Nikon Training for the Ti2 A1RHD25 System

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- Calcium ratiometric imaging (Ca/FRET software module required)*

Hardware – components

A1RHD25 Components - Scan head, Controller and detectors
Ti2 – controller and microscope main body
Laser Bed
PC

A1RHD25 confocal scan head

Controller and Detector stack

Press the power button on the left hand side

Ti2E Microscope main body

Ti2 Controller

Laser Bed

Press the power button on the rear of the laser unit and then turn the key

Z4 PC

Turning on the Equipment

1. Confocal controller (button on left side of the unit at the bottom of the stack)
2. Laser Bed – Press power switch at the back of the unit (in the middle of the stack) then turn the key at the front.
3. Ti2 – controller and microscope main body – Turn on controller then the microscope. DO NOT leave a long gap between this.
4. PC
5. Start NIS Elements software

Turning off the Equipment

1. Turn off the NIS Elements software
2. Turn off the micrcoscope main body. Once the lights have stop flashing on the front of the microscope turn off the controller
3. Turn the key on the laser bed and then turn off the power button at the back
4. Turn of the button on the side of the controller
5. Turn off the PC

Ti2 Controls

Ti2 Main Body

Objective Changer switch

Rotating the switch changes the objective position left or right. Depressing the switch puts the associated optical element in the light path

Limit Button

Pressing the limit button will create a firmware per session upper limit for z travel at the current z position.

When a limit is activated the limit switch will illuminate.

It will flash when the focus reaches the limit position.

Escape Button

Pressing the escape button will move the objective to the escape position.
The z will not move until the escape is released by pressing to move back to the original position
Holding down the escape button will clear position memory and turn off the escape
The escape position can be set in the Ti2 Control

Bertrand Lens

The Bertrand lens allows viewing of the back aperture for adjustment of phase rings and viewing of the TIRF beam using the camera on the Eyepiece Base Unit. It is placed in and out of the light path by the larger outer dial, and focused using the inner dial.

Optical Path changeover buttons/ indicators

The light path can be switched between the left port, right port, eyes, and a 20%eye/80% left port.

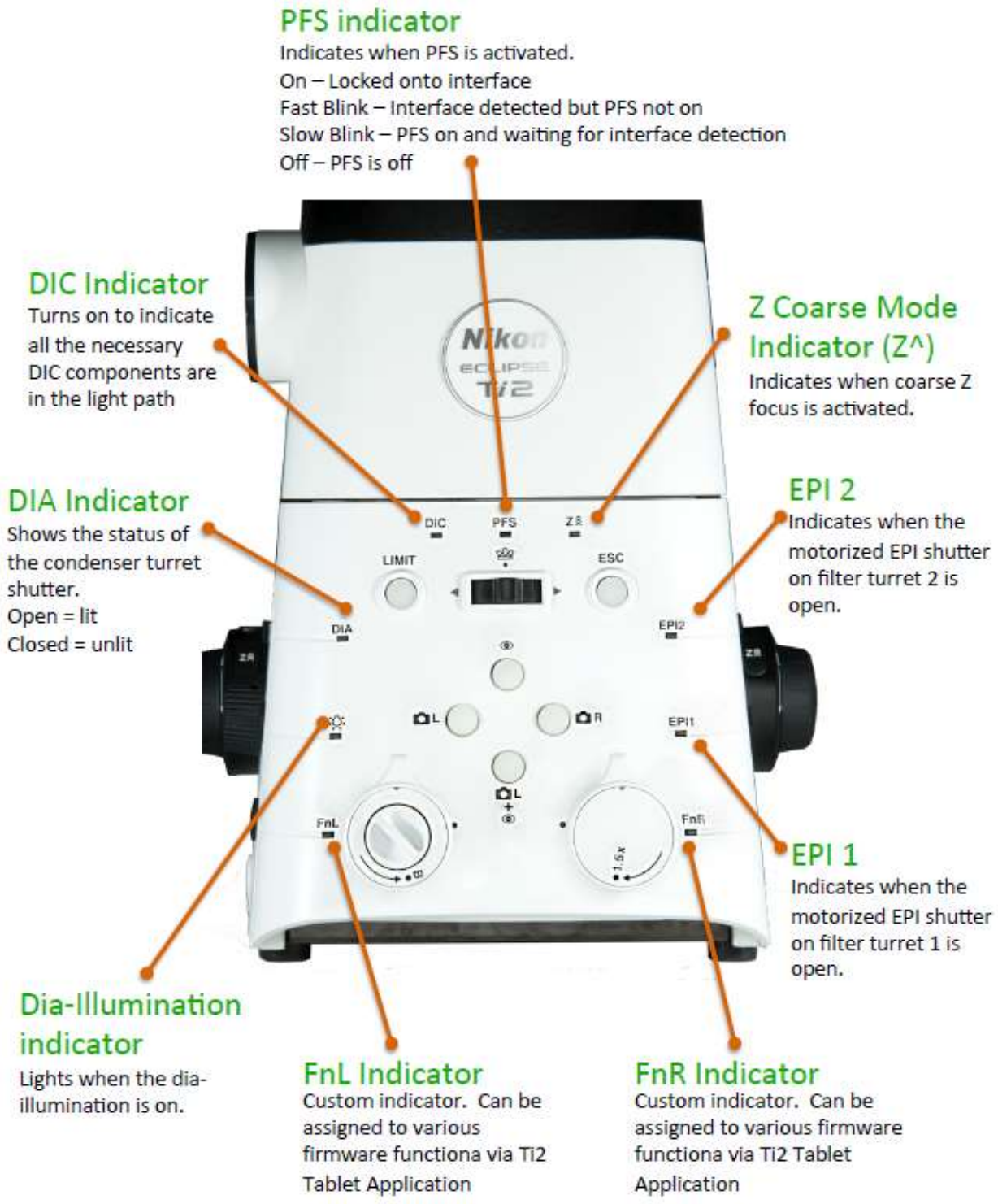
A bottom port Ti2-E is an option but requires purchase of a specific bottom port Ti2-E Base and that will replace the 20/80 beam splitter.

Tube Lens Magnification Changer

The Tube lens can be switched between 1x and 1.5x. It is manual but intelligent so it will be recognized in software.

The 1.5x can be replaced with a 2x lens. This must be quoted at time of order and replaced by the service department.

Ti2 Main body indicator lights



Ti2 left side buttons

PFS Button
Turns the PFS system on and off.

Hold down to insert or remove the PFS dichroic mirror from the light path.

Z Coarse Mode
Toggles coarse Z movement on or off.

Holding down activates Coarse Z, while letting go sets fine focus.

Alternatively in Ti2 Control the button can set to toggle between coarse and fine.

Condenser module changeover switch
Moving left and right moves the position of the condenser modules sequentially in a motorized condenser.

Pressing will engage and disengage the shutter position on the condenser.

DIA illumination ON/OFF switch
Toggles on/off for the diascopic lamp
LED or Halogen.

Z Focus knob
Focus the objective up and down using this knob.
The speed of the focus knob changes with the NA of the objective in the optical path
Standard movement moves the objective up when moving the top of the knob towards the operator, but can be changed in Ti Control.

Dia-illumination brightness adjuster
This control adjusts the brightness of the diascopic illumination lamp.

FnL
Left Function Button. Can be programmed to perform a variety of firmware functions or NIS Elements Macros. Default function is Main Body LED on or Off

Ti2 Right Side Buttons

Z Coarse Mode

Same Function as the Z coarse mode button in Pg. 6.

Camera Base Unit Path Selector

Choose between eyepieces and the camera base unit camera port. Only on the camera base unit and external phase unit eyepiece base units. Manual but intelligent.

PFS Button

Same Function as the PFS Button on Pg. 6.

Turns the PFS system on and off.

Hold down to insert or remove the PFS dichroic mirror from the light path.

Filter Turret 2 changeover switch

The button changes the position of filter turret 2. pressing the button will open/close the filter turrets motorized shutter. Holding the button will turn positions continuously.

Filter Turret 1 changeover switch

The button changes the position of filter turret 1. pressing the button will open/close the filter turrets motorized shutter. Holding the button will turn positions continuously.

FnR

Right Function Button. Can be programmed to do a variety of firmware functions or NIS Elements Macros.
Default function is Control Box I/O 1 output

Z Focus knob

Focus the objective up and down using this knob.
The speed of the focus knob changes with the NA of the objective in the optical path
Standard movement moves the objective up when moving the top of the knob towards the operator, but can be changed in Ti Control.

Ti2 Joystick Controls

Backlight Brightness Adjuster

Dial on side of controller that changes the brightness of the Joystick display

DISP button

4 way switch that changes what information is shown on the display

LC display

Displays chosen information about the position of microscope components

Function Buttons (6)

Programmable buttons that can drive a firmware function or a NIS Elements Macro

PFS Button (both sides)

Same Function as the PFS Button on Pg 6.

XY Coarse Mode

Toggles the The speed of the XY Movement of the stage.
Holding down activates Coarse XY, while letting go sets fine focus.
Alternatively in Ti2 Control the button can set to toggle between coarse and fine.

Reset Z and XY buttons

Resets the relative position indicators to zero on either the Z drive or XY drive

Z Coarse Mode (both sides)

Same Function as the Z Coarse Mode button in Pg. 6.

Joystick

Moves the stage in X and Y.
Moving the Joystick further increases the speed of movement
The speed of movement also changes depending on the magnification of the objective in the optical path

Z Focus Knobs (both sides)

Focus the objective up and down using this knob.
The speed of the focus knob changes with the NA of the objective in the optical path
Standard movement moves the objective up when moving the top of the knob towards the operator, but can be changed in Ti Control.

NIS Elements Software

Workspace Overview

NIS Elements has a highly customisable user interface. The workspace is based around a system of control panels that can be floating or docked into panes.

Control Panels

Control panels in NIS Elements are grouped in one of four major categories: Analysis, Acquisition, Visualisation and Macro. The panels available will depend on the level of package (D, Br, Ar) and the optional modules loaded onto the HASP.

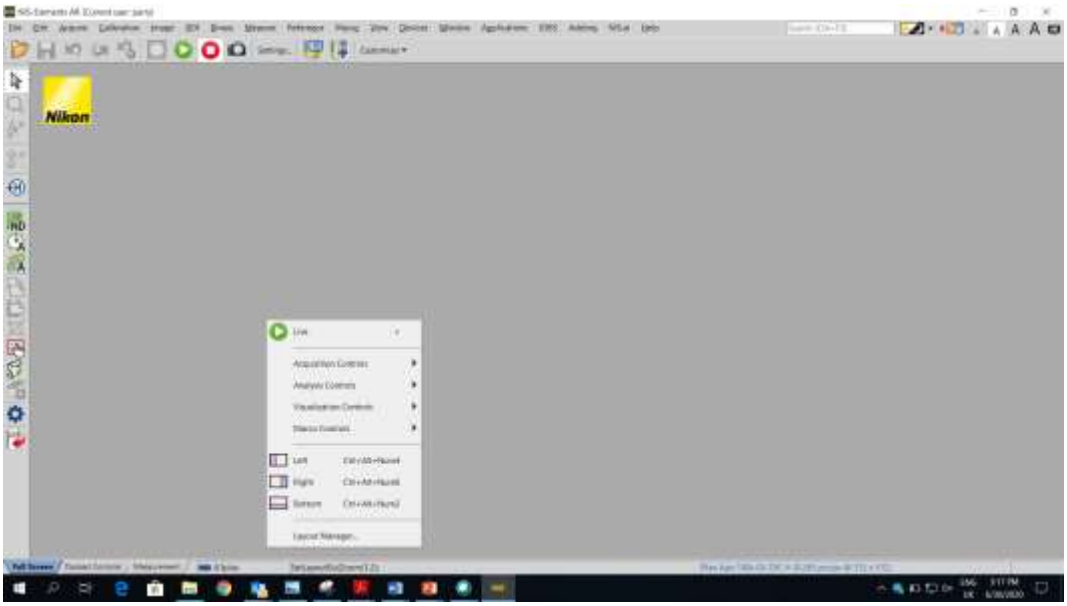
Acquisition controls contains control panels related to the capture of images, such as the camera settings, microscope pad, XYZ navigation, and ND acquisition.

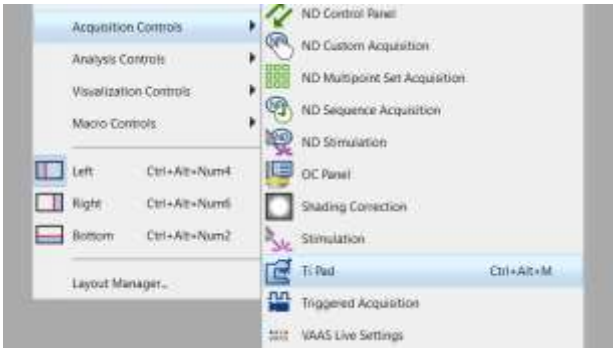
Analysis controls contains control panels for post-acquisition measurement, such as annotations and measurements, automated measurement, co-localization, time measurement and tracking.

Visualisation controls contains control panels that can be used both during and after acquisition, such as LUTs (lookup tables), synchronizer, spectral profile and system information.

