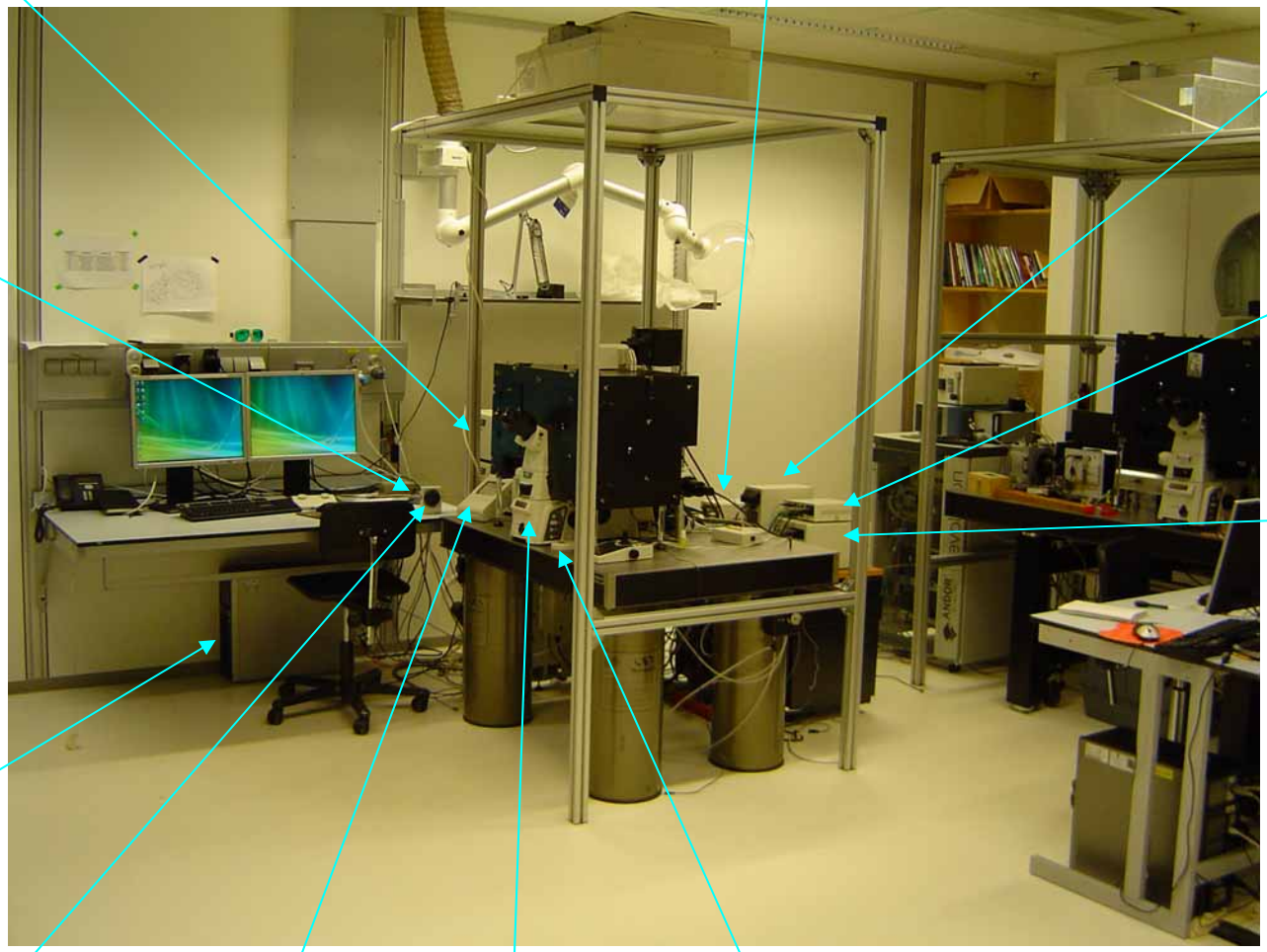
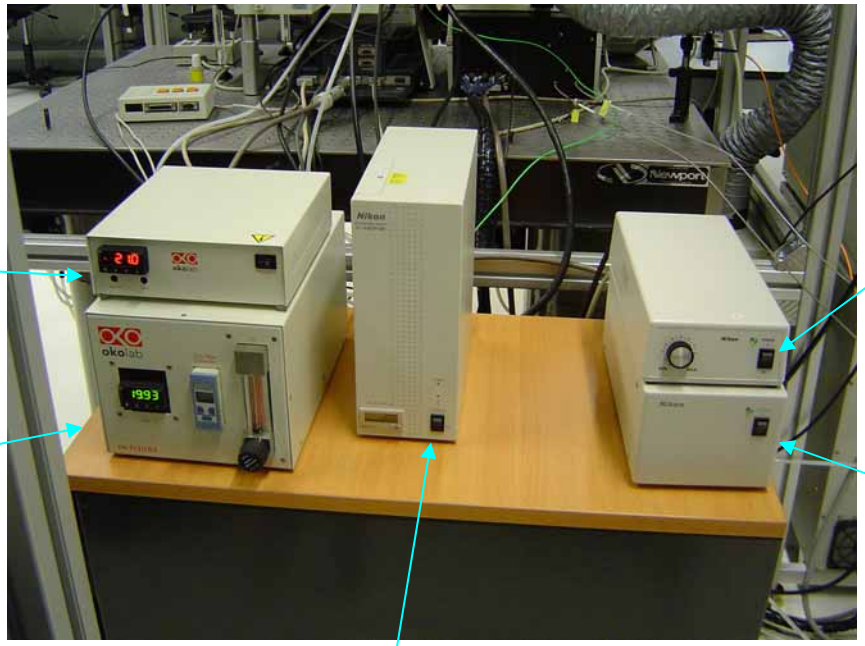
Overview A1 Microscope



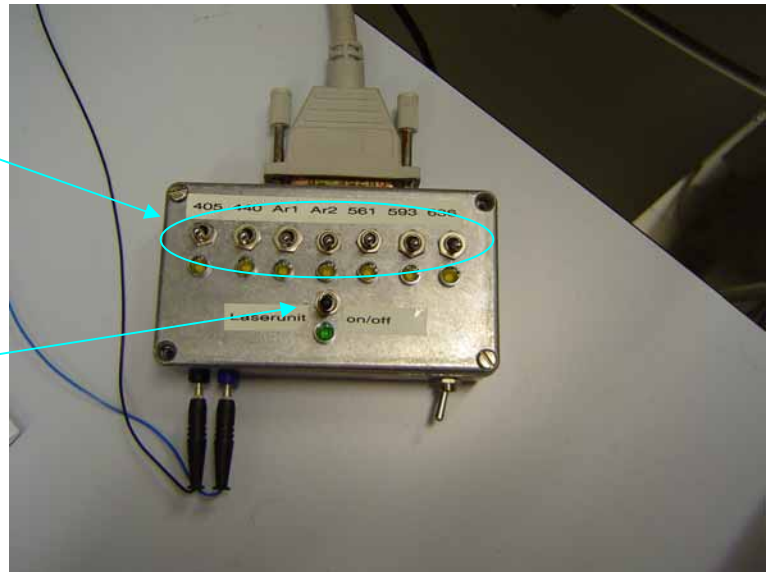
Switch on procedure

- Switch on halogenlamp powersupply (1)
- Switch on xyz driver unit (2)

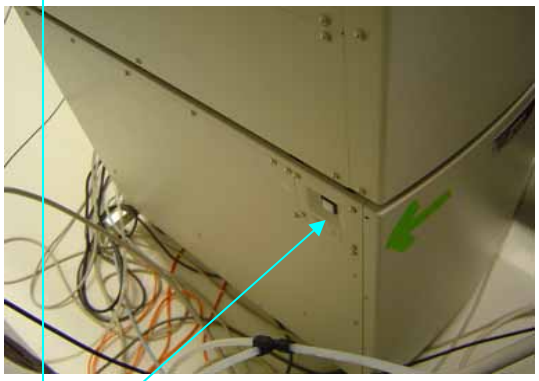


- Switch on control unit (3)
- Switch on laserunit (4)
  - First switch on the laserlines you need (Yellow LED's)
  - Secondly switch on the laserunit (green LED)

