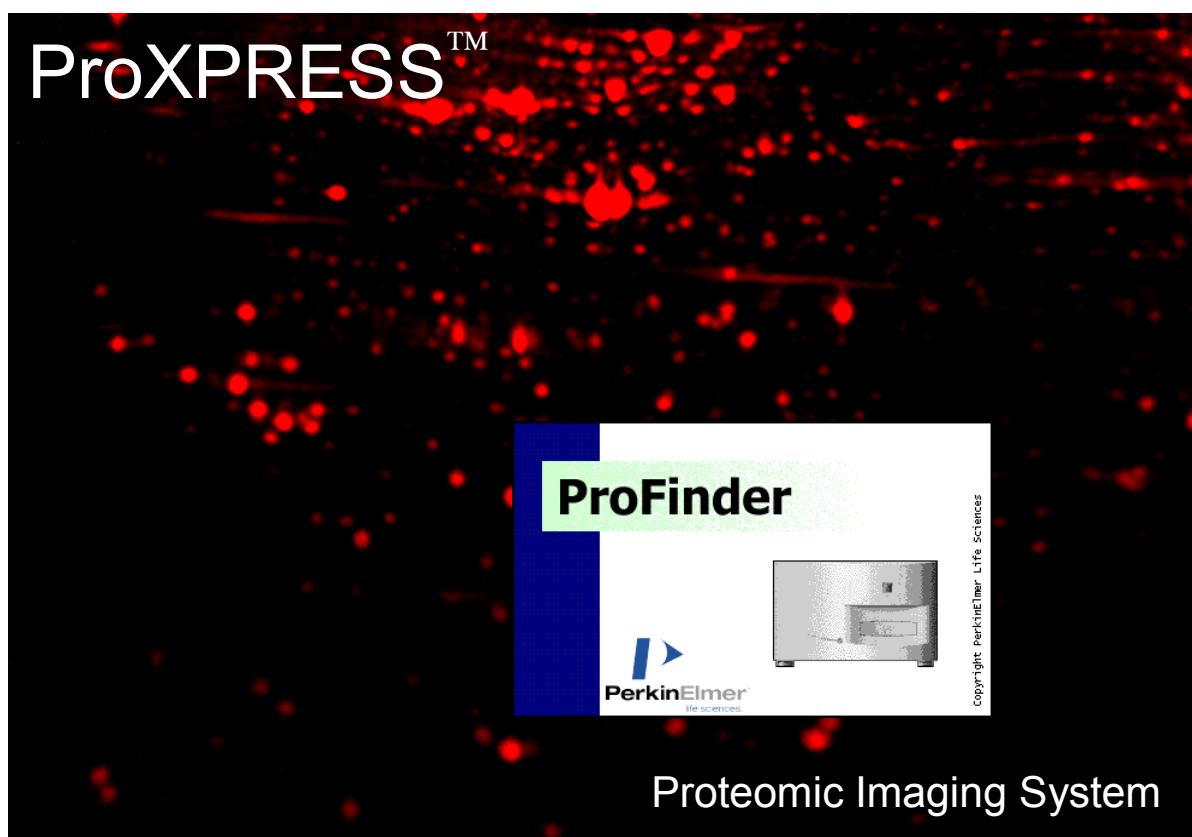


ProXPRESS™ ProFinder



USER MANUAL

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Introduction

The ProXPRESS Proteomic Imaging System is a multiwavelength fluoroimager capable of optical density and fluorescence measurements on a variety of media including gels, microscope slides, tissue culture plates and exposed film. It has a maximum scanning area of 230 x 280 mm at resolutions of up to 50 microns. Fluorescence measurements may be made using any combination of up to six software selectable excitation and emission wavelengths within the range 400 to 750 nm. It is also possible to select "No illumination" for luminescence applications and "transillumination" for applications requiring ultraviolet excitation or white light excitation. ProXPRESS is supplied with a high specification PC with Windows 95 or Windows NT software and multi-format image export capabilities.

The sample is placed horizontally on the plate holder which is automatically loaded into the instrument when a scan is requested. Adapter plates are used to locate different media samples in the focal plane of the camera. A cooled CCD camera scans the required area by capturing a number of frames, which are combined to produce an image of the full sample. Each frame is of fixed size (approx 50 x 70 mm) and the total number of frames required to scan the full sample is easily set in the software.

A high-resolution image is produced by illuminating the sample in one of the following ways:

- Below:** with an ultraviolet light source for UV excitation.
- Below:** with an ultraviolet light source and UV-white light converter plate for densitometry (transmission mode).
- Above:** with a high power xenon light source with interference filters for fluorescence measurements (top excitation mode).
- Edge:** with a high power xenon light source with interference filters for fluorescence measurements (translusion mode).
- None:** for glow-type luminescence applications.

A split tail light guide travels with the scanning head to illuminate the media from above. The arrangement concentrates the excitation light frame by frame.

The edge illumination light guide is fixed in position at the rear edge of the sample. This illumination method is especially applicable to gels where it distributes the available light between the glass plates.

In addition to simply setting the illumination method and wavelengths via the software, it is also possible to set up protocols to capture multiple images of the same sample for multi-probe fluorescence applications or multiple exposures of the same sample.

Images may then be subject to simple manipulation and analysis and either saved to disk or exported in a variety of standard file formats for more detailed analysis by other software packages.

Safety

This instrument produces internal **hazardous voltages and UV light**. Please read and obey all labels and safety notices applied to the instrument. They are there for **YOUR SAFETY**.

A warning sign on the rear panel warns to disconnect from the power supply before the rear cover is removed. In addition to the obvious risk of electric shock from hazardous voltages, there is also the additional hazard of exposure to Ultra Violet radiation.

Particular attention should be paid to potentially hazardous operations such as changing the lamp. It is especially important to allow the instrument (and lamp) to cool for at least 15 minute prior to removing the lamp for replacement. The xenon lamp used contains gas at extremely high pressure and presents an explosion hazard when removed from its lamphouse. The power supply for this lamp produces starting voltage in excess of 20 kV which is potentially lethal if not properly isolated before removing the covers.

A warning sign on the lamp- house cover is supplied to prevent inadvertent removal and exposure to these hazards:

WARNING!
UV LIGHT AND
LAMP EXPLOSION RISK
**Disconnect and allow to cool before
removing cover to change lamp**
WEAR EYE PROTECTION
SEE MANUAL FOR DETAILS

New lamps are supplied with a manufacturers safety data sheet which **MUST** be read before handling lamps.

Particular attention should however be paid to the following:

The lamp radiates **UV light** which is harmful to the eyes and skin.

Do not look at the lamp while in operation without adequate Ultra Violet eye protection.

Proper skin protection must be worn or avoid direct exposure.

The lamp reaches **very high temperatures** during operation:

Do not place flammable materials in the vicinity of the lamp while it is in operation.

Do not touch the lamp while it is operating or while it is still hot.

The lamp has very **high internal gas pressure**:

Do not subject it to shock, stress or scratches which may result in the explosion of the glass envelope.

The lamp must be correctly installed in its housing and the cover replaced before operation.

Wear a protective visor and gloves when handling an uncovered lamp.


The lamp is started by a **very high voltage** pulse:

Ensure the supply is disconnected before exposing the internal terminals.

IF IN DOUBT, call your Service Engineer or the Hotline number at the start of this manual for advice.

Terminology and controls

NOTE: Important tips or notes for special attention when using the software.

 **WARNING:** *Attention should be paid to the information contained here. Information regarding misuse or carelessness is advised.*

Keyboard combinations: A plus sign (+) used between key names indicates those keys must be pressed at the same time. For example Press **Alt+F** means you press both the **Alt** key and the **F** key at the same time.

Using the mouse: **click** - press and release the left mouse button, **double click** - press and release button twice in quick succession, **click and drag** - press button, hold whilst moving the mouse, then release.

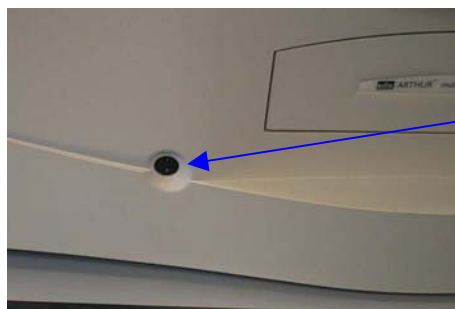
Menu commands: These are referred to in the form: Menu name/Menu command e.g. Select **File/New** and use the mouse to select the **File** menu and then the **New** function from the menu, or pressing the **Alt** key and the key corresponding to the **underlined character** in the name at the same time i.e. **Alt+F**, followed by **n**.

Operation

Instrument startup

Switch on ProXPRESS and allow it to stabilise for about 15 minutes.

NOTE; This will stabilise the Xenon /Arc lamp source. If UV excitation is required it is necessary to open the ProFinder software and take a single image using bottom illumination. This action switches on the UV-transilluminator and allows it to stabilise, before taking your sample images.



On/Off button

WARNING: when the instrument is started up the transport carrier comes out automatically. Make sure you leave space for this in front of the instrument.

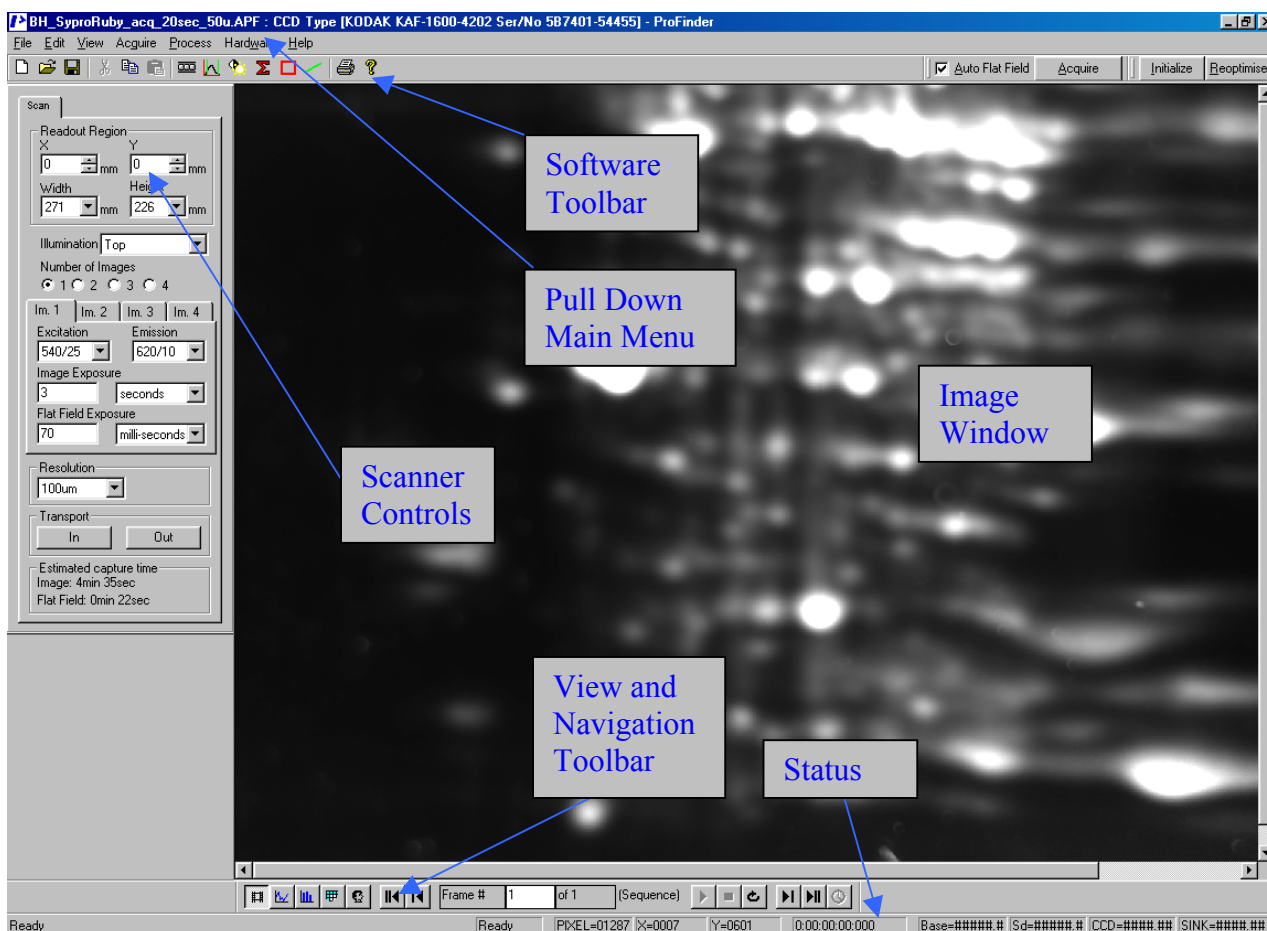


Press the green button on the side of the instrument to drive the transport carrier into the instrument. Note that you have to hold the button down all the time until the carrier is in fully in the instrument.

In normal operation the carrier is controlled from the In and Out buttons in the ProFinder acquisition software.

Software startup

Double-click on the ProXPRESS ProFinder icon on the Windows desktop, or start the program from the Windows Taskbar. The following screen will appear:

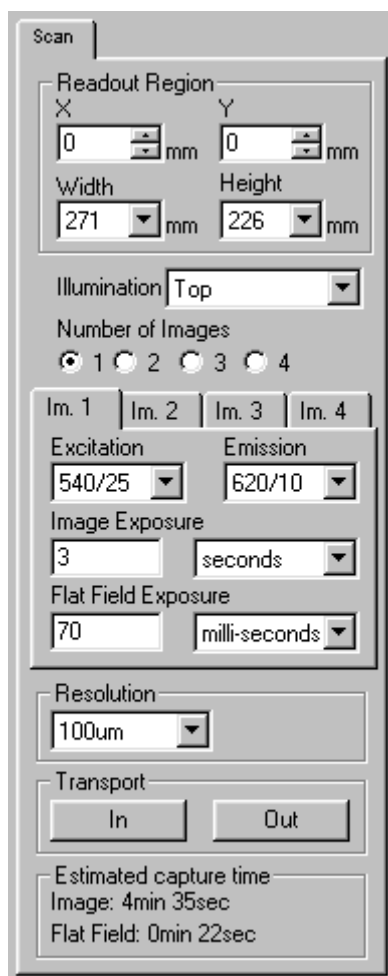


NOTE: The Software toolbar is a flexible dockable/undockable toolbar. It can be picked up and moved left, right, top or bottom, docked or left floating for your convenience. The Scan and Camera control tabs and View & navigation toolbar are also left or right dockable or can be floating.

This window can be maximised using the standard Windows™ functions, found in the top right hand corner of the screen. Click on the underline in the top right hand corner of the menu to reduce the application to the Windows™ 95 bottom menu bar. Click on the double screen to allow greater detail of the image to be observed, or click on the X to exit the application and return to the main Windows™ 95 screen.

For information about the command menus see the appendix.

Setting Scan parameters




The 'Scan' dialog box contains the following controls:

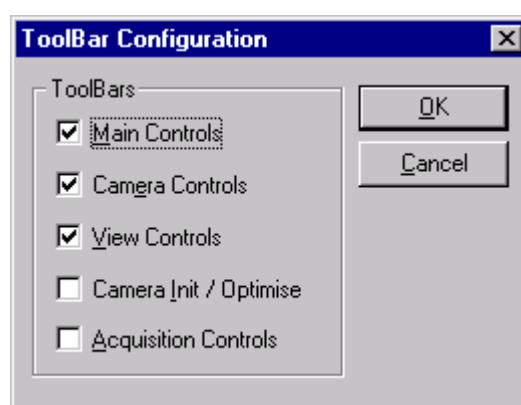
- Readout Region:** X (0 mm), Y (0 mm), Width (271 mm), Height (226 mm).
- Illumination:** Top (dropdown).
- Number of Images:** 1 (selected), 2, 3, 4 (radio buttons).
- Im. 1 | Im. 2 | Im. 3 | Im. 4:** Tabs for image selection.
- Excitation:** 540/25 (dropdown).
- Emission:** 620/10 (dropdown).
- Image Exposure:** 3 seconds (text and dropdown).
- Flat Field Exposure:** 70 milli-seconds (text and dropdown).
- Resolution:** 100um (dropdown).
- Transport:** In, Out (buttons).
- Estimated capture time:** Image: 4min 35sec, Flat Field: 0min 22sec (text area).

Before you can run a sample you must set the parameters used to control scanning. This is done with the SCAN tab.

The parameters to be set are described below.

Note: if the Scan tab is not visible select Toolbars either from the View menu or from the Toolbar icon .