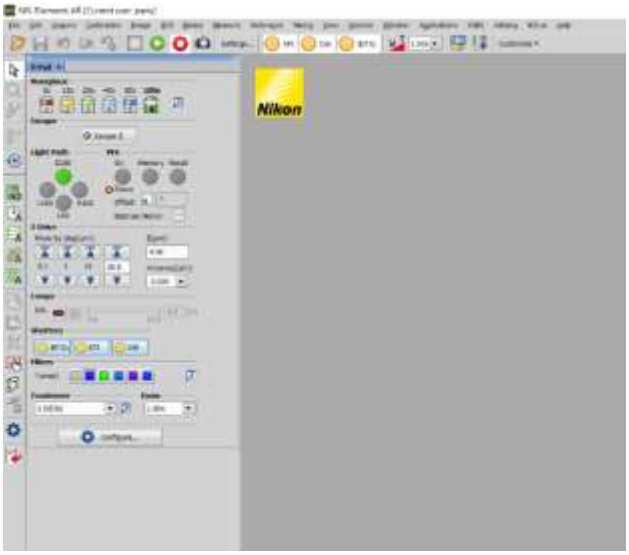
Macro controls contains control panels specifically for use in macro programming, such as command history and variables.

By right clicking in the empty workspace you can select left right and bottom docking panes and access the available windows under each of the 4 categories.

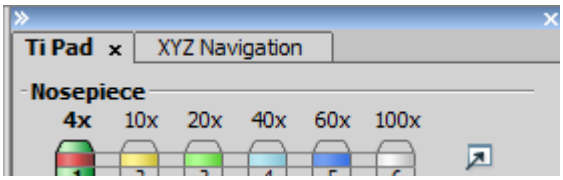




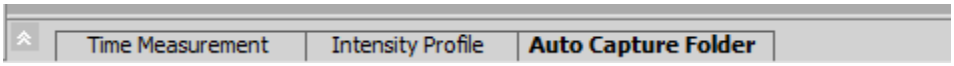
You can select the required control pad and then drag and drop the resulting window into a docking panel.



When docked, control panels can be layered in a tab system.



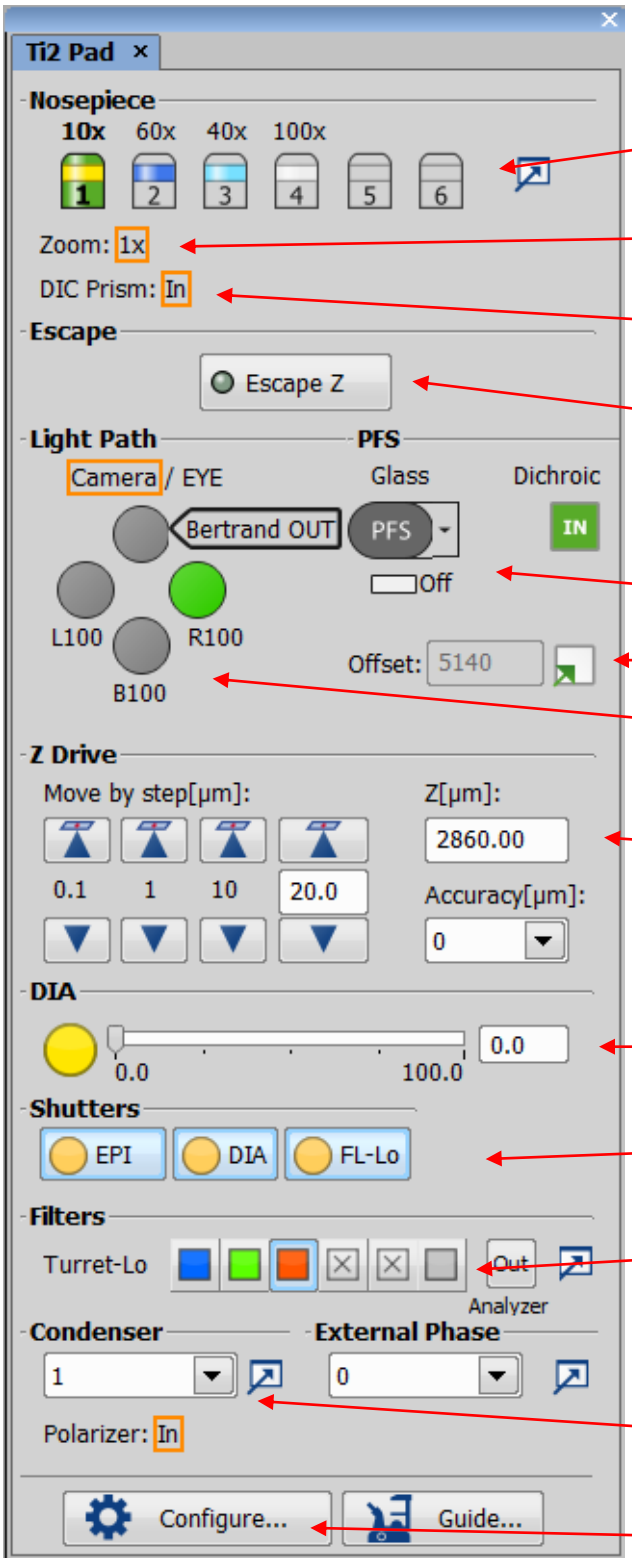
Dockers can also be collapsed by clicking the double arrows to increase the available workspace without removing control panels. Clicking the double arrows a second time will restore the docker and the control panels to their previous size and positions.



Collapsed bottom docker with control panels

Tabs at the bottom of the workspace allow the user to toggle through several different workspaces, each with different selections and arrangements of control panels.

Microscope Control pad



Nosepiece panel, listing currently installed objectives

1x or 1.5x magnification changer (intelligent not motorised)

Whether or not the objective has a DIC prism in the nosepiece (not motorized)

Lower the nosepiece into the escaped position. De-press to return to previous position

PFS on/off button

PFS offset –set to current focus position

Lightpath selector (100% to eyepieces, left or right ports, 20% eye and 80% left port split)

Z position and focus speed

Brightfield lamp software control. On/Off and voltage control slider

Shutter open/close buttons

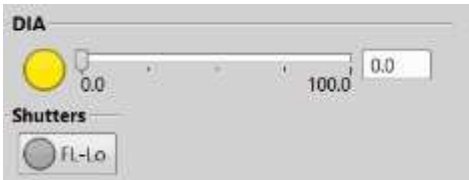
Filter turret, listing installed filter cubes

Condenser, listing currently installed modules e.g. phase and DIC.

Configure setup of the Ti2 including short cut buttons

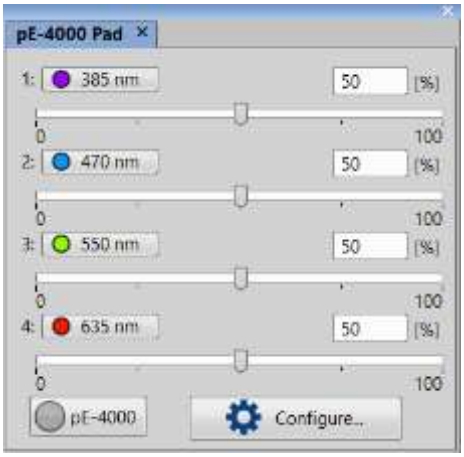
Light Source

Widefield – Brightfield illumination



The Ti2 has an LED white light for bright field illumination. The intensity of the LED can be controlled in the software using the slider on the Ti2 pad. The LED can be turned on or off by pressing the yellow button pictured here.

Widefield - Fluorescence



An LED light source is provided for the Epi fluorescence. This can be used to look by eye and for wide field imaging with the camera. The wavelength and intensity can be set in the control panel. The PE4000 button turns the light source on and off. There is a physical shutter in the Ti2 main body names the Fl-Lo button on the control panel in the software. This must be open for the fluorescence light to travel through the system.

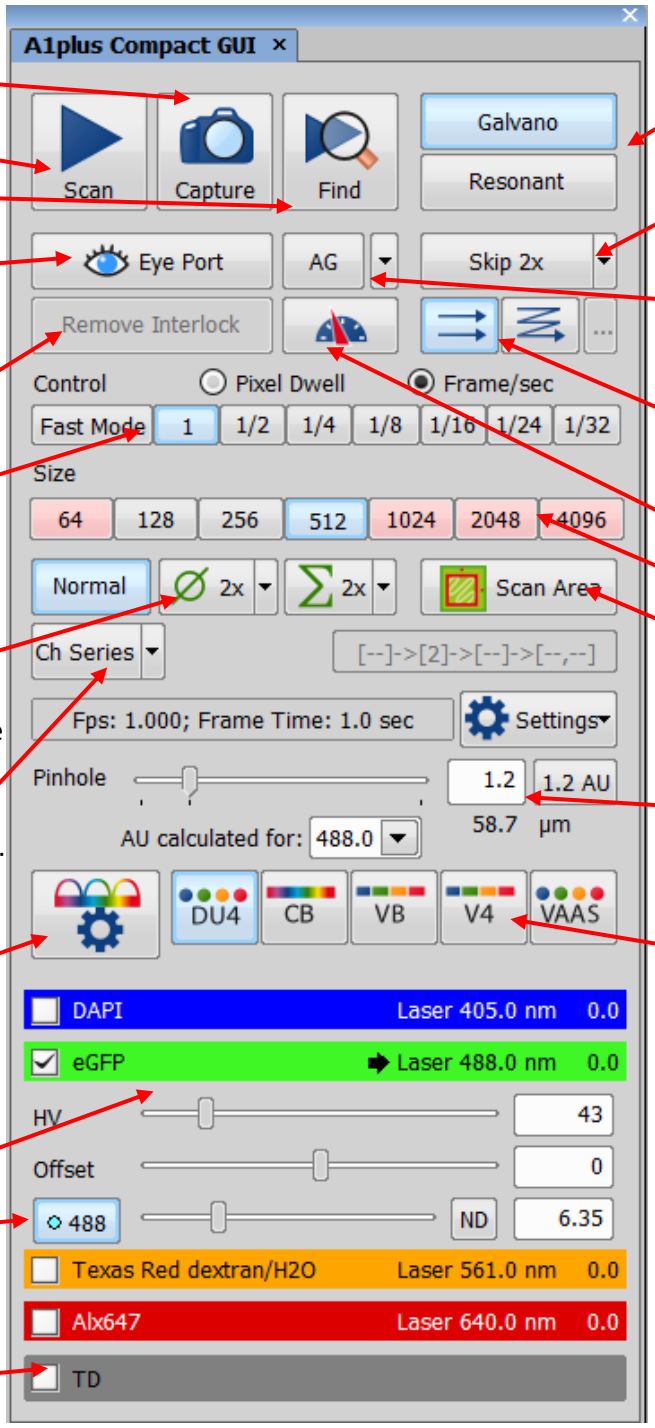
Confocal – Fluorescence

A laser bed is provided as the light source for the confocal. The Diode lasers can be controlled in terms of power and on/off state using the sliders at the bottom of the confocal control window (see below).

Confocal control pad

The A1RHD25 has two scanning options: Galvo and resonant. The confocal pad changes depending on which option you choose.

Galvo Confocal Mode



Captures a single image

Starts/Stops scan

Find mode

Changes optical path to eye port. Lasers are interlocked when selected

Removes interlock (when light path not to eye port)

Scan speed. Set by pixel dwell time or frames/second. Fast mode is a preset fast scan with 8x zoom

To improve signal to noise you can select normal, average (Ø) or integration (Σ). Integration changes the image depth to 16 bit (from 12 bit)

Channel series. To avoid bleedthrough.

To set the channel order

Opens Optical path window

Select laser/detector combination . Detector gain and offset.

Laser on/off and power

Transmitted detector

Select Galvano or Resonant scan mode.

Line skipping. Can set to none, 2x, 4x, 6x, or 8x.

AG Automatically adjusts the HV value (HV gain) of the currently selected channel to the optimum values.