Switches
laserlines

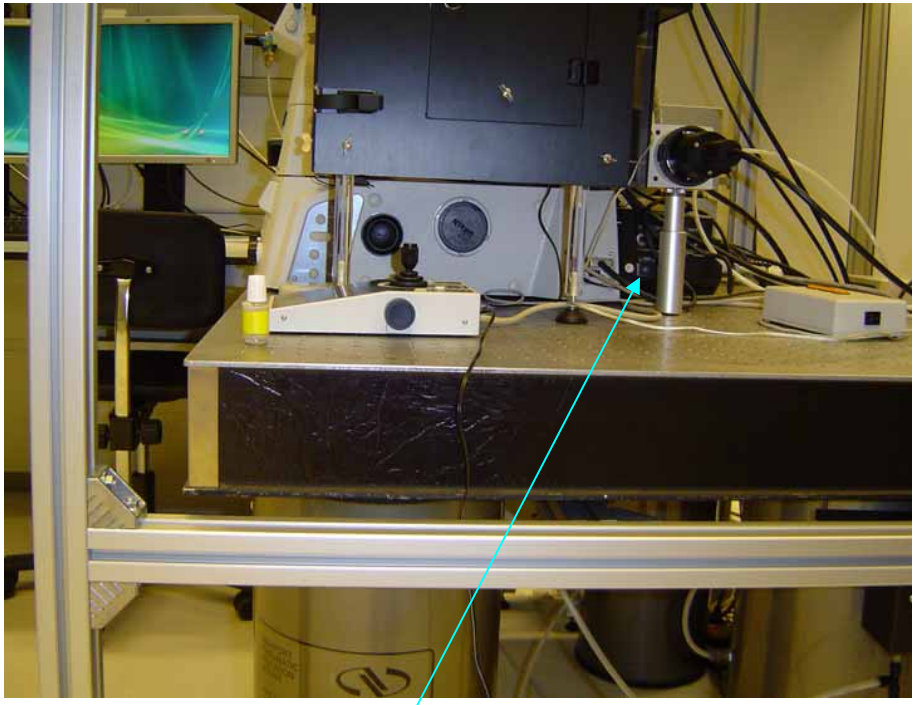


Switch
Laser unit (4)



Switch
Control unit (3)

- Switch on microscope body (5)
- Start NIS software (6)
- If needed switch on:
  - Hg lamp (7)
  - Temperature controller (8)
  - CO2 regulator (9)

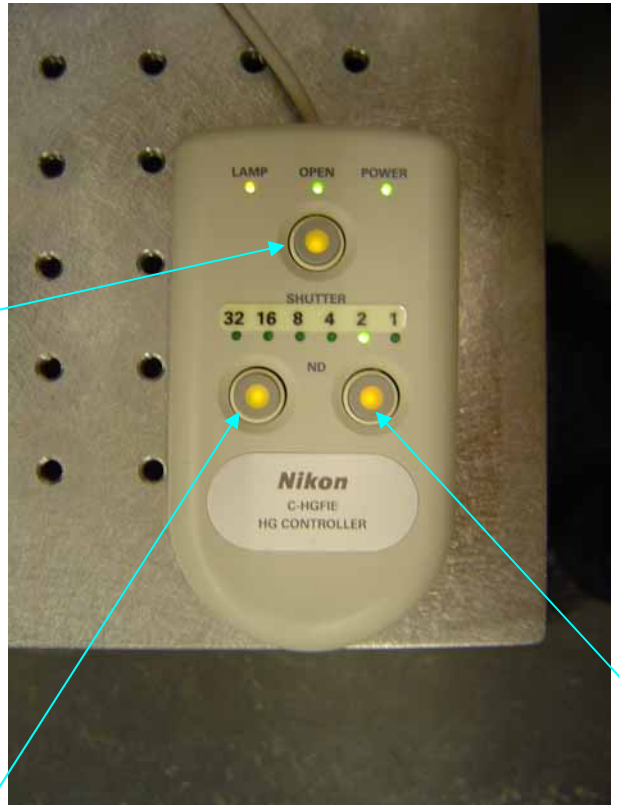


Switch
Microscope body (5)

Switch off procedure

- Shut down NIS software (6)
- Switch off laser unit (4)
- Switch off Hg lamp (7)
- Switch off CO2 regulator (9)
- Switch off contol unit (3)
- Switch off microscope body (5)
- Switch off halogen powersupply (1)
- Switch off XYZ driver unit (2)

Hg lamp remote control

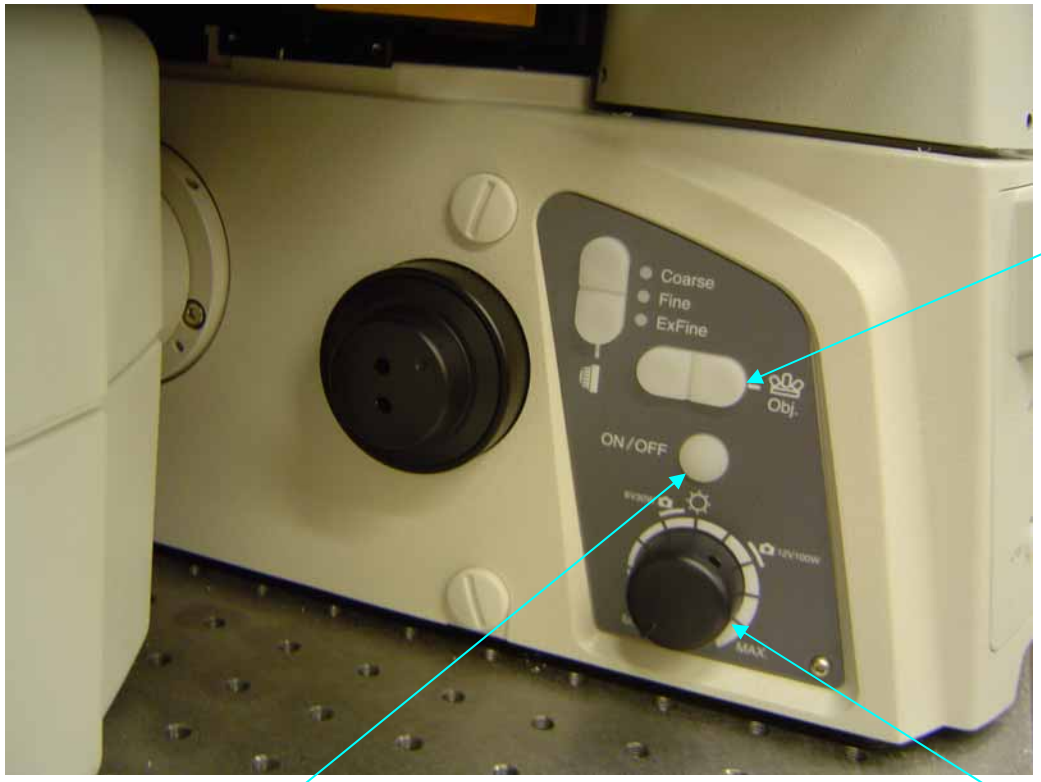


Shutter

Decrease light intensity

Increase light intensity

Switch on Halogen lamp



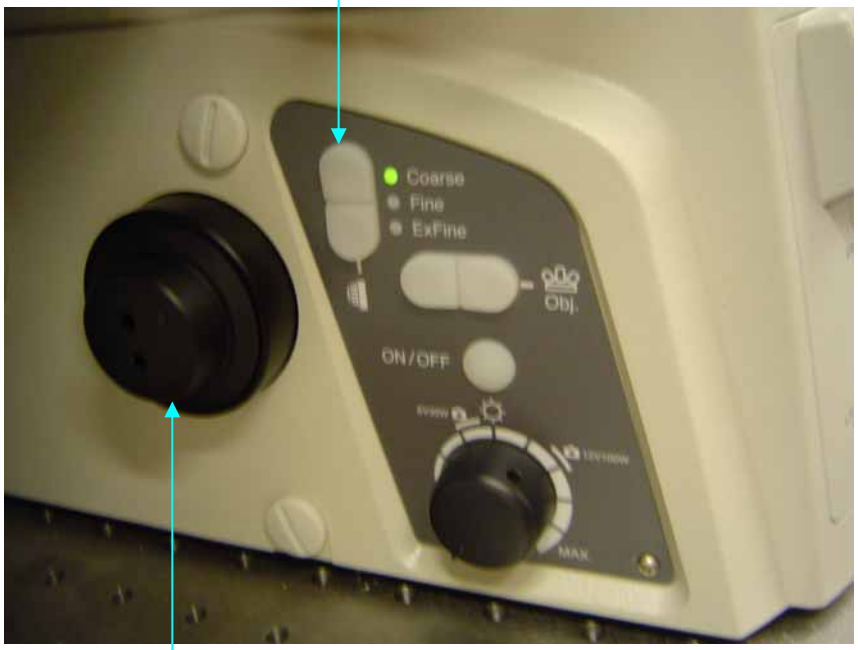
Change objective

Switch on Halogen lamp

Regulate intensity
Halogen lamp

Focus

Select between coarse/fine/extrafine



Focus knob

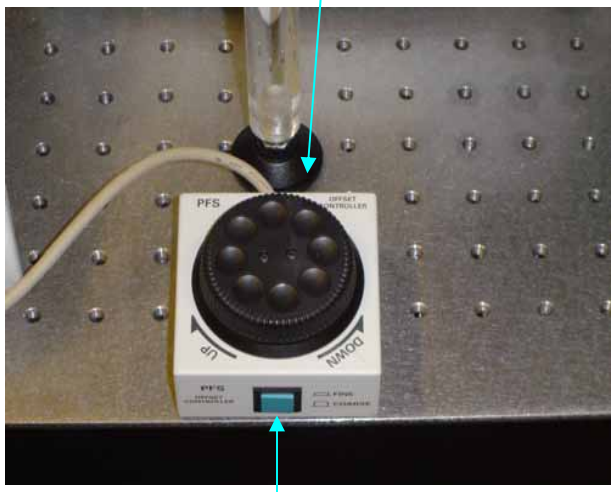
Select between coarse/fine/extrafine

Focus knob

- Or focus in the NIS software

Perfect focus

Focus adjust when perfect focus is switched on



Toggle between coarse and fine

Switch on perfect focus

Picture pfs dichroic

To be able to use PFS the dichroic Needs to be in the Light path

Port control

Eye port

Scan port (100)

