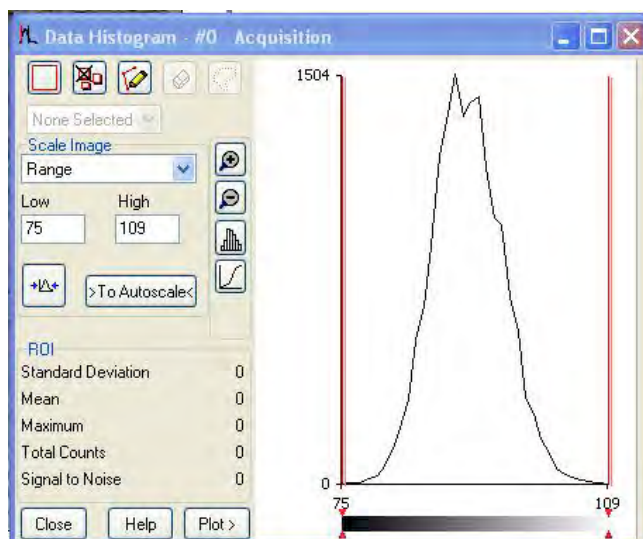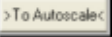


By clicking on an open data window, its histogram will be displayed on the Data Histogram dialog. When plotting the histogram, the focus is transferred to the new data window and the displayed histogram is that of the plotted histogram. The data from which the histogram is displayed is indicated on the 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, e.g.:
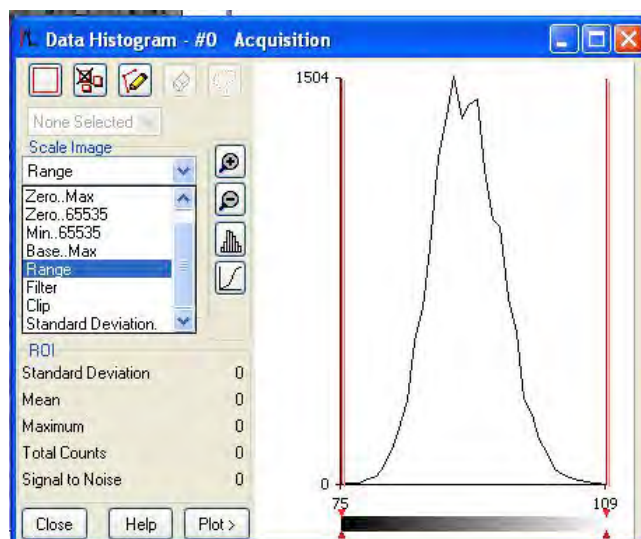
Values can be modified either by typing in the new values in the Low and High text boxes or by dragging the red arrows and bars using the slider below the histogram, e.g.:
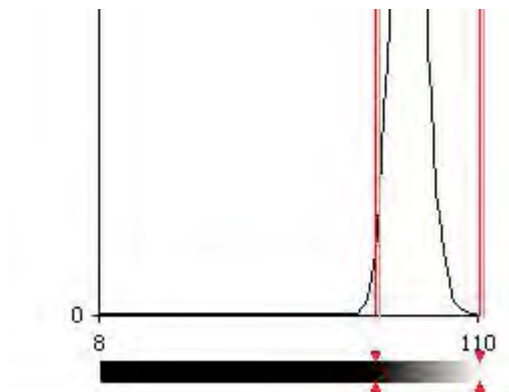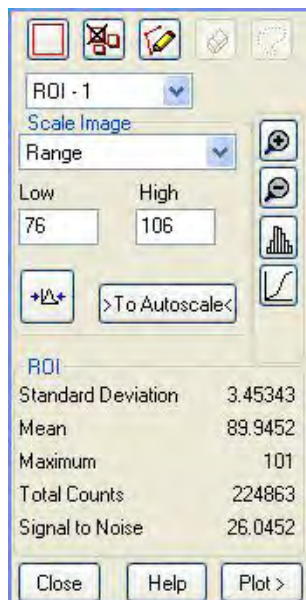


- **Update**: Any change on the mode and/or the Low/High values is updated when the Update button ⌐⌐ is clicked.

- **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 on 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.

## 4.11 - 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, using the corner handles.
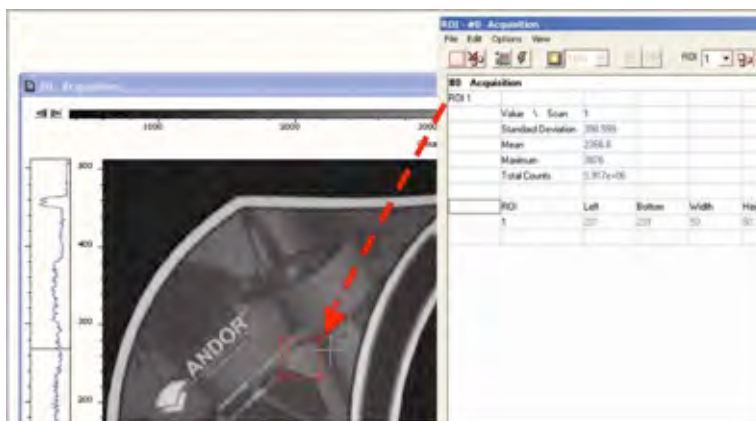