Scanning can be set to unidirectional or bidirectional

Laser Power monitor

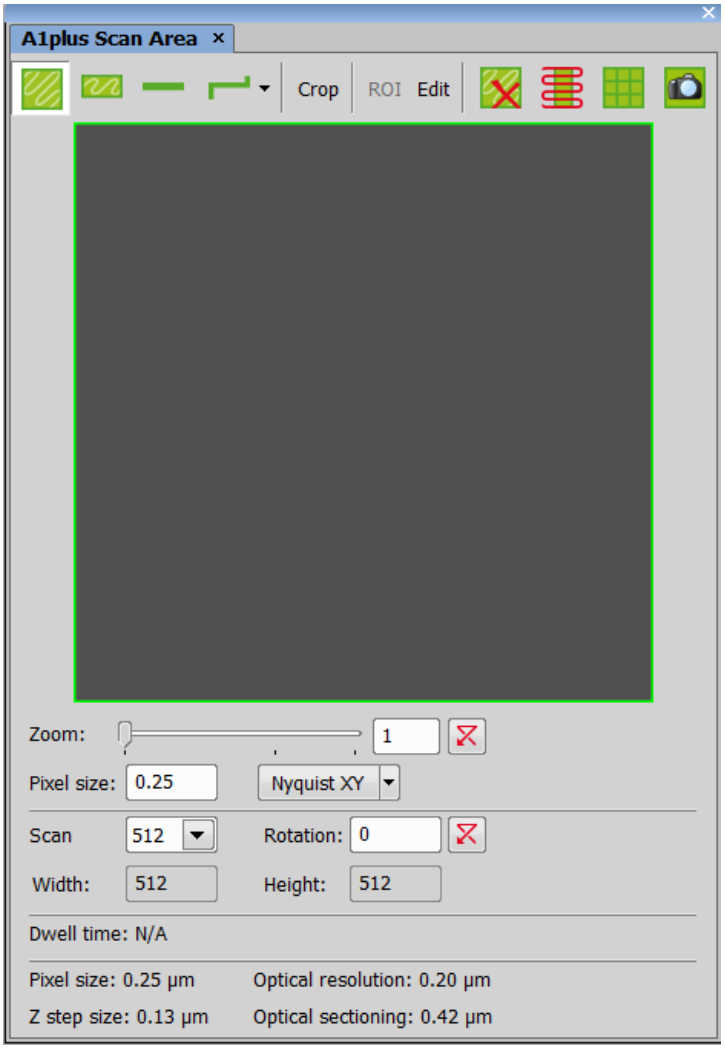
Scan size (pixel number)

Opens scan area window

Pinhole size. Can set for each wavelength but only one size when imaging with multiple wavelengths

Select detector

Scan Area



 Square scan area

 Band scan area

 Line scan area

 Poly line scan area (and settings)

 Crop scan area within square area

 Use ROIs inside square scan

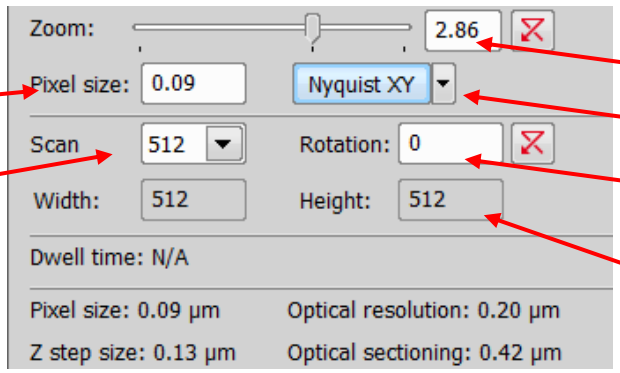
 Edit the ROIs. First need to draw ROIs on live or captured image

 Reset scan area

 Update preview

 Scan current scan area as large image

 Scan full FOV at Nyquist zoom



Pixel size

Scan area size (no. of pixels in X and Y)

Zoom 1-1000

Set the zoom to give a Nyquist pixel size

Square or band scan area can be rotated up to 180 degrees

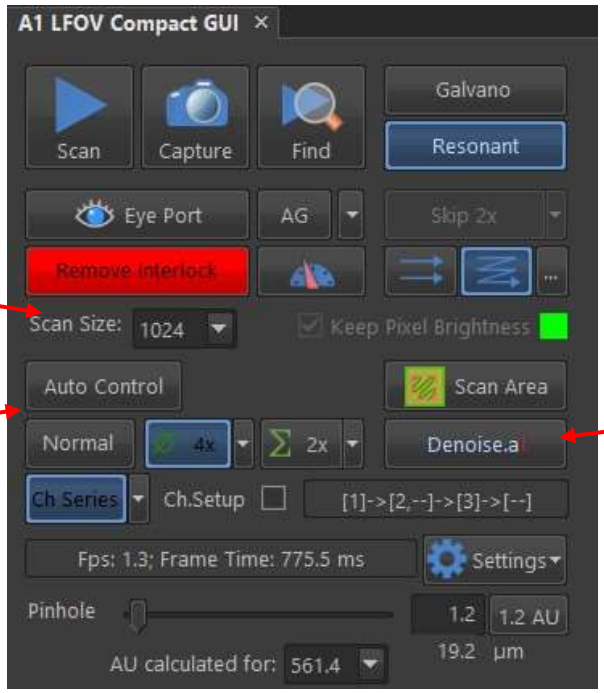
Height can be varied in a band scan and width is set by the scan area

Resonant Scanner Mode

The Resonant scanner runs at a set speed in terms of pixel dwell time and as such the confocal pad window has fewer options in terms of scan size and speed. At 512x512 it will image at 15fps in unidirectional and 30fps in bi-directional mode.

Scan Size 1024, 512 and 256 pixel options

When selected, Auto control pauses the scan and stops the laser when there is no movement in X,Y or Z.



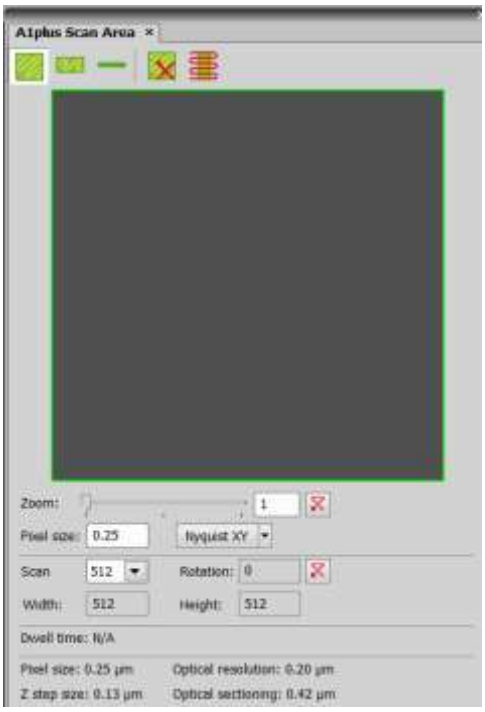
Live denoise

Resonant Scan area

The A1 scan area has reduced options when resonant scan is selected. The scan size is limited to 256, 512 and 1024.

The Zoom has a maximum of 8x

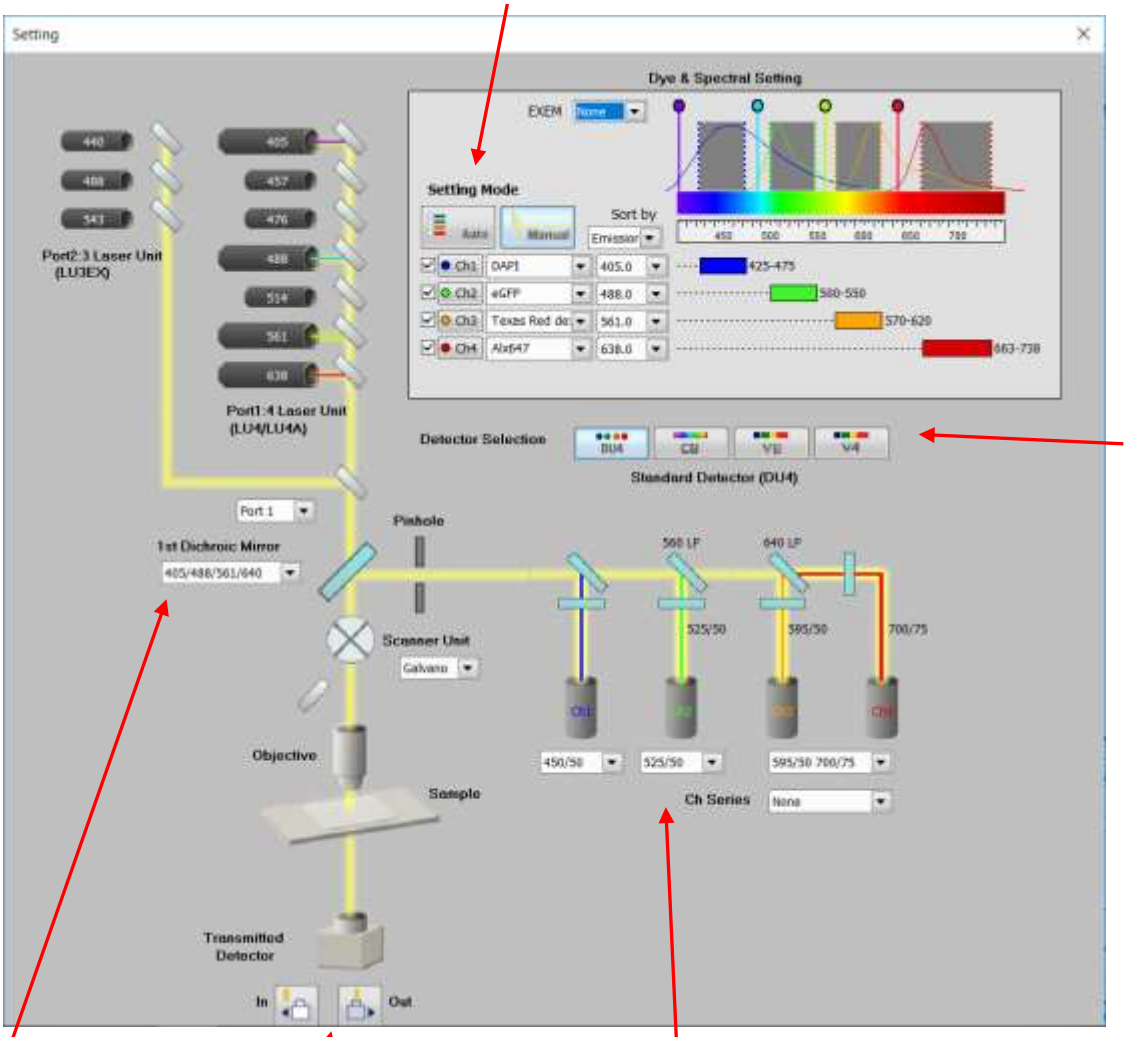
Rotation of the scan area is not possible



Light path settings 

In this window you can select the lasers you want to use and the emission path way. If you have multiple filters in the detectors you can manually select the corresponding one or by choosing the auto settings and a listed fluorophore and it will set the light path for you. There are a number of 1st dichroic mirrors in the scan head that should be selected to match the lasers used and you can move the transmitted light path in and out here too.

Excitation lasers - choose manually or set automatically by fluorophore



Optional detector units (if multiple are on the system)

1st dichroic mirror in scan head

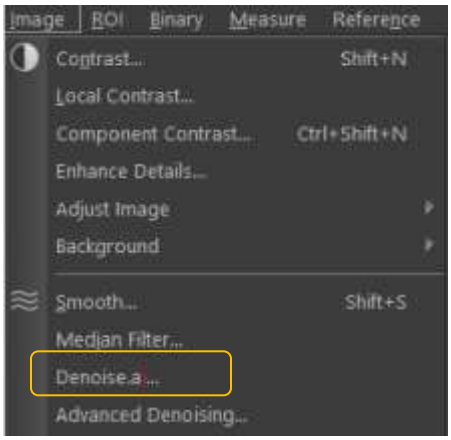
Transmitted detector. Select in or out

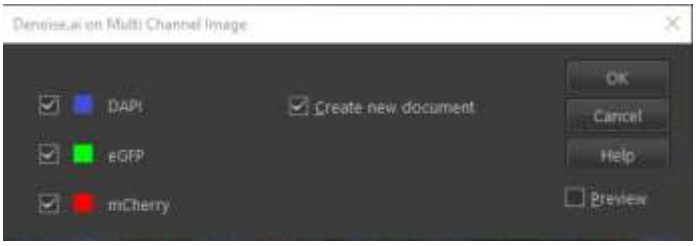
Confocal Episcopic detectors. Select optional filters here

Post-Acquisition Image processing

Denoise.ai

An optional processing method is called “denoise.ai”, an artificial intelligence filter that has learned to denoise resonant scanner images. Click on Image>Denoise.ai and the software will open a new window asking which channel you want to denoise and whether you want to create a new document. .





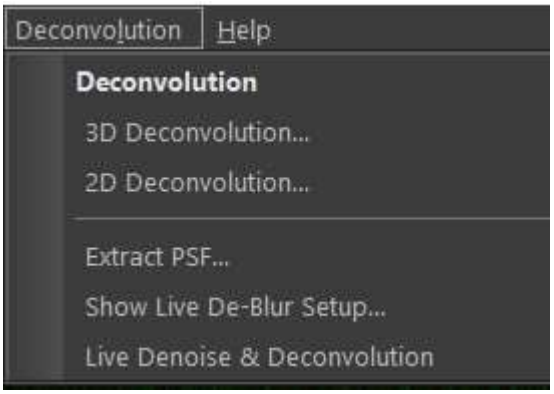
Deconvolution

An optional image processing method is an in-built deconvolution algorithm.

Top of the screen click on Deconvolution and select 2D or 3D deconvolution

Auto deconvolution or the Richardson-Lucy algorithm are preferred methods.

A new window will open and give you a series of choices for the deconvolution process. These are sample specific. You should keep the same parameters for images that will thereafter need to be compared.



