

# Overview A1 Microscope

Control/Detection unit (3)

Halogen lamp  
And XYZ  
Controlbox (1,2)

Hg lamp (7)

Temperature  
control unit

Co2-air regulator

lasercontroller (4)

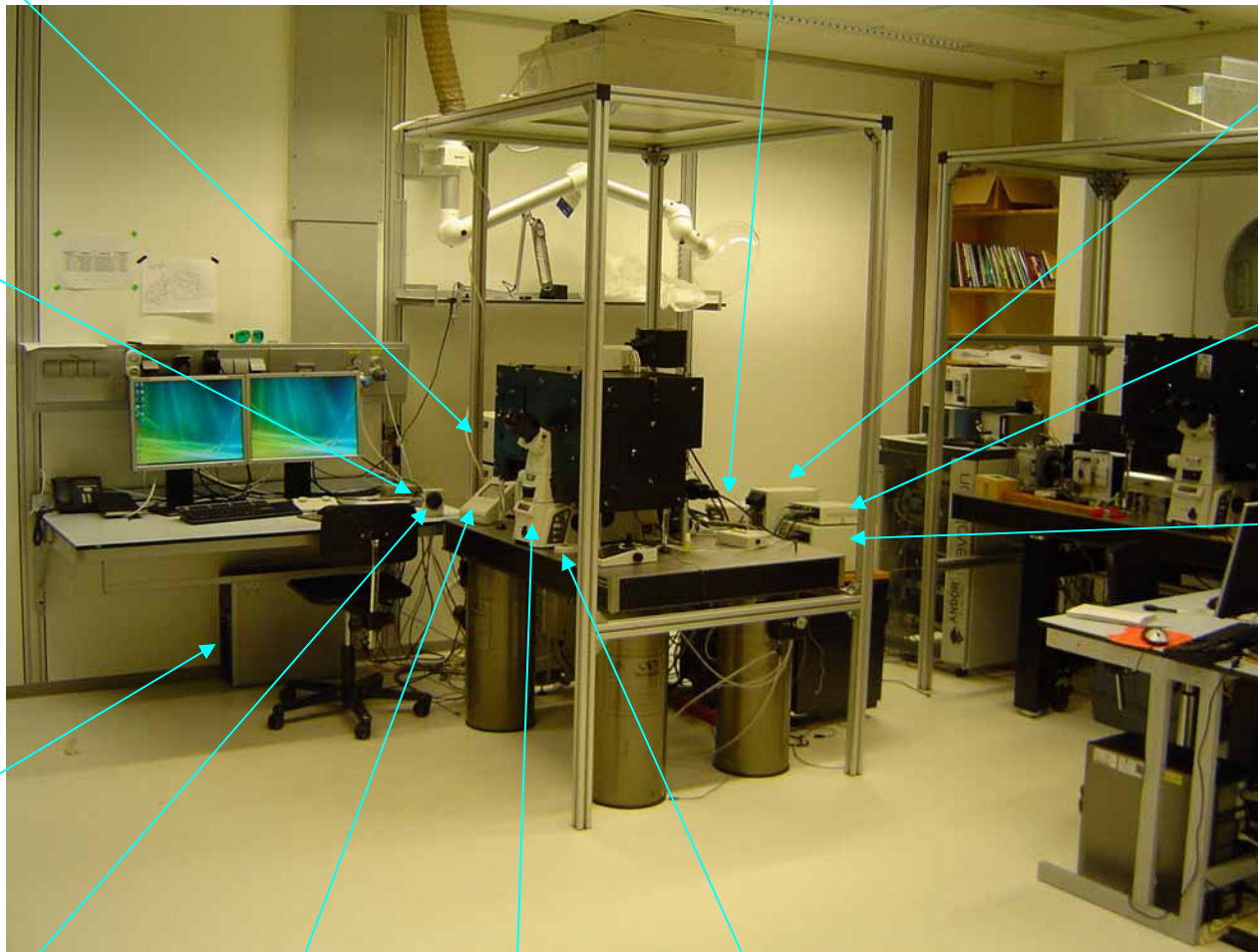
Pc (6)

Z-control for  
Perfect focus

Remote control  
Microscope

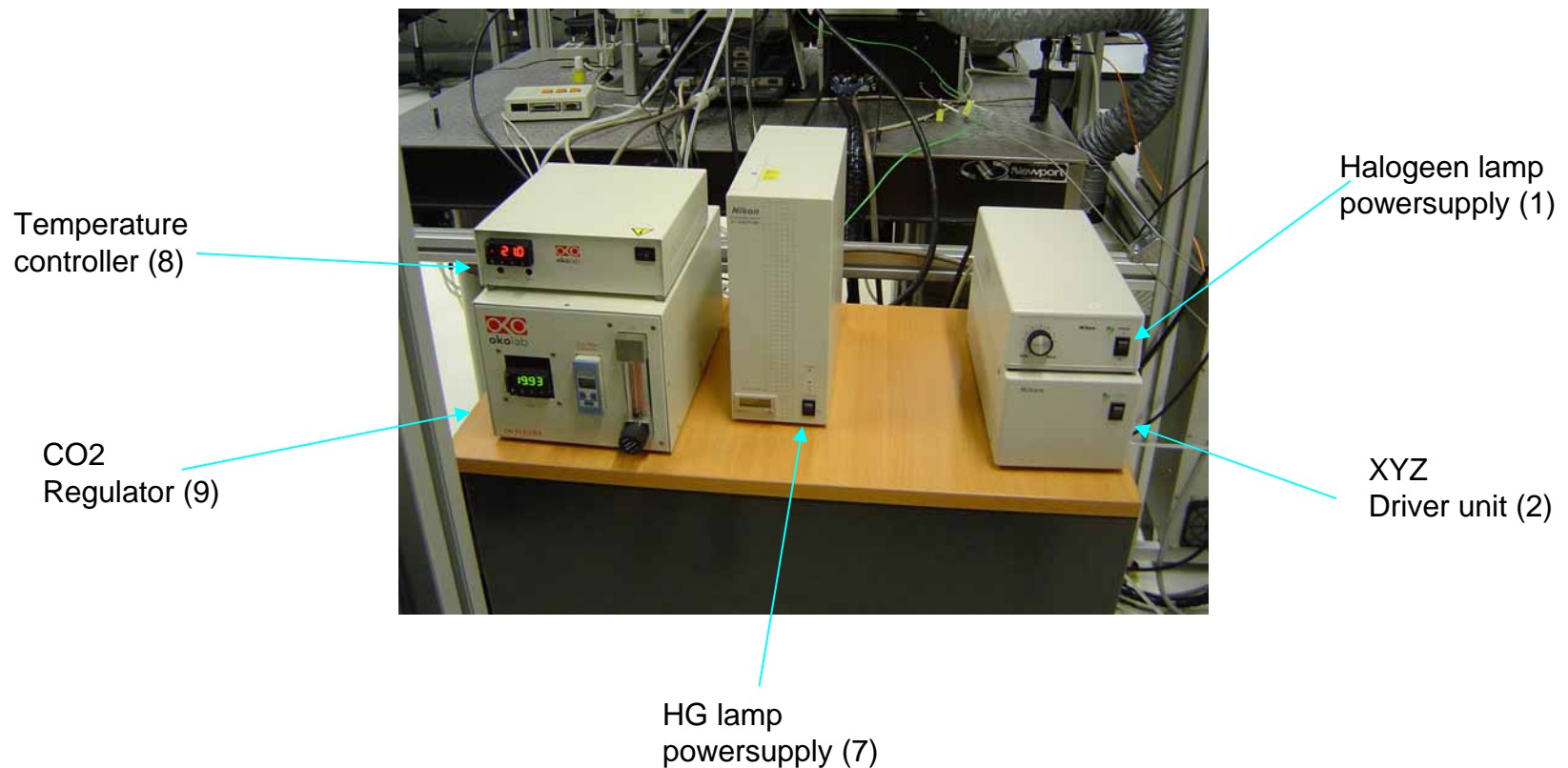
Microscope  
body (5)