You can use this tool to select and draw multiple ROI"s onto your image, then use the ROI data set to compare the values obtained, e.g.:

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.:
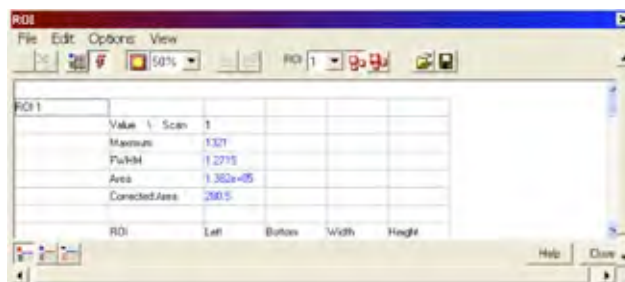


- Clicking the **Scan** button will present and group your ROI data, according to individual data scans. It will also display the pixel co-ordinates for all the ROI"s for that scan, e.g.:
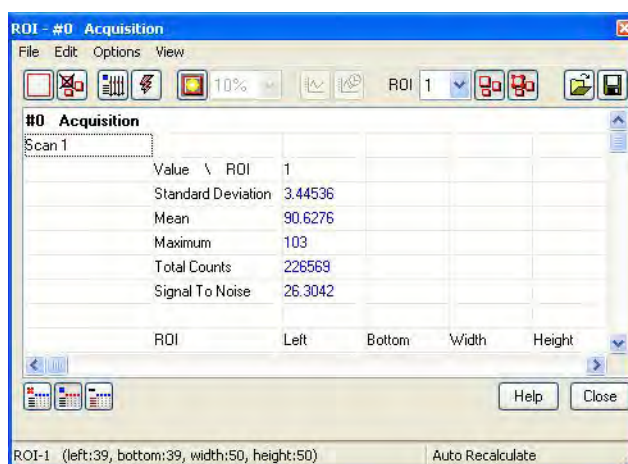


- Clicking the **Property View** button will present and group your ROI data, according to value regions, i.e. Mean, Range and Standard Deviation. It will also display the pixel co-ordinates for all the ROI"s for that scan, e.g.:
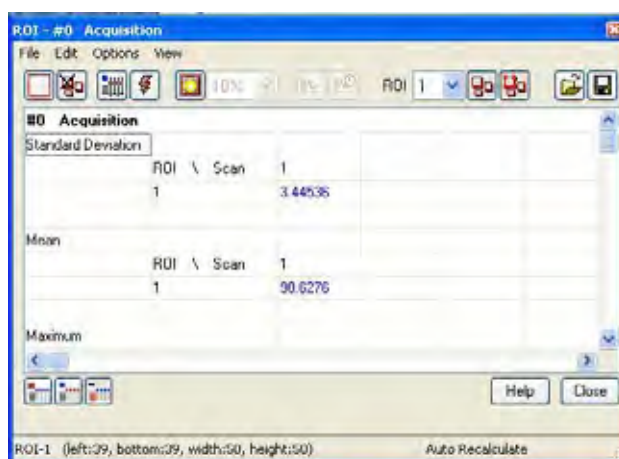
The ROI can be switched on and off by clicking the [icon] **(Show ROI)** button. When Show ROI is selected **ON**, the selected ROI will be displayed and outlined by red boxes, e.g.:
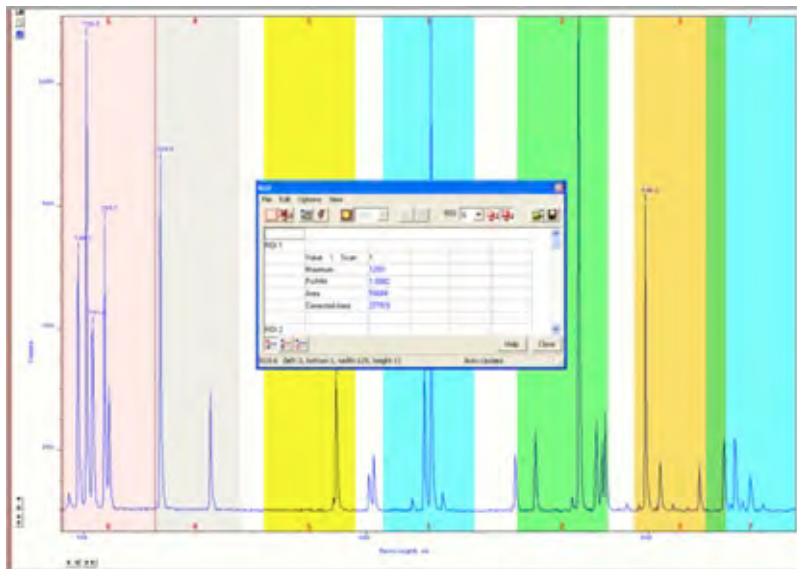


When **Show ROI** is selected **OFF**, the red ROI boxes are hidden.

**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 [icon] **(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 [icon] **(Delete current ROI)** button.

## 4.11.1 - 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.

## 4.11.2 - 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 [icon] 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.

### 4.11.3 - Recalculate

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

### 4.11.4 - 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 [icon] button. The software is then able to acquire data and at the same time calculate and tabulate ROI data.