External phase
Contrast selector

Not used

Not used

Intermediate magnification (1.5 – 1.0x)

Remote

Switch on pf

Objective selection

Filter selection for viewing with HG lamp

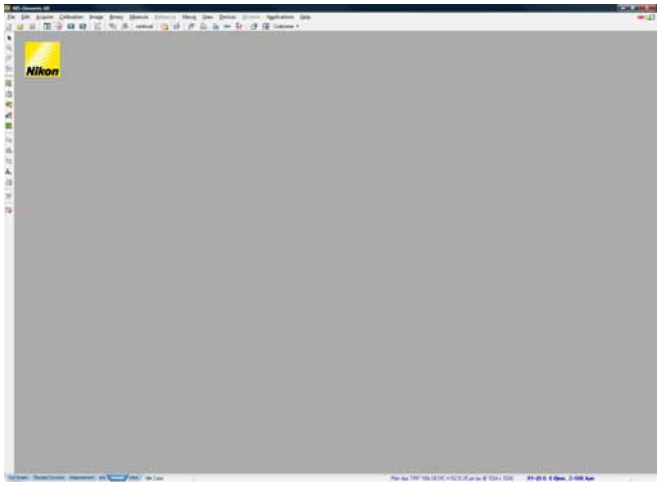
Phasering condensor selection

Software

Double click the NIS Elements icon

When the software has started the main screen appears.

Note: If a fault appears close software, see if microscope and Control unit are switched on and restart the software



Control software for the microscope

Objective selection

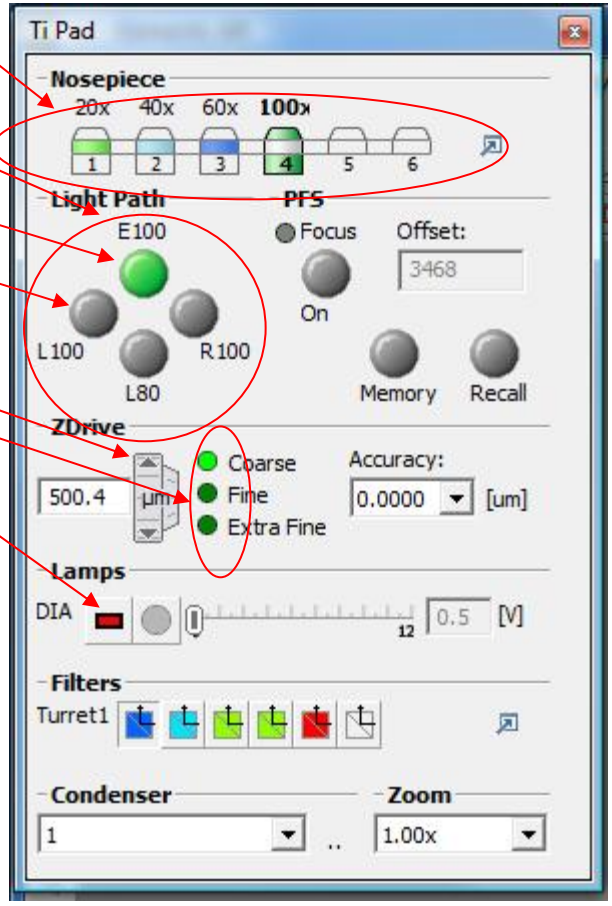
Port selection
Eye port
Scan port

Z control
Change speed

Halogen lamp control
Remote
On/Off
Intensity

Filter selector for Hg lamp

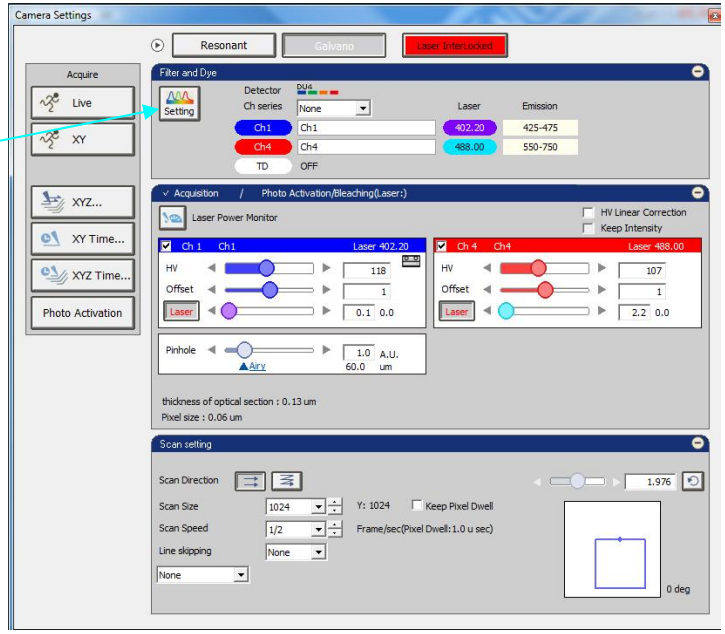
Codensor selector



How to setup a configuration

In the camera setting menu click on settings

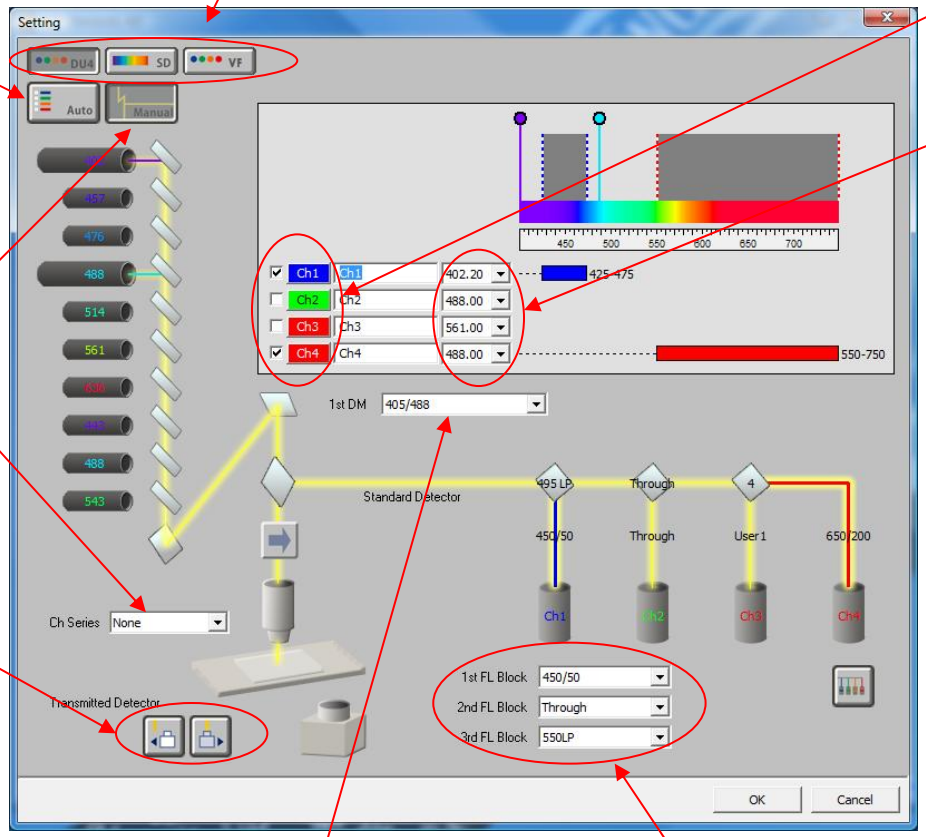
Setting



Select the 4 PMT detection unit, spectral detector or variable filter (in this example the 4 PMT detection unit has been selected)