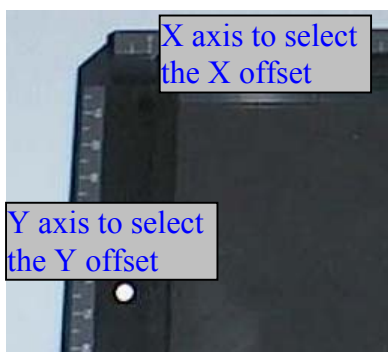
Then check the Camera Controls check box, see below.



The 'ToolBar Configuration' dialog box shows the following settings:

- ToolBars:**
 - ☒ Main Controls
 - ☒ Camera Controls
 - ☒ View Controls
 - ☐ Camera Init / Optimise
 - ☐ Acquisition Controls
- Buttons:** OK, Cancel.

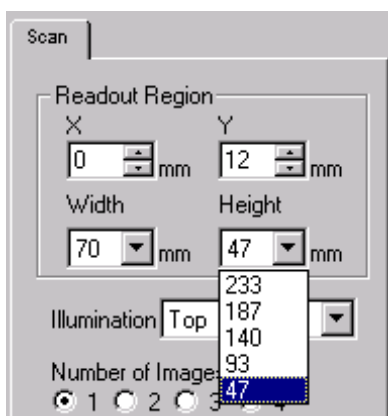
Scan size and position



Setting the X and Y offset

You can use the scroll buttons or type in the X and Y offsets for the top left-hand corner of the scan (refer to the scale on the adapter plate, see the picture).

Note: If you type a number too large for the current size, your number will be replaced automatically with the largest offset available.



Setting the width and height

Use the drop-down lists to select the image width and height.

Note: if you select a size which is too large for the current offset (X and/or Y), the largest size possible will be selected automatically. To scan the largest area possible make sure the X and Y settings are set to 0.

Scanning takes place by rows and columns where each column is approximately 50mm wide and each row 70mm high. To scan the whole area an image with 5 rows by 4 columns will be taken.

Illumination

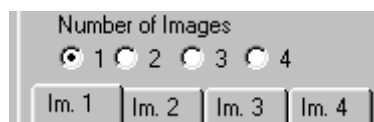


You can define how the sample will be illuminated.

There are five options:

- Top** with a high power xenon light source and interference filters for fluorescence measurements. (top excitation mode).
- Top (big FF)** Top illumination using a full image flat field image to correct for uneven illumination.
- Edge** with a high power xenon light source with interference filters for fluorescence measurements (translusion mode).
- Bottom** with an ultraviolet light source for fluorescence mode and with the UV-white light converter plate in position for densitometry (transmission mode).
- None** for luminescence applications.

Number of images

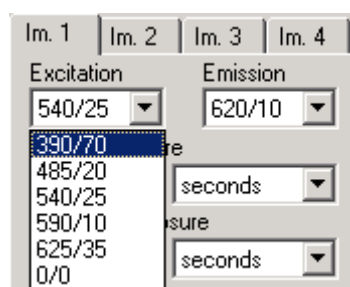


You can have multiple images of a single gel (up to 4), using different filter sets for different fluorophores. This is called multiplexing. The image tabs below allow you to select the parameters for each image.

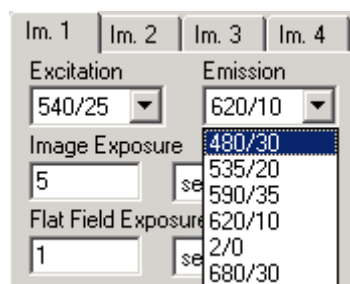
Selecting filters

Use the drop-down menu for each excitation filter and emission filter. For example, the filters for Fluorescein are: excitation filter = 485/20 (i.e. a peak at 485 nm and a bandwidth (full width at half height) of 20 nm), and emission filter = 530/25 (i.e. peak at 530 nm and a bandwidth of 25 nm):

Selecting the excitation filter

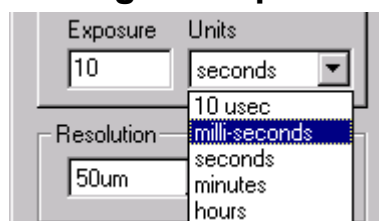


Selecting the emission filter



Note: a value of 0/0 means the position is empty, and /0 represents a long-pass filter i.e. 485/0 is a long-pass filter).

Setting the exposure time



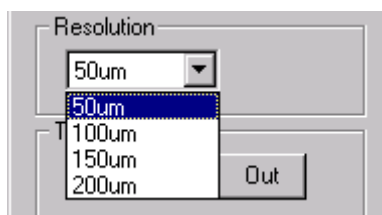
Use the drop-down menu to select the units.

Change the exposure length by editing the value in the box at the left (in this case, 10 milliseconds has been selected).

The exposure time used will depend on many factors such as the efficiency and quantity of the fluorescent probe loaded, the sensitivity of the camera to the emission wavelength and the selected resolution (degree of binning, see image resolution below).

For most gels stained with the commonly used dyes, the exposure time required is somewhere in the range 10-30 seconds. This will vary depending on the sample brightness and background.

Selecting the image resolution

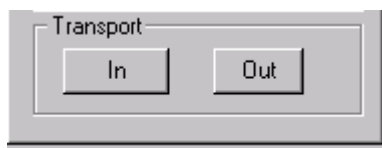


Select the desired image resolution from the drop-down menu.

Reducing the image resolution increases signal to noise and reduces the total amount of data to be stored.

For example, decreasing the resolution from 50 microns to 100 microns will increase the signal to noise by a factor of four as each four pixels on the camera are summed to produce one pixel on the image. There will also be a reduction of a factor of four in the data to be stored as there are four times less pixels displayed. This procedure is known as binning.

Transport In/Out



These two buttons "In" and "Out" respectively allow you to cause the transport carriage to be taken into or sent out from the instrument.

Normally you do not need to click the "In" button because the transport carriage is taken in automatically when scanning is to start. However, as a safety measure, the "Out" button must be clicked to get the transport carriage open.

Illumination sources

Introduction to flat-field correction

Flat-field correction is a function that is performed to correct for uneven illumination across a frame in the field of view of the camera. To correct for this uneven illumination, a uniform fluorescent sample is first imaged. The software then calculates the mathematical correction that has to be made to the actual image. This is the flat-field correction (sometimes called the shade correction). When actual samples are then imaged, the correction is applied to the image to get the final image. This image can be collected and saved in the computer for subsequent use or it can be imaged just before measuring the samples.

On deciding to acquire an image the software will prompt you to either create a new flat-field file, use a previously saved one or continue without performing the flat-field correction (for some samples eg: luminescence) no shade correction is required.

There are a number of criteria that must be met to acquire the most representative flat-field correction for your sample. The ProFinder acquisition software will guide you through the process of taking an appropriate image, however, a brief explanation of the principles are as follows:

- The image must be taken using the same resolution as your chosen sample
- The same number of images as the number in the sequence you are taking (ie 1-4)
- Preferably the same emission filter as the probe you are measuring.

The process of flat-field correction is different for the different modes of illumination.

Note: the following paragraphs describe the principle of flat-field correction for each case. Detailed information about how to load the instrument and make the measurements is given later in this manual in the chapters beginning with "Sample loading" on page 26.

Top

Top illumination involves illuminating the sample frame by frame from above. In this instance the camera moves with the illumination source. Therefore, the uneven illumination is the same in each frame. For this reason, a single frame image is taken of the uniform acrylic fluorescent sheet, provided with the instrument for use as the flat-field sample

NOTE; It is recommended that the same emission filter as the fluorophore you are measuring is used to take the flat-field measurement. For this reason 2 colours of the acrylic sheet are provided with the instrument (red and green). To avoid saturation of the camera, for emission wavelengths between 400-550 use the red sheet and for wavelengths above this use the green sheet as your flat-field sample. These sheets should be carefully looked after to avoid scratching of the surface, which will create distortions on your image. They are also prone to fading if left in the light so should be stored in a dark place or bag.

Edge

Edge illumination is used for the imaging of pre-labelled fluorescent bio-molecules within a gel, where the gel remains sandwiched between 2 low-fluorescent glass plates. Its primary application is to measure protein samples run on 2D gels which are labelled with multiple fluorescent probes (ie; 3 samples each labelled with a different fluorophore run on the same

gel) to make comparative measures of the relative amounts of the same protein spots under different conditions. This application allows the measurement of changes in protein expression levels or patterns and minimises the experimental variability inherent in running multiple 2D gels and gel-gel matching. The gel is then imaged sequentially on each appropriate wavelength. The relative positions and therefore illumination of these spots are the same between gels. For this application no flat-field correction is required.

Bottom

Bottom illumination is used for the measurement of either fluorescent samples excitable by UV or for the measurement of colourimetric samples such as silver and Coomassie® stained gels, using the UV-white light converter sheet.

If quantitation is required with UV excitation, then it is necessary to perform flat-field correction.

Flat-field correction must be performed when imaging colourimetric samples.

None

The “No illumination mode” is used for the measurement of luminescent samples. As there is no illumination, NO flat-field correction is required. We recommend the use of chemiluminescent kits that extend the duration of light emission from the sample (sometimes up to 24hrs). Kits that are compatible with measurement on the ProXPRESS include Supersignal (Pierce Chemical, Rockford IL, USA) ECL Plus (Amersham Pharmacia Biotech) and NEN kits.

Criteria for a good flat-field image

The software intuitively ascertains if the flat-field correction image you are going to use will be suitable for your sample. However some criteria to optimise the image are as follows: The resolution of the flat-field correction image must be the same as the sample you are imaging. Aim for a mean signal of between 20,000-30,000 grey levels. You can see this in the Statistics function on the tool bar or by drawing a line across the image and looking at the plot view on the bottom tool bar of the image window (see pages xx). If you are taking multiple images (up to 4 at one time) with different filters then you need to take the same number of flat-field correction images.

When using bottom and edge illumination the height and width settings and x any start co-ordinates of the flat-field correction have to match those of your sample or it will be unable to correct the data. This is because, unlike with top illumination, the camera moves independently from the illumination source and therefore, the illumination over each frame is different and needs correcting on a frame-by-frame basis. Use the same criteria as with top illumination i.e. signal around 20 000-30 000 grey levels, same resolution and emission filter.

Guide to collecting a flat-field image for specific samples

Top illuminated fluorescent stained gels

Select Top illumination mode. Set the same resolution and emission filter as the sample you wish to measure. Use the fluorescent acrylic sheet (red or green) provided with the instrument. Place this sheet either in the wet gel cassette or in between 2 glass plates and place in the instrument on the **white magnetic pucks** (see page). It is preferable to set the emission filter to match the label you are measuring and use sufficient exposure to give grey levels (visualised in the statistics window) of between 20 000-30 000.

Top illuminated fluorescent stained membranes

Select Top illumination mode. Membranes used for blotting techniques have different fluorescent properties. It is recommended to achieve a representative flat-field image that a blank piece of the membrane you have selected is used in place of the fluorescent acrylic sheet. Because of the variable fluorescent properties it is important to use the **same** emission filter as the label you are measuring. The membrane should be placed between 2 low-fluorescence glass plates and mounted on the **red magnetic pucks** (see page). As above, aim to achieve a mean signal between 20,000-30,000 grey levels.

Bottom illuminated Silver and Coomassie® stained gels

Select Bottom illumination mode. Set the scanning parameters to take an identical sized image (from the same x and y start position), resolution and filter combination to your gel image using only the UV-to-white-light converter sheet on the carriage (see page 28 for the correct positioning of the converter plate in the instrument).

As a general rule for absorbance measurements the flat-field correction will require a shorter exposure time than your sample as in this application your gel sample is darker because you are measuring light absorption rather than fluorescence emission.

You should aim for a flat-field correction image with maximum grey levels between 20 000-30 000. This will probably be around 100-200 milliseconds using 100-micron resolution but may differ. The image you obtain will have a bright centre and be darker around the edges.

Examples of Imaging proteins in gels using some common stains

SYPRO Ruby

SYPRO Ruby™ binds to the basic residues of proteins (Arg, Lys and His) and to a lesser extent the hydrophobic amino acids. This dye is reported to show minimal variation of staining capability between different proteins.

Follow the labelling protocol provided with the dye. This is a simple protocol involving the submerging of the gel in the undiluted SYPRO Ruby solution (10x volume of sample i.e. 500ml for large 2D gel) for between 0.5 to 3 hours (preferably overnight) with gentle agitation protected from light. It is advised that the proteins in a 2D-gel be fixed in 7% acetic acid, 10% methanol for 30 minutes to an hour prior to staining. In a 1D gel fixing is not generally required and can result in a slight loss of sensitivity. After staining, the gels are destained for around 15-30 minutes in 7% acetic acid 10% methanol. For this step the acetic acid concentration can be reduced down to 2 %. Alternatively the gel can be rinsed in water for a couple of minutes.

NOTE: To minimise the background levels and improve the signal to noise optimisation of the duration of the destaining step may be required.

NOTE: Prolonged washing in water will result in a reduction in sensitivity as SYPRO Ruby™ fluorescence is affected by pH. An acidic pH is required for maximum fluorescence.

Place the gel into the wet gel cassette to prevent drying and mount in the instrument using the white magnetic pucks (if your sample is 075-1mm thick) and image using Top illumination.

NOTE: UV transillumination can also be used for typical scans but it may result in bleaching of your sample after prolonged exposure (20-30 minutes).

Gels should be protected from light during staining to prevent bleaching of the dye.

The manufacturers (Molecular Probes Inc, Oregon USA) do not recommend multiple usage of the SYPRO Ruby dye.

Other SYPRO post labelled protein gels

SYPRO Orange™ and SYPRO Red™ dyes bind to the SDS micelles surrounding each protein during electrophoresis.

Follow the labelling protocol provided by the supplier (Molecular Probes Inc, Oregon USA). This is a simple protocol involving fixing (optional) and then submerging the gel in the stain (diluted 1:5000 in 7.5% acetic acid) for 1 hour with gentle agitation.

After a short wash (a few minutes) in either water or 7.5% acetic acid, the gel is placed in the wet gel cassette to prevent drying and imaged using Top illumination. Prolonged destaining will wash the SDS out of the gel resulting in a loss of sensitivity. For best results, the duration of the washing step may be optimised.

NOTE: it is recommended that gels be protected from light during staining to prevent bleaching of the dye. The nature of these dyes is such that the gels can appear slightly speckled with fluorescent dust-like particles. Filtering of all solutions may reduce this dust. The dust will also be more apparent on UV excitation.

The process of imaging these stains is the same as with Sypro Ruby described above, using the appropriate filter combination for the dye and imaging using top illumination

Silver and Coomassie® Blue stained protein gels

The principle of imaging these gels is different from imaging fluorescent gels because absorbance is being measured rather than emission.

What you are trying to achieve is a good contrast between the background and spots. The background (areas of gels where no spots are detectable) will therefore be brighter than the spots. It is important that you do not allow this background area to saturate the camera. Spots in this case will appear as troughs in the line profile as opposed to peaks as with fluorescence.

PROXPRESS™ can provide images within the range of 2.0-2.5 OD units.

For absorbant dye measurements, i.e. silver and Coomassie® Blue stained gels, it is best to use bottom illumination with the Perspex UV-to-white-light converter plate.

NOTE: this plate is an accessory part for ProXPRESS and has to be ordered separately.

Both stains are imaged using Bottom illumination with the UV-to-white-light converter plate placed under the black sample tray with the square open window.

This white phosphorescent perspex sheet effectively converts the UV light to white/blue light. This provides good even illumination over the sample and when flat-field corrected, provides images with good contrast.

NOTE: a green emission filter (530 nm) should not be used because this saturates the camera using UV illumination. For visualisation of Coomassie stained gels use either a 680nm emission filter or an ND/3 filter. A neutral density ND/3 emission filter is used for silver stained gels.

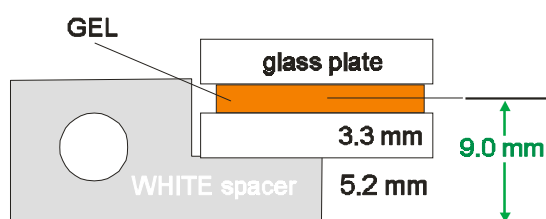
If the UV-white light converter plate is not available, these gels can be visualised using Top illumination in reflectance mode. This is performed by placing a sheet of white paper under the gel and performing a flat-field correction using the paper or by placing a piece of white paper placed directly over the UV light box. Neither method is recommended for routine use.

Sample loading

Use of spacers

The *height of the sample is crucial* due to the fixed focus position and stitching of the individual frames to create the large format high-resolution images. Spacers to adjust the height are provided. Those with red spots are mainly for flat-field samples and with white spots for 1 mm thick gel samples. This information is valid if you use the 3.3 mm glass plates provided with ProXPRESS.