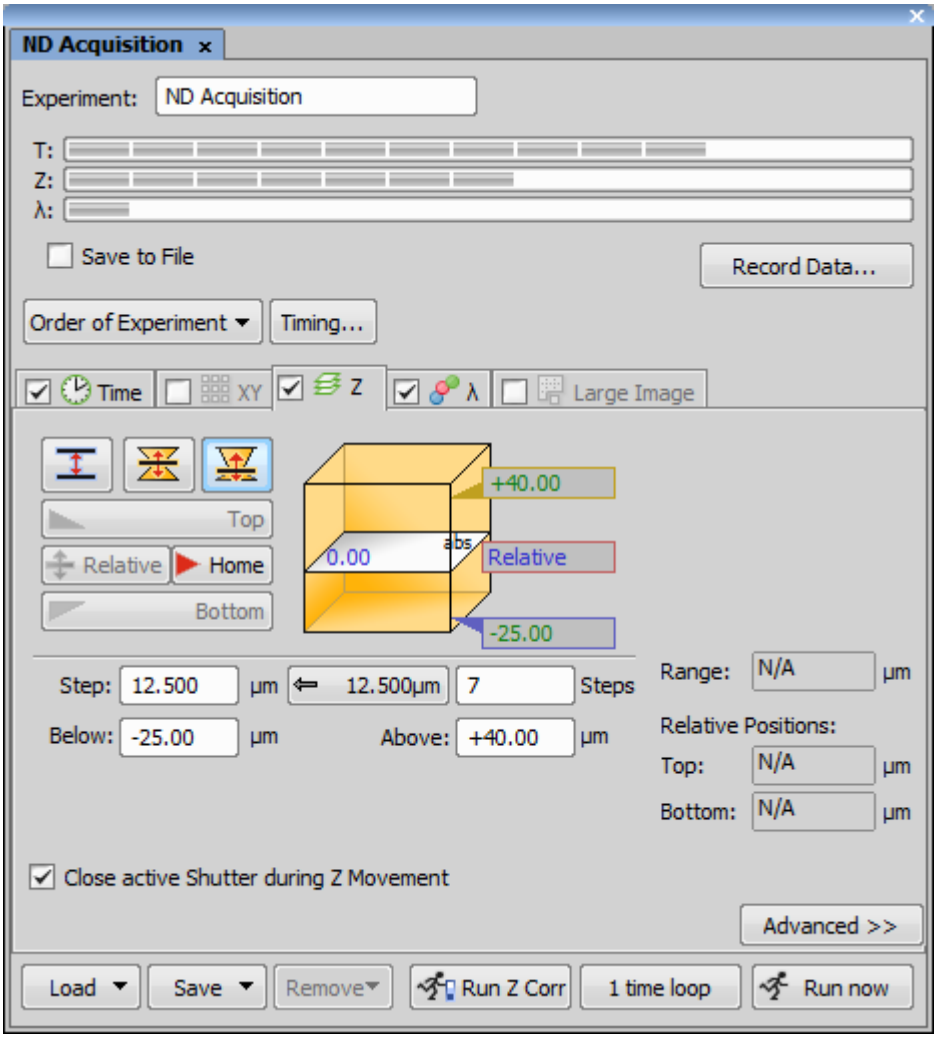
The software will open a new window with the deconvolved image.

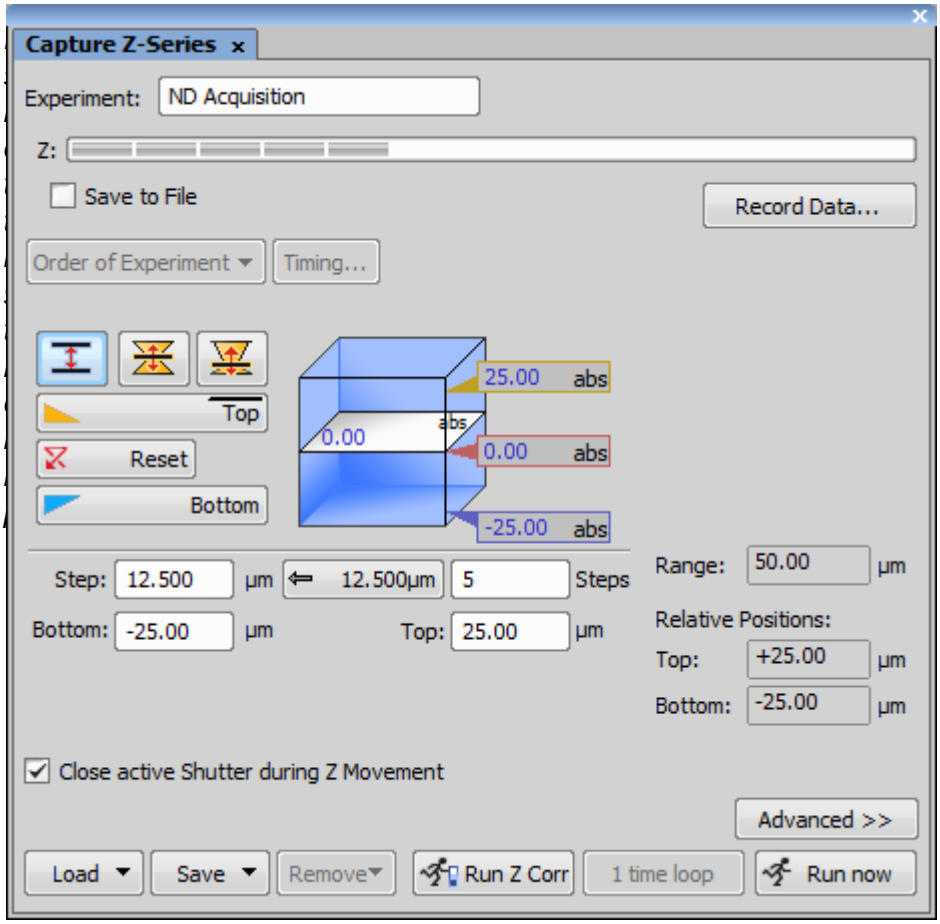
ND Acquisition – Z Stack

Z stacks allow the collection of images at multiple planes of focus. These can be used to image specimens which span a large range of focus or to create a 3D image of a specimen.

ND Experiments with Z Stack

Go to Applications and choose <Define/Run ND Acquisition…> and ensure that the Z tab is ticked.





The range of the z-stack is set and then the microscope will move through the steps automatically. There are three methods for setting the range.

To set Top/bottom (blue cube) move the focus to the top and click Top to store, and repeat for bottom position. The centre button shows the Nyquist recommended step size, calculated from the NA of the objective. To set this suggested value, click the middle button. To choose another value, type the desired step size into the <Step> box and press enter. The total number of steps is calculated and shown in the <Steps> box. Alternatively the number of steps can be typed in and the step size will be automatically calculated.

To set a symmetrical range around a mid-point (symmetrical yellow cube), type in the desired range in microns. Clicking <Home> will set the current absolute z position to be the centre of the Z stack. The number of steps will always be an odd number, as the home position will always be captured, plus an identical number of steps each side.

All three methods for setting the z range can be used in an ND experiment, however the most appropriate choice will depend on the other dimensions present.

When the XY tab is active and includes a Z position, or when the Perfect Focus System is engaged, one of the two range (yellow cube) methods must be used, and the mid-point must be set to <Relative> rather than an absolute <Home> value. This means that the current z position based on XY point or PFS will always be taken as the home position. The range can initially be set by using the top/bottom (blue cube) method, and this defined range will then be automatically carried across when switching between the range types.

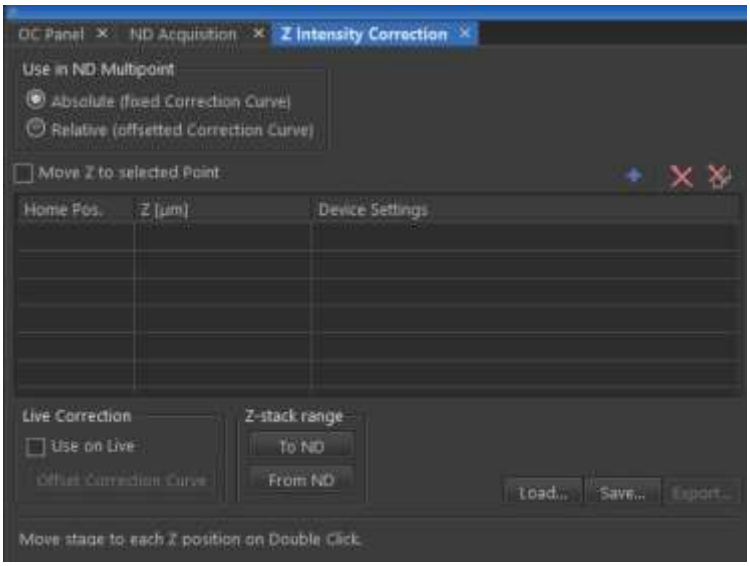
To set the relative position to be in the centre of the z range, select the symmetrical range (symmetrical yellow cube).

To set the relative position to not be the mid-point of the range, select the asymmetric range (asymmetric yellow cube). Type in the range below and above the home position to set the total range and relative position of the reference plane. This type of z-stack is very useful for imaging cells that will start at or near the coverslip or bottom of a well and will proliferate or migrate upwards in z.

Z intensity correction

If you have a thick sample you may find the image is dimmer on the far side of the sample furthest from the objective. To correct for this you can define a Z correction which will automatically adjust the laser power and/or gain as you move in Z through the sample. This should allow you to obtain a brightness that is visually comparable throughout your sample.

Start by going to the brightest part of your sample, closer to the lens, and define your channels. It's worth looking at the Histogram to have an idea of the pixel intensity distribution. You will aim to have a similar histogram when you go to the other side of your sample. Click on "Z Intensity Correction" and click on "+". Go to the other side of your sample and repeat defining the channels. When you are happy with the result and the histogram is as good as can be comparable, click on"+".

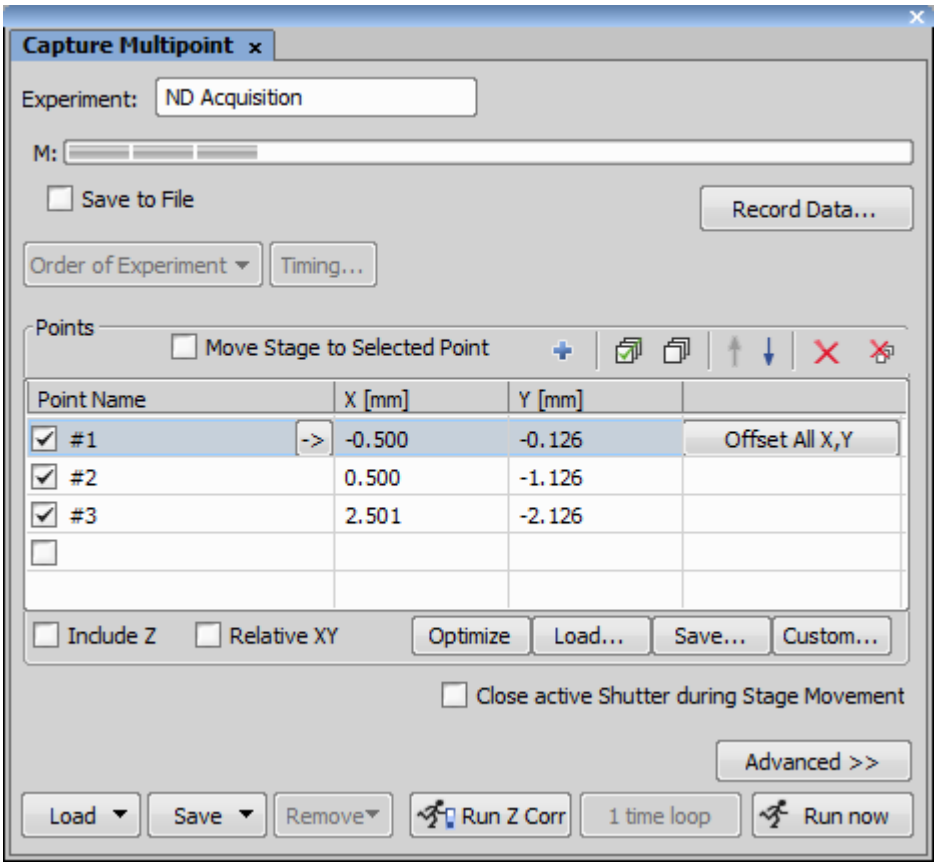


Tick "Use on Live" and review your sample.

Go back to the ND Acquisition panel and click on "Run Z Corr" 

ND Acquisition - Multipoint

Multipoint acquisition is used to move between pre-defined points on a specimen, such as wells in a well plate, points in a dish, or samples in a tissue microarray. Multipoint acquisition is only possible when the system is equipped with a motorised stage which has been correctly calibrated.



Choose <Close active Shutter during Stage Movement> to close the shutter between points.

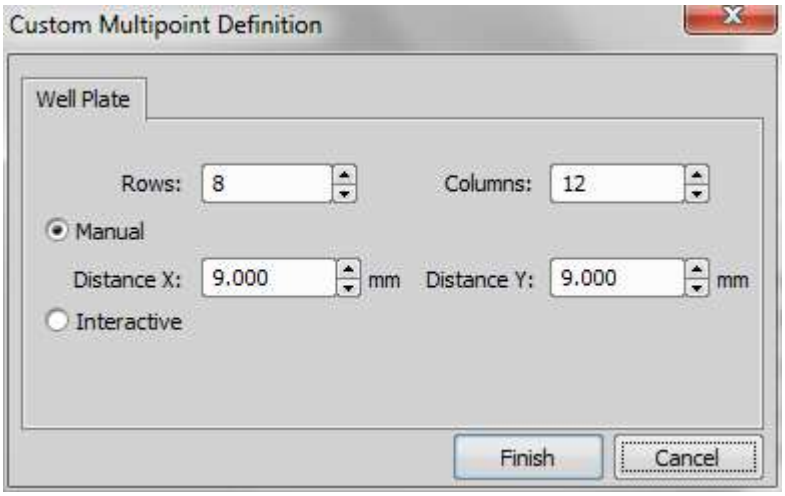
Custom Multipoint

When working with a sample with a set geometry (most often a well plate or chamber slide) it is possible for NIS Elements to calculate the coordinates of each well by knowing the number,

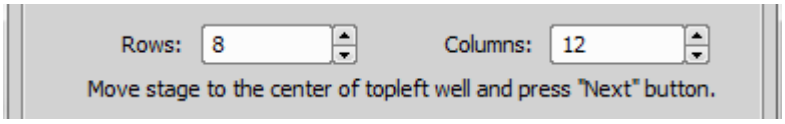
layout and spacing of the wells. To access this function, click the <Custom…> button on the Capture Multipoint window to bring up the <Custom Multipoint Definition> window.

First, enter the number of Rows and Columns on the well plate.