Select what pmt(s) to be used

Automatic settings for standard dyes



Laser selection

Manual configuration

Sequential scanning

Transmission detector

Select the filters you want to use

Select main dichroic

Using the autosetup

Select auto

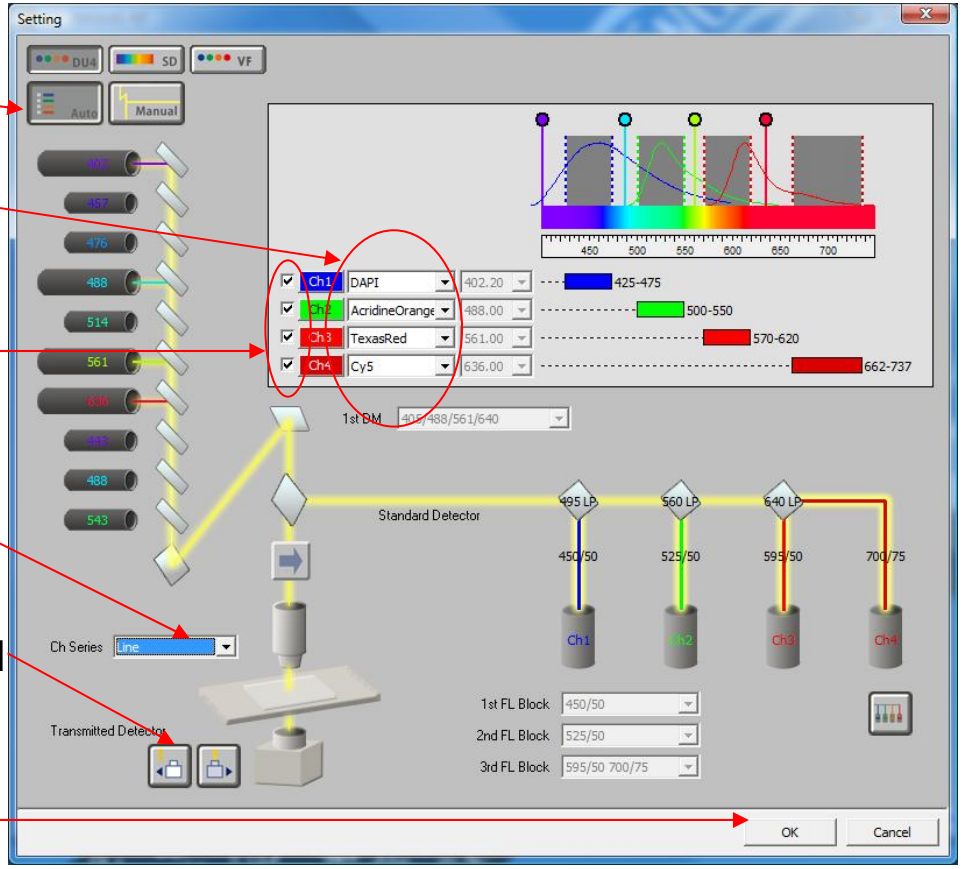
Select the fluophore (from blue to red)