The spacers used will depend on the thickness of your sample. *It is essential that the height of the plane you wish to image is 9 mm \pm 0.5 mm above the black carrier plate.*

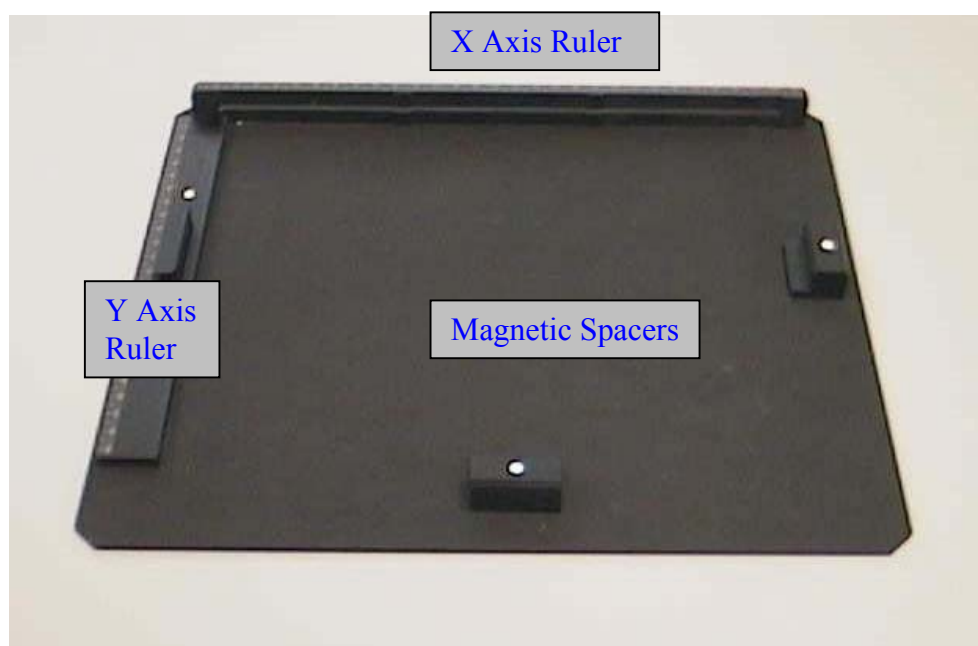


Various combinations of plate and spacer are described below. Choose the arrangement that suits your situation.

For wet gels with top illumination

The gel is mounted in a glass cassette called the "wet gel cassette".

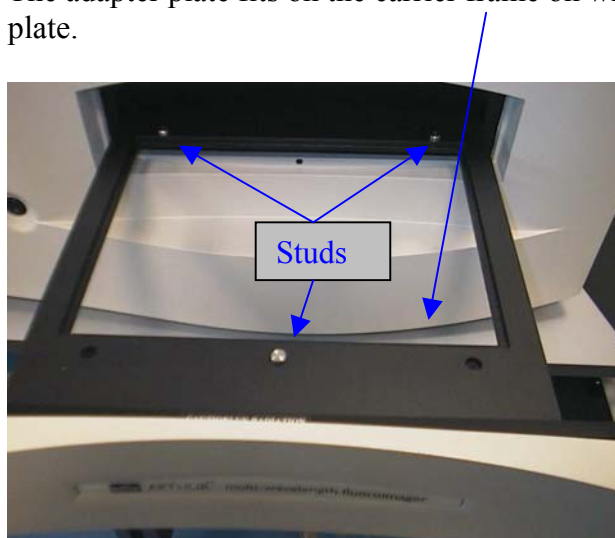
The sample carrier plate is a metal plate on which the wet gel cassette with the gel in it is placed. The correct height is determined by magnetic spacers and a Y axis ruler; each is marked with a WHITE dot, see the picture below.



The picture below shows a wet gel cassette loaded on the adapter plate.



The adapter plate fits on the carrier frame on which there are three studs to locate the adapter plate.



On the back of the adapter plate are three recesses which fit over the studs. It is easiest to load the adapter plate at an angle so that you can see under it to locate the rear studs and then lower it to locate the third stud at the front.

For flat-field correction of Top illuminated gels

A fluorescent acrylic plate is used for the flat-field correction. This sample is placed in the wet gel cassette or between 2 glass plates and mounted on the white pucks.

For edge illuminated samples

The light is channelled through the slit at the back of the sample carriage plate. The gel, still between the glass plates* is then mounted on the white pucks. It is important that the gel is abutted against the slit for maximal excitation. If you do a flat-field correction then the same spacers (white spots) can be used.

* Pyrex low fluorescence glass plates are recommended to give optimal sensitivity.

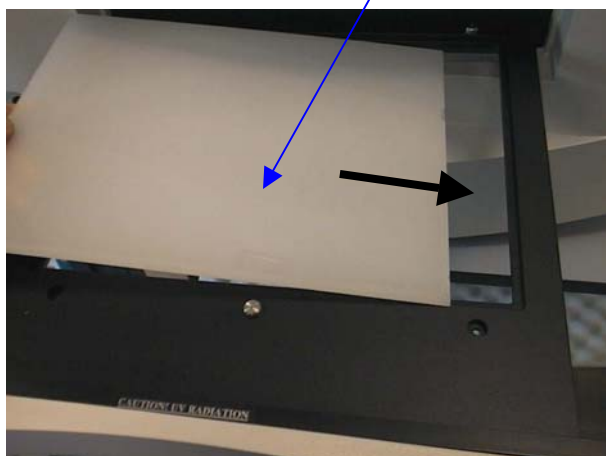
For bottom illuminated samples with UV light

An open sample carrier plate is used. The sample is mounted with the magnetic spacers as with the closed sample carrier plate described above.

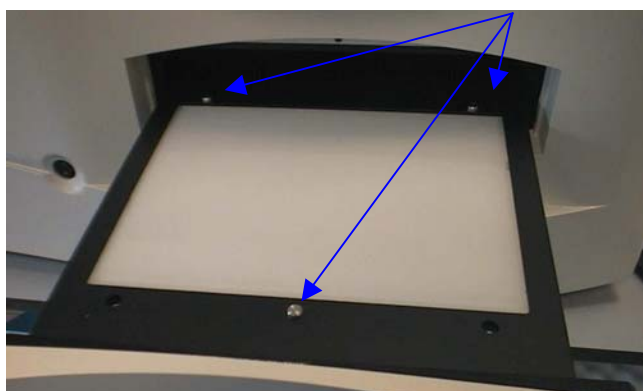


For bottom illuminated samples with white light

Remove any sample carrier plate from the transport carrier and place the UV-to-white light converter plate, shiny side up, in the recess in the transport carrier.



For flat-field correction you can use this as it is. For sample imaging, use the open sample carrier plate shown on the previous page. Place it on the transport carrier. Make sure it is located on the three studs.



For microscope slides

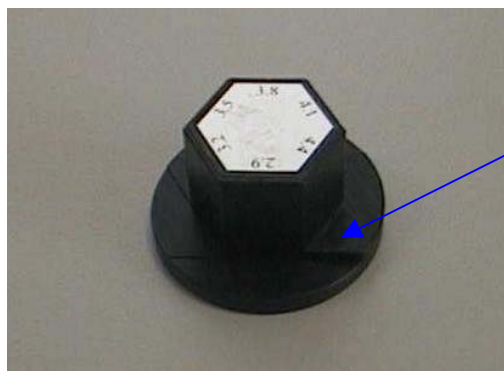
A black adapter plate is fitted on top of the normal sample carrier plate and the spacers with a red spot are used. The slide is positioned on this plate.



For non-standard samples

For non-standard samples use:

Hexagonal spacers that allow a variety of heights to be selected:



Beveled edges allow different heights to be selected by turning the spacer.

The following figure shows the hexagonal spacers in use. It is important that the same setting is used for each of the three spacers so that the sample is level. Note that there is no Y axis ruler but instead a third hexagonal spacer is used.

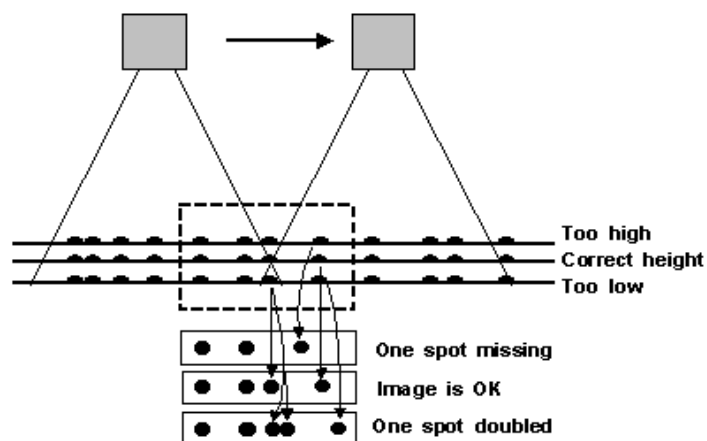


You can check the correct height by eye. The centre of the sample should be in line with the slit at the back of the sample carrier plate. This slit is exactly 9mm above the plate.

For most work with standard thickness (2.8mm and 3.3mm) glass 2D gel plates the normal red and white spacers are accurate.

The technical note following shows what happens if the sample is not at the correct focus height.

Technical note –the correct sample height is crucial



The camera moves a predefined distance, which is defined by the pixel sizes in the 'scanner.ini' file. If the sample is positioned too high up there will be a gap between each frame and parts of the image will be missed. If the sample is too low there will be an overlap between frames and part of the sample will be imaged twice.

Parameters for dyes commonly used in ProXPRESS

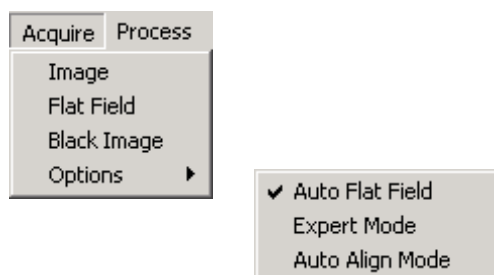
Dye	Excitation	Emission	Illumination	Exposure*
MBB	390	480	Edge	1-60s
SYPRO Orange	480	590	Top	1-30s
SYPRO Red	540	620	Top	1-30s
SYPRO Ruby	450-480 or UV	620	Top (480ex) or UV trans-illumination	100ms-30s
SYBR Green	480	530	Top	1-2s acrylamide gel 10-15s agarose gel
Coomassie	0/0	680 or ND/3	Bottom with light converter	2-5s depending on darkness of sample
Silver	0/0	ND/3	As above	100ms -5s depending on darkness of sample
EtBr	UV or 520	605	Bottom (UV trans-illumination) or Top with 520ex	1-2s acrylamide gel 10-15s agarose gel

*Exposure times are given as a rough guide only. On obtaining an image check the actual pixel values. If the image is saturated (65536 = 16 bit) then decrease the exposure appropriately. The exposure required will depend on the intensity of the sample. Use these values as a starting point and work from there. Except where stated otherwise these exposure times are based on images at 100 µm resolution.

Starting the scan

When you have loaded the plate and set the Scan parameters you are ready to start the scan.

Note: in order to get good results from a sample scan you must have a suitable flat-field correction reference image. This will either be a correction just obtained using the current scan parameters or it will be a correction obtained earlier which you have selected from the memory. Flat-field corrections have the extension ".aff". The software can remind you to have a flat-field correction ready, make sure Auto Flat-field is checked see below.



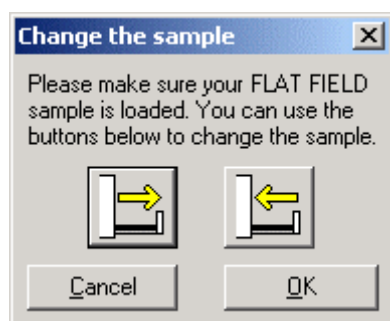
This is the same as selecting the Enable Auto Flat-field item in the Acquire menu on the right hand side of the screen.

Click on Acquire and you will be given information about the current flat field image loaded or prompted to obtain a new one.



Acquiring a flat-field image

You must have a flat-field sample loaded. Click OK and you will be given the opportunity to open the drawer to load a flat field sample. Follow the on screen menus until you have a suitable flat field image loaded.

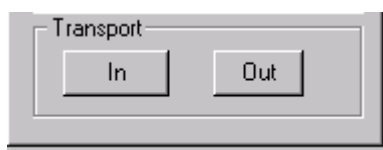


Scanning

Click OK and the transport carriage will be taken in (if it had not been sent in by clicking the icons on the menu), the instrument will be initialized and the scan performed with the current scan parameters.

Note: the first time this is done after the software is run will cause the camera to be initialized as well. This process may take a minute or two.

A dialogue box in the centre of the screen will display "Acquiring images" and a process indicator control will display the progress. When all the frames have been captured, the system resets and displays the full image on the screen.



When the scanning has been completed the sample may be unloaded immediately by clicking the "Out" button in the Transport section of the SCAN tab.

Note: plates are not ejected automatically. You must click the "Out" button.

Estimated times for capturing flat field and sample images are displayed below the transport control buttons.

Viewing results

When you have obtained an image, there are some parameters which you may wish to check prior to exporting the image for detailed analysis. These are described later in this chapter. There are also other data handling functions available with the ProXPRESS ProFinder software which will probably not be used in routine work. These are described in the appendix at the end of this manual.

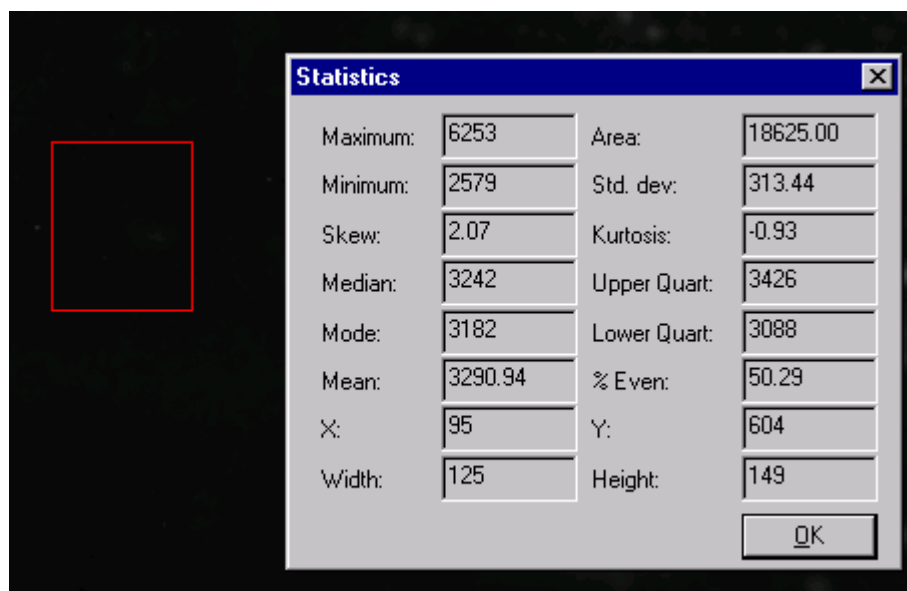
Checking signal intensities

If only a small part of the 65536 grey scale range has been used it would be better to use a longer exposure time to maximise the dynamic range.

Statistics	
Maximum:	65435
Minimum:	43

You can check the range used by means of the statistical view, see page 56. If no rectangular area (see page 51) is selected then the whole maximum and minimum from the whole range is shown.