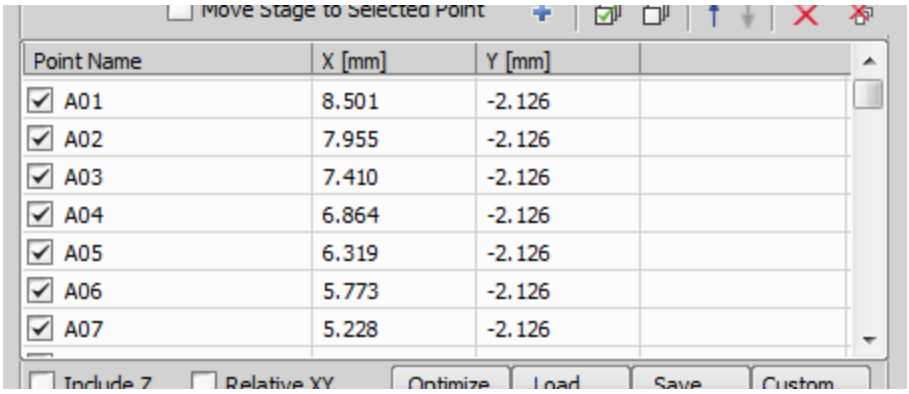
To define the wells manually, select <Manual> and enter the spacing of the wells horizontally and vertically in millimetres.



For NIS to calculate the spacing automatically, select <Interactive>. Move the stage to the centre of the well in the top left corner of the plate and press <Next>. It is easiest to do this on a low power objective so the centre of the well can be easily located.

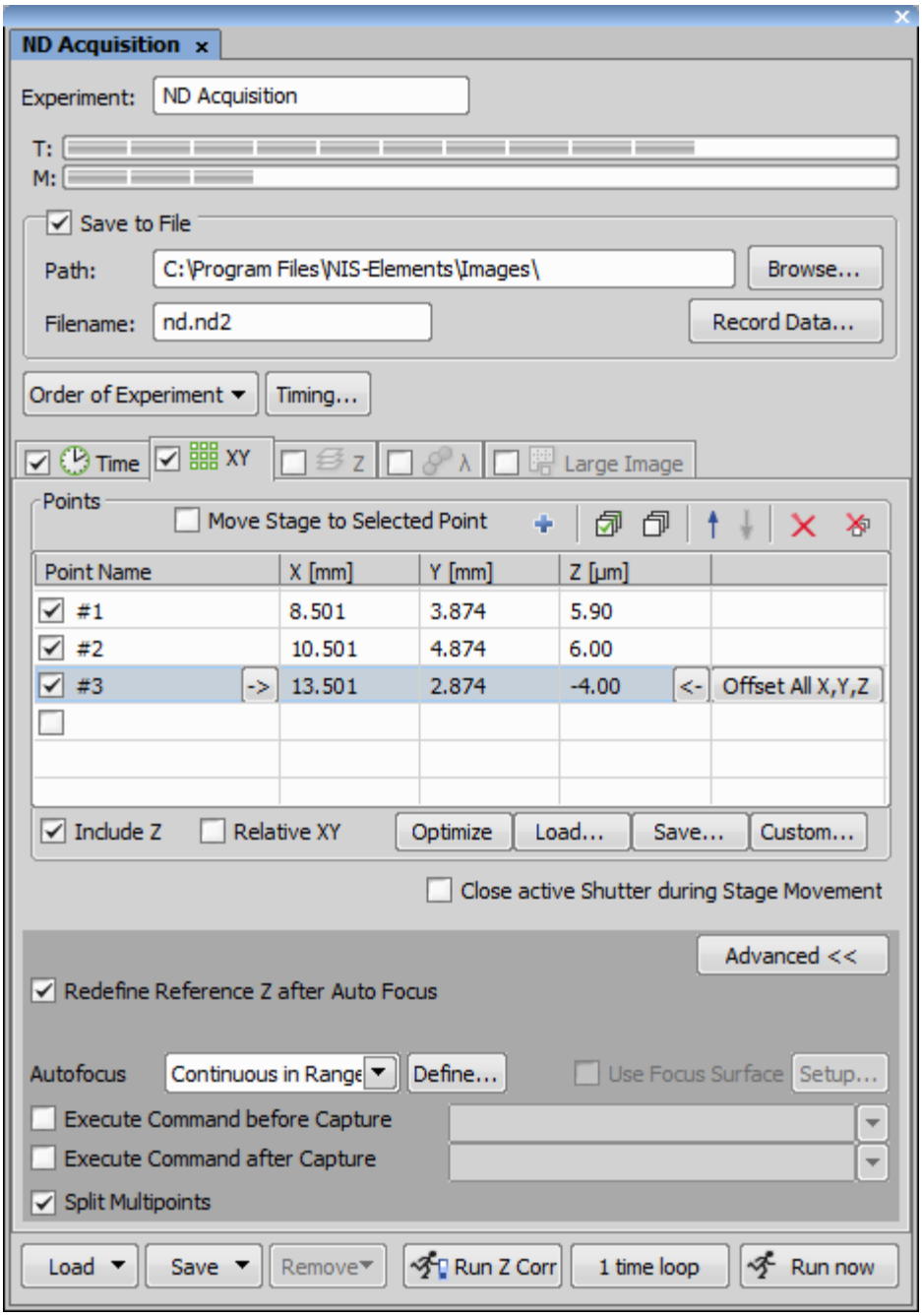


Move the stage to the centre of the well in the bottom right corner of the plate and press <Finish>. The positions of all the wells will be calculated and populated into the multipoint list. Wells are named and can be selected or deselected for imaging as desired.



ND Experiments with Multipoint

Go to Applications and choose <Define/Run ND Acquisition…> and ensure that the XY tab is ticked.



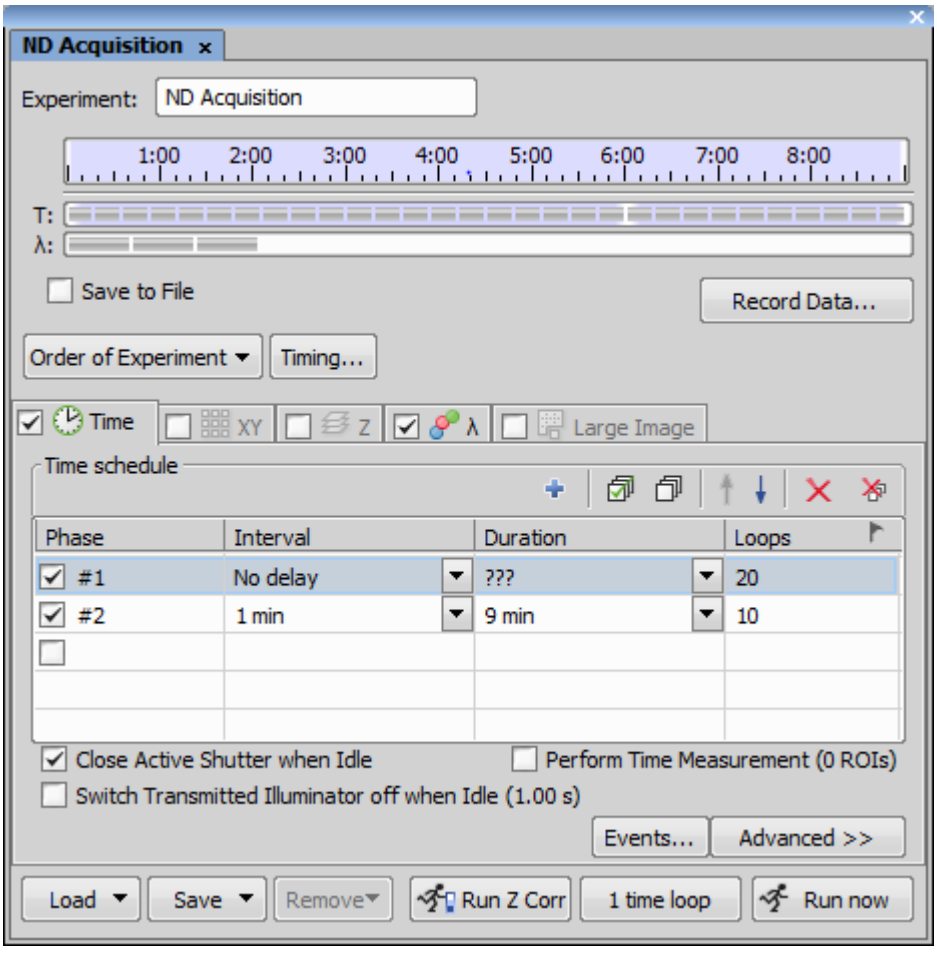
During a timelapse, PFS or autofocus can be used to keep the reference z postion as the cells change focus position by selecting <Redefine Reference Z after Auto Focus/PFS> from the <Advanced> options. In this section, <Split Multipoints> can also be selected when <Save to File>is on. This results in a separate file being generated for each position in the multipoint list.

ND Acquisition – Time-lapse

Time-lapse experiments allow the imaging of live specimens over time to record dynamic events.

ND Experiments with Time

Go to Applications and choose <Define/Run ND Acquisition…> and ensure that the Time tab is ticked.



To add several phases to the time-lapse with different capture speeds click the next line to add another phase.

If the system has a motorised shutter, ensure that this is set as an active shutter and that <Close active Shutter when Idle> is selected to close the shutter between images. This is especially important for time-lapses with long intervals. When running an experiment with <No delay> this box can be unticked to speed up acquisition.

The <Advanced> options allow setting of specific commands or macros before or after each capture and at the beginning of selected phases.

When setting the interval, take special care to ensure that all the other dimensions can be captured within the interval time. This can be checked by pressing the <Timing…> button, which gives an estimated capture time for a single time point. This can also be empirically tested by clicking <1 time loop> and running through a single capture of the experiment.

Using the Perfect Focus System in ND Experiments

When using Perfect Focus with NIS-Elements it is important to consider how different dimensions in a multi-dimensional experiment will impact the performance of Perfect focus.

General Notes

It is important that the objective lenses are correctly programmed into NIS Elements, the RCP Control Pad or Ti Tools. If you have the wrong objective identified, PFS may work but in sporadic ways.

When creating an optical configuration, the default is for "PFS Offset" to not be included in microscope settings. This is the recommended setting. If you change this and add it to microscope settings then the PFS Offset at the time the optical configuration is created will be stored. Please bear this in mind to avoid conflicts when setting up an ND experiment.

If you are doing a single point experiment and want to run as fast as possible you can run PFS in “hardware-only” mode. To do this, remove PFS from <Manage Devices> by un-ticking the box next to PFS under Ti. PFS will now run stand alone via hardware and firmware control only. This will reduce total system overhead.

Using PFS in ND Experiments

Time-Lapse

No special considerations are needed. PFS will use the offset that is currently in place when you start the experiment. This type of experiment can be run in “hardware-only” mode.

Lambda

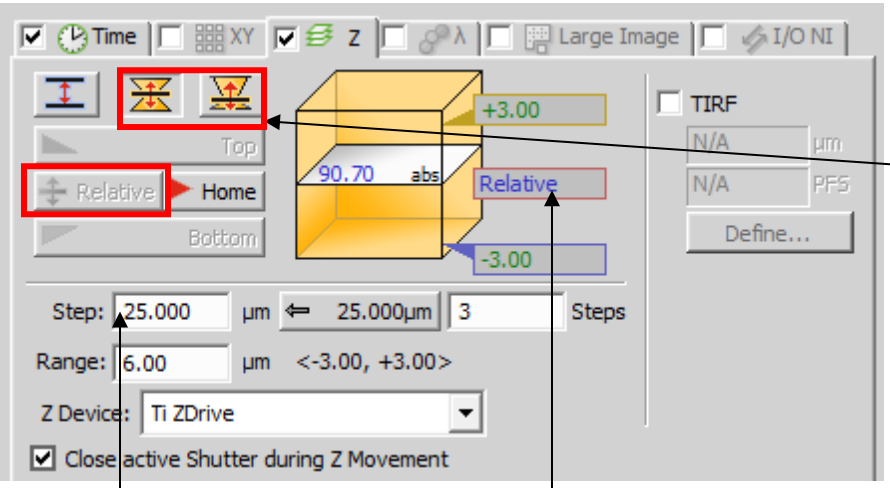
No special considerations are needed. This type of experiment can be run in “hardware-only” mode. PFS will use the offset that is currently in place when you start the experiment unless you have memorized PFS offsets for each optical configuration (channel). Do this ONLY if each channel is located in a different Z plane. Remember to assign the correct PFS Offset to each optical configuration every time you use this routine. When using Apo VC objectives this should not be necessary as all channels should be located in the same Z plane.

Z Stacking

PFS is constantly trying to adjust Z position whenever there is drift in the specimen/coverslip interface. Any stored Z values in a Z stack set-up will override any Z correction made by the PFS. If you want PFS correction to be active during a Z series (usually when doing Z over time) you should always use a relative Z stack set-up.

The following are the recommended Z accuracy and speed settings for the Ti internal Z drive: Speed: 2.25mm/s Accuracy: 0.0125um

To alter these settings go to [Manage Devices] > [Configure Device] (see image below).