Remote control  
Hg lamp

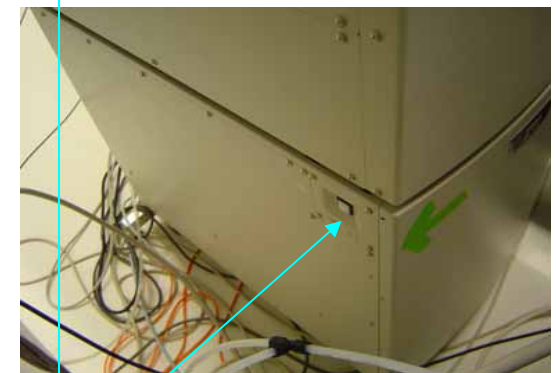
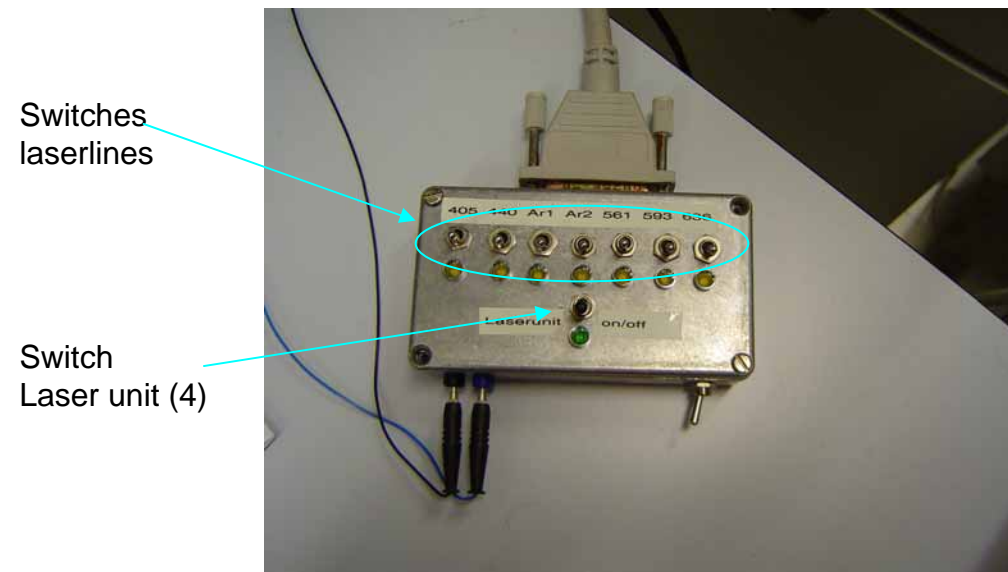


# Switch on procedure

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

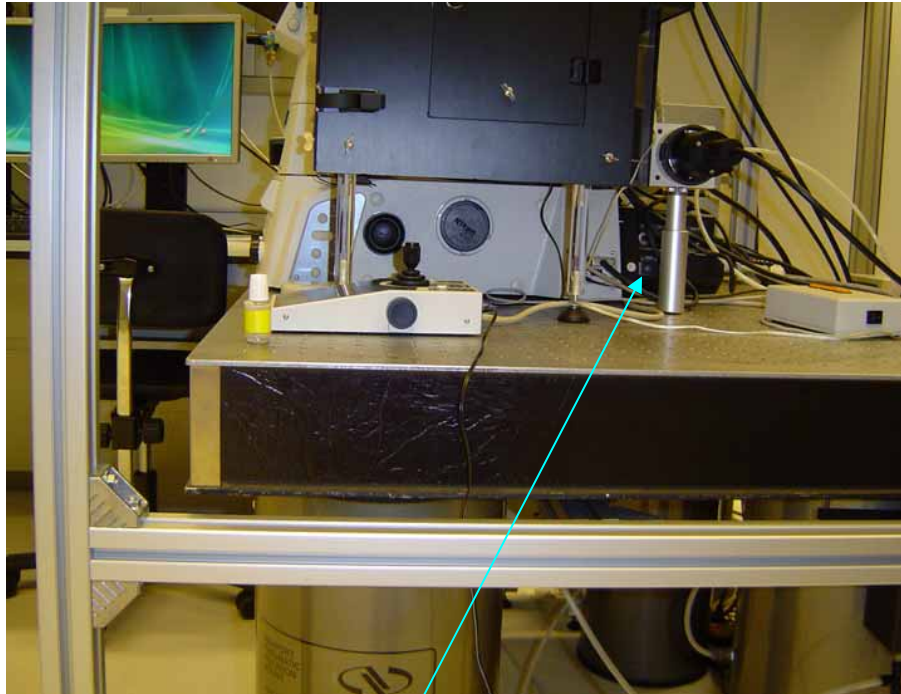


- Switch on control unit (3)
- Switch on laserunit (4)
  - First switch on the laserlines you need (Yellow LED's)
  - Secondly switch on the laserunit (green LED)



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)

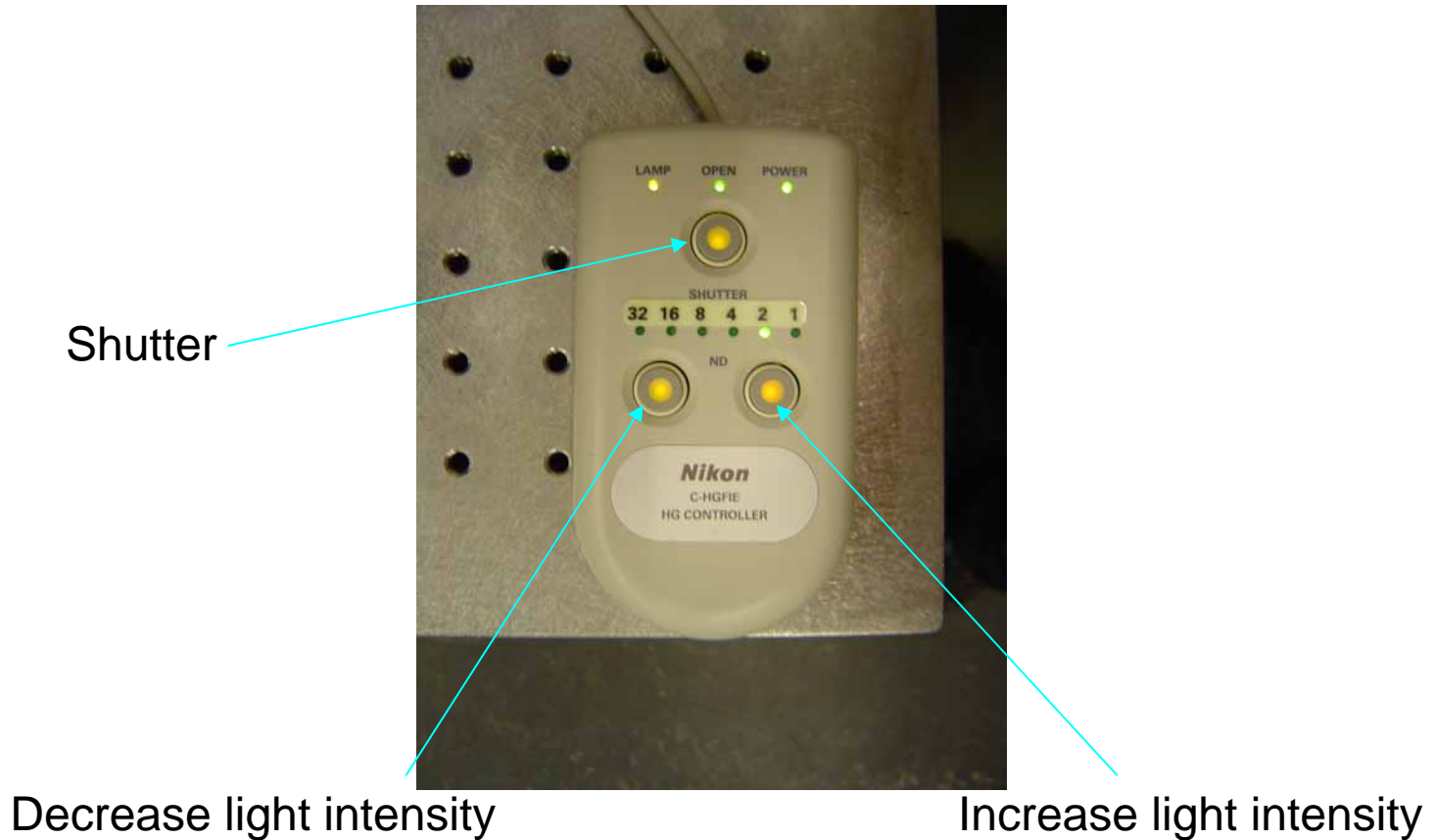


Switch  
Microscope body (5)