Select the channel for this fluophore

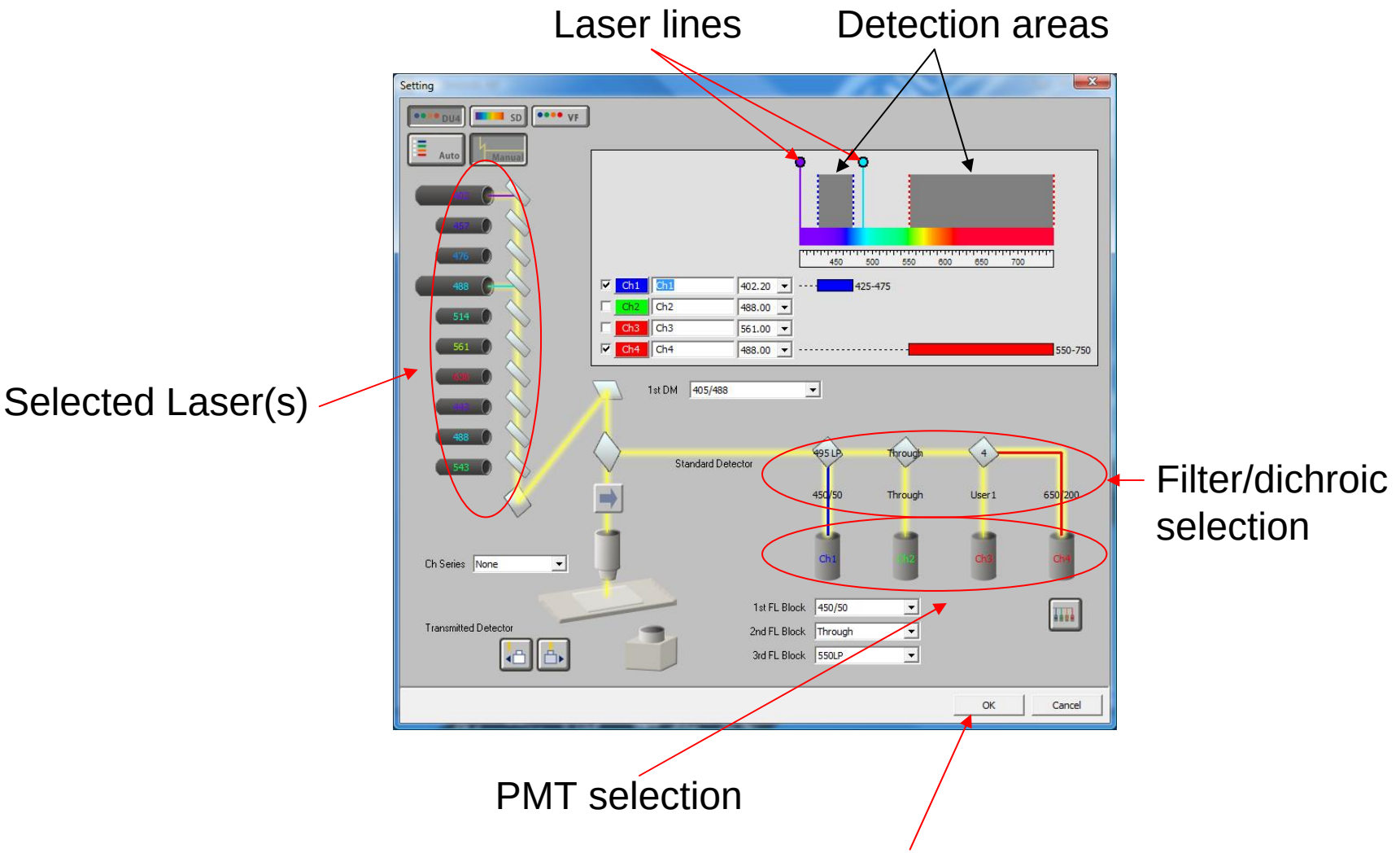
Select sequential scanning if needed

Select transmitted light detector is needed

Select ok



Visualisation of your configuration



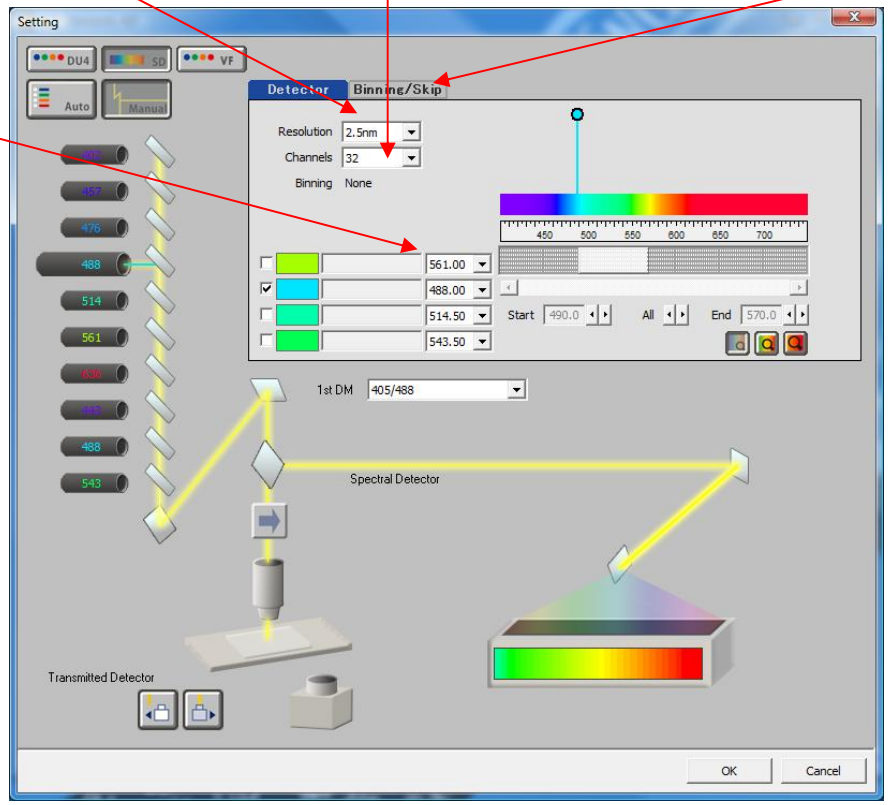
Spectral detector setup screen

Resolution per pmt

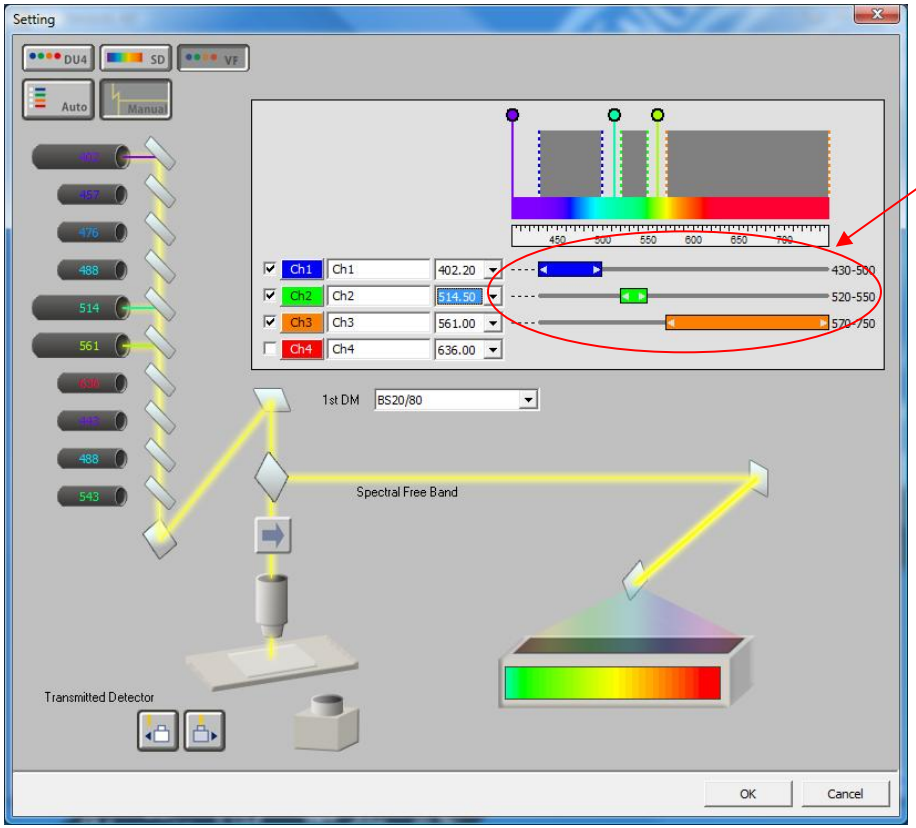
Number of pmt's to be used

Possibility for binning or skipping pmt's

Laser selection

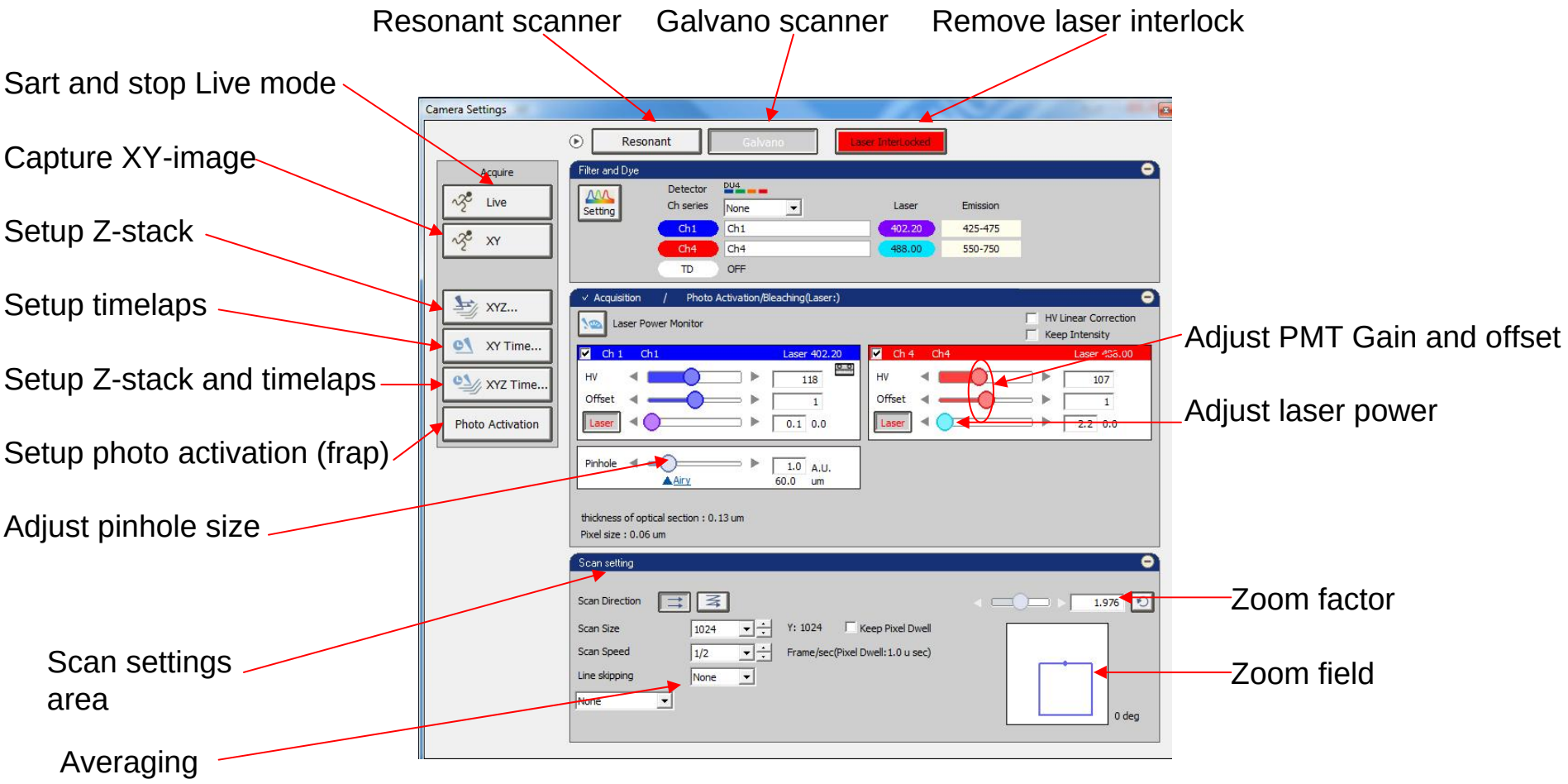


Using the variable filter mode



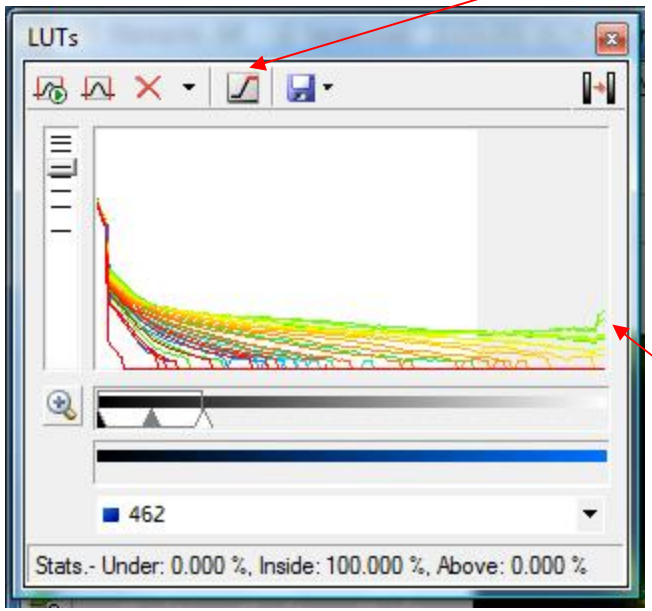
The detection bands can be adjusted in with and position.

The camera settings menu



Check quality of your image

Mark overexposed pixels another colour



The LUTs screen gives an overview of the greylevel distribution. If there is a peak
On the right side of the table you have pixels that are overexposed.