### 4.11.5 - Maximum Scans

When the Maximum Scans is selected from the 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.**

### 4.11.6 - 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.

## 4.12 - TIME STAMP

When the  (Time Stamp) button is clicked, the **Display Preferences** dialog box opens as shown:



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.:
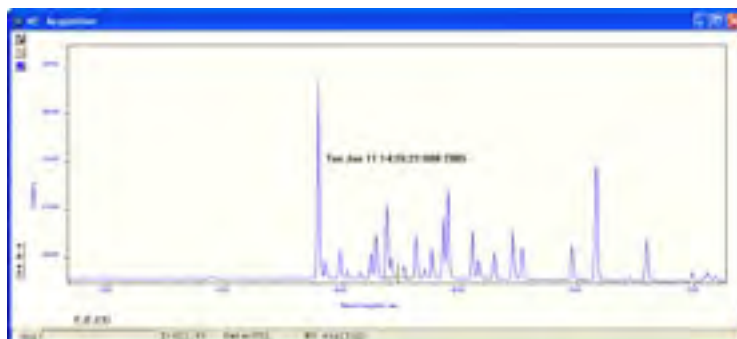


- Time & date information, or time relative to the start of the acquisition can be displayed by selecting Enabled, then selecting the appropriate option in the Style drop down list

- 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

## 4.13 - 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 the next section for further details on Handling Files.**

## SECTION 5 - HANDLING FILES

## 5.1 - MENU OPTIONS

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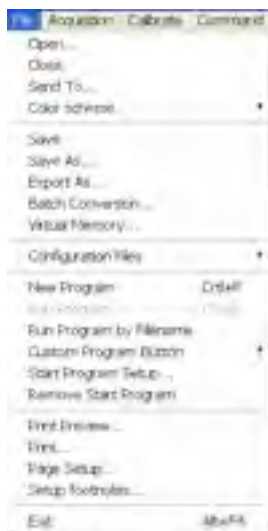


Some of the options available are typical "Windows facilities" to Open, Save, Print files, etc. but some are specific to Andor Solis software to let you create or run programs.

Selecting **Open**... from the drop-down menu or clicking on the button opens a standard dialog box, e.g.:
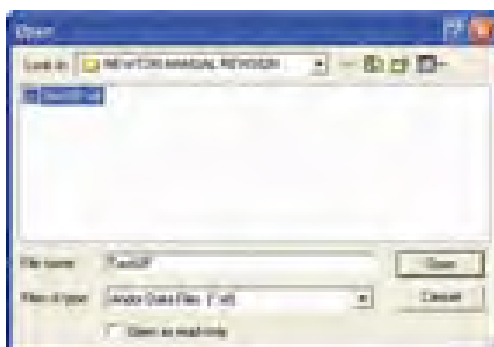
If you select a Data file (.sif), the system launches a data window with the appropriate file displayed, e.g.:



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 E**diting Programs** in **Section 7**), e.g.:
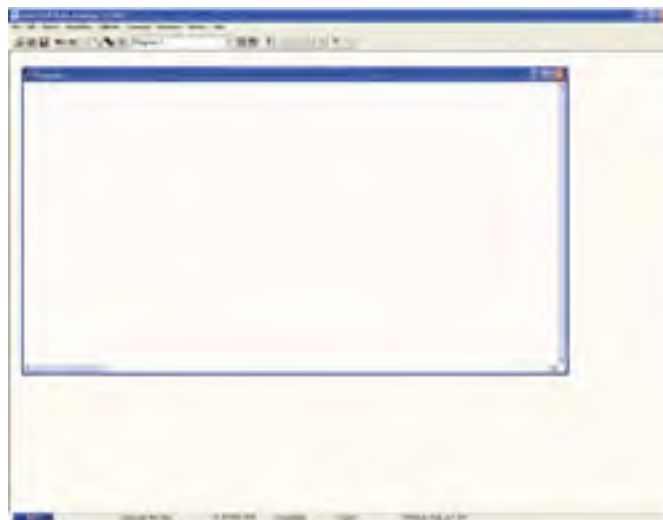
## 5.1.1 - Close

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.

## 5.1.2 - Save

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

## 5.1.3 - 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.**

## 5.2 - EXPORT AS

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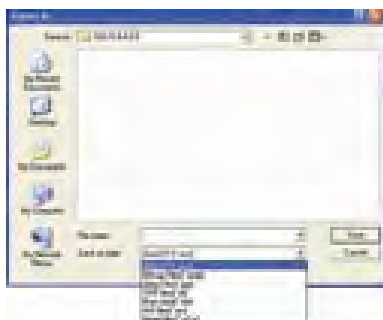


Depending on the Display & Readout modes selected, the file can be saved in one or more of the following formats:

- **ASCII (*.asc)**
- **Bitmap (*.bmp)**
- **Jpeg (*.jpg)**
- **TIFF (.tif)**
- **Raw data (.dat)**
- **AVI (*.avi)**
- **Mpeg (.m1v)**

The table below shows a matrix of the actual combinations available:

| READOUT MODE | DISPLAY MODE | ASCII | BITMAP | JPEG | TIFF | RAW DATA | AVI | MPEG |
|---|---|---|---|---|---|---|---|---|
| Image | Image | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |

## 5.2.1 - ASCII

**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.:
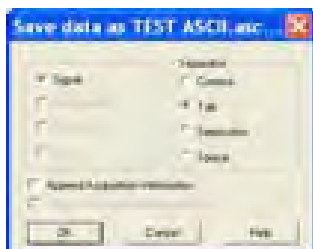


- The radio buttons on the left allow you to choose whether you want to save the Signal, Background or Reference data from the live data set.