# Switch off procedure

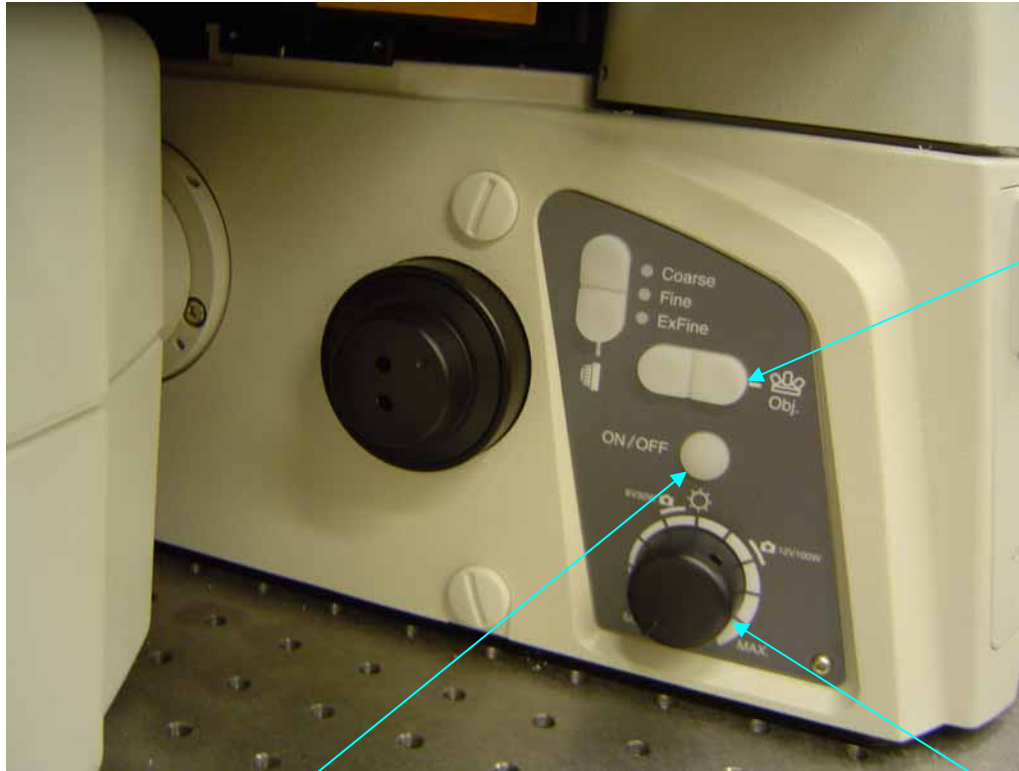
- Shut down NIS software (6)
- Switch off laser unit (4)
- Switch off Hg lamp (7)
- Switch off CO2 regulator (9)
- Switch off control unit (3)
- Switch off microscope body (5)
- Switch off halogen powersupply (1)
- Switch off XYZ driver unit (2)

# Hg lamp remote control





# 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



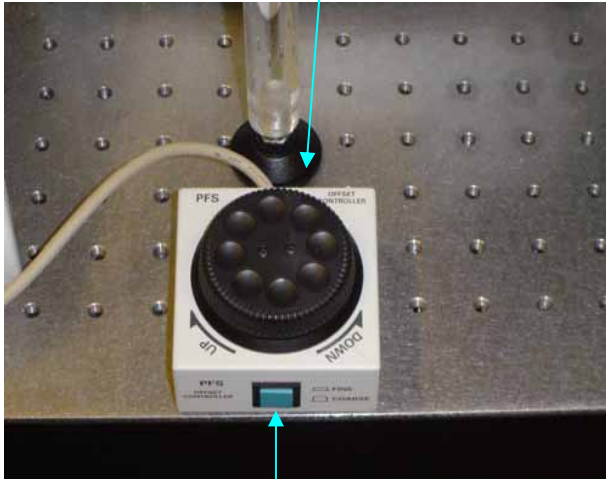
Focus knob

- Or focus in the NIS software



# Perfect focus

Focus adjust when  
perfect focus is switched on



Toggle between  
coarse and fine

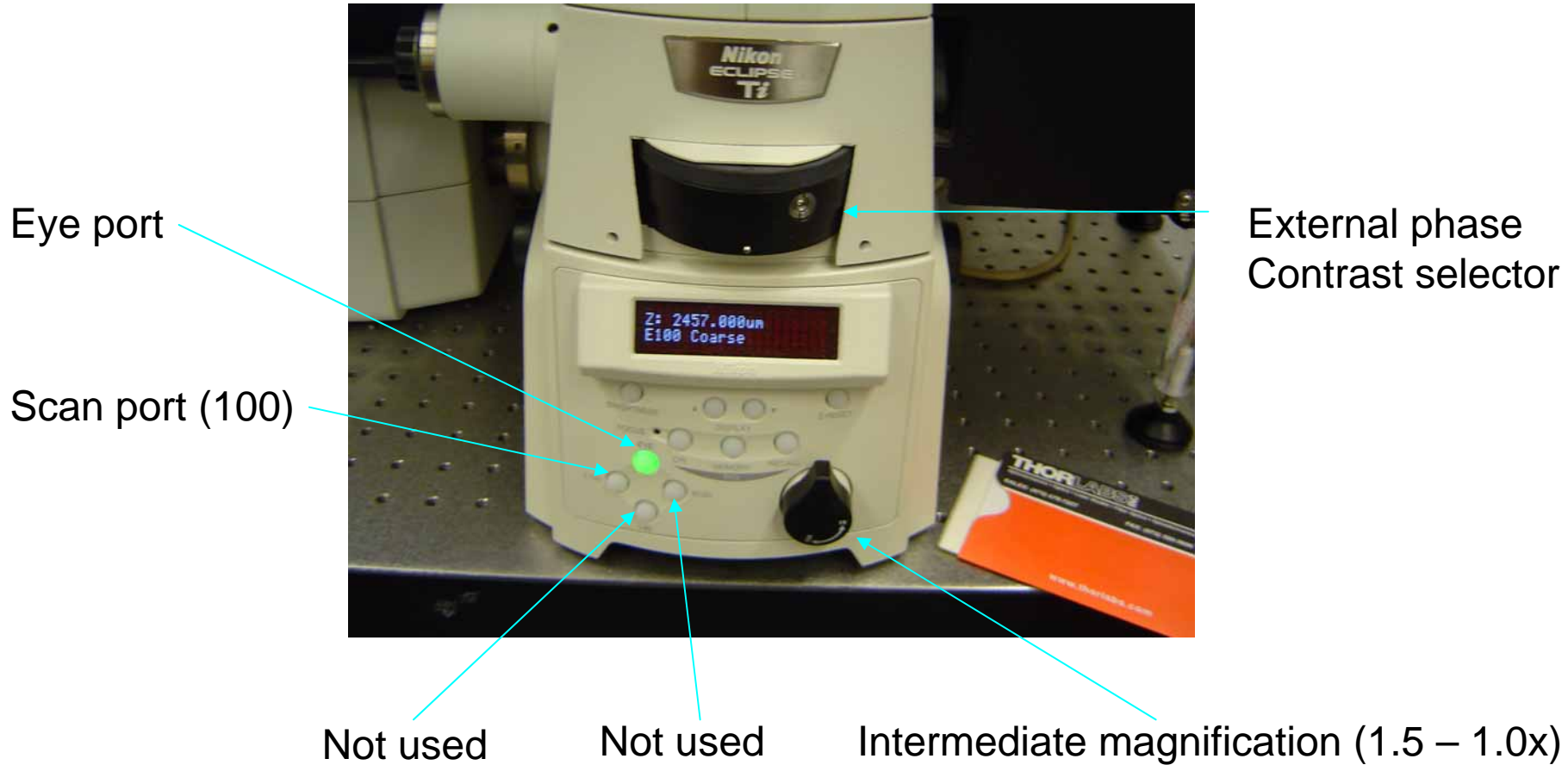
To be able to use  
PFS the dichroic  
Needs to be in the  
Light path