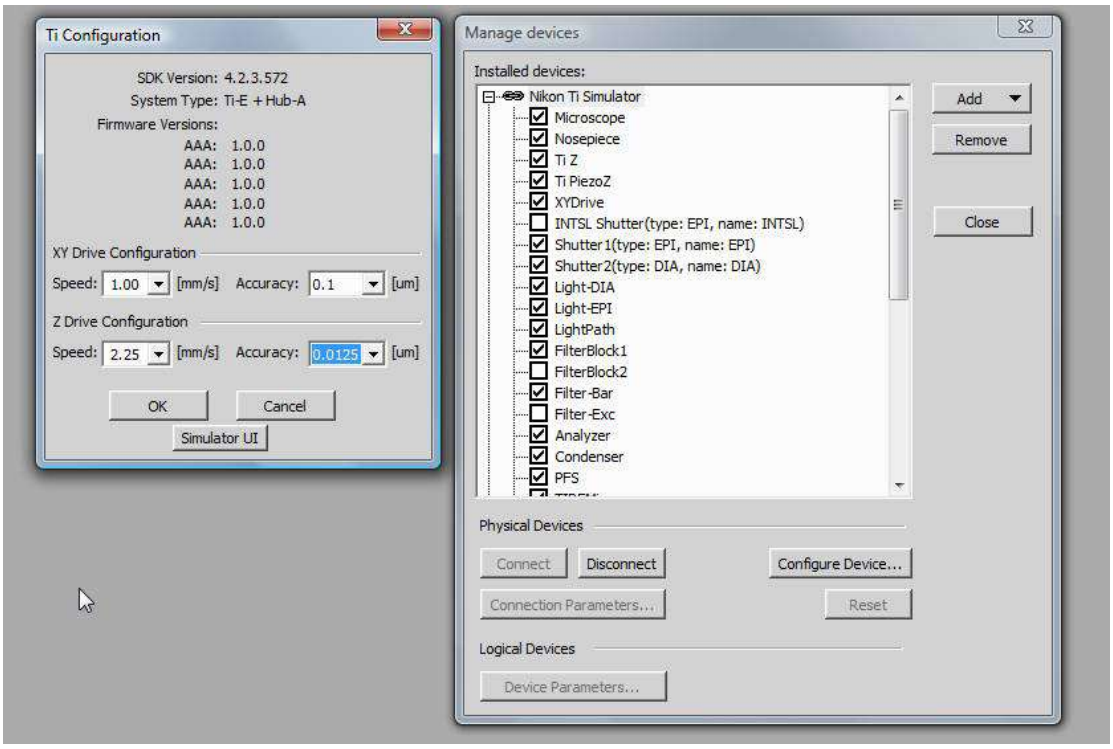


Select one of the two types of Relative Z stack: symmetric or asymmetric

Press the <Relative> Button to switch between relative and absolute Z stacks

XY Multi Point

The recommended accuracy setting is 0.1 um. If you are using the motorized stage with immersion optics we are recommending that you set the stage speed to 1.00 mm/s. These settings are especially important when using PFS and also ensure the best XY repeatability accuracy.



XY Multi Point when using PFS with immersion optics on normal slides/dishes

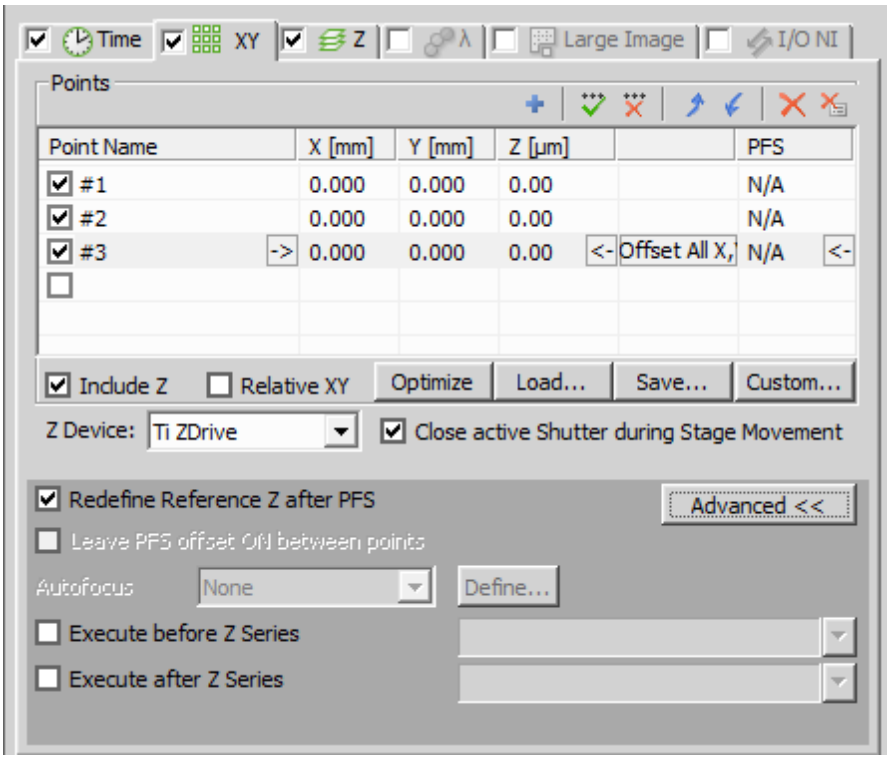
1. Use above recommended stage settings

2. Return to point 1 before starting the experiment and make sure PFS has reacquired

3. Turn off <include Z > found on XY tab in ND Acquisition Dialog

4. Turn on <Leave PFS Offset ON between points> found in Advanced Section XY tab in ND Acquisition Dialog

Note: Leave PFS Offset ON Between points can only be selected if include Z is not selected.



XY Multi Point when using PFS with immersion optics and chamber/divided slides

When using divided slides PFS can lose the refractive interface when traversing the division.

1. Use above recommended stage settings

2. Turn on <include Z > found on XY tab in ND Acquisition Dialog

3. Turn on <Redefine reference Z after PFS> found in Advanced Section XY tab in ND Acquisition Dialog

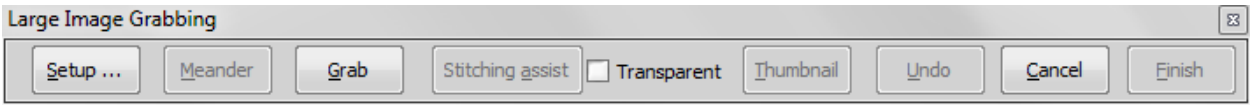
4. Return to point 1 before starting the experiment and make sure PFS has reacquired

Note: Redefine reference Z after PFS can only be checked on if "Include Z" is selected.

ND Acquisition – Large Image

Large Image allows several fields of view to be acquired and stitched together as a single high resolution image. Automatic acquisition of this image requires a motorised stage which has been correctly calibrated.

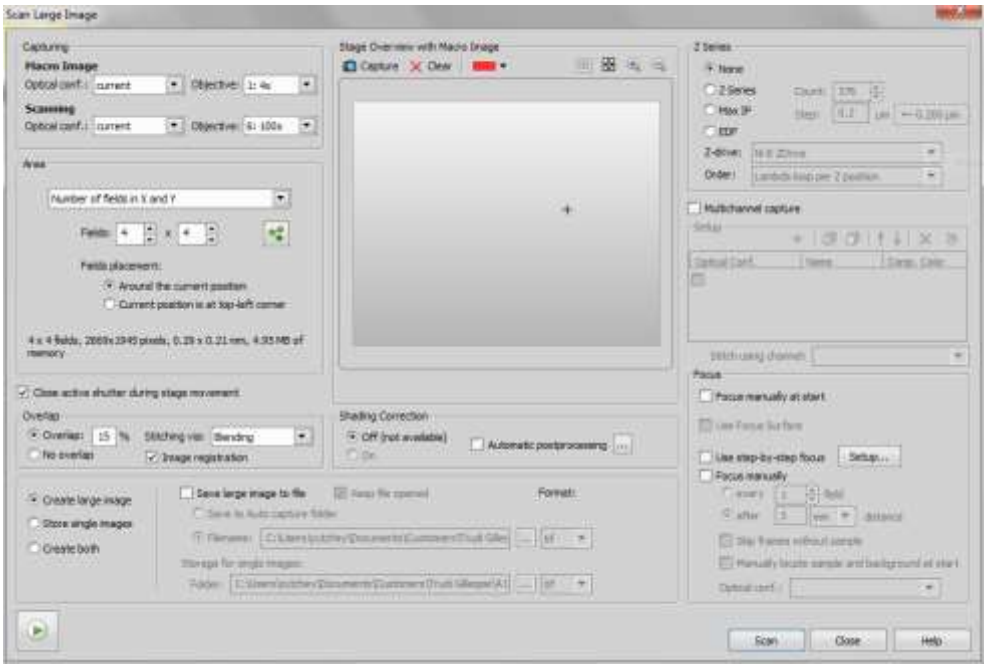
Standalone Grab Large Image
To capture a large image with a manual stage, go to Acquire and select <Grab Large Image…>



The overlap of the images can be defined in the <Setup> menu.

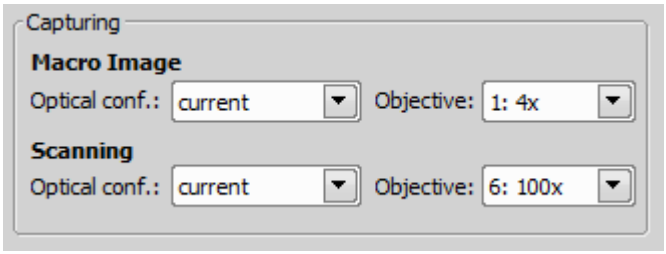
Position the specimen with in the Live window and click <Grab>. The image is captured and a thumbnail appears in the overview window. Clicking <Stitching assist> will show part of the previous image. Align the specimen with this image and click <Grab> again. Continue to the end of the row. To move down to start the next row, click <Meander>. When the large image is completed, click <Finish>.

Standalone Scan Large Image
To capture a large image with a motorised stage, go to Acquire and select <Scan Large Image…>.

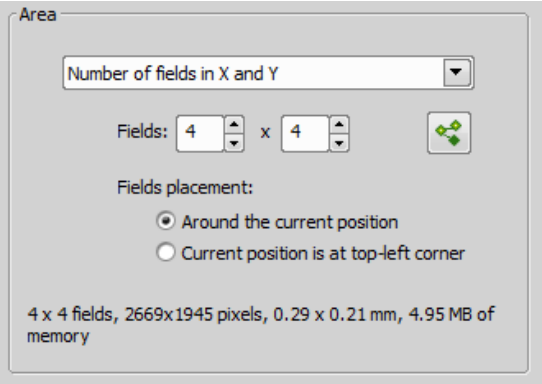


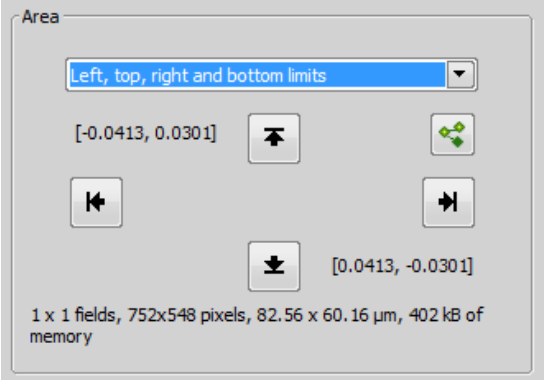
The Scan Large Image window is comprehensive, and allows the capture of a large image in combination with Z or Lambda dimensions, but not Multipoint or Time. The important sections to note are <Capturing>, <Area> and <Overlap>.

<Capturing> determines the lens used for the optional preview (Macro Image) and the actual captured image (Scanning). Ensure <Scanning> is set to the desired lens, and optical configuration if desired.

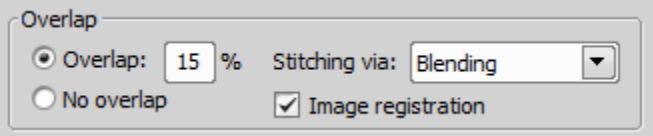


<Area> controls the total area covered by the large image. This can be defined by number of fields or by moving the stage to the top, bottom, left and right limits of the area to be scanned and clicking to store each limit. Once defined the number of fields, real size, pixel size and disk space required will be displayed.





<Overlap> controls if image registration is used, the amount the images overlap (used to align the images) and the stitching method (Optimal Path or Blending).