Checking background

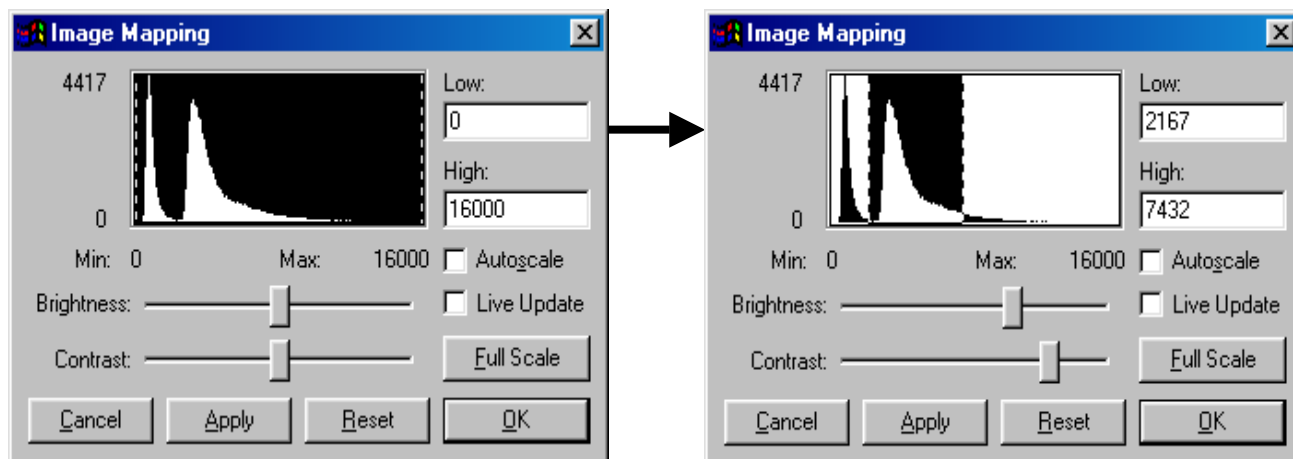


Statistics			
Maximum:	6253	Area:	18625.00
Minimum:	2579	Std. dev:	313.44
Skew:	2.07	Kurtosis:	-0.93
Median:	3242	Upper Quart:	3426
Mode:	3182	Lower Quart:	3088
Mean:	3290.94	% Even:	50.29
X:	95	Y:	604
Width:	125	Height:	149
OK			

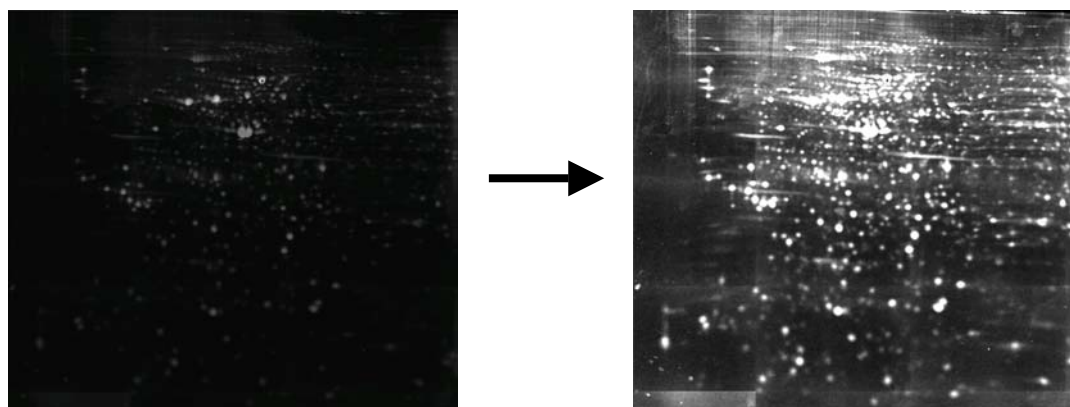
If you draw a rectangle on a dark area, you can then use the same statistics function as above to check the background. If the minimum is too high, you could try de-staining your sample for a longer time and then imaging again.

Adjusting grey scales

You can use the Mapping function, see page 48, to adjust the maximum and minimum grey scales to help you to see the image better.

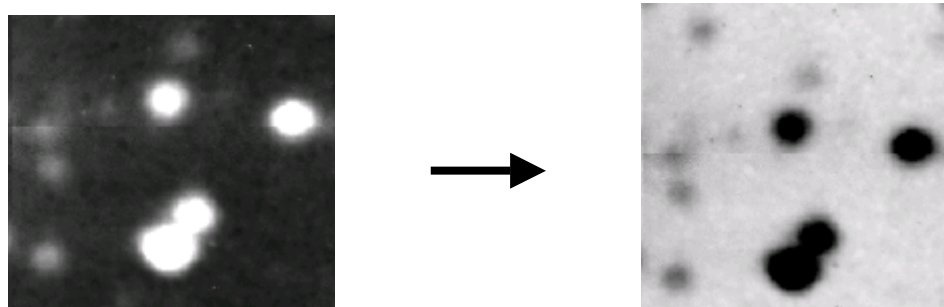


Note: this function has no effect on the raw data it simply helps the visualization of the image on the screen. See **Mapping** later.




Inverting the image

If you prefer to view the image as dark spots or bands on a lighter background you can do this with the Invert sub-item in the View menu/ Look up tables, see page xx.



These functions are now described in more detail.

Image view

The contents of the Image view can be selected by clicking  Image View in the View controls toolbar at the bottom of the window (or by selecting the item from the View menu). A tick mark will indicate which mode is chosen.



When selected, the button on the View controls toolbar will depress on the bottom left corner of the display area.

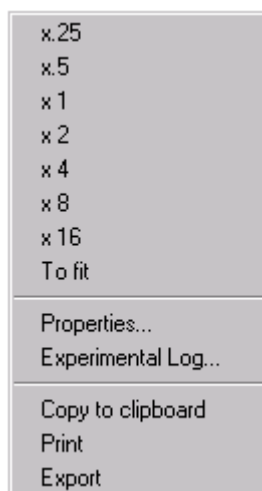
Image view shows the actual flat-field corrected image in the main window. There can be a sequence of images if you chose to have more than one scanned.

If the image displayed is larger than the window, use the window scrollbars located on the right hand and lower side of the main window to see the rest of the image.

The Status bar (bottom right-hand) shows the current cursor position and pixel intensity when the cursor is moved within the image.

Zooming

Clicking the right hand mouse button will open an option box which allows access to: Zooming in and out, Properties, Copy to the clipboard, Print and Export.



Select the required magnification.

x1 means the image is the same size as the original. Other settings magnify or reduce the image.

To fit allows the whole image to be displayed at the maximum possible magnification.

Clicking on the chosen option will change the image and close the zoom in/out option box. To close the zoom in/out option box without selecting an option, click the left mouse button outside the option box.


Properties

The properties dialogue box provides information on the sequence title, when the image was created and the image size details. The current line selection and current rectangle selection are accessible for checking or changing. Check boxes allow vertical or horizontal line selections to be made. Data may be typed as required.

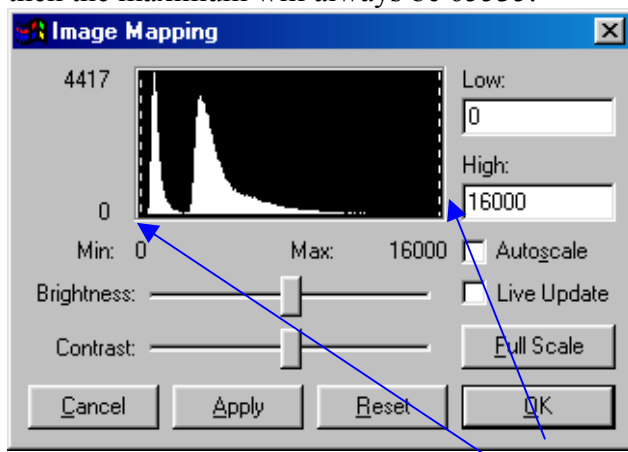
Output items

The last three items in the menu allow you to copy the area to the clipboard, print the image and export the image to external software.

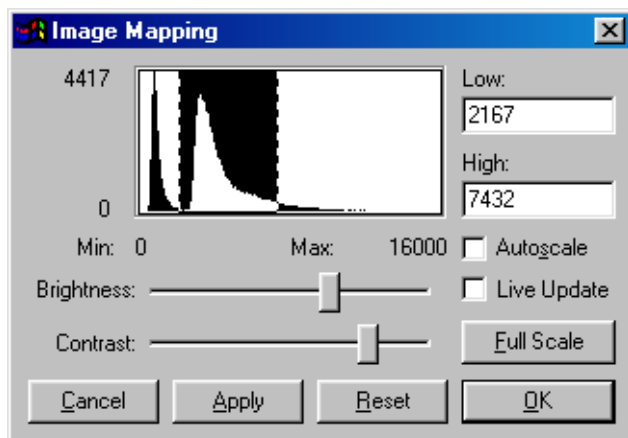
Mapping – Improving the contrast of the image

Select View/Mapping from the main menu or use the mapping icon  on the software toolbar to adjust contrast and brightness.

A mapping is a histogram of the frequency of occurrence of each grey level (pixel intensity) within the image. The picture below shows an example when Autoscale is selected. In this case the actual maximum grey scale value will depend on the image. If Full Scale is selected, then the maximum will always be 65535.

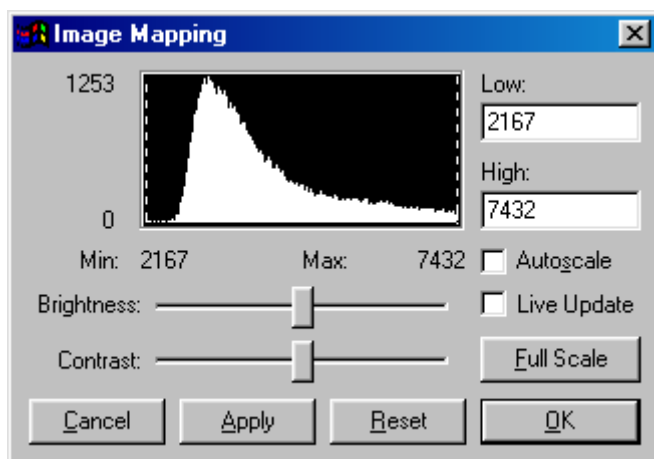


On either side of the histogram there are dashed lines showing the limits of the current range of pixels displayed. These limits can be dragged with the mouse to new settings (or you can type in the new values for Low and High). The position of the slide bars for Brightness and Contrast will also change. Alternatively you can change settings by moving the slider or clicking the arrow buttons either end of the slide scale.



The example below shows the range of grey scales set to include the main part of the histogram.


Any changes made can be applied by clicking the Apply button. The histogram is expanded to fill the new window.




The image appears a lot brighter as shown in the pictures on page 43.

The "Reset" button will cancel any changes made and revert to the original AutoScale image values.

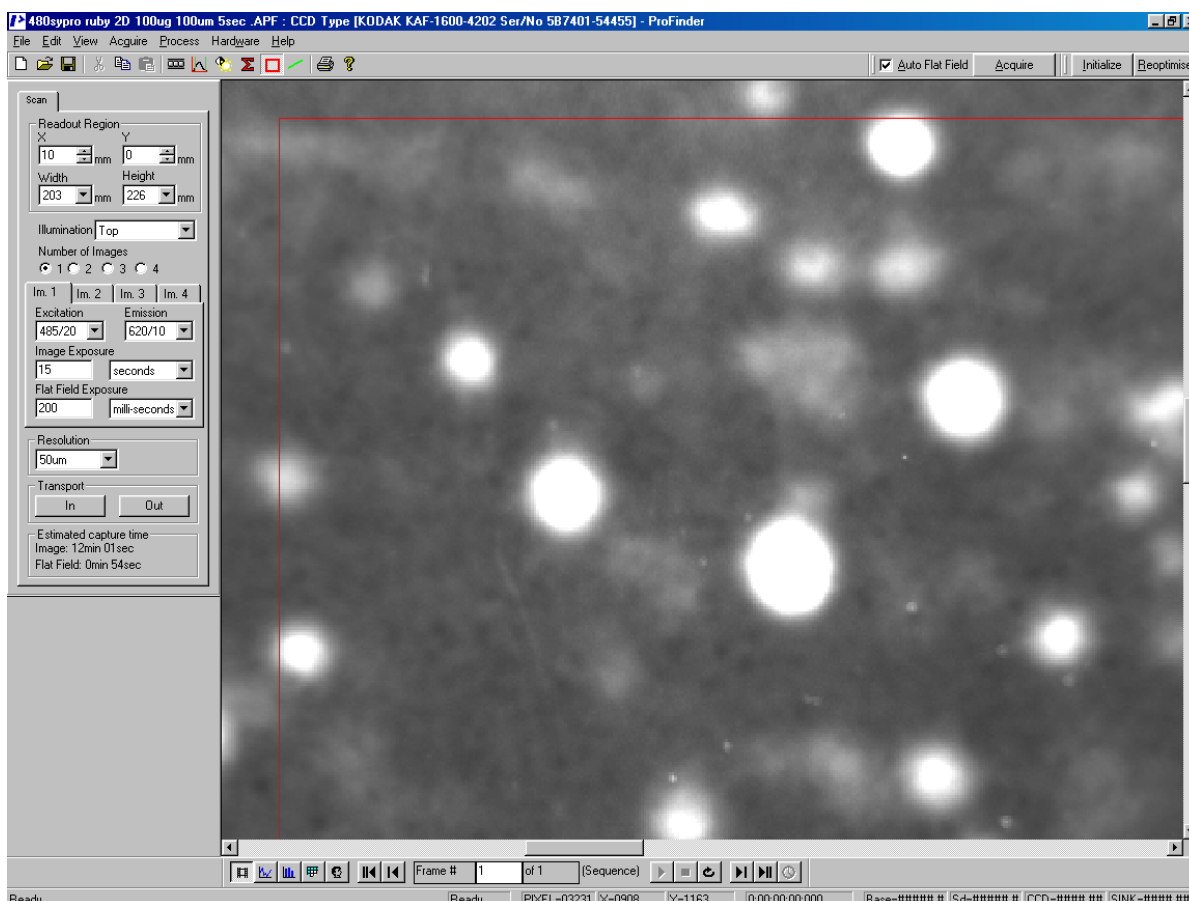
Mapping is only available when the Image view is selected.

 **WARNING:** ProFinder is a multi-tasking program. The mapping functions are used by the data capture procedures. If you also use the mapping functions during data acquisition, serious confusion can occur. *It is best to only adjust mapping once data acquisition is complete.*

Selecting an area

Click the  icon to allow you to select an area of interest for viewing. There are two ways to do this:


1. By positioning the cursor at a suitable point on the image and pressing and holding the **Ctrl**-key while dragging the mouse with the left button pressed a rectangle will be drawn about the desired area. When you release the left button, the area outlined in red will move as close to the centre of the window as possible keeping the same magnification as the whole image. If you increase the magnification of the whole image as described above, this will also increase the magnification of the selected rectangle.
2. By positioning the cursor at a suitable point on the image and pressing and holding the **Shift**-key while dragging the mouse with the left button pressed a rectangle will be drawn about the desired area. When you release the left button, the area outlined in red will expand to fill as much of the window as possible up to a x16 magnification. This is a "zoom-in" function.

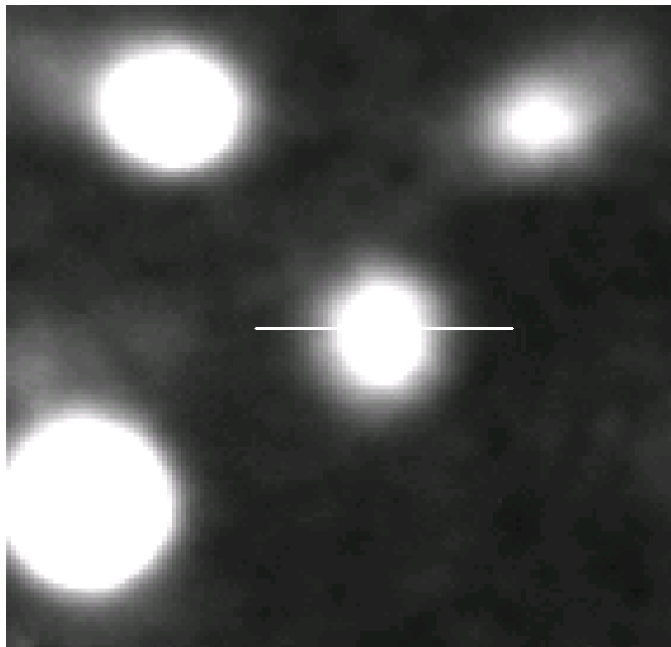


In the figure the same rectangular area has been selected as in the previous figure but now it is magnified.

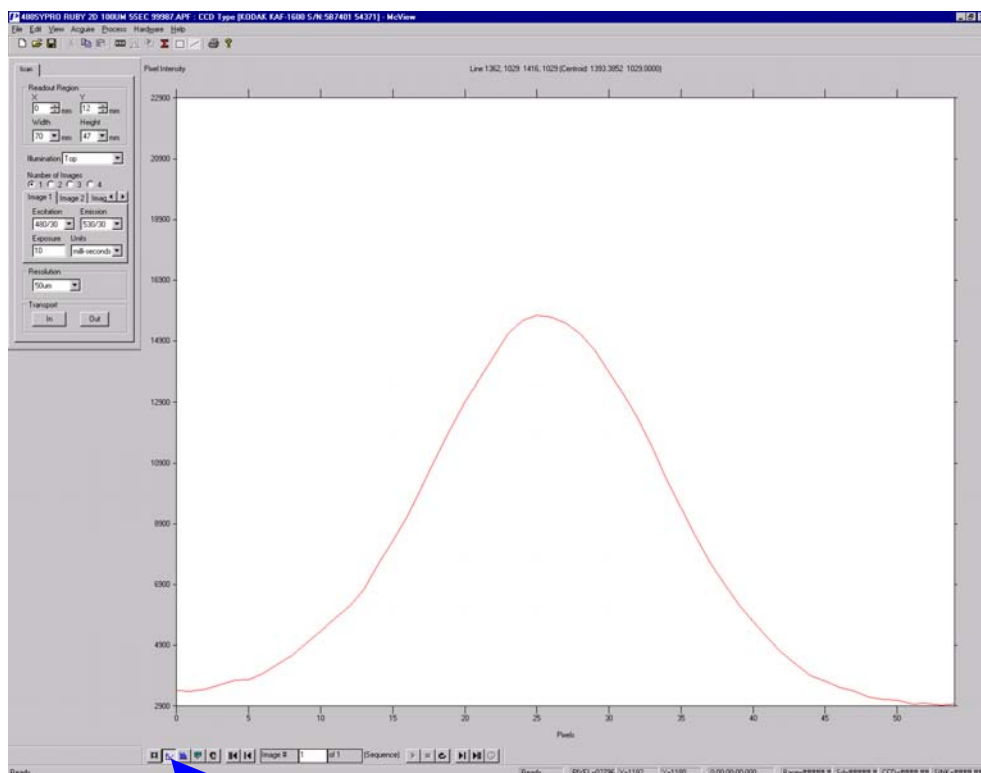
To revert to the original full image, click the right hand mouse button to activate the zoom in/out box. Select x1 to revert back to the original sized image or choose another option from the list shown.