Picture pfs  
dichroic



Switch on perfect focus

# Port control



# Remote

Switch on pf

Objective selection

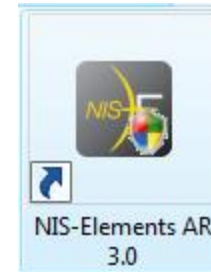
Filter selection for  
viewing with HG lamp

Phasing condensor  
selection



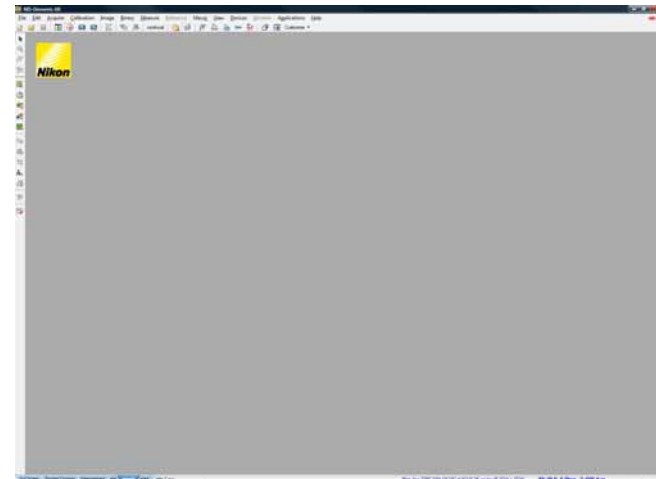
# Software

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

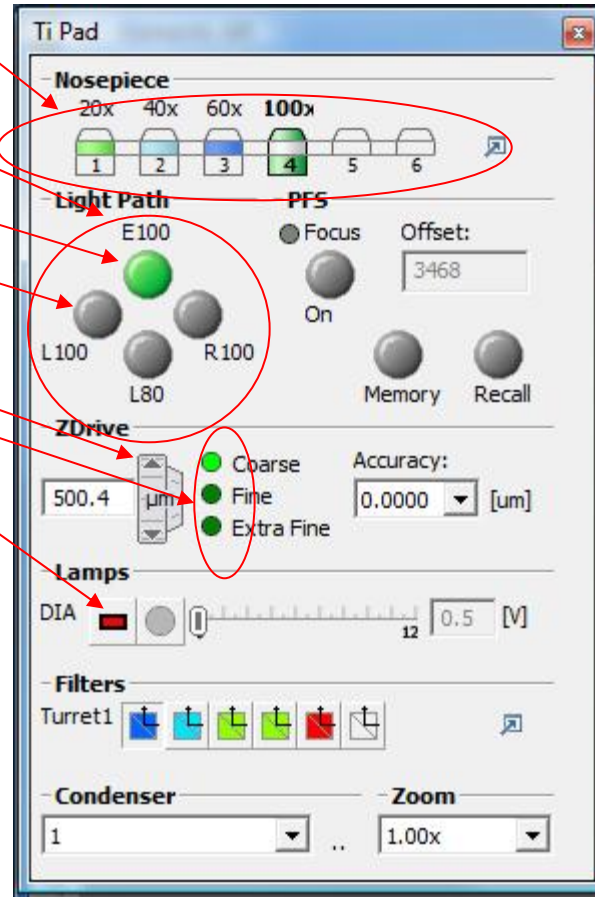
Remote

On/Off

Intensity

Filter selector for Hg lamp

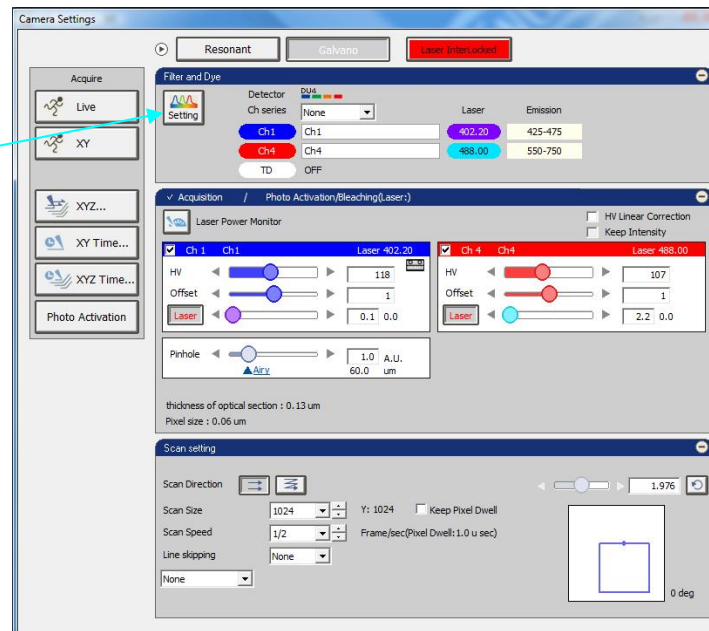
Codensor selector



# 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

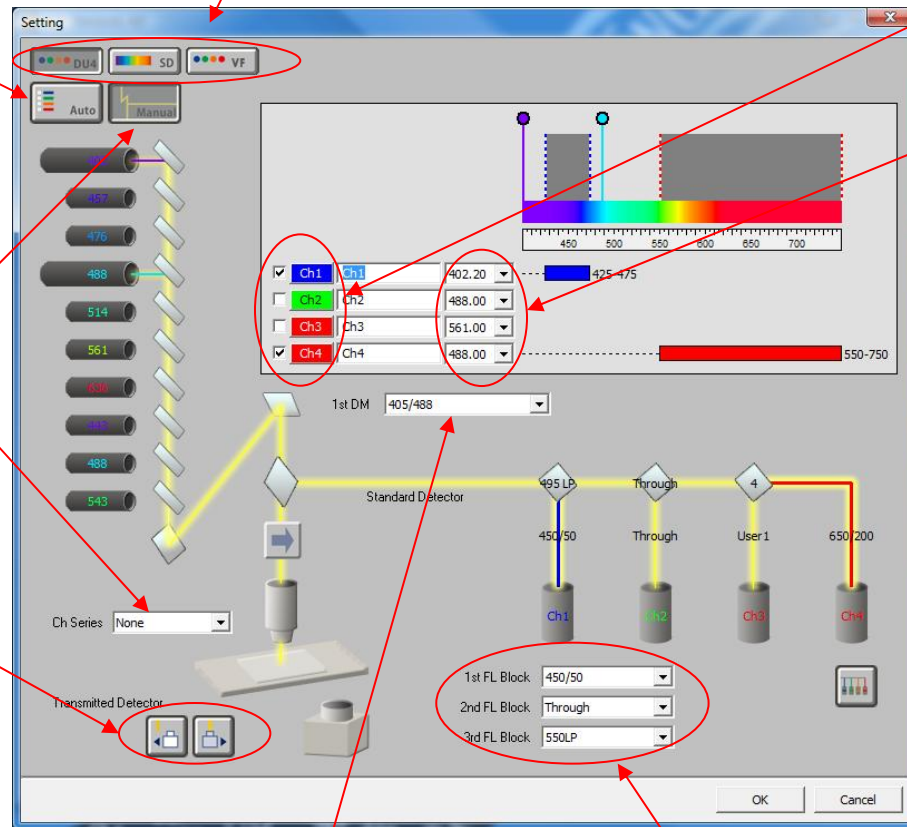
Automatic settings for standard dyes

Manual configuration

Sequential scanning

Transmission detector

Laser selection



Select the filters you want to use

Select main dichroic

# Using the autosetup

Select auto

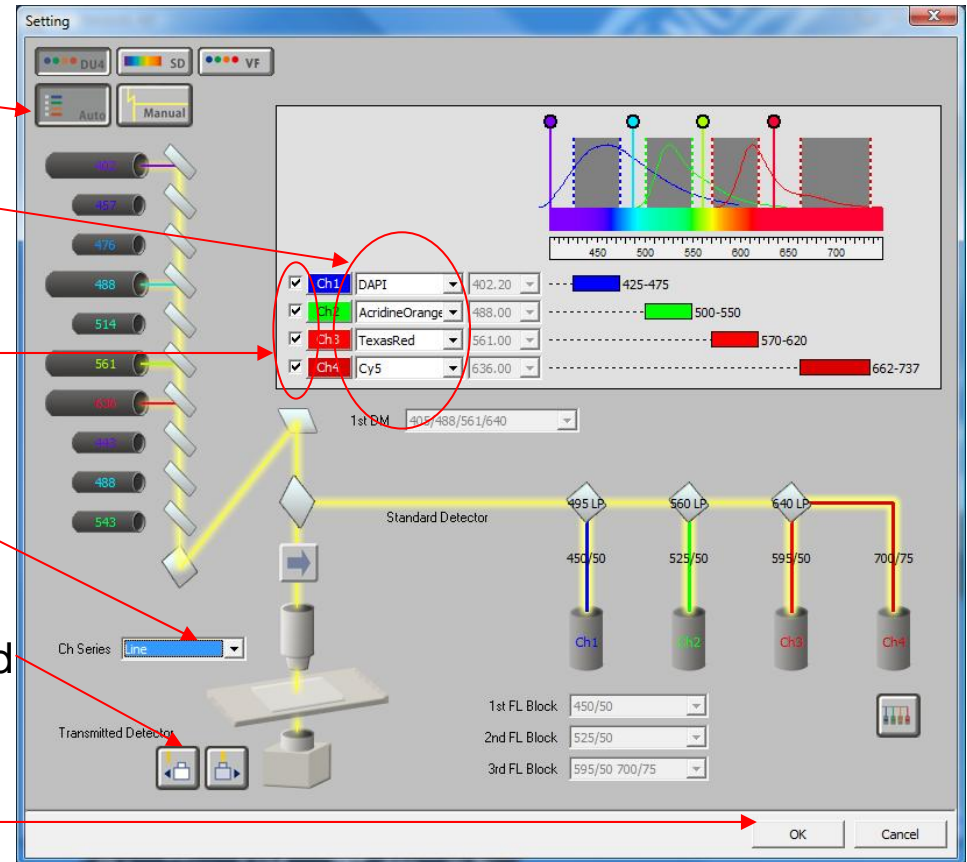
Select the fluophore (from blue to red)

Select the channel for this fluophore

Select sequential scanning if needed

Select transmitted light detector is needed

Select ok



## Visualisation of your configuration

Laser lines

Detection areas

Selected Laser(s)

Filter/dichroic selection

PMT selection

If the settings are ok click on the ok button

The screenshot shows a software window titled "Setting" for configuring a microscope. On the left, a vertical list of laser lines includes 405, 407, 476, 488, 514, 561, 630, 640, 688, and 543. A red oval highlights the 488 and 561 nm lines, with a red arrow pointing to the label "Selected Laser(s)". In the center, a diagram illustrates the optical path from a laser source through a dichroic mirror (DM) and filters to a standard detector and a transmitted detector. A red oval highlights the filter/dichroic selection area, which includes a 495 LP filter, a 450/50 dichroic, and four emission filters labeled Ch1 (blue), Ch2 (green), Ch3 (red), and Ch4 (yellow). A red arrow points from the label "Filter/dichroic selection" to this area. On the right, a table lists the detection areas for each channel:

Channel	Wavelength (nm)	Detection Area (nm)
Ch1	402.20	425-475
Ch2	488.00	
Ch3	561.00	
Ch4	488.00	550-750

Below the table, the "1st DM" is set to 405/488. At the bottom, the "1st FL Block" is set to 450/50, the "2nd FL Block" is set to "Through", and the "3rd FL Block" is set to 550LP. A red arrow points from the label "PMT selection" to the "1st FL Block" dropdown. At the bottom right, there are "OK" and "Cancel" buttons. A red arrow points from the label "If the settings are ok click on the ok button" to the "OK" button.