- The radio buttons on the right allow you to choose a character that will serve as a Separator between the numeric values in your raw data.

You can then configure the importing application to recognize this separator (or delimiter) and in the case of a spreadsheet, display the data in a suitable configuration of rows and columns. In 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 EMCCD-chip

## 5.2.2 - 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

## 5.2.3 - Jpeg

**File extension = .jpg**

**JPEG** (**J**oint **P**hotographic **E**xperts **G**roup) is an organization of people that develops and maintains standards for a suite of compression algorithms for computer image files. JPEG is a term used for any graphic image file produced by using a JPEG standard.

When you create a JPEG or convert an image from another format to a JPEG, you are asked to specify the quality of image you want. Since the highest quality results in the largest file, you can make a trade-off between image quality and file size.

After you have selected Jpeg Files (*.jpg) from the Save as type drop-down menu, allocated a filename and clicked Save, the file is saved.

## 5.2.4 - TIFF

**File extension = .tif**

**TIFF** (**T**agged or **T**ag **I**mage **F**ile **F**ormat) is used for storing bitmapped images and is widely supported by commercial publishing packages. After you have selected the option from the Save as type drop-down menu and allocated a filename, the TIFF export dialog box appears, e.g.:
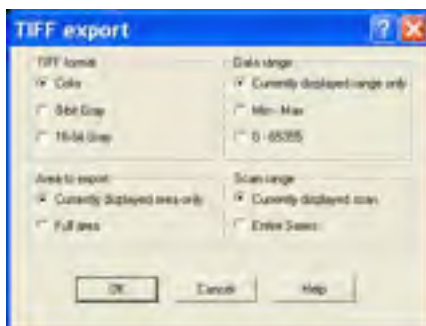


You can then choose the parameters you require to be displayed.

## 5.2.5 - Raw data

**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 EM 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 EMCCD, the second 1024 data values correspond to the second row, etc.

The .dat types range is shown in the table below.

| | 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 x 10-38 to 3.4 x 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.

## 5.2.6 - AVI

**File extension = .avi**

An **AVI** (**A**udio **V**ideo **I**nterleaved) file is a sound and motion picture file that requires a special player. After you have selected AVI files (.avi) from the Save as type drop-down menu, allocated a filename and clicked Save, the AVI Export dialog box appears, e.g.:
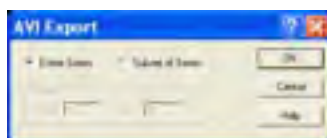


You can then select which series of data to export to the file.

## 5.2.7 - MPEG

**File extension = .m1v**

**MPEG** (**M**oving **P**icture **E**xperts Group) is a standard for digital video and digital audio compression. MPEG standards are an evolving series, each designed for a different purpose.

After you have selected Mpeg (*.m1v) from the Save as type drop-down menu, allocated a filename and clicked Save, the file is saved.

## 5.2.8 - Configuration files

**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 own 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.

## 5.3 - PROGRAM SELECTION

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



**Working with Programs is explained in more detail in Section 7.**

# SECTION 6 - SPECTRAL CALIBRATION

## 6.1 - INTRODUCTION TO SPECTRAL CALIBRATION

The iXon Ultra may be coupled to spectrographs such as the Andor Shamrock range in order to carry out spectroscopy, typically in combination with Full Vertical Binning or Multi-track readout modes.

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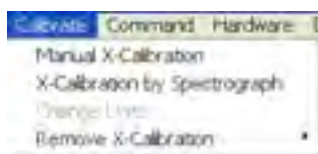


- **Manual X-Calibration**: lets you calibrate the x-axis of data displays by manually setting values (time, pixel number, wavelength, Raman shift or spatial position) against recognizable features of a particular 2D data trace

- **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:

- **Current Data** returns the data in the active Data Window to its default pixel x-calibration

- **New Acquisitions** causes future data to be acquired with the default pixel x-calibration
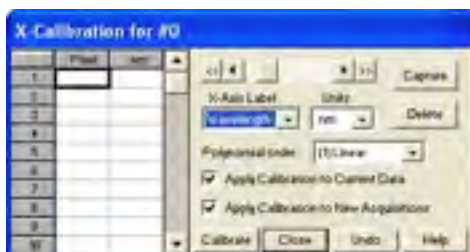
## 6.2 - MANUAL X-CALIBRATION

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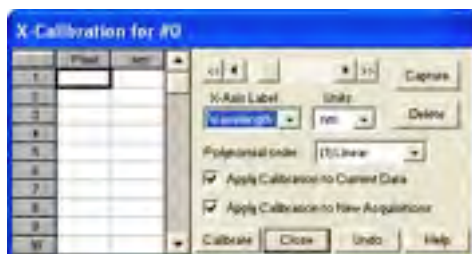


The number (#n) of the data window (or #0 in the case of an Acquisition Window) appears on the title bar of the dialog box.

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 here:

| 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 |

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.

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 centre of the PDA sensor. |
| Cubic | A cubic polynomial produces the best calibration fit if the known spectral features are evenly distributed across the PDA sensor. |
| Time | ms, secs |
| Pixel | pixels |

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.