Selecting a line profile

Click the  icon to allow you to draw a line across an area of interest for viewing. Within the image view, press the left hand mouse button and drag to draw a line through the area you want to get a plot of, see the next page.




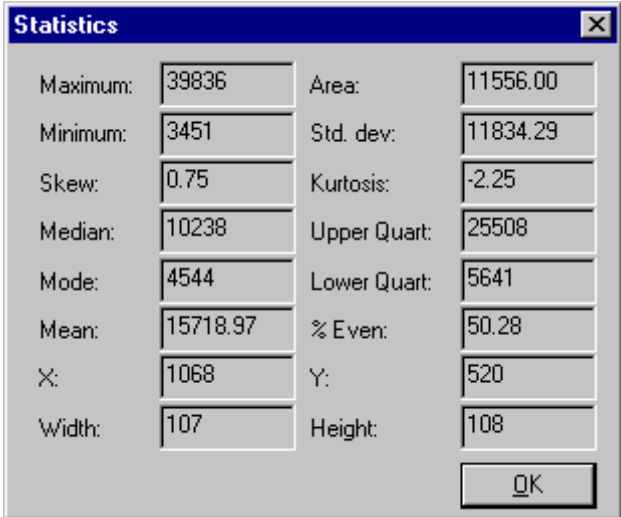
Plot view



To display a pixel plot of the image, select the plot icon. The icon will be activated. Alternatively, click on View/Plot from the main menu. The main window will show a line graph with values corresponding to the grey level pixel fluctuation along the current *line* selection. Data from the Pixel values can be cut and pasted into most Windows™ compliant software for further analysis and display. Place the cursor on the plot line and the corresponding value will be displayed. To revert to the selected image click on the Image icon.

Statistics

Clicking on View/ Statistics in the main menu, or the statistics icon , will show the statistics dialogue box. This gives statistical information for pixels in the whole image if *no rectangle has been selected*. If you have at least once selected a rectangle then the statistics view will show the information for the *currently selected rectangle* even when the rectangle is not visible. If you want to get statistics for the whole image after a rectangle has once been selected you must draw a rectangle for the whole image.



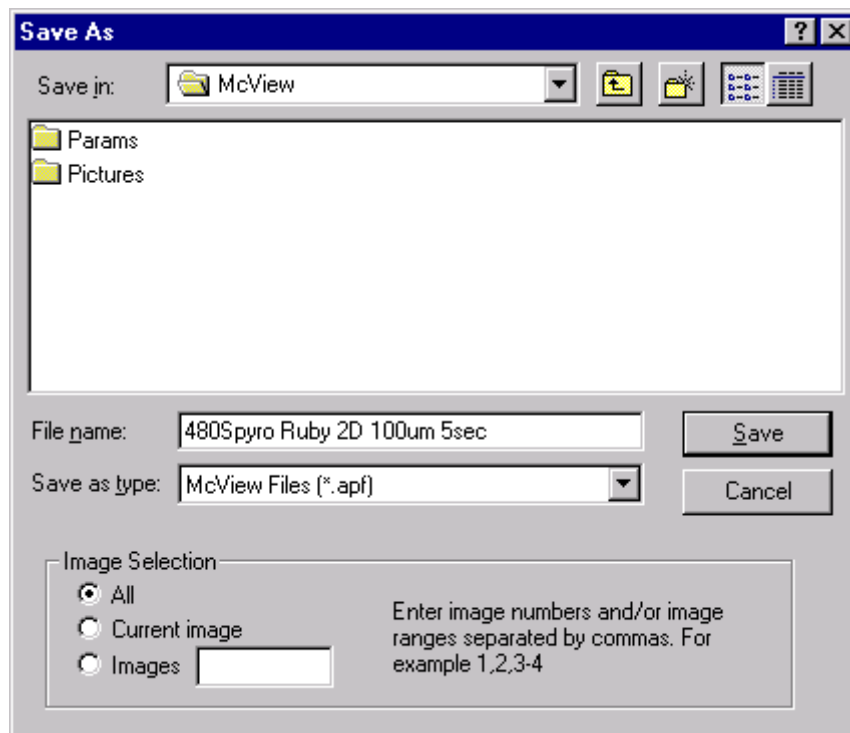
Statistics			
Maximum:	39836	Area:	11556.00
Minimum:	3451	Std. dev:	11834.29
Skew:	0.75	Kurtosis:	-2.25
Median:	10238	Upper Quart:	25508
Mode:	4544	Lower Quart:	5641
Mean:	15718.97	% Even:	50.28
X:	1068	Y:	520
Width:	107	Height:	108
OK			

The following calculated data values are shown: Maximum, Area, Minimum, Std dev, Skew, Kurtosis, Median, Upper quartile, Mode, Lower quartile, Mean, % Even, as well as the following information about the rectangular area: the X and Y co-ordinates of the origin of the rectangle (top left-hand corner) and its height and width. The units are the number of pixels). The figures are not editable in this table, but can be copied individually to the clipboard. Click OK to close the dialogue box.

Exporting results

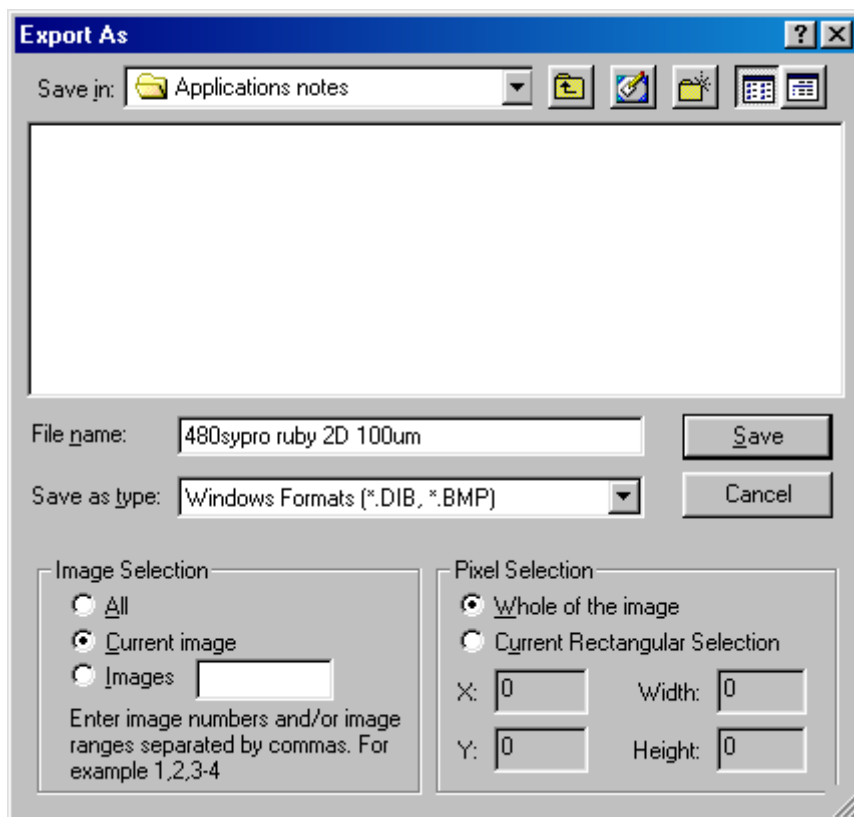
Saving the image

Once you are happy with the image it is advisable to save it in ProFinder ".apf" format. Click Save from the File menu and then give a name for the file and click the Save button.



Exporting an image

To export an image into an external image package*, proceed as follows. Click Export in the File menu. A dialogue will open allowing you to define the path for the export file.



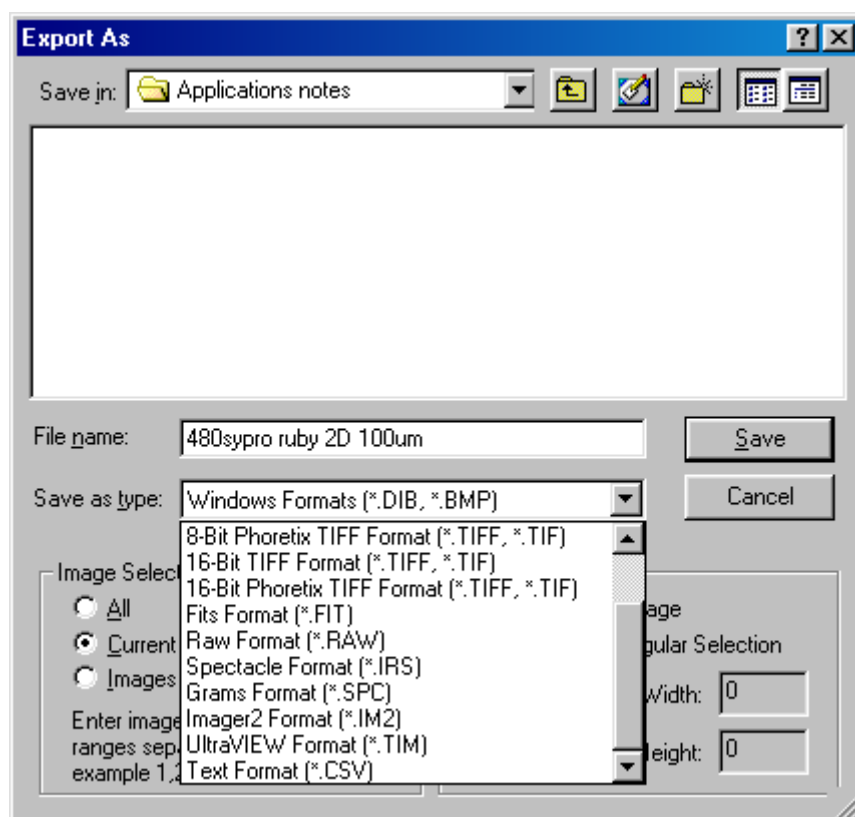
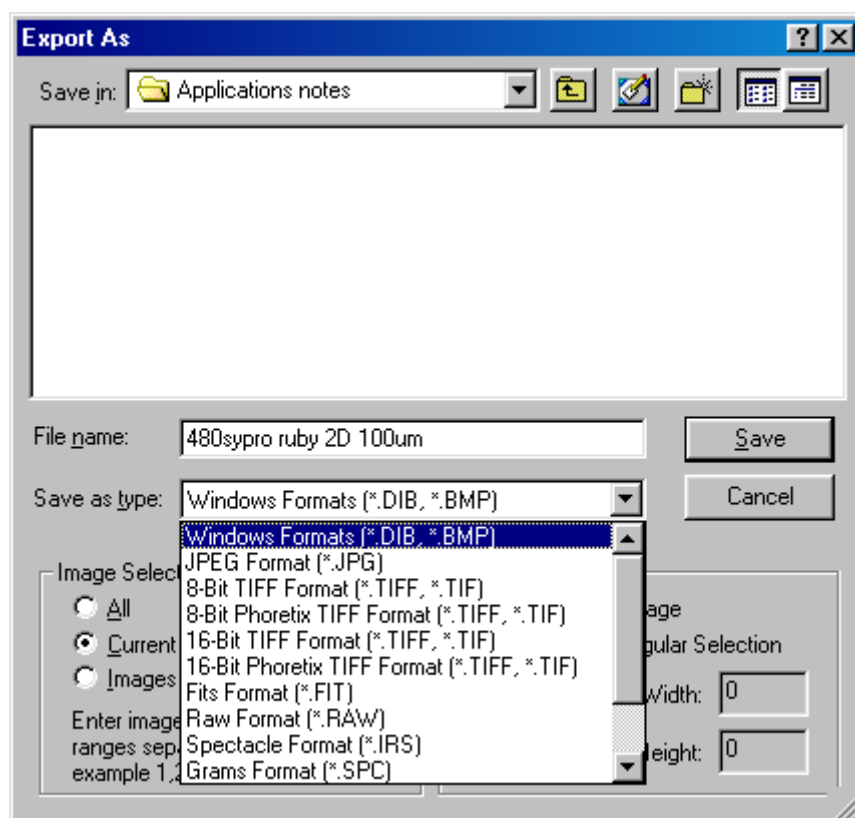
*: the recommended image analysis package is Phoretix 2D by Nonlinear Dynamics. In addition to the name you should define the following:

Image selection – if you have taken a sequence of images (up to four) you can select to export all of them, just the current one, or select from the sequence the ones you want using commas to separate individual images or a hyphen to separate the beginning and end of a range. If you select to export all four images 4 individual tiff files will be created

Pixel selection - you can select to export the whole image or just the part defined by the rectangle. The coordinates and size of the rectangle are also shown but you cannot change them (except by re-drawing the rectangle).

File format – there are several options but for general use it is recommended that you choose only 16-bit Tiff.

Save as type- select the .tiff option in the pull down menu



“Phoretix” versions of 6 and 16 bit TIFFs are grey level inverted for ease of import into Phoretix analysis software.

Troubleshooting

Problem	Probable Cause	Remedy
No Image , image area completely white or black.	Grey level mapping set too low or too high.	Set to autoscale by checking the AUTOSCALE box under the mapping icon.
No Image , image area alternate black and white stripes.	Camera saturated by too much light.	Reduce the exposure time.
No Image . Image area black.	Wrong illumination type set.	Check illumination type is correct for sample in use.
No Image . Fully white, black or stripes.	Wrong filters in use.	Check the parameters of the probe and select filters to match.
Unable to boot camera error message on screen	Camera lead disconnected or loose	Check connections and click OK . Stop scan and restart .
LOAD error returned from reader box on screen	Transport carriage obstructed.	Clear obstruction and click OK .
Park Timeout waiting for reply from reader error	RS 232 connection with instrument failed. Lead may be loose or wrong COM port in use.	Check RS 232 lead connections, and click OK . If necessary, restart software and/or restart instrument.
Park Timeout waiting for reply from reader error	Reader switched off	Power up instrument, allow to warm up and restart scan.
Low sensitivity or ripples on edge illuminated image	Sample height incorrect	Check correct spacers are in use and that the sample carrier plate is properly located on its pins.
Other problems		Call Service engineer and/or Hotline for advice. Hotline.cambridge@perkinelmer.com

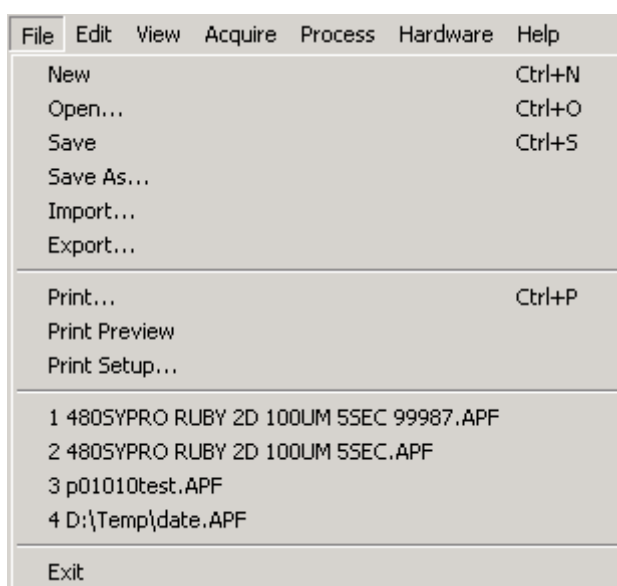
Appendix 1: Command line menus

Using the 'Pull down' menu bar

File Edit View Acquire Process Hardware Help

The Main menu bar is found at the top left area of the screen and comprises File, Edit, View, Acquire, Analyse, Hardware and Help. Use the mouse to click on the word or use the keyboard i.e. Alt+F to obtain the File option on the menu, further options are available from a drop down box. A tick mark where appropriate, indicates the option is selected.