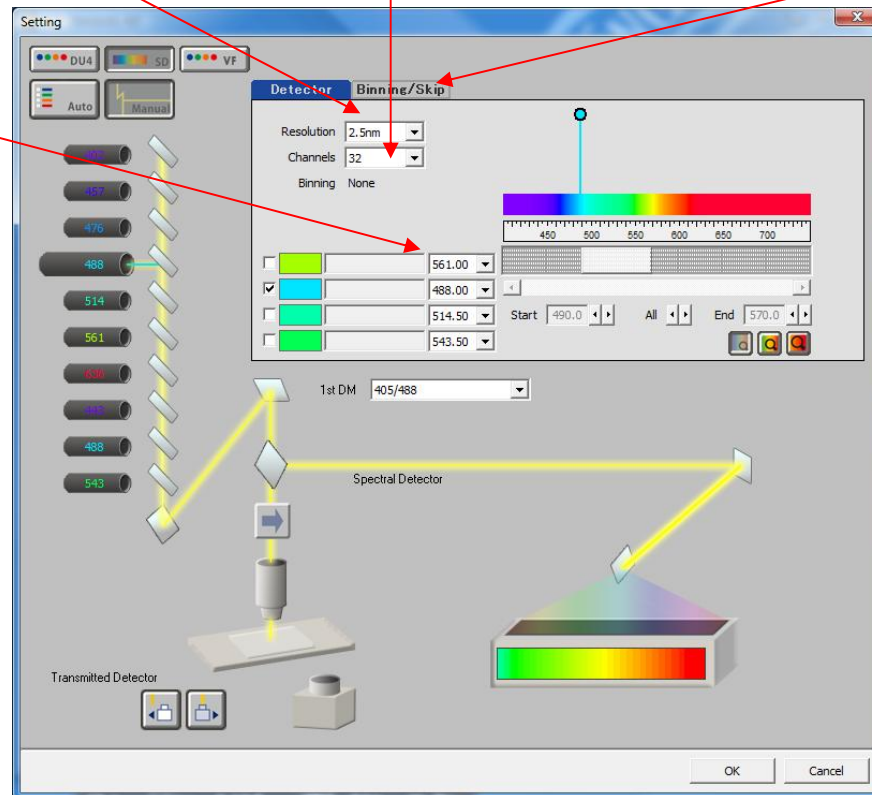
# Spectral detector setup screen

Resolution per pmt

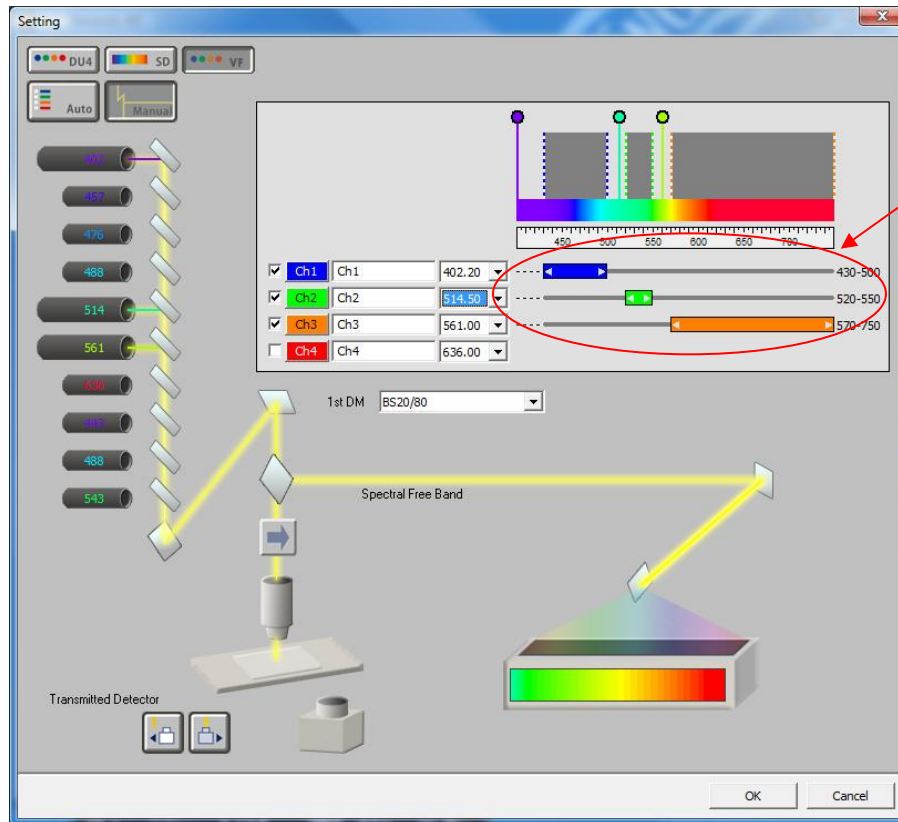
Number of pmt's to be used

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

Resonant scanner Galvano scanner Remove laser interlock

Start and stop Live mode

Capture XY-image

Setup Z-stack

Setup timelaps

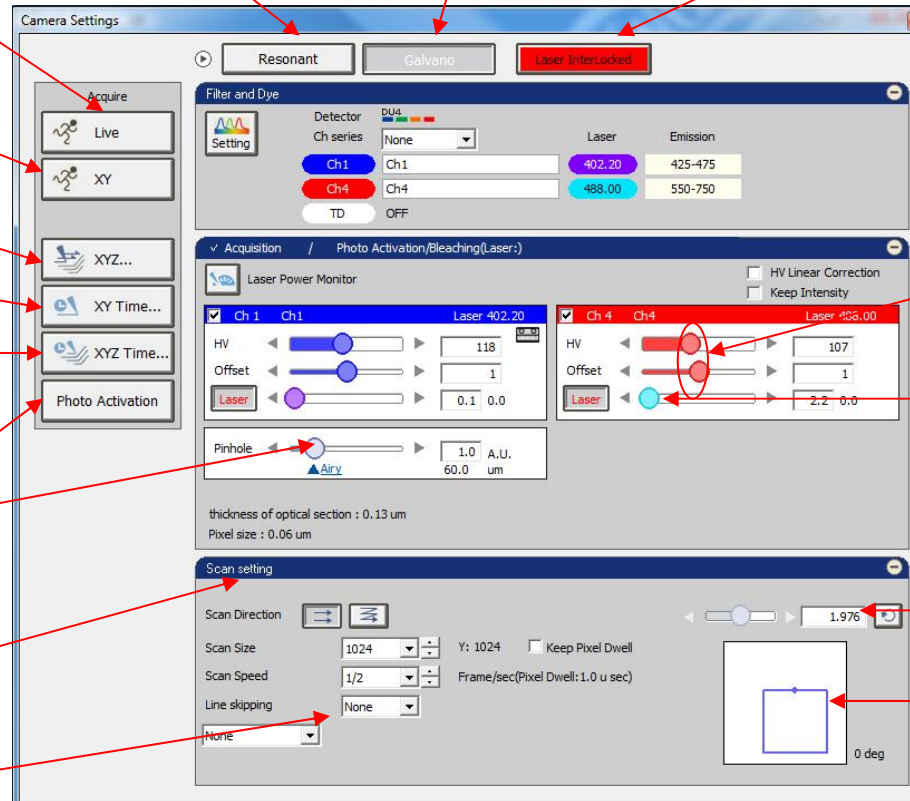
Setup Z-stack and timelaps

Setup photo activation (frap)

Adjust pinhole size

Scan settings area

Averaging



Adjust PMT Gain and offset

Adjust laser power

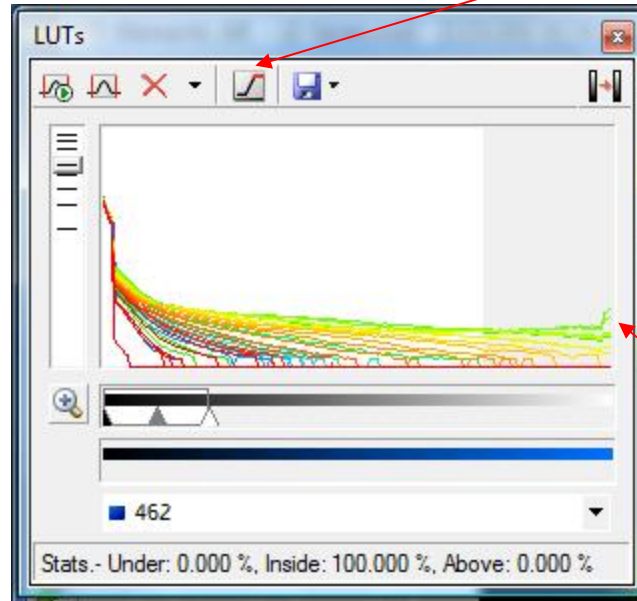
Zoom factor

Zoom field



## Check quality of your image

Mark overexposed pixels another colour

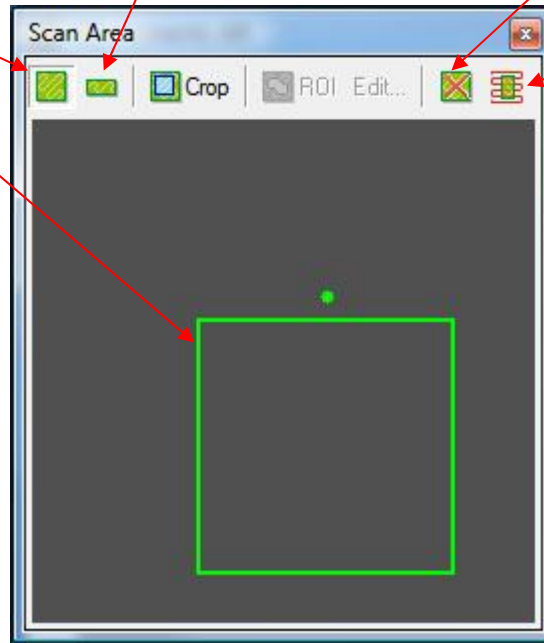


The LUTs screen gives an overview of the greylevel distribution. If there is a peak On the right side of the table you have pixels that are overexposed.

## 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

Symmetrical volume

Asymmetrical volume

Slice thickness

Absolute values

Number of slices

Start experiment

The screenshot shows the 'Capture Z-Series' window with the following settings and annotations:

- Experiment Setup:**
  - Z:** A progress bar representing the Z-stack range.
  - ☒ **Save to File**
  - Path:** C:\Users\A1\Pictures\Images\Ronald
  - Filename:** Sipchart.nd2
  - Record Data:** A dropdown menu.
- Volume Selection:**
  - Top:** 4396.18 abs
  - Bottom:** 4381.00 abs
  - Reset:** A button to reset the volume selection.
- Steps:** 52 (Number of slices)
- Step:** 0.30  $\mu\text{m}$  (Slice thickness)
- Bottom:** 4381.00  $\mu\text{m}$  (Absolute values)
- Top:** 4396.18  $\mu\text{m}$  (Absolute values)

Advanced >>

Load Save Remove 1 time loop Run now

## Setting up a timelapse

Progress bar

File setting

The screenshot shows the 'Capture Timelapse' dialog box. A red arrow points from the 'Progress bar' label to a timeline at the top. Another red arrow points from the 'File setting' label to the 'Path' and 'Filename' fields. A third red arrow points from the 'Time interval selection' label to a table of phases. A fourth red arrow points from the 'Live statistics of ROI' label to checkboxes for 'Perform Time Measurement' and 'Use Ratio'. A fifth red arrow points from the 'Start experiment' label to the 'Run now' button.

Experiment Setup

T: 05:00 10:00 15:00 20:00 25:00 30:00 35:00 40:00 45:00

☒ Save to File

Path: C:\Users\A1\Pictures\Images\Ronald Browse...

Filename: Sipchart.nd2 Record Data ▼

Phase	Interval	Duration	Loops
<input checked="" type="checkbox"/> #1	5 min	30 min	7
<input checked="" type="checkbox"/> #2	1 sec	1 min	61
<input checked="" type="checkbox"/> #3	1 min	10 min	11

☐ Perform Time Measurement (0 ROIs)

☐ Use Ratio Define Ratio...