Repeat steps 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.

## 6.2.1 - Applying Calibration

Two check boxes on the Manual X-Calibration dialog box allow you to choose how the system should act upon the calibration details you have provided. You may select either or both of the following options:

1. **Apply Calibration to Current Data:** lets you change the x-axis calibration on an active data window whose filename (or #0 in the case of an Acquisition Window) appears on the title bar of the Manual X-Calibration dialog box

2. **Apply Calibration to New Acquisitions:** the calibration details you have supplied in the Manual X-Calibration dialog box will be applied to any subsequent data acquisitions

## 6.2.2 - 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

## 6.2.3 - When Manual X-Calibration goes wrong

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:
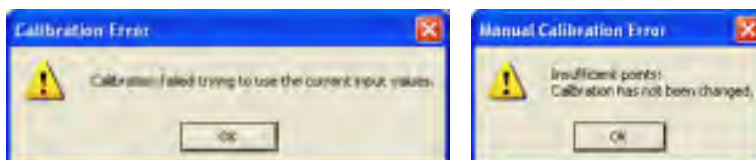
## 6.2.3.1 - Data are Non-Montonic

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:
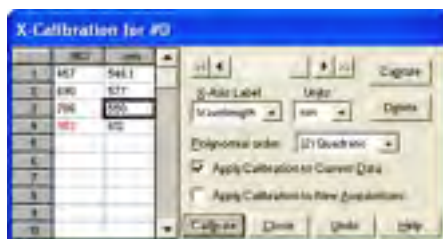


From Pixel 706 to Pixel 953 the user has indicated a fall in wavelength, despite the preceding rise in pixel 690. In such a case, an illegal non-monotonic calibration (as shown on the graph below) results.

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).
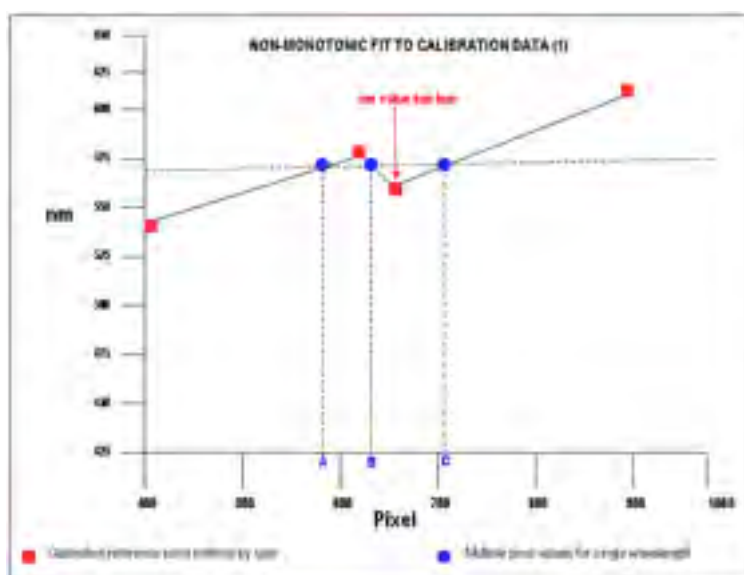
However, a non-monotonic calibration may come about even in cases where your data are not as grossly inaccurate as those shown in the example above. A non-monotonic calibration sometimes results if you attempt to calibrate points that are very close together on your trace, even if, for example, you are 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 point on the extreme right being at slightly too low a wavelength value.
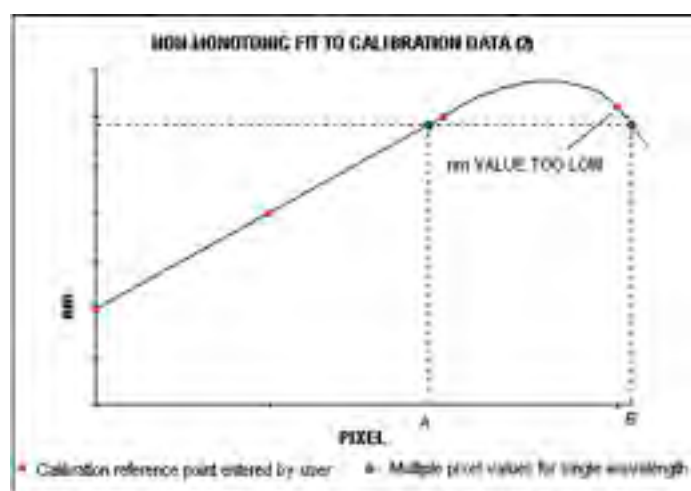


## 6.2.3.2 - Too few points

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 PDA sensor.

## 6.2.4 - 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.

## 6.2.5 Close

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

## 6.3 - 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: This dialog box can also be opened by selecting the Setup Spectrograph option from the Hardware menu on the main window.**

## 6.3.1 - 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.:
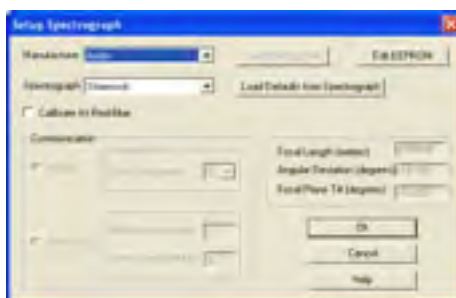


From the Manufacturer drop-down list, select the appropriate company name and from the Spectrograph drop-down list, select the model type being used, then click OK. The Spectrograph X-Calibration will appear again, with the details of the selected spectrograph.