File



New: Select the New file icon to clear the image area and start a new image. Alternatively select from the main menu File/New or Ctrl+N.

Open...: Select the Open file icon to open an existing image file held on disk. Alternatively, select from the main menu File/Open or Ctrl+O. When the Open dialogue box appears, select the image from the File name list and choose the OK button. If the image is not listed, then a search of the directories can be conducted by selecting the appropriate directory, file type and/or file name. Either double click the item or highlight the option and click the Open button to select the file.

NOTE: only .APF or .TIF files which have been saved using the Save file option can be opened with this method.

Save: To Save an image in the software internal file format, select the Save file icon. Alternatively, select from the main menu File/Save or Ctrl+S. If the image has been previously saved, all new changes will automatically be saved to the original file name and location, and the original file data will be overwritten.

Save As...: Use Save As... when saving for the first time or saving to a different file format or directory. The Save As dialogue box will appear allowing you to select a directory in which to save the file, type a file name, and select a file type. Within a sequence the images can be saved as All for the sequence as a whole, Current frame for the image currently in view on the screen, or a particular frame or frames within a sequence. Select and check against the corresponding requirement and enter the frame numbers if appropriate.

NOTE: The saved file can be in .APF format which contains additional information about how the image was collected, or in normal .TIF format.

Import...: Not currently available.

Export...: See page 58.

Print: To print the current view, click on File/Print. The shortcut action is to press Ctrl+P. Selection of the printer, print range and number of copies are available from the Print dialogue box. After selection is made, click on the OK button to print or the Cancel button to cancel the option. You may use the standard Windows™ facilities to select the page layout. In particular changing from portrait to landscape layout can be helpful.

Print Preview: Print preview is used to view the document and how it will be positioned. To access Print Preview, click on File/Print Preview. The image or sequence of images is displayed as it will be printed. You can then select the following options:

Print: to access the Print dialogue box to print the page(s) (Ctrl+P).

Next page: to move to the next page if multiple pages.

Previous page: to move to the previous page if multiple pages.

Two pages: to view two pages together on the screen.

Zoom In: to zoom in for finer detail. (Alternatively, by clicking within the page, the zoom in and out modes can be accessed automatically. The cursor will change to a magnifying glass to indicate the zoom mode)

Zoom Out: Zoom out to view whole page. (Alternatively, by clicking within the page, the zoom in and out modes can be accessed automatically. The cursor will change to a magnifying glass to indicate the zoom mode)

Close: to close Print Preview and return to the main image window.

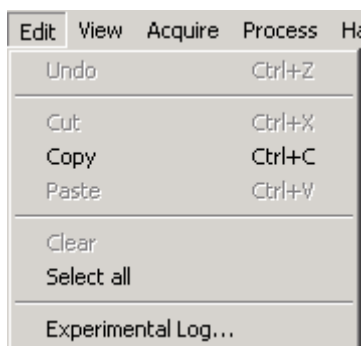
Print Setup: Print Setup gives you access to the Printer setup dialogue box and allows selection of the Printer type, type of paper and orientation. Click on **File/Print Setup** to access the Print setup dialogue box. Selection of the printers available to you in your computer configuration is available by clicking on the down arrow button. To view the Properties available to the selected printer, click on the Properties button and edit as required. To revert to the Print Setup dialogue box

click on the OK button, or to abandon any changes made in this action and revert to the Print Setup dialogue box, click on the Cancel button.

Recent file List: This area lists the recent files by name which have been opened, saved and/or changed. They are listed 1, 2, 3 etc showing the most recent as 1. This feature allows quick access to the most recently opened files.

Exit: To Exit the program, click on File/Exit. The option will automatically prompt you to save the image if not previously saved. Click on Yes to save the image, or No to Exit the program or Cancel to revert to the main image window.

Edit

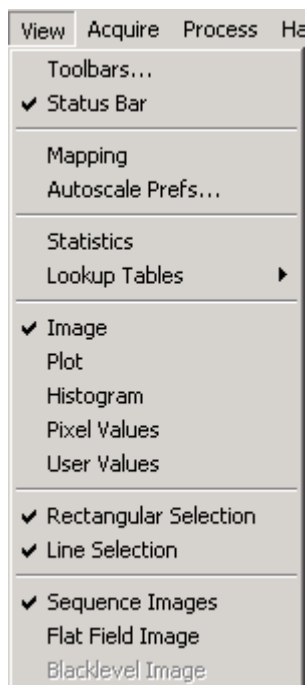


Copy: Click on Edit/Copy (Ctrl+C) or the Copy icon to copy the current rectangle selection to the clipboard for retrieval in another package e.g. Word, Excel etc.

Select all: Use Edit/Select all to set the current rectangle selection to the whole image.

Experimental Log: Allows editing and display of the experimental log associated with each image.

View



Toolbars...: The Toolbars allow one click access via icons to a variety of operations. Click on View/Toolbars.. and the Toolbar configuration dialogue box will appear. Select the toolbars to be viewed.

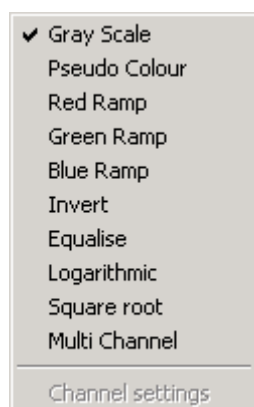
Status bar: This provides information on the camera acquisition status and the cursor position. A tick indicates that the Status bar is visible. To hide or reveal the Status bar, click on View/Status bar.

Mapping (brightness/contrast): This allows the brightness and contrast to be adjusted. Click on View/Mapping. The Image Mapping dialogue box will appear. Changes or adjustments can be made and then applied by clicking the Apply button. Autoscale preferences can be accessed by checking the Autoscale box. The Reset button will cancel any changes made and revert to the previously applied settings, see page 48.

Autoscale Prefs...: This enables the automatic selection of a number of high and low pixel grey values and adjusts the settings according to the values found. See page 81 for more information.

Statistics: Statistics provides statistical information about the image. Click on View/Statistics to view the statistics dialogue box or the statistics icon on the software toolbar. The figures are not editable in this table, but can be copied individually to the clipboard. See pages 55 and 81 for more details.

Lookup Tables: Lookup tables (LUT) can be selected by clicking View/Lookup tables.



First select the Image icon on the software toolbar to show the current image, then select the lookup table you wish to use.

A choice of Grey scale, Pseudo colour, Red ramp, Green ramp, Blue ramp, Invert, Equalise, Logarithmic or Square root, can be made by moving the cursor to the required option and clicking. A tick mark indicates which lookup table is in use.

It is also possible to merge either two or three images in a sequence and display them as particular colours. Use channel settings to change the colour for each image.

Use the First/Last/Next/Previous/Frame #/Play and Stop buttons on the view & navigation toolbar to move from one image to another in the sequence.

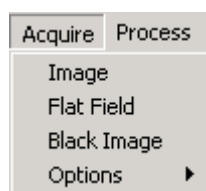
Image, Plot, Histogram, Pixel values, User values: You can switch from one screen to another by clicking on View, and either image (page 45), plot (page 54), histogram (page xx), pixel values (page xx) or user values (page xx). A tick mark will indicate which mode is chosen and the main image window will change accordingly. Also, the appropriate button on the View controls toolbar will be activated on the bottom left corner of the display area.

Sequence Images: Views the images captured from the sequence camera controls.

Flat-field image: The Flat-field image function corrects for non-uniform illumination or sensitivity. Select View/ Flat-field image to display the image. You can use the standard viewing techniques e.g. plot, image, histogram, statistics, mapping etc. with the Flat-field image. This Flat-field image will be applied to all images taken within this particular sequence. The Flat-field image is obtained using the Acquire facility in the Main menu toolbar.

NOTE: this function is disabled until Flat-field images are taken using Acquire/Flat-field reference, see page 37.

Acquire

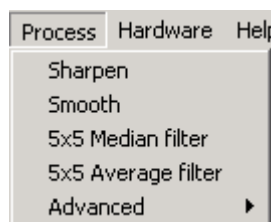


Enable Auto Flat-field: if this is ticked it means that a sample image cannot be acquired if a flat-field image is not currently selected. A flat-field image may be obtained either by clicking the **Flat-field** Corr. (Share Corr.) button or by recalling a stored image recalled from disk.

Acquire: takes a dark current corrected image and automatically performs flat-field correction. This facility is also available on the software toolbar.

Flat Fijeld : The Flat-field (Shade Corr.) function captures a Flat-field image using the current scan parameters. This allows the user to correct non-uniform illumination or sensitivity. Select View/ Flat-field image to display the image. Standard review techniques can then be applied i.e. plot, image, histogram, statistics, mapping etc. This image will be used as a reference for all images taken within this particular sequence.

Process



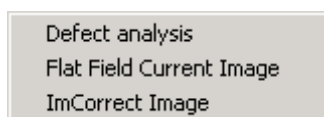
Sharpen: Invokes the sharpening filter and improves the edges of the image

Smooth: Invokes the smoothing filter and softens the image

5x5 Median filter: Removes high frequency changes in the image

5x5 Average filter: Alternative method of removing high frequency changes

Process Advanced

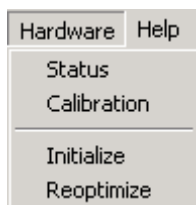


Defect Analysis: Locates pixels below a threshold value

Flat-field Correct Current Image: Performs a manual flat-field correction using the flat-field image loaded.

ImCorrect Image: automatically corrects shading and alignment of image frames

Hardware



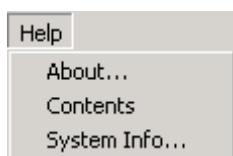
Status: Gives the current camera in the Camera hardware dialogue box. Hardware status, Recorder, Voltages/Temperatures and AT-Link configuration give information about the Camera setup and allows access to AT-Link configuration. Click on OK to accept any changes made and to exit the Camera Hardware dialogue box and return to the main image window.

Calibration: This is the pixel size calibration wizard used to recalculate the pixel size using an image if the calibration plate supplied with the instrument.

Initialize: This feature can be used to force initialization, for example if the camera was turned off accidentally.

Reoptimize: This feature enables the camera to be reoptimized if the CCD temperature has changed more than a few degrees since the last reoptimization or initialisation. If there is any evidence that the image quality has degraded because the CCD has not reached its operating temperature then reoptimization may help. NOTE: each time a new Acquire operation is started, the camera is automatically reoptimized, so simply stopping and starting will also reoptimize the camera hardware.

Help



About ProFinder gives information regarding physical memory and disk space available together with **ProFinder** version number and CCD serial number. Click on Help/About **ProFinder**.. the About **ProFinder** dialogue box will appear stating information.

Click the OK button or the exit icon in top right hand corner of the box to return to the main window.

Contents: Contents holds the on line Help to assist you with the **ProFinder** package. Click on Help/Contents to access a version of this manual in electronic form.

System Info...: Gives standard Microsoft Windows system information.

Appendix 2: Using the Software toolbar



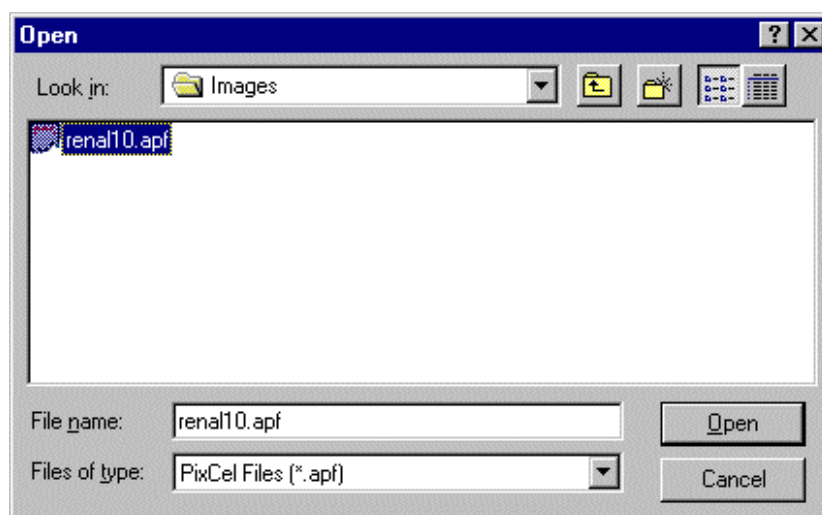
The Toolbar is located along the top left area of the screen. This is a dockable toolbar allowing it to be moved left, right, top or bottom, docked or left floating. It can also be switched off and removed from the screen by accessing View/Toolbars option in the main menu. The toolbar has a range of icons that are explained below in detail from left to right:



New file: Select the New file icon to clear the image area to start a new image. Alternatively select from the main menu File/New or Ctrl+N.



Open file: Select the Open file icon to open an existing image file held on disk. Alternatively, select from the main menu File/Open or Ctrl+O.

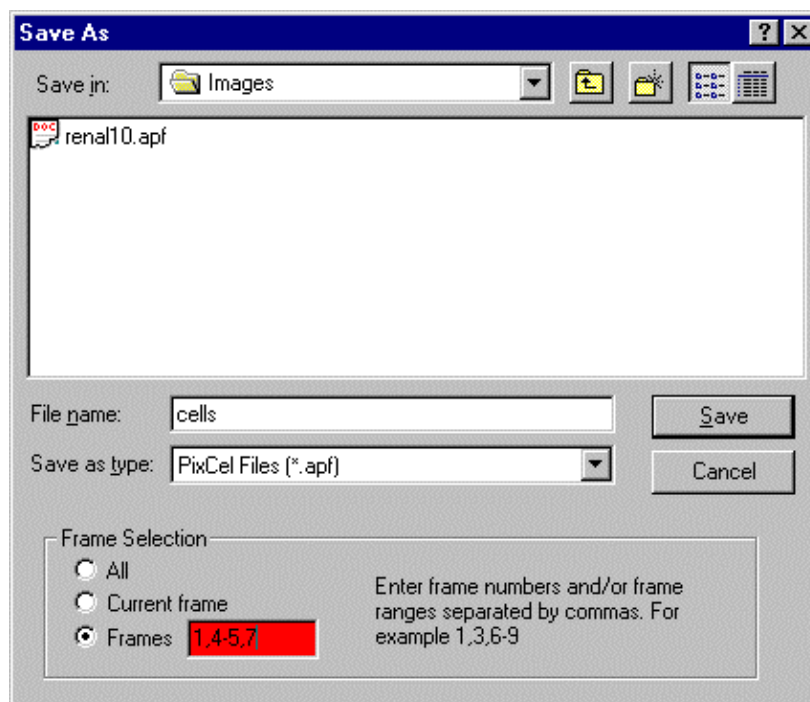


When the Open dialogue box appears, select the image from the File name list and choose the Open button. If the image is not listed, then a search of the directories can be conducted by selecting the appropriate directory, file type and/or file name. Either double click the item or highlight the option and click the Open button to select it.

NOTE: only .APF which have been saved using the Save file option or .TIF files can be opened with this method.



Save file: To Save an image in the software internal file format, select the Save file icon. Alternatively, select from the main menu File/Save or Ctrl+S.



If saving for the first time, the Save As dialog box will appear allowing you to select a directory in which to save the file, type a file name, and select a file type. Within a sequence the images can be saved as All for the sequence as a whole, Current frame for the image currently in view on the screen, or a particular frame or frames within a sequence. Select and check against the corresponding requirement and enter the frame numbers if appropriate. If the image has been previously saved, all new changes will automatically be saved to the original file name and location, and the original file data will be overwritten.

NOTE: The saved file is in ProFinder format i.e. .APF or .AFF, which contains additional information about how the image was collected.



Cut: Not available.



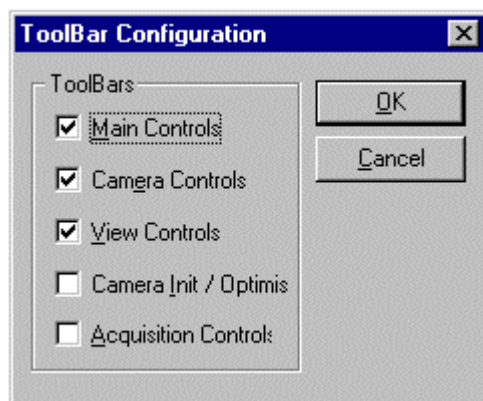
Copy: The Copy feature allows the image view or a current rectangle selection to be copied and placed on the Windows™ 95 clipboard for retrieval by another package i.e. Word, Excel, etc. To create a current rectangle selection, click in the image view with the left mouse button, press the Control keyboard button simultaneously and drag a rectangular area. Select the Copy icon to copy the selected area or image and to paste it onto the clipboard. Alternatively, select from the main menu Edit/Copy or Ctrl+C.



Paste: Select the Paste icon to paste the selected area from the clipboard to the area or into a document or image of your choice. Alternatively, select from the main menu Edit/Paste or Ctrl+V.