Advanced >>

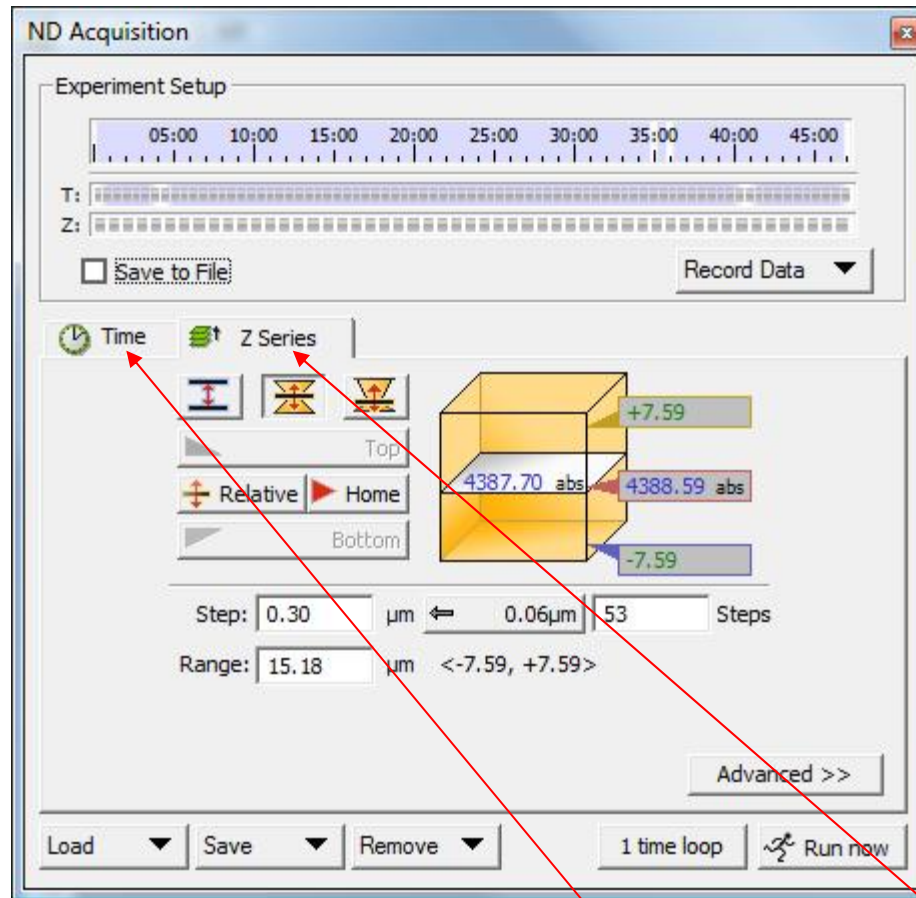
Load ▼ Save ▼ Remove ▼ 1 time loop Run now