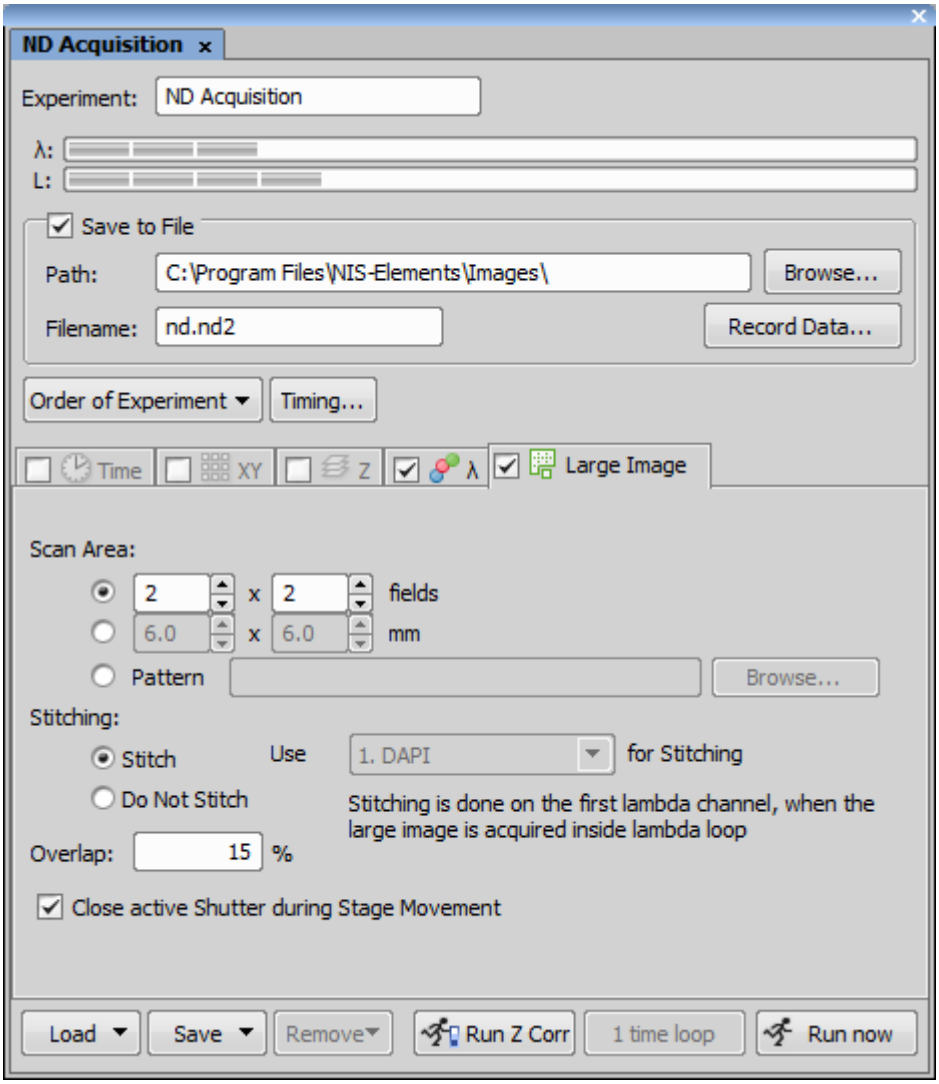


This dialogue allows the saving of the final stitched large image, the raw individual images, or both. The images can be saved direct to a specified folder.

To capture the large image, ensure that stage is free to move and click <Scan>.

ND Experiments with Large Image

This requires a motorised stage. Go to Applications and choose <Define/Run ND Acquisition…> and ensure that the Large Image tab is ticked. Please be aware that Large Image must be combined with at least one other dimension in order to be captured inside an ND experiment.



The large image will be captured around the middle position (either the current position or each position defined in the XY tab). The size of the image can be defined by number of fields or size in millimetres, or can be loaded from a previously stored pattern (e.g. from Grab Large Image Free Shape).

Stitching turns image registration on or off. <Stitch> will use image registration to align the images, and it will use the first channel in the Lambda tab for this purpose. <Do Not Stitch> will still give a single large image, but will rely only on the position of the motorised stage to align the tiles. <Overlap> shows the amount of overlap between each field in the large image. The default setting is 15%, which is optimal for most samples.

It is important to pay particular attention to the order of experiment with Large Images, as the order in which the parts of the image are acquired can have a significant impact on the quality of the reconstruction of the large image.

Optical Configurations

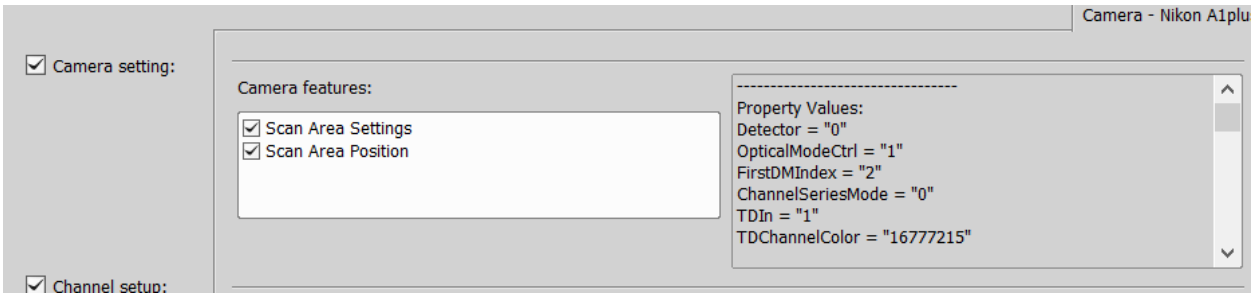
Optical configurations are stored settings that can be used to recall the camera and microscope to particular state with a single button click.

Optical Configuration List

The list of optical configurations can be accessed under the <Calibration> menu, under <Optical Configurations>. An optical configuration must consist of at least one of Camera Settings, Channel Setup, Microscope Settings or Objective.

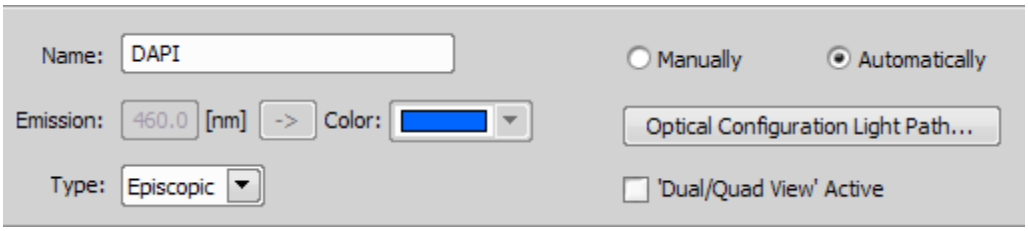
Confocal Settings

Adding the confocal settings to an optical configuration will mean that all confocal settings including scanner selection, detectors, lasers and scan area will be saved to the OC button.



Channel Setup

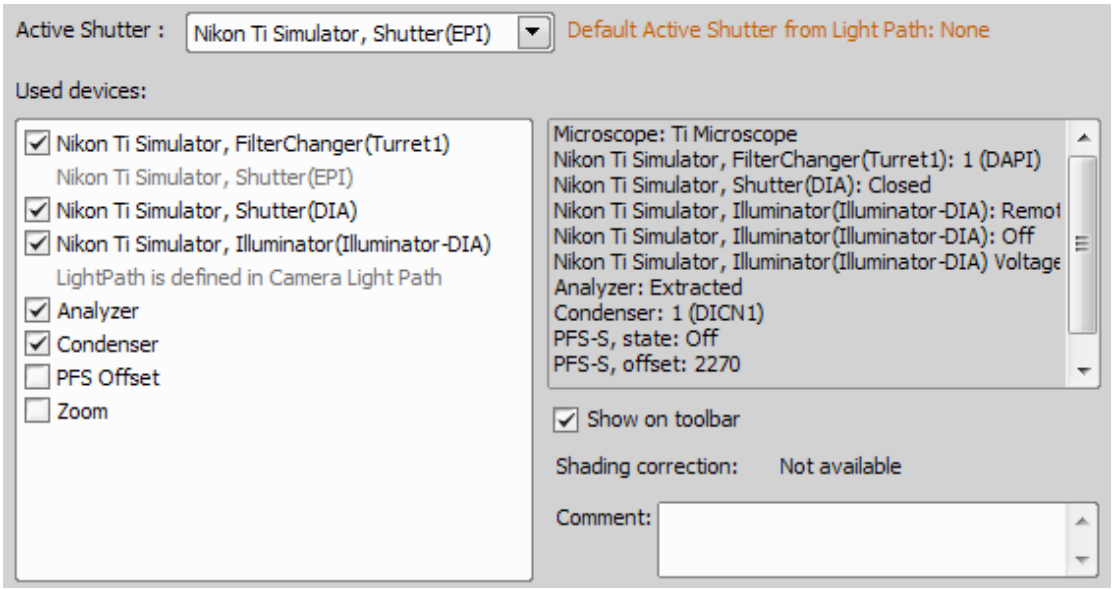
Channel settings determine the name of the channel and how it is pseudo-coloured when the optical configuration is selected. These settings can be defined automatically, based on the configuration name and filters selected, or manually by selecting a colour and name of the user's choice.



Microscope Settings

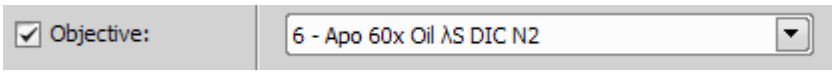
Optical configurations can change some or all of the microscope settings when activated. The Active Shutter is the shutter that will be opened when the camera is live or capturing and will close when the camera is idle. The other information stored in the optical configuration may include filter positions, light path, condenser and other shutters. The settings should be kept to the minimum number of components necessary to minimise the software overheads of changing between configurations.

Comments can also be added to the configuration for reference.



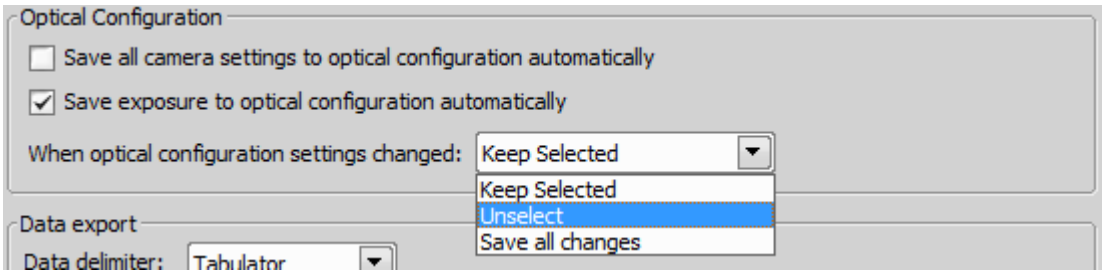
Objective

Optical configurations can be associated with a particular objective. Any objective currently installed on the nosepiece can be selected from the drop down list. This is not necessarily advised.

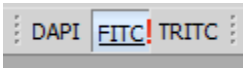


Update Settings

The update behaviour of the optical configurations can be controlled in Edit > Options under the <General> tab. Either all camera settings or just exposure time can be automatically saved to the optical configuration each time they are changed by selecting the appropriate tick boxes.



When any other changes are made, there are three behaviour options. <Keep Selected> keeps the optical configuration selected but displays an exclamation mark to show the settings have changed. Right clicking an optical configuration with an exclamation mark and selecting <Show Differences> shows the changes between the current state and the settings saved to the optical configuration.

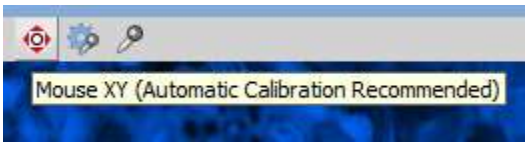


<Unselect>deselects the optical configuration as soon as a change is made. <Save all changes> automatically saves any changes made when the optical configuration is active, including microscope settings.

Mouse XYZ control

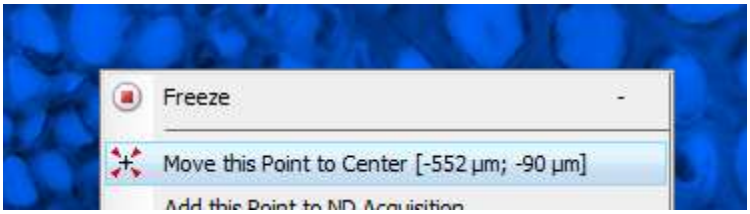
Microscopes equipped with a motorised z drive and motorised stage can be controlled directly by the mouse when a live image is open in NIS Elements.

Mouse Joystick XY
Mouse XY is enabled from the button on the live image window



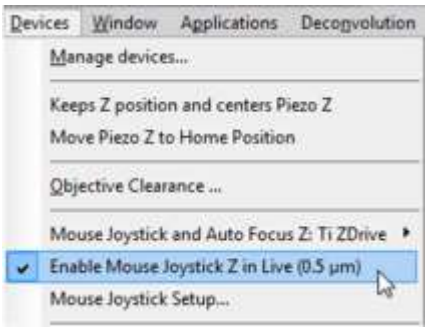
When active the Mouse XY button is highlighted in pink. The cursor turns into a four-pointed red cross. When this cursor is active the image can be clicked and dragged to move the stage. Please note that the current objective must be correctly calibrated for this to work.

The mouse can also be used to move the stage by right-clicking a point in the image and selecting <Move this Point to Center>.



Mouse Joystick Z

Enable Mouse Joystick Z by selecting it in the <Devices> menu in NIS-Elements. If you have more than one Z motor on the microscope, you can also define which motor is active as the mouse joystick here.



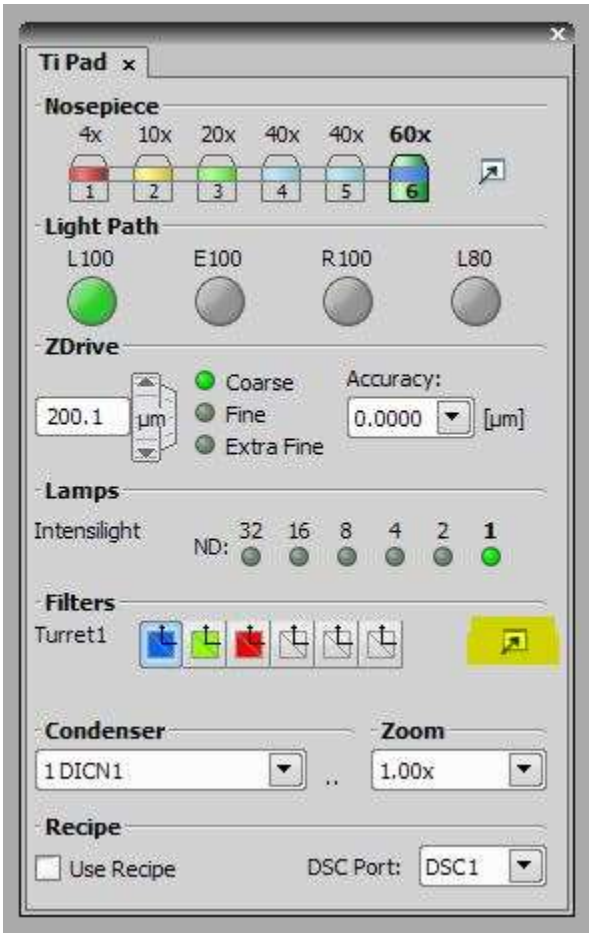
You can define the step size of the mouse joystick under the same menu by selecting <Mouse Joystick Setup> and choosing the step size for each movement of the mouse wheel.