Zoom window

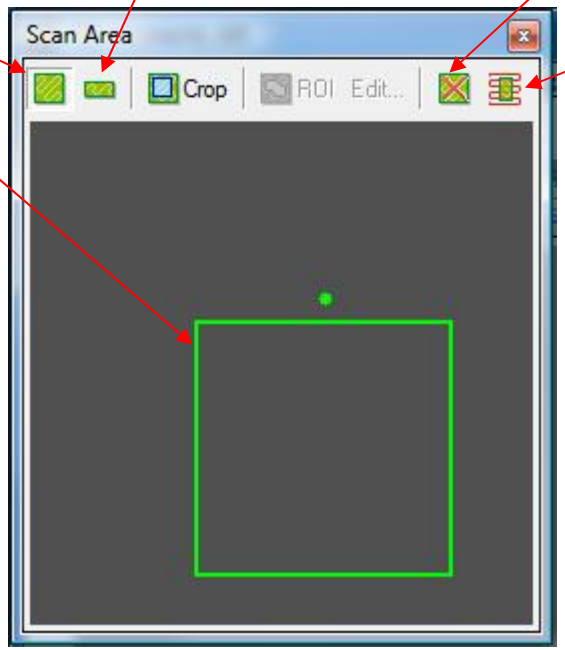
Keeps 512x512 pixels

Adjusts number of pixels

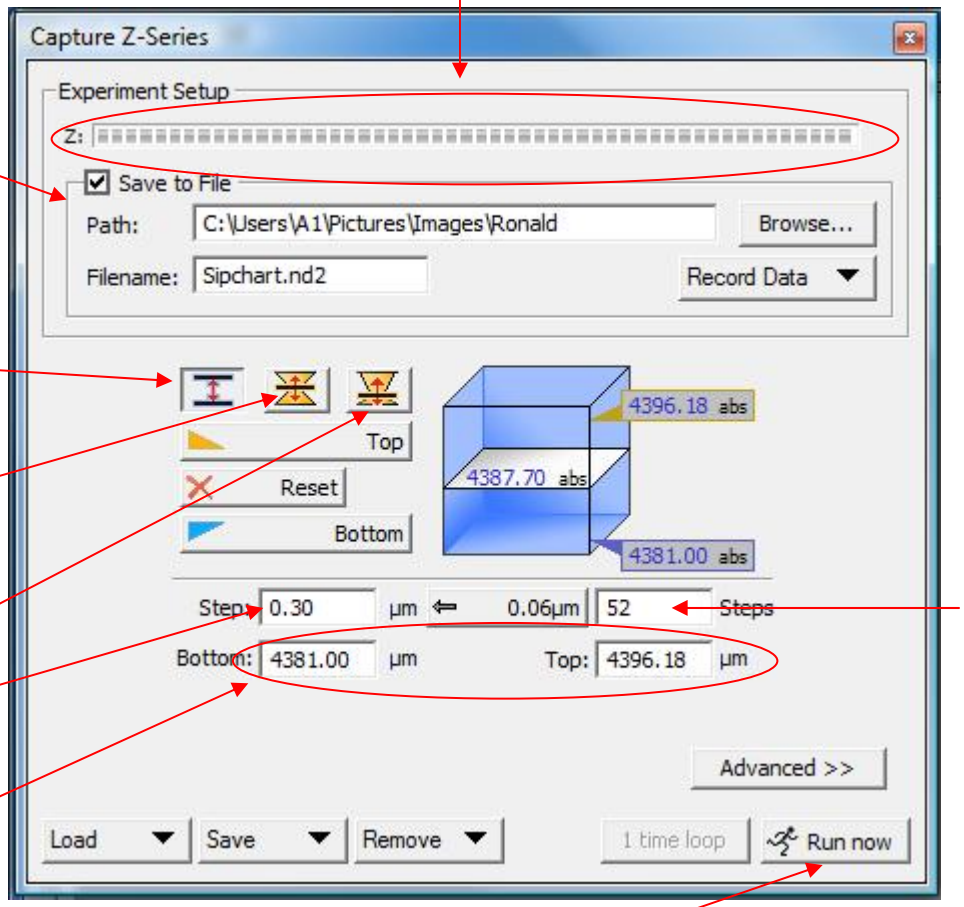
undo zoom

refresh zoom screen

Click on the green line and drag it to the size you need
Then rightclick to activate



Setting up a Z-stack



Progress bar

File setting

Mark top bottom

Symetrical volume

Asymetrical volume

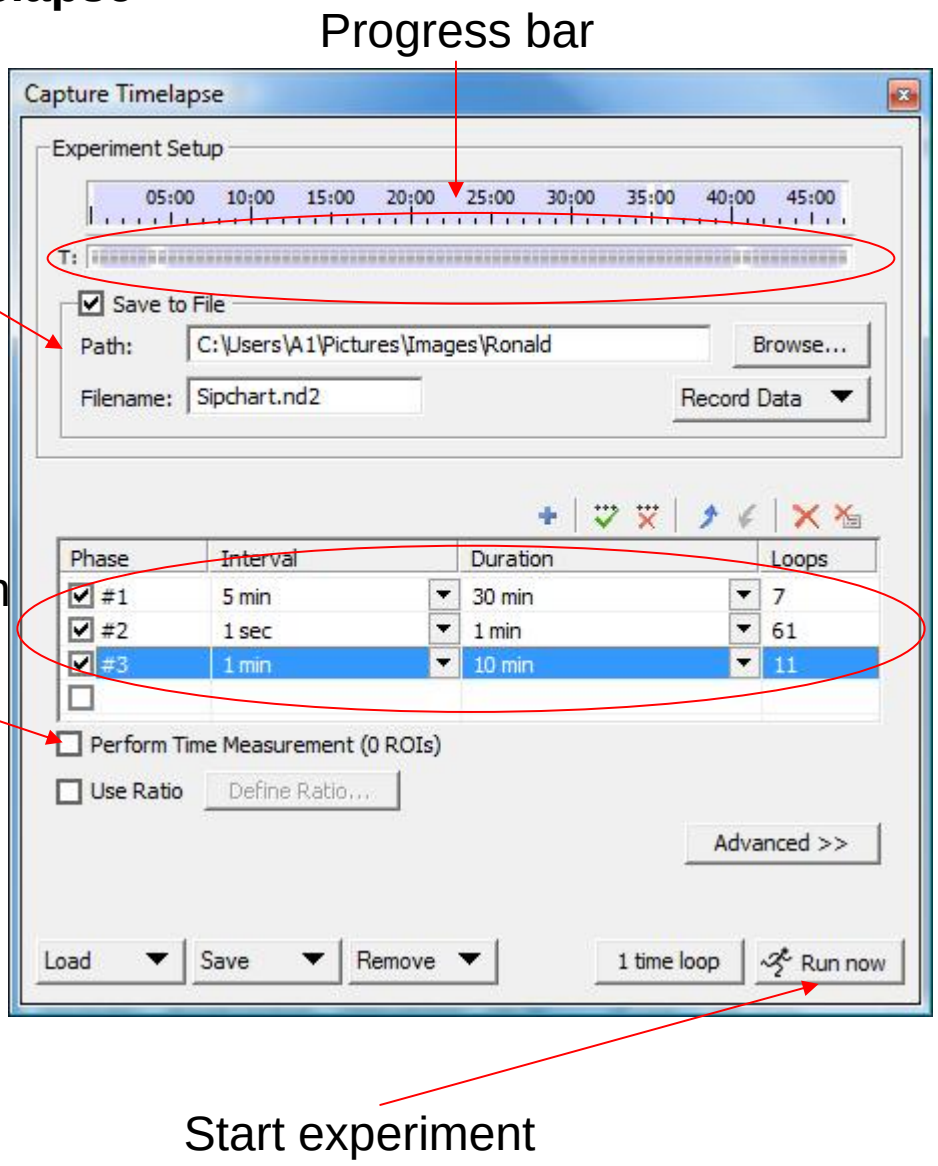
Slice thickness

Absolute values

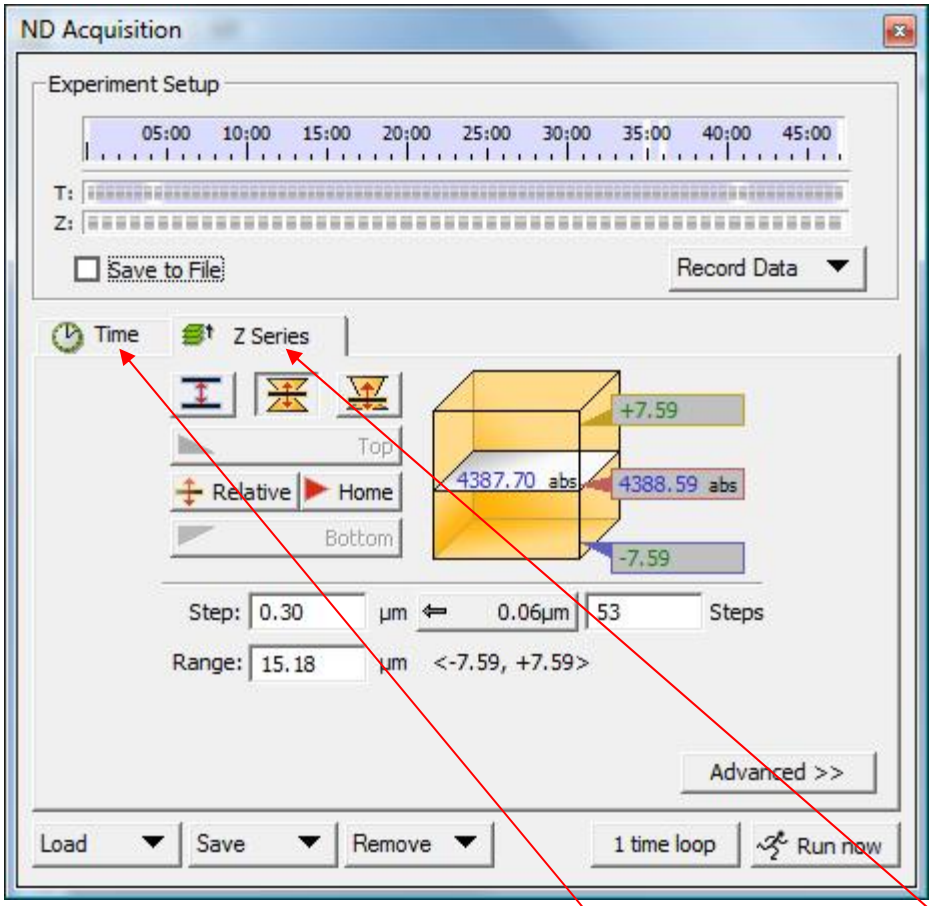
Number of slices

Start experiment

Setting up a timelapse



Setting up a timelaps of a Z-stack



It’s a combination of the two previous slides with the tab for timelaps and one for the Z-stack