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 Communication section of the dialog box.

## 6.3.1.1 - Calibrate As Red-Blue

Some PDA detectors readout data in the reverse direction to Andor cameras. In this case, the longer wavelengths (red) are to the left (as viewed from the detector), and shorter wavelengths (blue) are to the right.

If you tick the **Calibrate As Red-Blue** option in the Setup Spectrograph dialog box, the system then uses software to "reverse" the output of the detector when it generates a data window, thus presenting the display in the more usual orientation.

## 6.3.2 - Communications

The radio buttons in the **Communication** 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 I2C control links. Select Shamrock Control from the Hardware drop-down menu and the Shamrock Control dialog box appears e.g.:
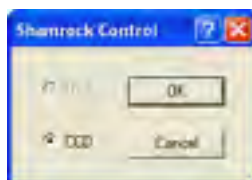


Select USB for USB control or for I2C control select CCD, then click OK.

N**OTE: When a Shamrock is connected, the Shamrock icon;**  **appears in the menu bar of the main window.**

In the event of an error in communication occurring, you will be prompted by a message, similar to the following:

## 6.3.2.1 - Other Spectrographs

If you have selected the Oriel MS257 spectrograph for example, 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 the Solis software, after which 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.

## 6.3.2.2 - 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 camera 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.

## 6.3.2.3 X-Axis Labels & 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 |

## 6.3.2.4 - Change Units

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.:
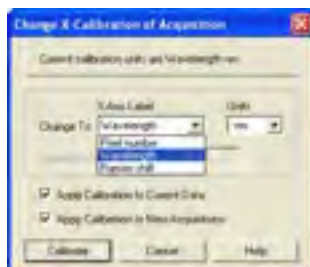


From the **X-Axis Label** drop-down list choose whether you want the x-axis to represent **Wavelength, Pixel Numbe**r or **Raman Shift**. From the **Units** drop-down list, choose the units that you want to use for your recalibration. The available combinations depend on how the data were previously calibrated. If for instance, the data were previously calibrated in **Wavelength** and **Nanometres**, the available combinations for recalibration are:

| X-AXIS LABEL | Units |
|---|---|
| **WAVELENGTH** | nm, µm, cm-1, eV |
| **RAMAN SHIFT** | cm-1 |

**Table 6** shows the available combinations for all modes.

Table 6: Label & Unit changes

| | X-AXIS LABEL | UNITS | CAN CHANGE TO: |
|---|---|---|---|
| 0 | User Type | - | |
| 1 | *Pixel Number (see NOTE 1. 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.**

## 6.3.3 - 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 PDA-sensor. These are referred to as **Center Wavelength** and **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.:
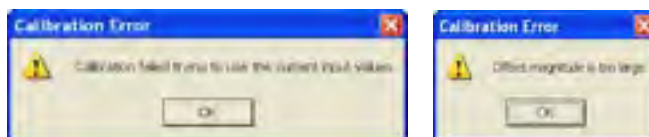


## 6.3.3.1 - Note on Raman Shift

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 = 107 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.

## 6.3.4 - 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.**

## 6.3.5 - 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.
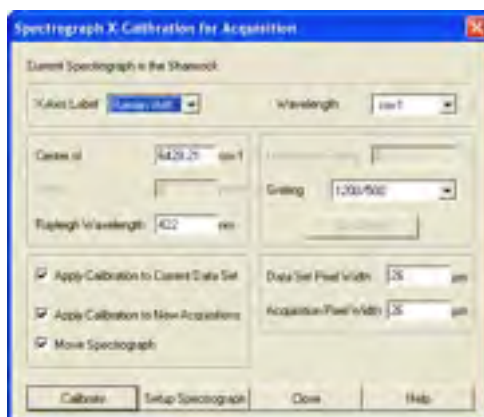


An error message will appear if you attempt to perform a calibration without having entered a valid Rayleigh Wavelength, e.g.:



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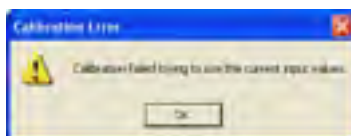
The Micrometer Setting option appears in a text box on the Spectrograph X-Calibration dialog box.  You should use this value to manually set the micrometer on the spectrograph.

**NOTES:**

1.  **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 PDA-sensor (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.**

2.  **If you choose Raman Shift as the X-Axis label, the Center Wavelength text box is relabeled Center of Raman Shift.**

3.  **In the case of motorized spectrographs, the wavelength drive is under direct software control.**

## 6.3.7 - 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.

## 6.3.8 - Close

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

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

## 6.3.9 - Processing Data via the Command Line

## 6.3.9.1 - 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 ":".

To 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:
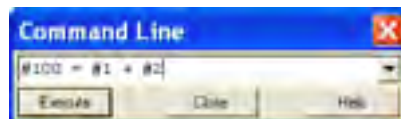


To run a command, click **Execute**. For further details of how to use the Andor Basic programming language, please refer to the User"s Guide to Andor Basic.