If you hold down the SHIFT key while scrolling the mouse wheel the mouse joystick moves in larger steps as long as SHIFT is held down.

If you hold down the CTRL key while scrolling the mouse wheel the mouse joystick is temporarily disabled; instead the mouse wheel reverts to its normal behaviour and the image is zoomed in

Changing Ti2 Filter cubes

Filter cubes on the Ti-2 are a user-changeable part and can easily be switched out to suit the needs of an experiment. When the filter cubes have been physically changed the software can be updated to reflect this

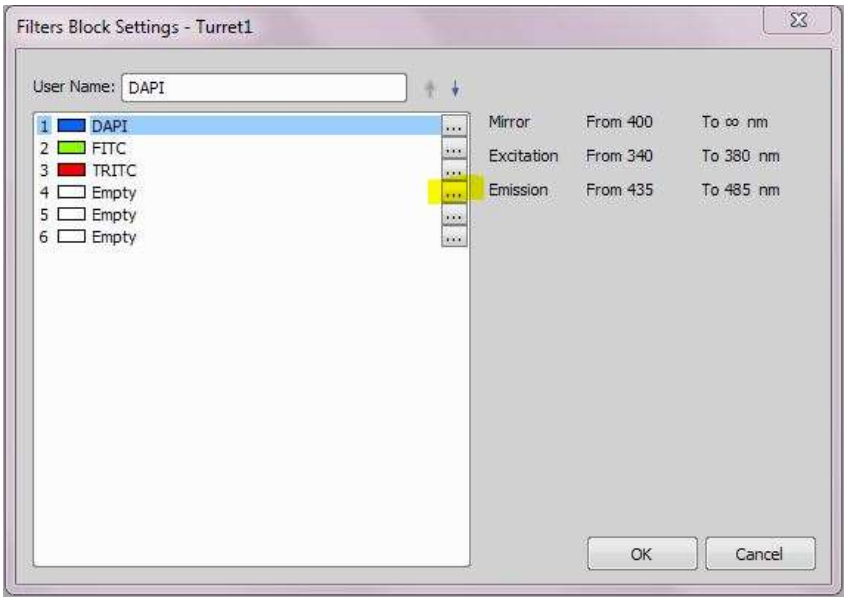


Accessing the Filter Block Settings

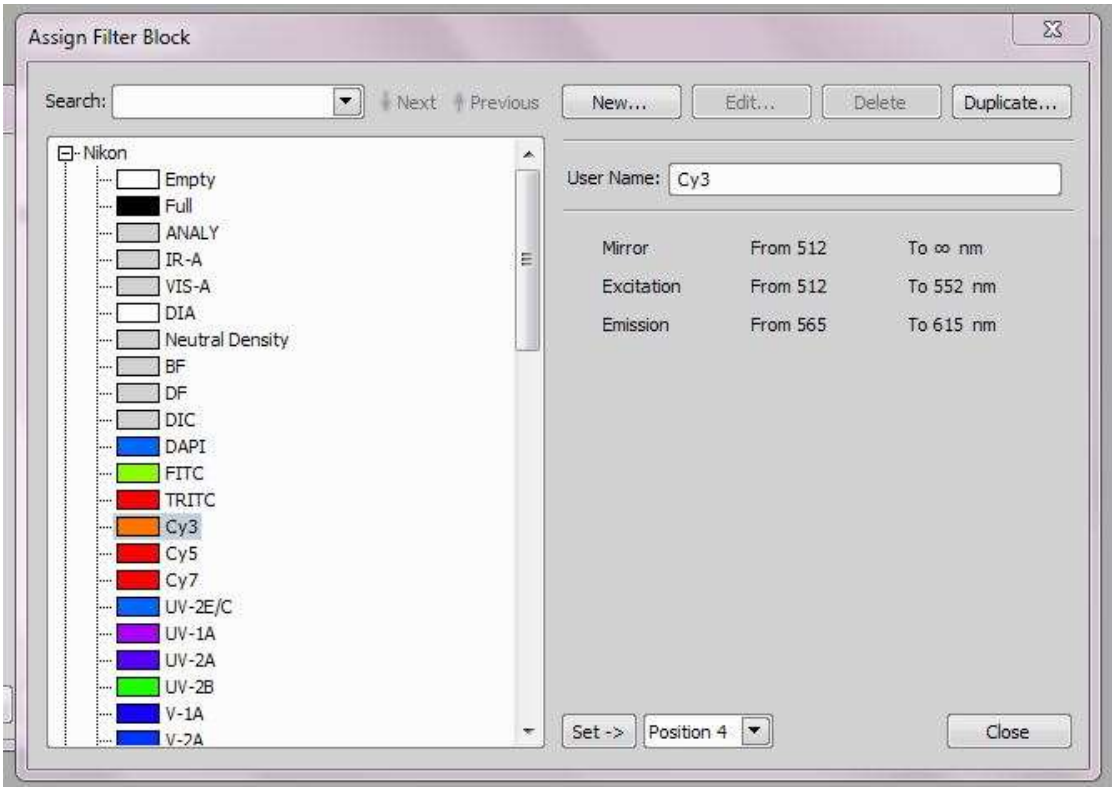
To access the settings menu for the filter blocks, open the microscope pad (Nikon Ti-E is used here) and look for the settings button, as highlighted in the image below.

Pressing this button will open the <Filter Block Settings> window. In order to add a new filter to an empty position, select the position that the filter is installed in (this can be found on the turret itself) and click the <…> button.

Filter cube



This will open the <Assign Filter Block> window. Select the manufacturer of the filter block (e.g Nikon, Chroma) and then select the block from the predefined list. Once selected click the <Set -> > button to assign the filter block to the desired position.



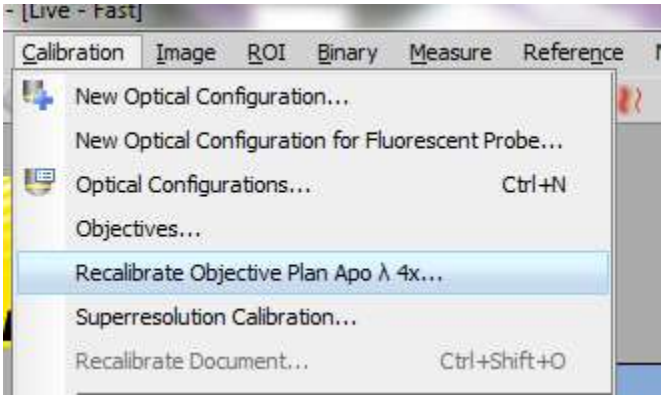
Once the filter block has been set, click the <Close> button. The filter block should now be visible in the Filters section of the microscope pad and is ready for use.

Objective Calibration

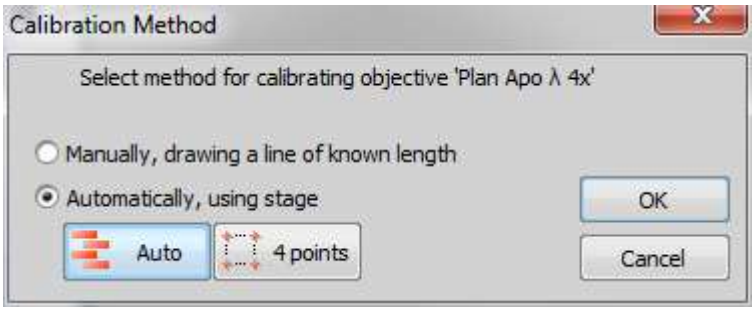
In order to give accurately calibrated images from which measurements can be taken, and also to perform functions that involve the motorised stage such as stitching or multipoint acquisition, the objective in use must be calibrated. Objective calibrations are stored separately for each imaging device (i.e. they are different for each camera)

Objective calibration with Motorised Stage

To start, ensure a specimen with clear structure and high contrast is on the stage and in good focus. To recalibrate the lens, choose <Recalibrate Objective … > from the <Calibration> menu.



When using a motorised stage, calibration should always be done automatically, because as well as determining the pixel size, this will also determine the camera angle and flip, which are required for all motorised stage functions.

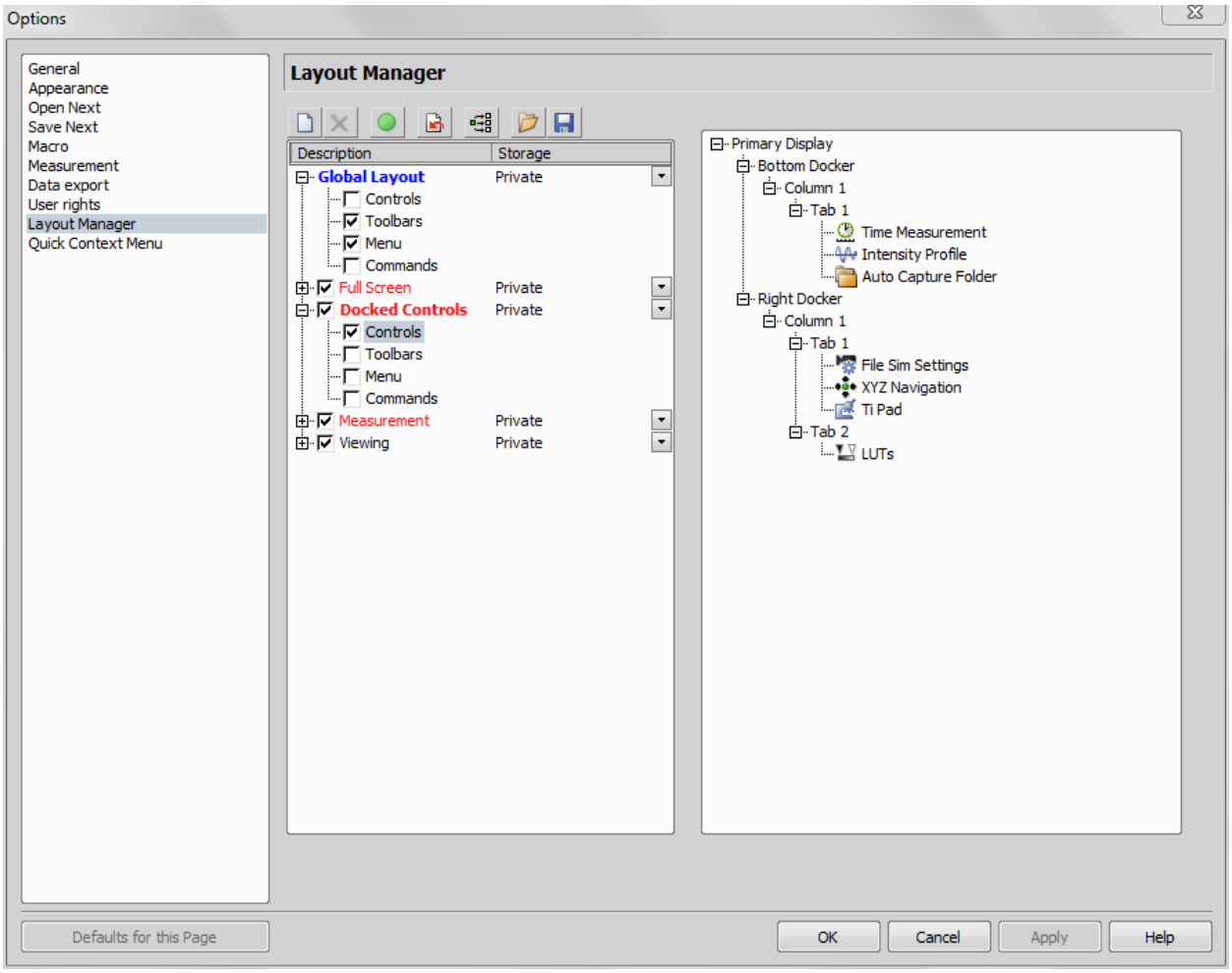


Select <Automatically , using stage> and <Auto>. Ensure the stage is free to move in all directions and click <OK>. The stage will move in an increasing spiral for several seconds. When completed a dialgoue box will display the results of the calibration. The stage is now ready to use.

Layout Manager

NIS Elements has three default workspaces as standard: <Full Screen>, <Docked Controls> and <Measurement>. Each layout can be customised with the preferred selection of control panels and dockers, as well as customised toolbars and menu items. These different workspaces are referred to as “Layouts”. Additional layouts can be created, and can be set to be private to each user login, or shared across all logins.

As well as selecting control panels in the workspace directly, panels, toolbars and menus can be edited in Layout Manager. Right-click the workspace background and select <Layout Manager…> to bring up the window.



Settings selected by checkbox under the <Global Layout> menu will remain constant when switching between tabs. All other settings are controlled on a tab-by-tab basis. Each tab appears under its name and a hierarchical menu appears on the right to illustrate the selected control panels and their positions in the workspace.