Toolbars: Select the Toolbars icon to access the Toolbar configuration dialogue box.



Individually clicking in the check boxes to select the Main, Camera, View, Camera Init/Optimise and Acquisition Controls will enable or disable the display of the toolbars and control boxes on the screen. Click on OK to accept changes, Cancel to abandon changes made or the X button in the top right hand corner of the dialogue box to close the toolbar dialogue box and return to the main image window. Default settings are Main Controls, Camera Controls and View Controls. To re-view the toolbars, select View from the main menu, select Toolbars and re-select the options available.

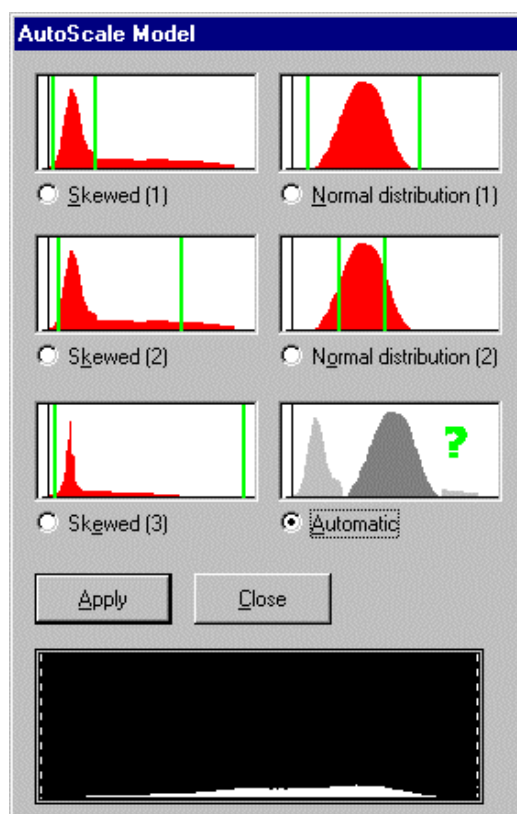


Mapping: The mapping function controls the display look up tables to adjust brightness and contrast, see page 48.



Autoscale Preferences: The Autoscale function can be customised, to allow closer autoscale data match to that of the image being captured. Autoscale initiates the automatic selection of a number of high and low pixel grey values and adjusts the settings according to the values found.

Click on Autoscale Prefs. to view the Autoscale Model dialogue box. Select the option required by clicking the appropriate radio button. Further adjustment can be made by placing the cursor on the dotted lines either side of the histogram indicated within the dialogue box. Click the Apply button to accept changes or Close to abandon changes and revert to the main window.



Statistics: Select the Statistics icon to access the Statistics information box. Alternatively, select from the main menu View/Statistics. See page 55 for more information.



Rectangle selection: The rectangle is the area indicated by clicking this icon or by holding the shift key down, clicking and dragging with the left hand mouse button between two points with the Image view window. The box created is the rectangle selection. It can also be changed by entering relevant details within the Properties dialogue box. Click on the Rectangle selection icon to enable or disable the display of the rectangle selection. See page 51 for more information



Line selection: The Line selection is a straight line on the image, indicated by clicking the left hand mouse button and dragging between two specific points with the Image view window. It may also be changed by entering relevant details within the Properties dialogue box. Click on the Line selection icon to enable or disable the display of the line selection. See page 53 for more information.

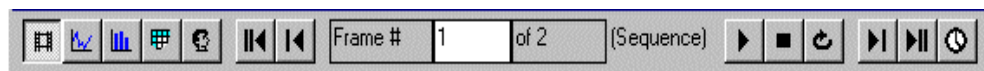


Print: Select the Print icon to access the Print dialogue box. Alternatively, select from the main menu File/ Print or Ctrl+P. The standard Windows printer dialogue box will appear which will allow the user to indicate which printer to use, which pages to print, and how many copies. Selecting the Properties button within the Print dialogue box will allow the further selection of the size and orientation of the paper, resolution and intensity of graphics, choice of fonts and device quality options. Select OK to accept changes or Cancel to abandon and return to the image window.



About: Select the About icon for access to information about the ProFinder software.

Appendix 3: Using the View and navigation toolbar



The View and navigation toolbar is located at the bottom of the screen. It is a dockable toolbar and can be moved left or right, left floating, or can be switched off and removed from the screen by using the View/Toolbars option. The toolbar comprises a range of icons that are explained below in detail from left to right.



Image: Click on the Image icon to display the image in the main window. See page 45 for more information.



Plot: Click on the Plot icon to view a line graph of the values under the current line selection. See page 53 for more information.



Histogram: Click on the Histogram icon to view the grey level values under the current rectangle selection as a histogram. See the next page.



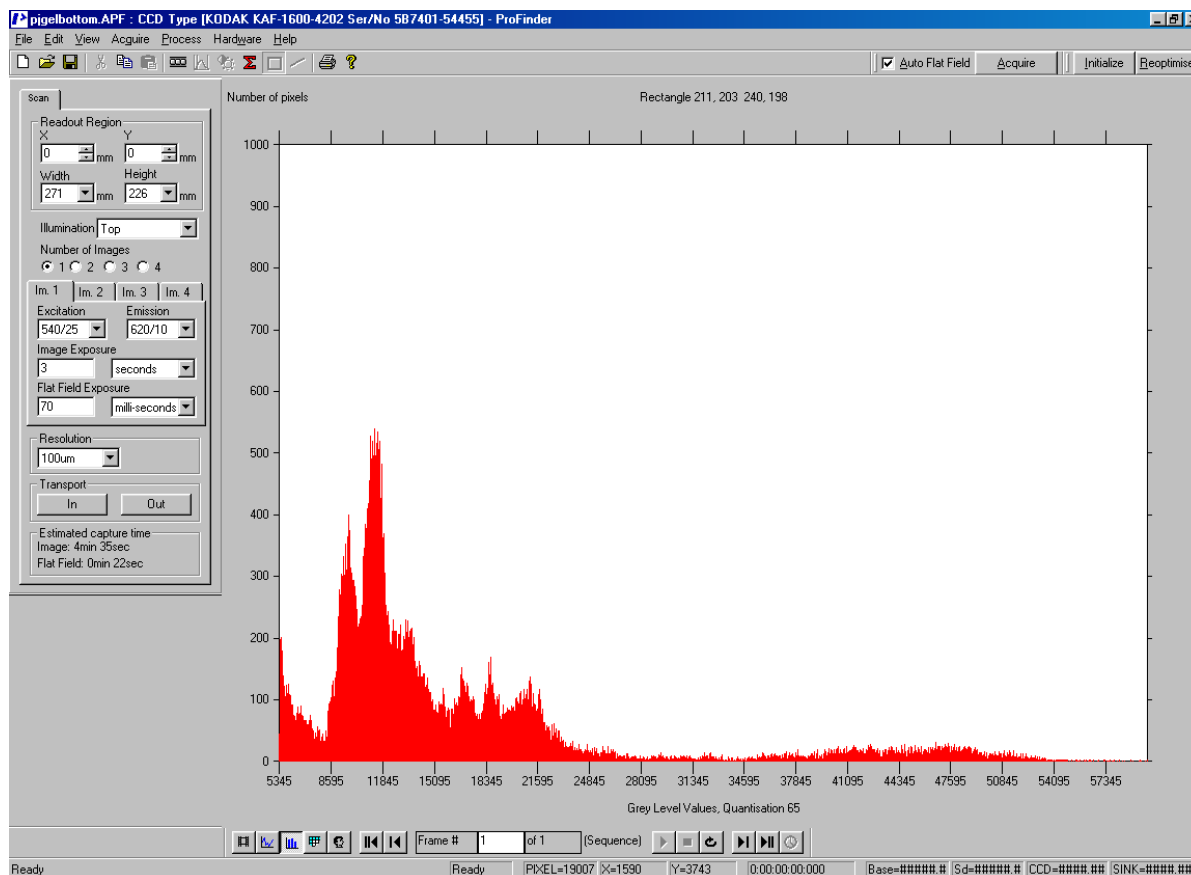
Pixel Values: Click on the Pixel Values icon to show the grey level values of the current rectangle selection in a spreadsheet format. See below.



User values: Click on the User values icon to view the current control parameters. See below.



Histogram view



A histogram is a graphical representation of the frequency of occurrence of each grey level (pixel intensity) within the *rectangle* selection. It can be calculated on the whole or just part of the image. Data from the histogram can be cut and pasted into most Windows™ compliant software, for further analysis and display.

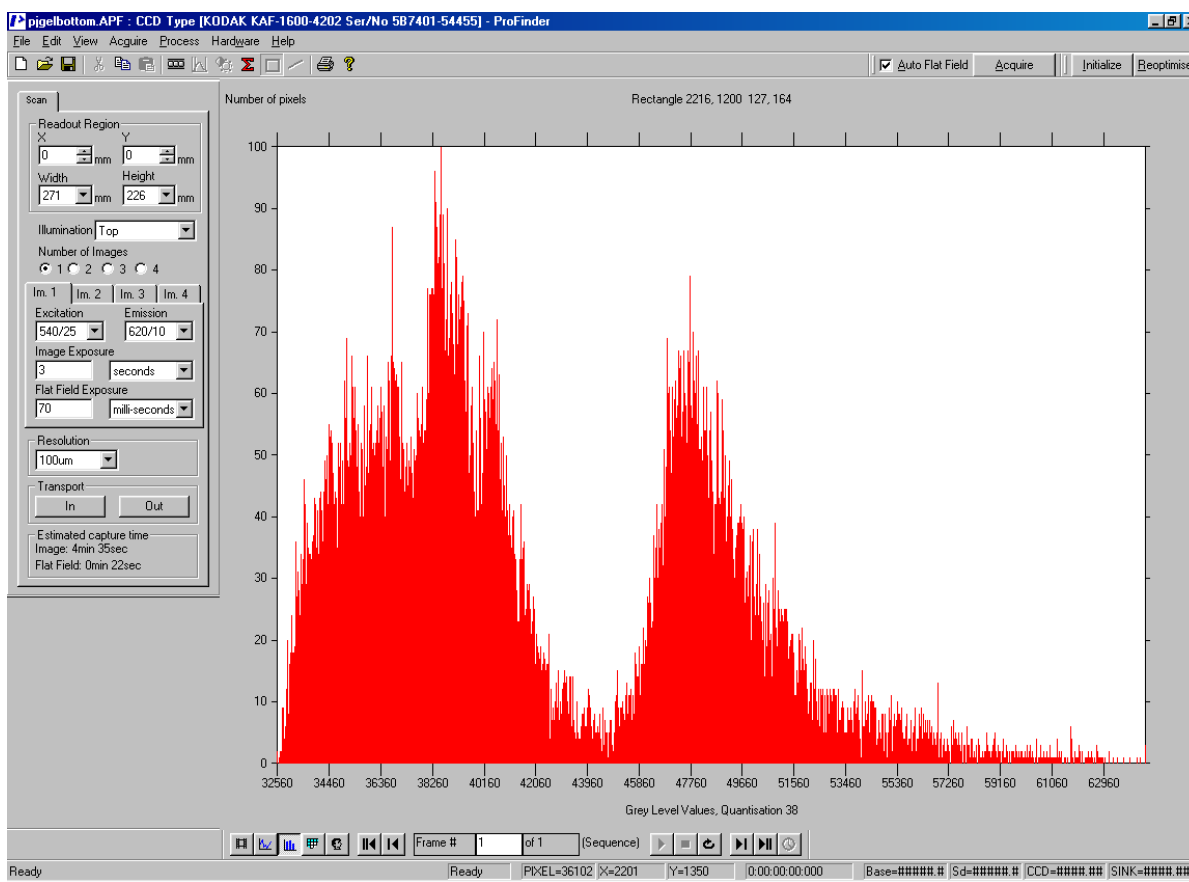
To display a Histogram of the image, select the histogram icon. The icon will be activated. Alternatively, click on View/Histogram from the main menu. The main window will show a bar graph histogram of the current rectangle selection. Place the cursor on the end point of any bar in the histogram and the corresponding value will be displayed.

To revert to the selected image click on the Image icon.

Use the First/Last/Next/Previous/Frame # controls on the view and navigation toolbar to move from one image to another in the sequence. The histogram is updated with the data from the new image under the rectangle selection.

Click the right hand mouse button and an option box will appear allowing the selection of either a bar or line histogram. A tick mark indicates the option currently chosen.

Outputting a histogram view



Use Edit/Copy, Ctrl+C, or click on the copy icon, to copy the graphical plot and values that make up the plot to the clipboard as a CSV (Comma Separated Value) and as a picture. This can be pasted (Edit/Paste Special) into Excel, Word or another package for further analysis. The values pasted in the output have as the first two numbers the grey level of the first bin of the histogram and the quantisation factor (the number of consecutive data numbers that are used to make a single point in the histogram).

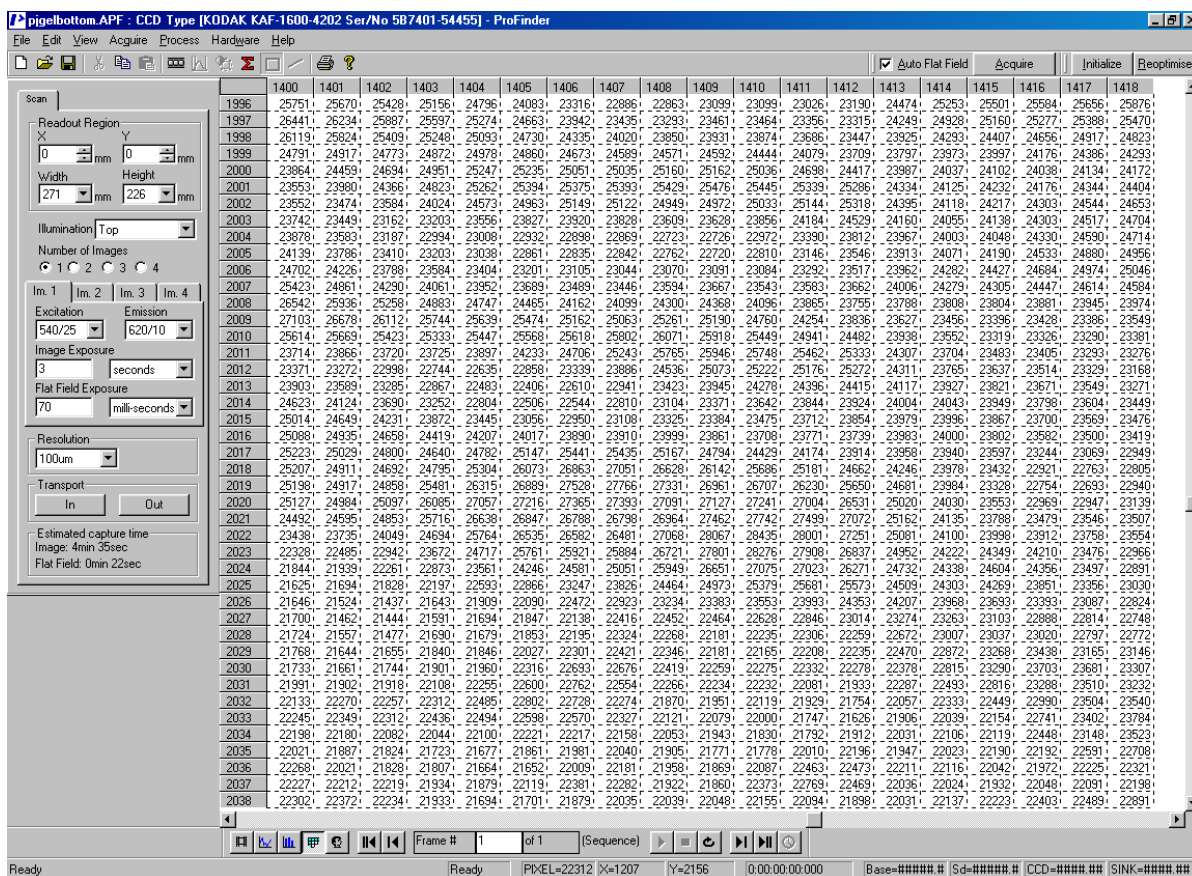
Select File/Print from the main menu, Ctrl+P, or the print icon to print the histogram. The histogram can be previewed before printing by using the Print Preview menu option. The page can also be changed from landscape to portrait, Letter to A4 etc. using the Page Setup option from the same menu.

Data from a plot can be dragged from ProFinder into a Microsoft Excel spreadsheet. Start both ProFinder and Excel. Arrange the ProFinder and Excel windows beside one another. Load a sequence into ProFinder, select the histogram view and press and hold the left mouse button in ProFinder. Move the cursor to the Excel window and release the cursor. The data has now been copied across. This saves time compared with using ProFinder Edit/Copy and Excel Edit/Paste special controls.