Analyse a timelaps

Under analysis select time measurement, create a region of interest (ROI) and select measure

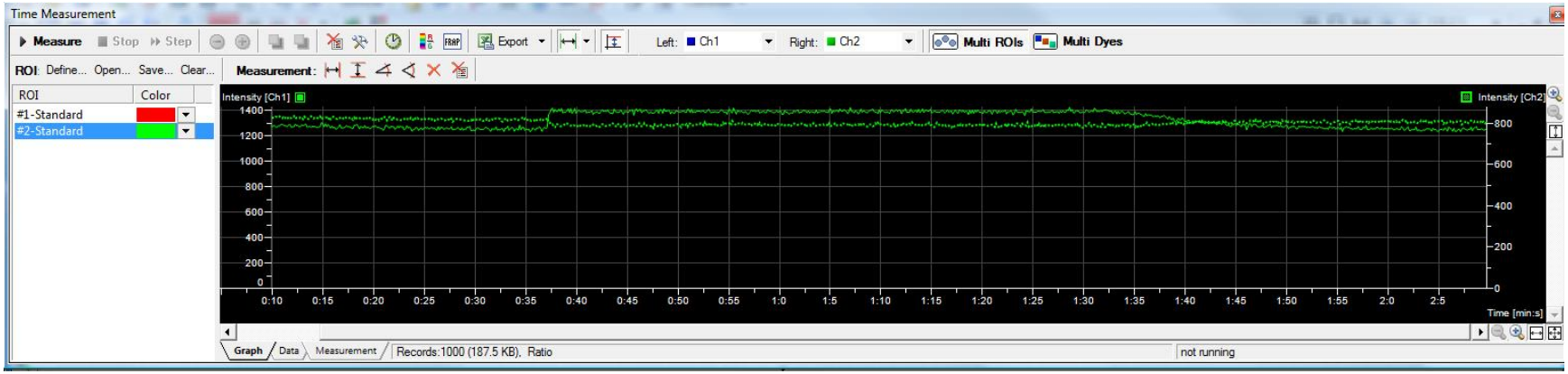
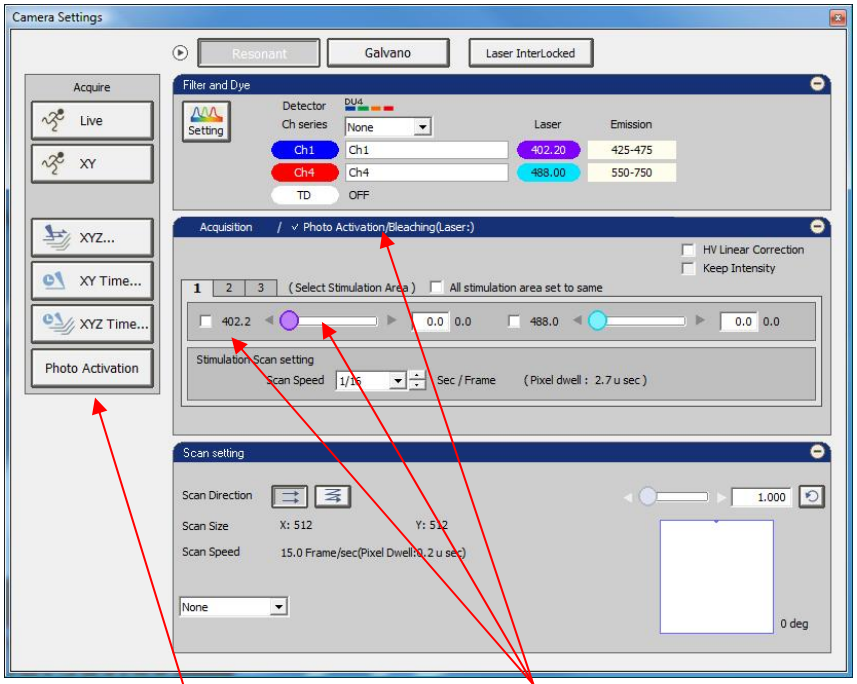
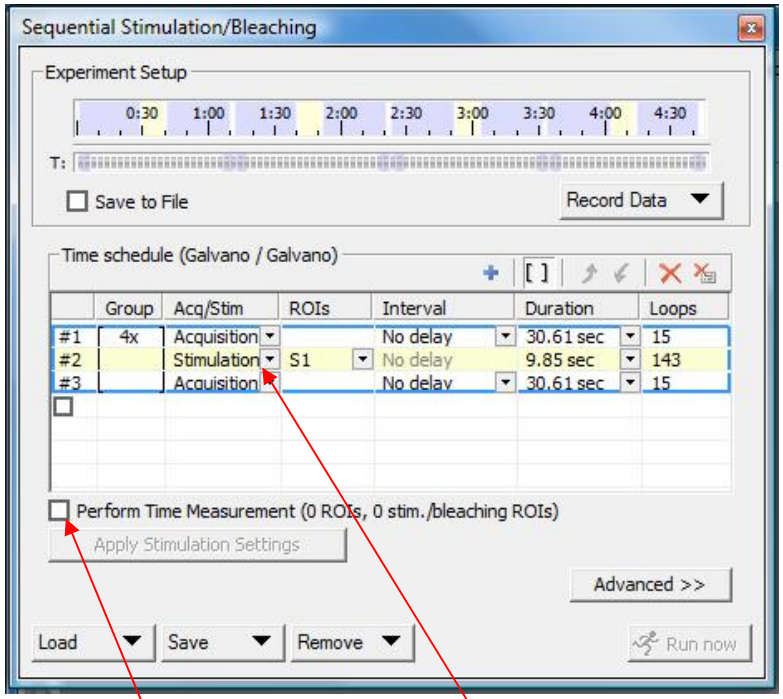


Photo activation (FRAP)