Time interval selection

Live statistics of ROI

Start experiment

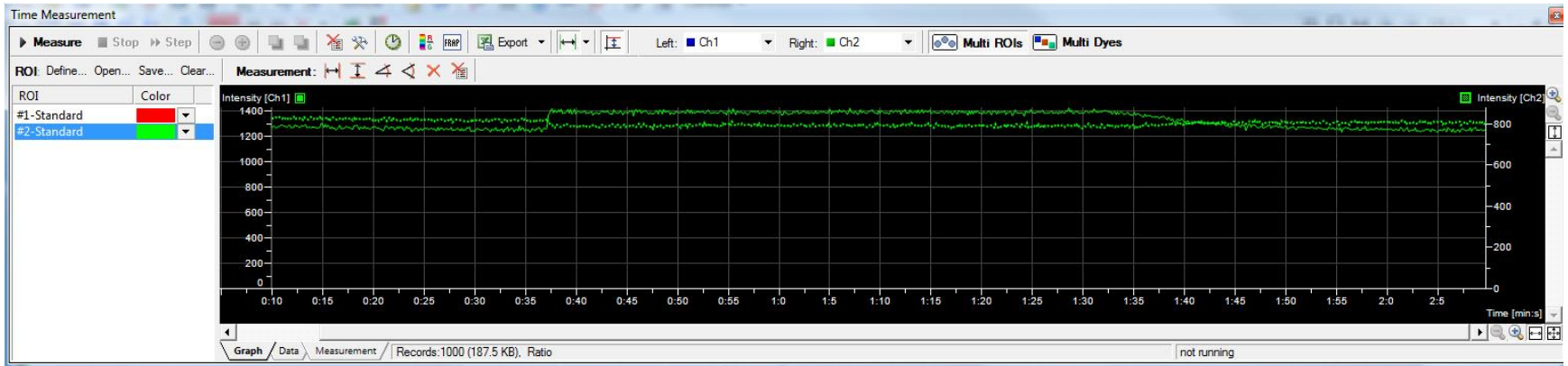
## Setting up a timelaps of a Z-stack



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

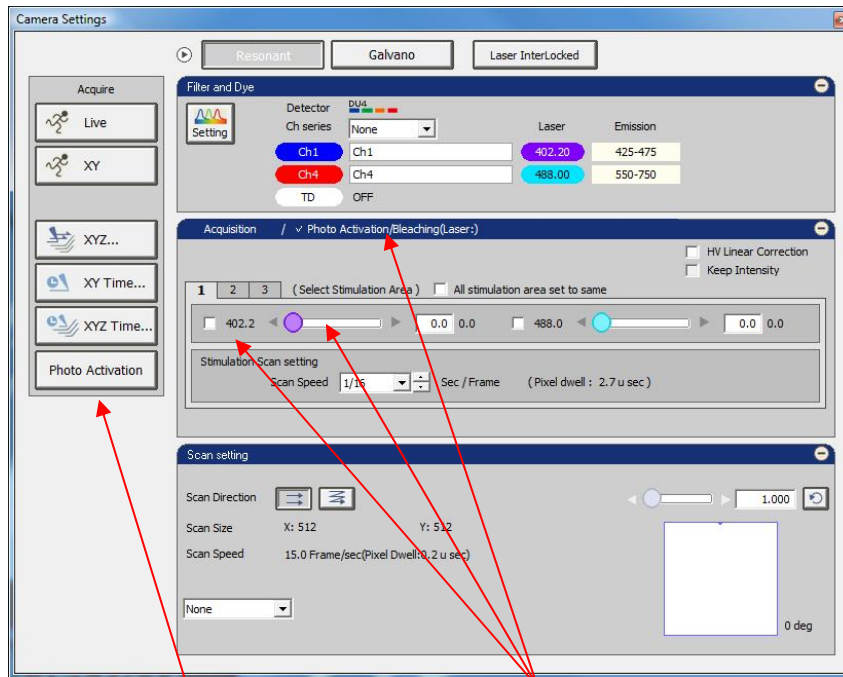
# Analyse a timelaps

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