## 6.3.9.2 - Calculations

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

## 6.3.9.3 - Configure Calculations

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 7 - WORKING WITH PROGRAMS

## 7.1- Working with Andor Basic programs

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:
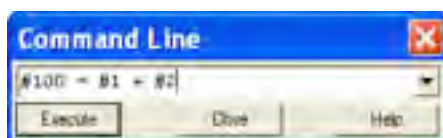


## 7.1.1 - Program Editor Window

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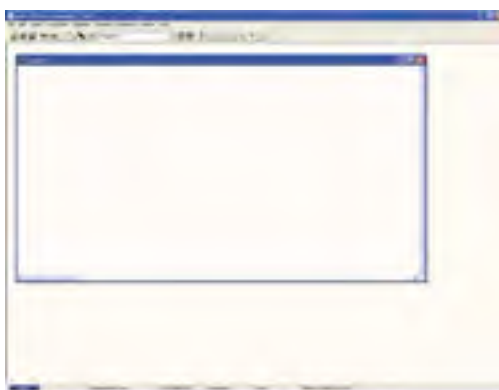


While you are working in the Program Editor Window, context sensitive help is available on the "reserved words" of the programming language: with the cursor on or immediately after a reserved word, press Ctrl + F1.

## 7.1.2 - 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 (i.e. Cut and 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.

## 7.1.2.1 - Cut, Copy, Paste, Undo

Cut; ▨ or Copy; ▥ text that you have highlighted, then paste the text into the desired 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.

## 7.1.2.2 - Search

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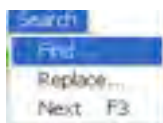


The **Find** dialog box appears:
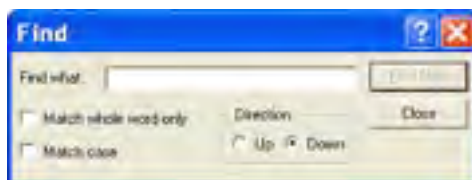


• In the **Find what** text box, type the word or phrase (the "search string") that you want to find

• 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

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

## 7.1.2.3 - Replace

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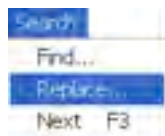


In the **Find what** text box, type in the search string and in the Replace with text box, type the word or phrase that you want to use instead, e.g.:
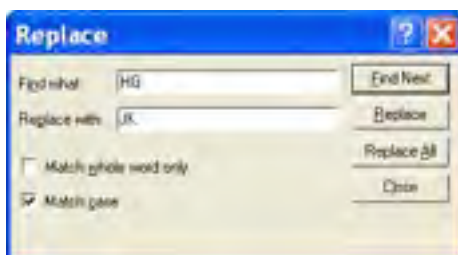


- Click the **Replace** button to change the next occurrence of the search string (or the highlighted search string if you have just used Find Next)

- Click **Replace All** to replace the search string wherever it occurs after the current cursor position

Check boxes lets you match the whole word only and/or match the case of the letters.

## 7.1.2.4 - 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:
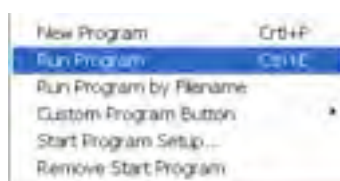


The program will now start. To change the name of the file you want to run, carry out one of the following actions:

- 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

## 7.1.2.5 - Run Program by filename

You can 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:
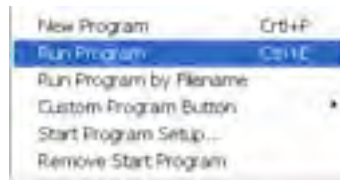


A standard Open dialog box appears, from which you may select the file whose contents you want to run. The file containing the program appears on screen as an iconized Program Editor Window and the program begins to execute immediately.