Select laser and amount of power

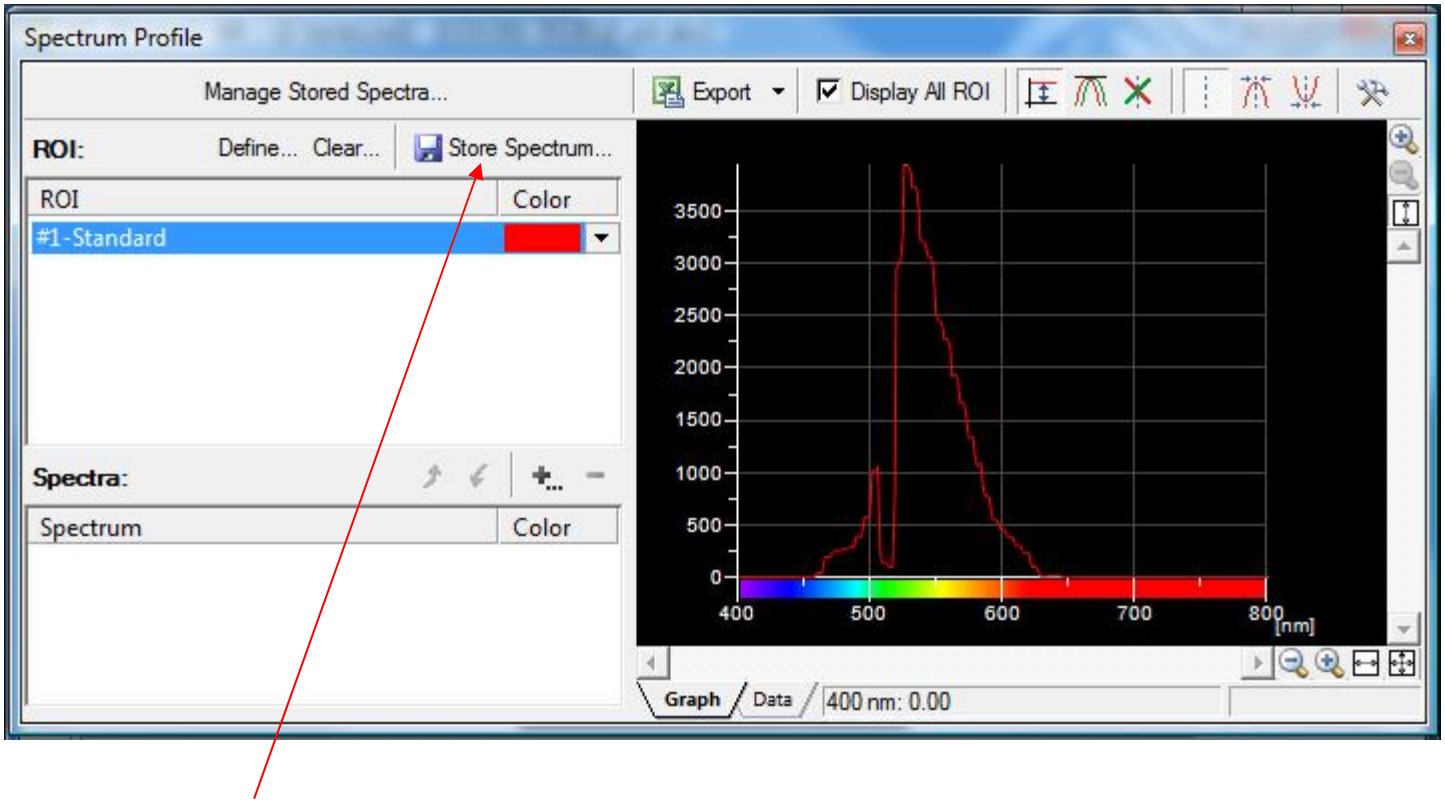
Photoactivation setup

Setup acquisition/bleaching

Live measurement but can slow down process

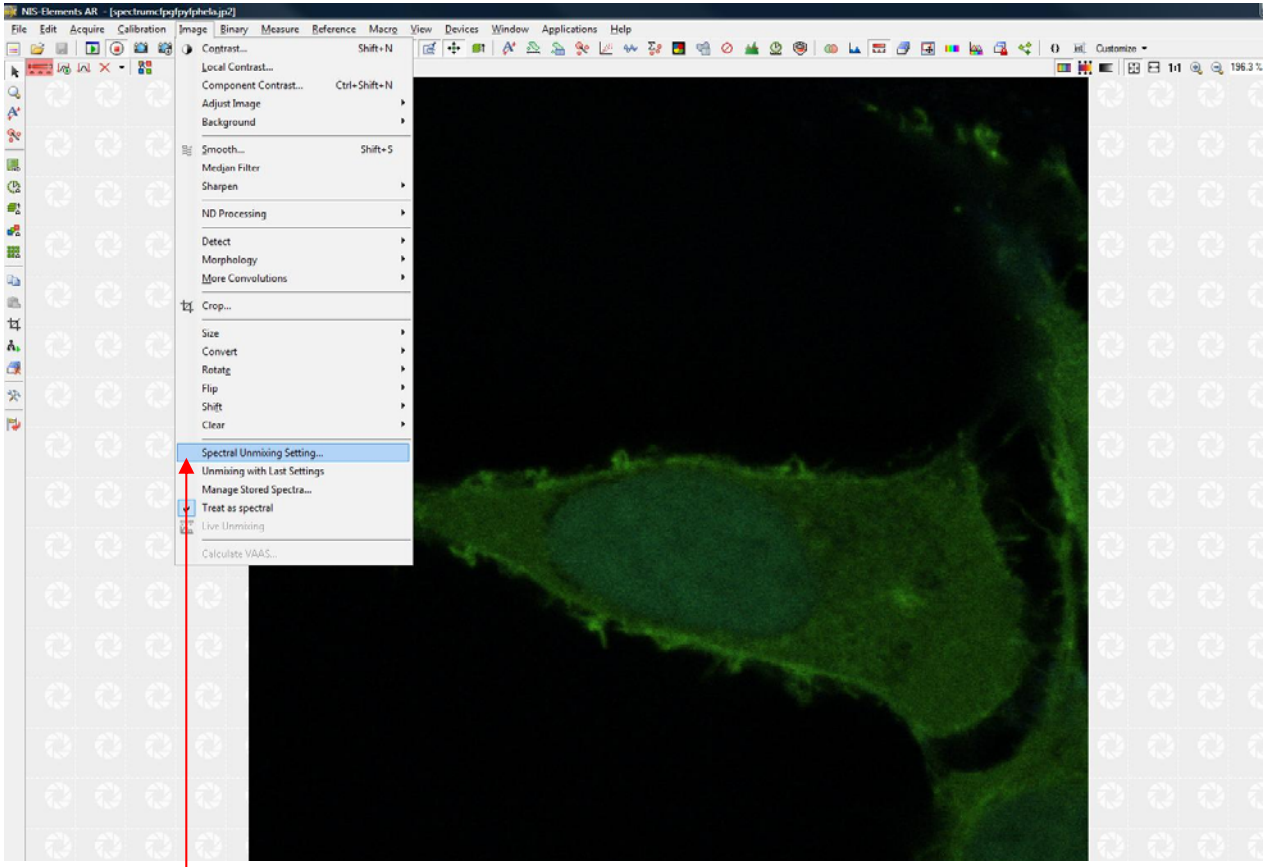
Using the spectral detector

To store a spectrum select Spectrum Profile



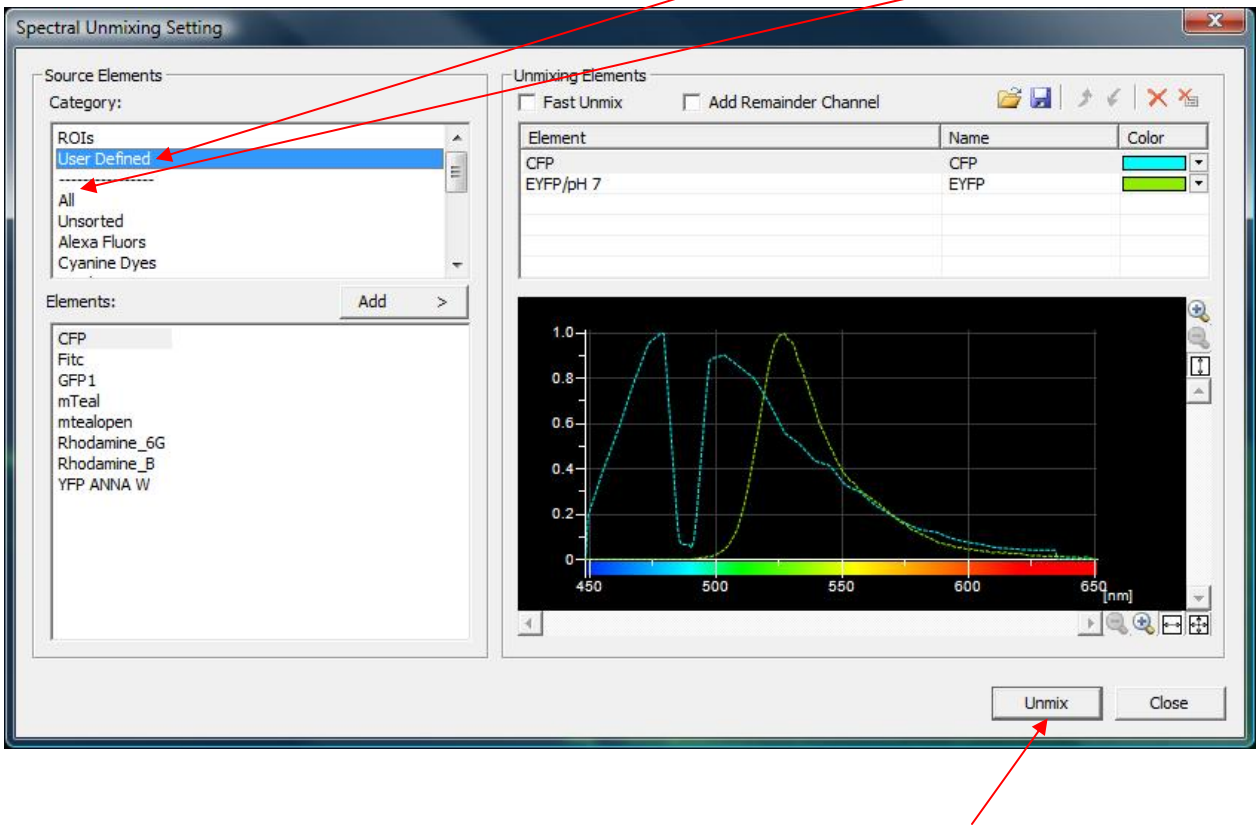
When an image is captured the spectrum of a ROI can be stored and recalled later for spectral unmixing.

Unmixing



Select unmixing settings from the image menu

Select the specra you want to unmix with (userdefined or standard)



To start the unmixing select unmix