Mapping is not available in this view.



Pixel values



The screenshot shows the ProXPRESS software interface with the 'Pixel values' window open. The window displays a spreadsheet of pixel intensity data. The data is organized by X and Y coordinates, with values ranging from approximately 1996 to 22302. The interface includes various controls for image acquisition, processing, and display.

To display the pixel values of the image, select the pixel value icon. The icon will be activated. Alternatively, select View/Pixel value from the main menu. The main window will show a spreadsheet of the pixel values at each X and Y location of the current rectangle selection. Data from the pixel values can be cut and pasted into most Windows™ compliant software for further analysis and display. The extreme pixel values of the image are highlighted in the table as red for the maximum and blue for the minimum values. To revert to the selected image click on the image icon.

Use the First/Last/Next/Previous/Frame # controls on the view and navigation toolbar to move from one image to another in the sequence. The spreadsheet is updated with the data from the new image under the rectangle selection.

Use Edit/Copy, Ctrl+C, or click on the copy icon, to copy the spreadsheet as a CSV (Comma Separated Value). This can be pasted (Edit/Paste Special) into Excel, Word or another package for further analysis.

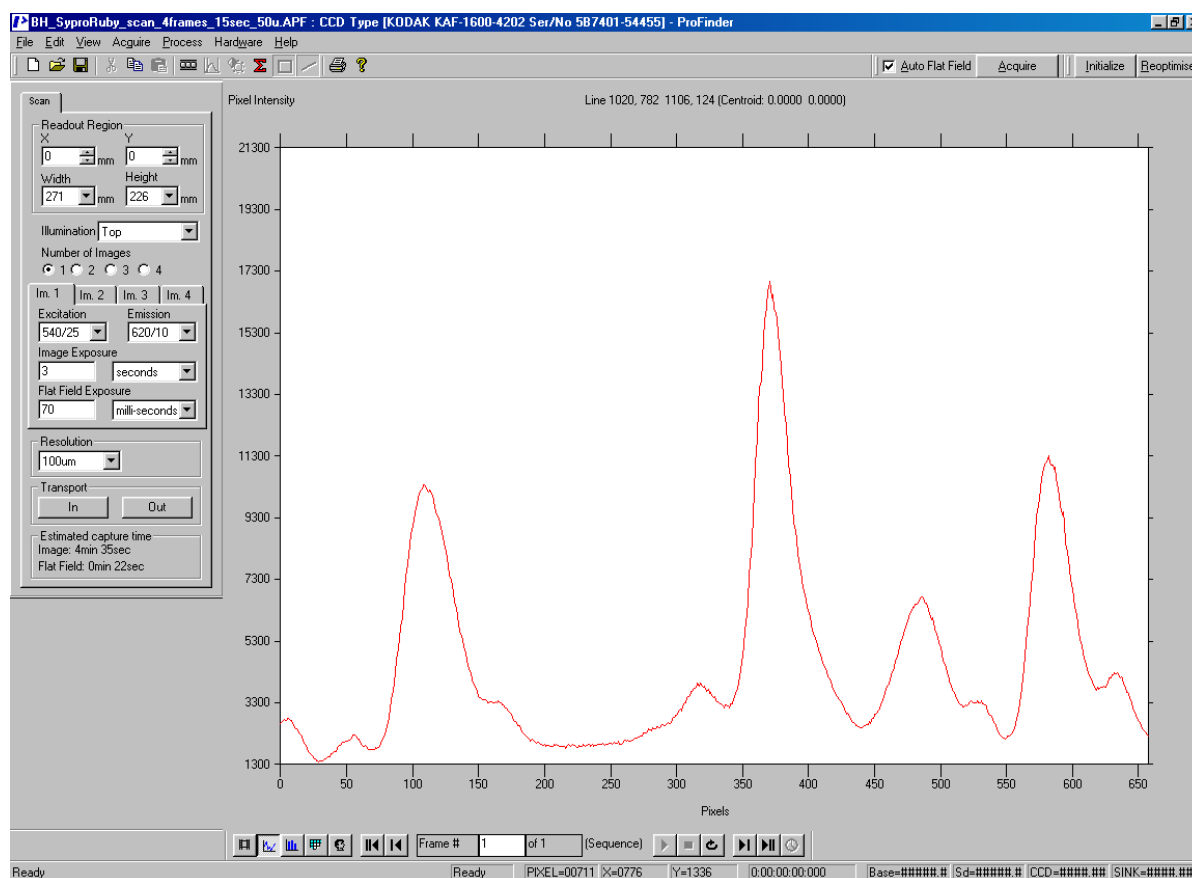
Select File/Print from the main menu, Ctrl+P, or the print icon to print the spreadsheet. The spreadsheet can be previewed before printing by using the Print Preview menu option. The page can also be changed from landscape to portrait, Letter to A4 etc. using the Page Setup option from the same menu.

NOTE: only the pixels in the rectangular area are printed. It is advisable to select the area of specific interest before copying or printing, as the amount of information in a full screen grab being copied to the clipboard will cause an extended delay in copying or printing. Also

avoid selecting much larger areas than you need. Programs such as Excel do not handle large data sets efficiently.

Data from a spreadsheet can be dragged from ProFinder into a Microsoft Excel spreadsheet. Start both ProFinder and Excel. Arrange the ProFinder and Excel windows beside one another. Load a sequence into ProFinder, select the pixel values view and press and hold the left mouse button in ProFinder. Move the cursor to the Excel window and release the cursor. The data has now been copied across. This saves time compared with using ProFinder's Edit/Copy and Excel's Edit/Paste special controls.

Outputting a Plot view



Use Edit/Copy, Ctrl+C, or click on the copy icon, to copy the graphical plot and values that make up the plot to the clipboard as a CSV (Comma Separated Value) and as a picture. This can be pasted (Edit/Paste Special) into Excel, Word or another package for further analysis.

Use the First/Last/Next/Previous/Frame # controls on the view and navigation toolbar to move from one image to another in the sequence. The plot is updated with the pixels from the new image under the line selection.

Select File/Print from the main menu, or Ctrl+P, or the print icon to print the whole plot. The plot can be previewed before printing by using the Print Preview menu option. The page can also be changed from landscape to portrait, letter to A4 etc. using the Page Setup option from the same menu. The print function is also available by clicking with the right hand mouse

button in the image window and choosing print from the zoom in/out option box. At this point the print dialogue box will appear.

Data from a plot can be dragged from ProFinder into a Microsoft Excel spreadsheet. Start both ProFinder and Excel. Arrange the ProFinder and Excel windows beside one another. Load a sequence into ProFinder, select the plot view and press and hold the left mouse button in ProFinder. Move the cursor to the Excel window and release the cursor. The data has now been copied across. This saves time compared with using the ProFinder Edit/Copy and Excel Edit/Paste special controls.

Plot is also very useful for focusing. Draw a line across an element within the image that should have sharp contrast (sharp transition when in focus), select plot mode, then use **scan**. The best focus is typically when the plot has the steepest transition in the plot mode.

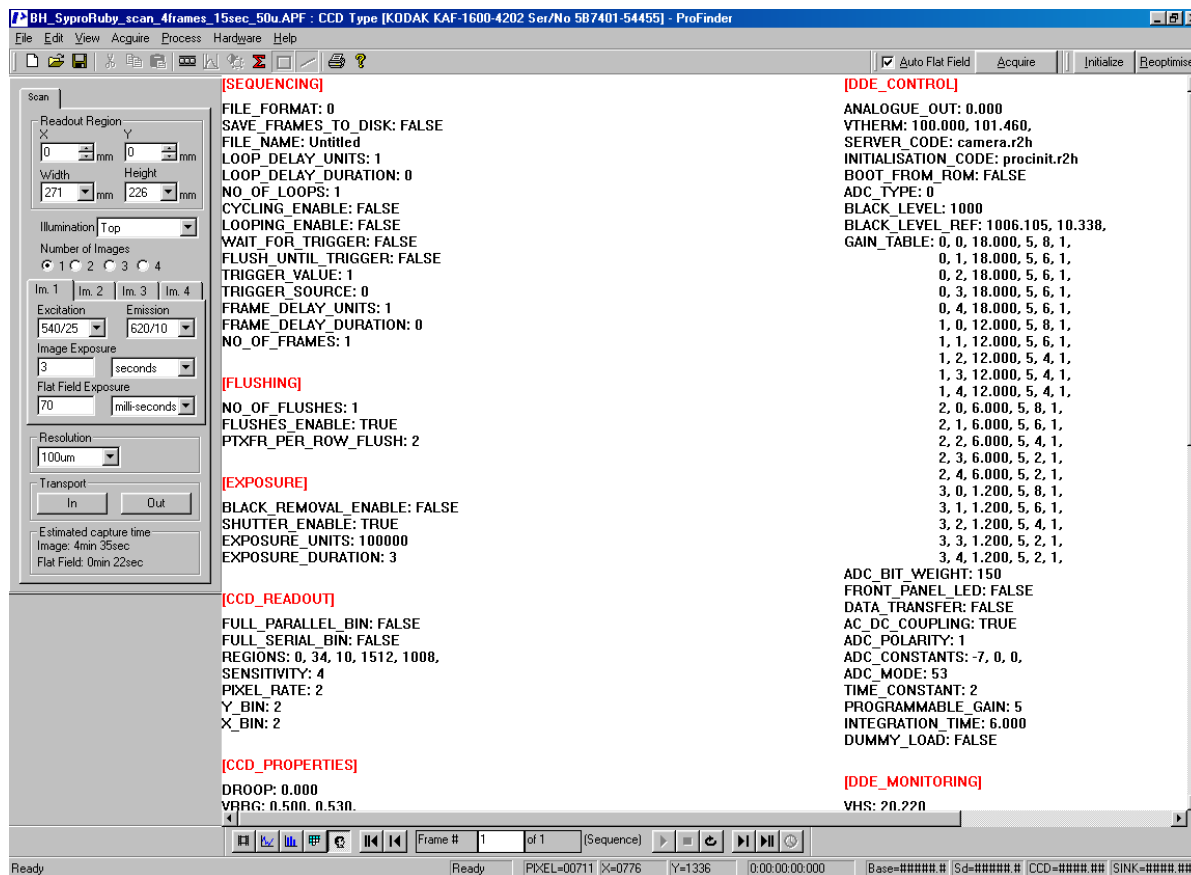
Mapping controls are not available in this view.

Use the Edit/Copy option from the main menu, Ctrl+C, or the copy icon to copy the current rectangle selection to the clipboard. The image can be pasted (Edit, Paste or Paste Special) into another application e.g. Word, Excel or another package. The copy function is also available by clicking with the right hand mouse button in the image window and choosing copy from the zoom in/out option box. When the copy option is clicked the image is transferred to the clipboard.

Select File/Print from the main menu, Ctrl+P, or the print icon to print the whole image. The image can be previewed before printing by using the Print Preview menu option. The page can also be changed from landscape to portrait, letter to A4 etc. using the page setup option from the same menu. The print function is also available by clicking with the right hand mouse button in the image window and choosing print from the zoom in/out option box. At this point the print dialogue box will appear.



User values



User values are the parameters used to acquire the image or sequence of images, including the camera control monitored voltages. This is a non-editable feature.

To display the User values, click on the user values icon. Alternatively, select View/User values from the main menu. Use the window scrollbars located on the right hand side and bottom of the main window to pan and scroll.

The editable values that were used when a sequence was captured are also displayed. They include the camera, sequence and focusing parameters as well as the state of the electronics.

Navigation controls



First Frame: Click on the First Frame icon to go to the first frame in a sequence.



Previous Frame: Click on the Previous Frame icon to go to the previous frame in a sequence.

Frame #	1	of 2
---------	---	------

Frame #: The Frame # box indicates the number of frames in a sequence and the number of the frame being viewed at the time e.g. Frame # 1 of 2 indicates you are viewing image one in a sequence of two. To view a particular image enter the image number in the Frame # box and press the enter key.



Start Playback: Click on the Start Playback icon to start the review of all the images in a sequence. Playback stops when the last frame has been viewed unless toggle looping has been enabled.



Stop Playback: Click on the Stop Playback icon to stop the review.



Toggle looping: Toggle looping is a review mode that starts at the first image again when the last has been reached etc. Click on the Toggle looping icon to select looping of sequences.



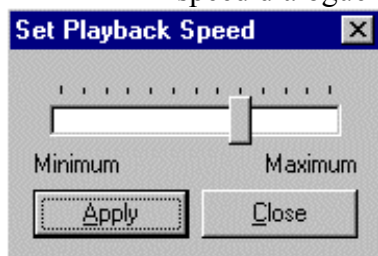
Next Frame: Click on the Next Frame icon to view the next frame in a sequence.



Last Frame: Click on the Last Frame icon to view the last frame in a sequence.

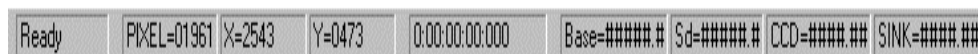


Playback speed: Click on the Playback speed icon to access the Set Playback speed dialogue box to vary the sequence playback speed.



Use the slide controls to vary the sequence playback speed and click Apply to apply the changes and Close to close the dialogue box and return to the main image window.

Appendix 4: Using the Status bar



The Status bar is located to the bottom of the screen. It is a static toolbar and cannot be moved within the screen although it can be switched off from the View main menu. The left side provides information about the state of the program. The boxes left to right provide information on the camera acquisition status together with cursor position information. It comprises a number of areas that are explained below in detail from left to right.

Ready

Camera Status: Indicates the mode in which the camera is operating e.g. Ready to grab an image, Re-optimize for optimisation, or Waiting when waiting for a trigger.

PIXEL=00020

Pixel: Grey level of pixel under the cursor.

X=0421 Y=0180

X position Y position: X and Y position of the image pixel pointed to by the cursor.

0:00:00:00:000

Frame Time: Shows the time from the start of acquisition of each frame in a sequence.

Base=#####.#

Base: Electronics base pixel value, typically 100-1000 and is updated periodically.

Sd=#####.#

Sd (Standard Deviation): Current readout noise in data numbers of the system and is updated periodically.

CCD= -273.0

CCD: CCD operating temperature (disabled with uncooled cameras) is indicated in this box, in degrees C.

SINK= -273.0

Sink: Heat sink temperature (disabled with uncooled cameras) is indicated in this box, in degrees C.

Appendix 5: Software Installation

The ProFinder software is normally pre-installed on the workstation and ready for use. It may however be necessary to re-install it or install a new release at a later time.

NOTE: This software relies extensively on parameters stored in initialization files. Two of these files are instrument specific and must be retained when new software is installed. There is a backup copy of these files in a subdirectory of the 'C:' drive called 'INIFILES'. It is however better to retain the original files and keep these for emergency use only. In the unlikely event of complete loss, these files are also stored in an LSR database and may be e-mailed on request by quoting the instrument serial number to the Hotline and the start of this manual.

Copying the original initialization files

Open WINDOWS EXPLORER and locate a sub-directory of the 'C:' drive in the tree C:\Program files\PerkinElmer software\ProFinder\

Locate the files called Pixel.ini and Scanner.ini and highlight them.

On the explorer toolbar click on EDIT, COPY (this will make a copy of them in the clipboard).

Place a formatted floppy disk in drive 'A:' and in Windows Explorer click the DRIVE A: symbol to make it active.

On the Explorer toolbar click on EDIT, PASTE (this will make a copy of them on the floppy disk).

Remove the disk from the drive and keep it safe for later to re-install these files.

There is also a copy saved in a directory called **C:\inifiles** on the hard drive should they be required.

Installing the software.

The software comes on three floppy disks labelled 1 to 3. With Explorer still active for DRIVE A: place DISK 1 OF 3 in the floppy drive and click again on the DRIVE A:-symbol to view the contents of the new disk.

Locate the file called SETUP and double click on it to run the program.

The installation wizard will guide you through the rest of the installation.

After you have clicked on FINISH you will need to replace the original initialization files.

Replacing the original initialization files

These files should still be resident in the clipboard so you should highlight the subdirectory
C:\Program files\PerkinElmer software\ProFinder\

On the Explorer toolbar click on EDIT, PASTE You will be asked if you wish to overwrite the files which are already in this directory. Click the button YES FOR ALL.

This will replace the new files with your original files.

If PASTE was not a highlighted option when you opened EDIT then it will be necessary to copy the files from the floppy disk you made at the start.

Click the DRIVE A: symbol. Explorer should display the two files Pixcel.ini and Scanner.ini. Highlight both of them and click EDIT, COPY.

Locate the program directory again:

C:\Program files\PerkinElmer software\ProFinder\

Highlight ProFinder

Click EDIT,PASTE and overwrite the new files by answering "Yes to All" to the question about overwriting files.

Close EXPLORER and run ProFinder as detailed below.