## 7.1.2.6 - 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

## APPENDIX

## GLOSSARY

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

## EMCCD READOUT SEQUENCE

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



Readout sequence of an EMCCD (Only subset of pixels shown)
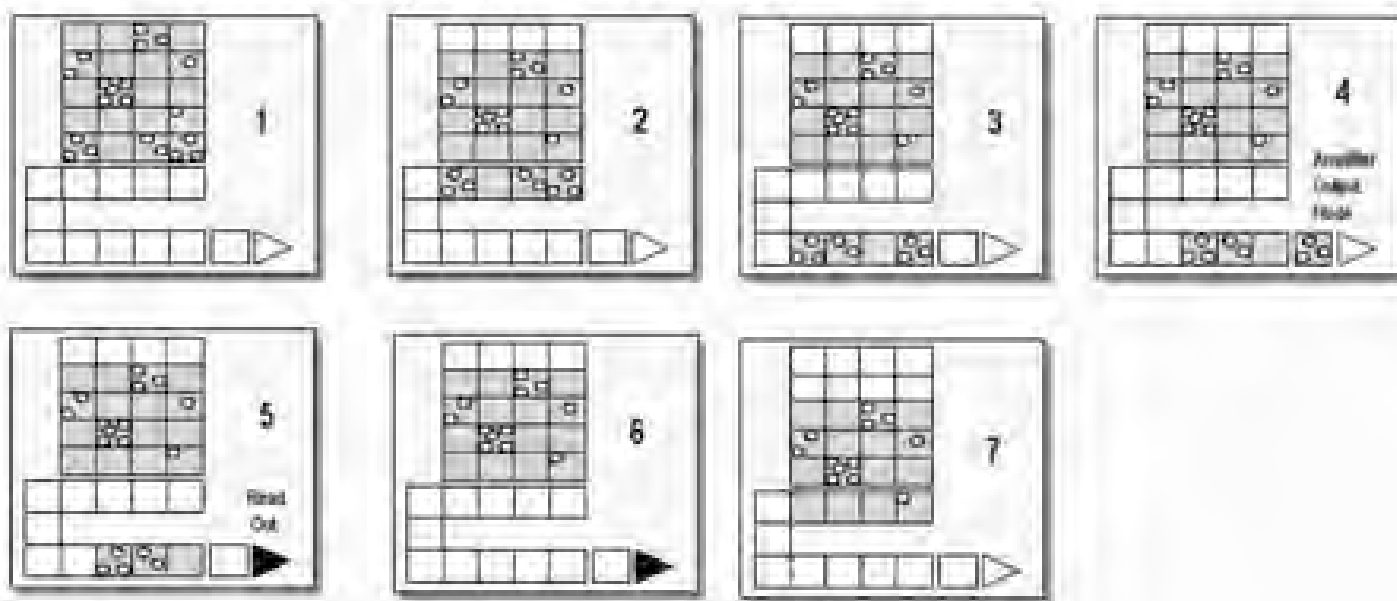
| 1 | Exposure to light causes a pattern of charge (an electronic image) to build up on the frame (or Image Area) of the EMCCD-chip. |
|---|---|
| 2 | Charge in the frame is shifted vertically by one row, so that the bottom row of charge moves into the shift register. |
| 3 | Charge in the shift register 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 EMCCD is initially read as an analog signal, ranging from zero to the saturation value. A/D conversion changes the analog signal to a binary (digital) 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 For further information on Binning, please refer to the iXon Ultra Hardware guide.

## 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 EMCCD.

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

- Shot noise of the signal itself

- Shot noise of any dark signal

- Readout noise

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

## Exposure Time

The Exposure Time is the period during which the EMCCD 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 EMCCD (FT EMCCD)**. An FT EMCCD differs from a standard EMCCD in 2 ways. Firstly, it contains 2 areas, of approximately equal size as follows:

1. The first area is the **Image Area,** which is located at the top and farthest from the Readout Register. This is the light sensitive area of the EMCCD.

2. The second section is the **Storage Area,** and is located between the Image Area and the Readout Register This section is covered by an opaque mask, usually a metal film, and hence is not sensitive to light
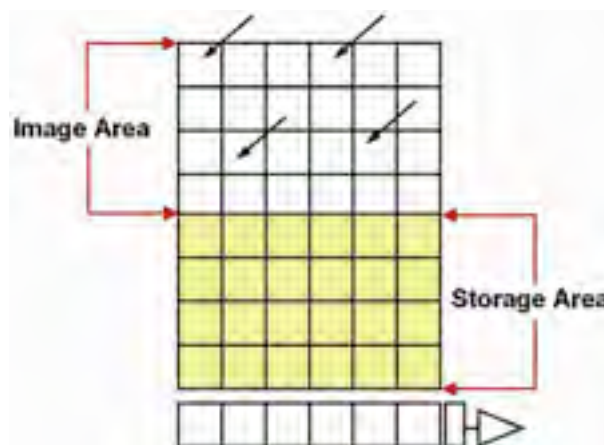


The second way in which a FT EMCCD differs from a standard EMCCD is that the Image and Storage areas can be shifted independently of each other. These differences allow a FT EMCCD to be operated in a unique mode where one image can be read out while the next image is being acquired. It also allows a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

## Noise

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

1. Pixel Noise

2. Fixed Pattern Noise

## Pixel Noise

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

- readout noise

- shot noise from the dark signal

- shot noise from the light signal itself

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

## Readout Noise

Readout noise (which in our detectors is, in any case, low) is due to the amplifier and electronics. It is independent of dark signal and signal levels and is only very slightly dependent on temperature. It is present on every read, as a result of which it sets a limit on the best achievable noise performance. Shot noise from the dark signal is dependent on the exposure time and is very dependent on the temperature; shot noise from the signal is additionally dependent on the signal level itself. If either the signal or the dark signal falls to zero, their respective shot noise also falls to zero. The total pixel noise is not, however, simply the sum of the three main noise components (readout noise, shot noise from the dark signal, and shot noise from the signal). Rather, the rms gives a reasonable approximation - **thus: total = sqrt (readnoise² + darkshot² + sigshot²)** 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

## 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.

## 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 Quantum Efficiency (QE) or **Spectral Response**.

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

## Readout

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

## 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

## Scans (Keep Clean & Acquired)

The CCD is continually being "scanned" to prevent its becoming saturated with dark current (**see Dark Signal on page 120**).

- If the Scan is being used simply to "clean" the CCD (i.e. it is a keep-clean scan), the charge from the CCD is discarded

- In an acquired scan however, the charge undergoes analog to digital conversion and is acquired into computer memory so that it can be used for subsequent processing and display: it is "read out" (see **Readout** above)

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

## 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 aluminum mask. The elements in the shift register have a greater capacity to store charge (i.e. a greater "well depth") than the other pixels on the CCD-chip.

## Shot Noise

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

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

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

## Signal To Noise Ratio

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

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

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