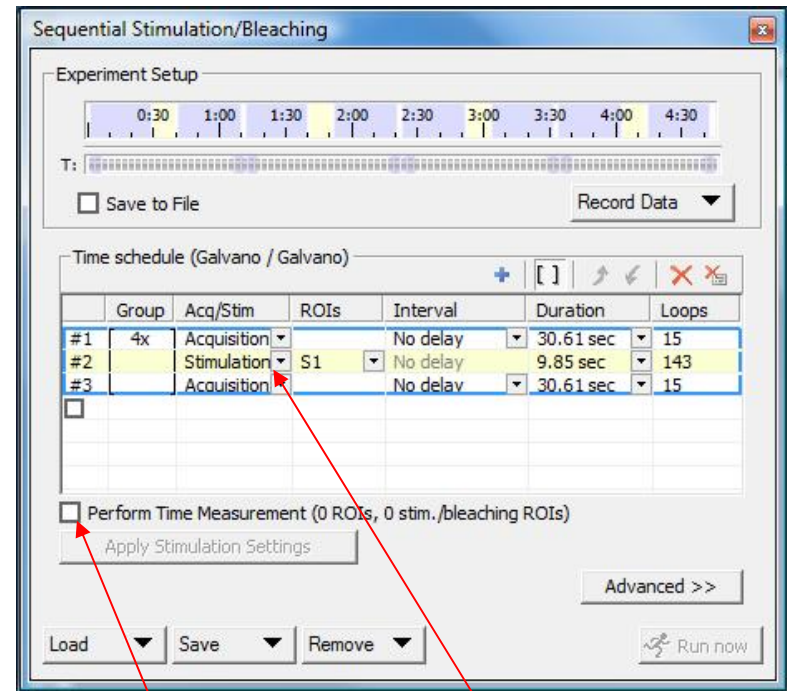


# Photo activation (FRAP)



Select laser and amount of power

Photoactivation setup

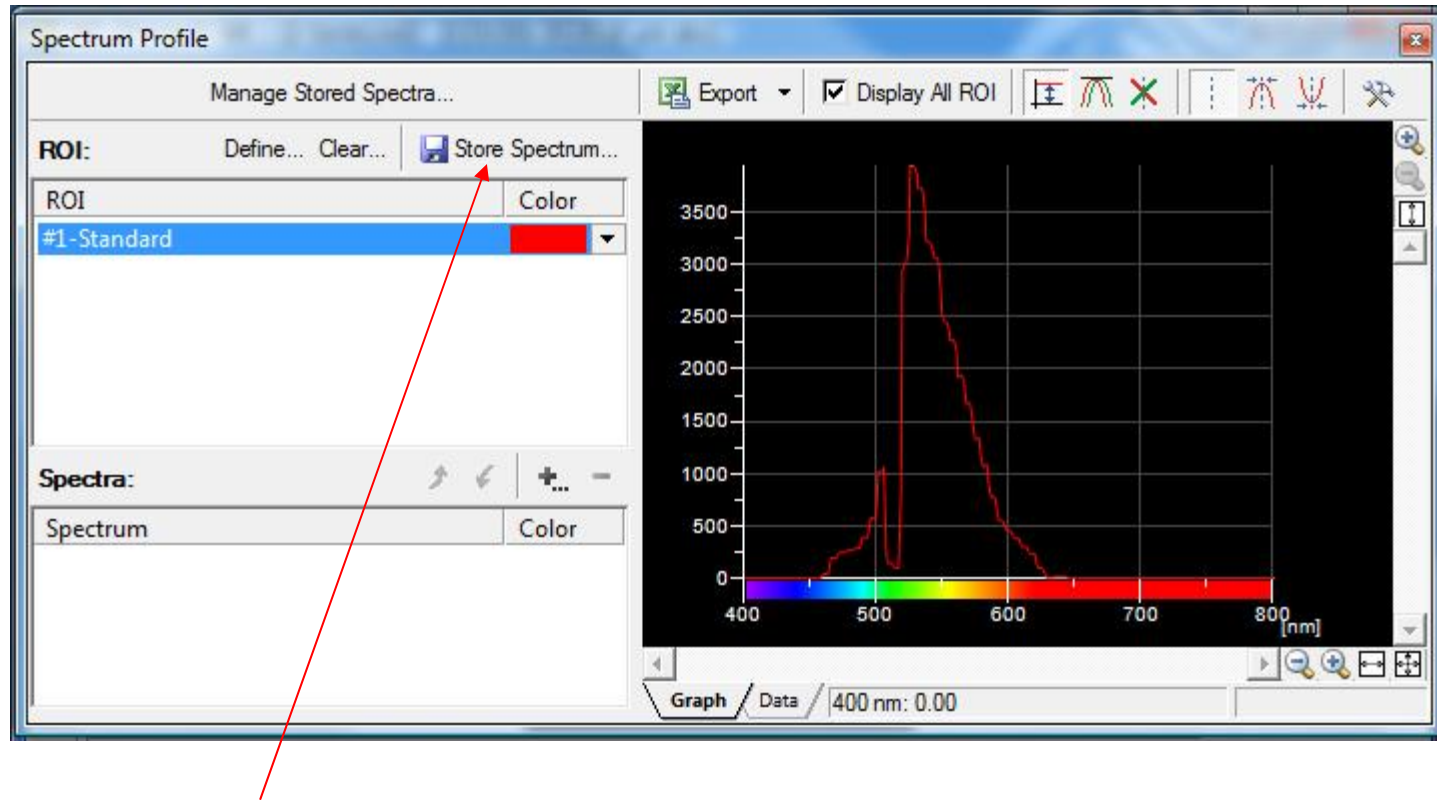


Setup acquisition/bleaching

Live measurement but can slow down process

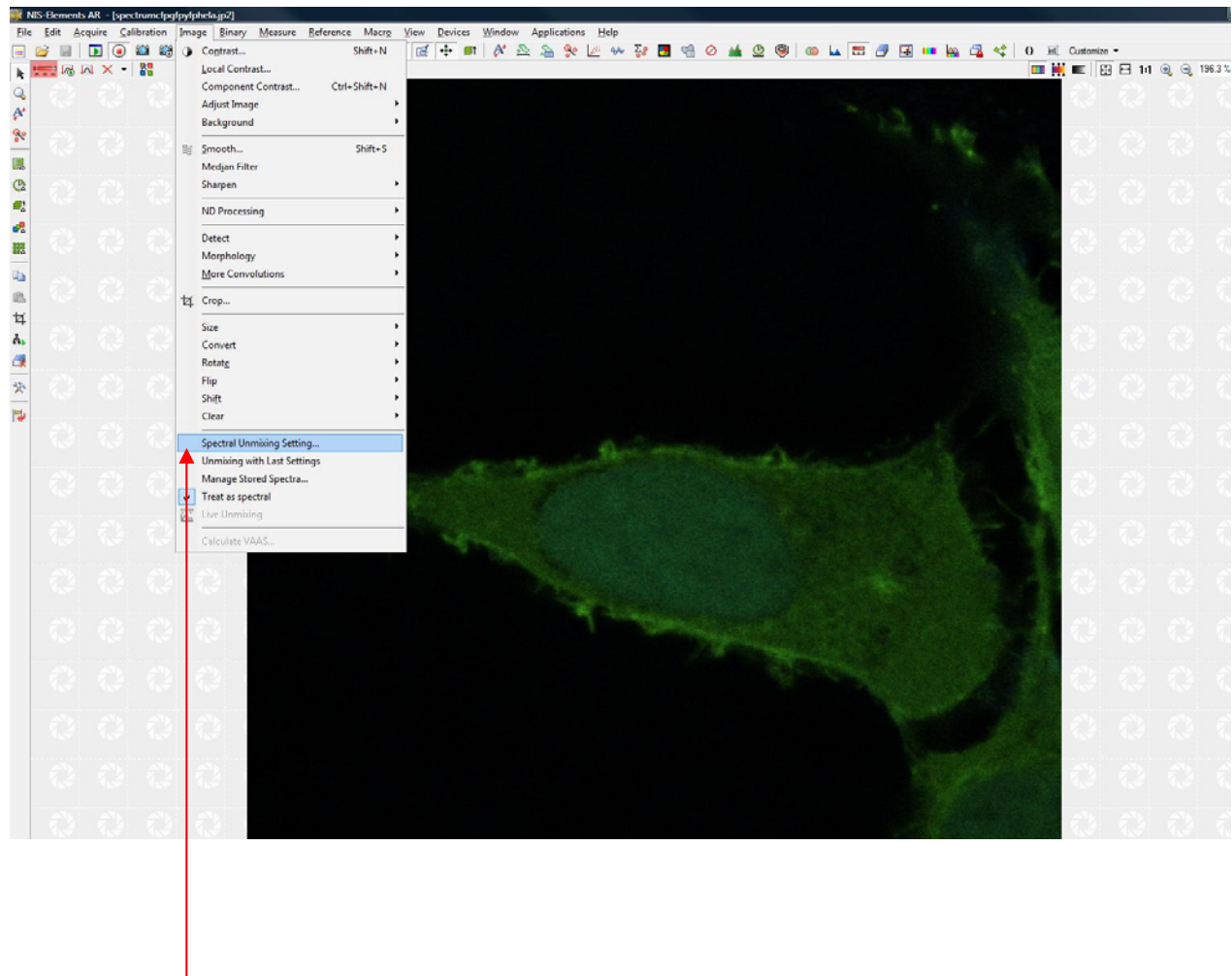
## Using the spectral detector

To store a spectrum select Spectrum Profile



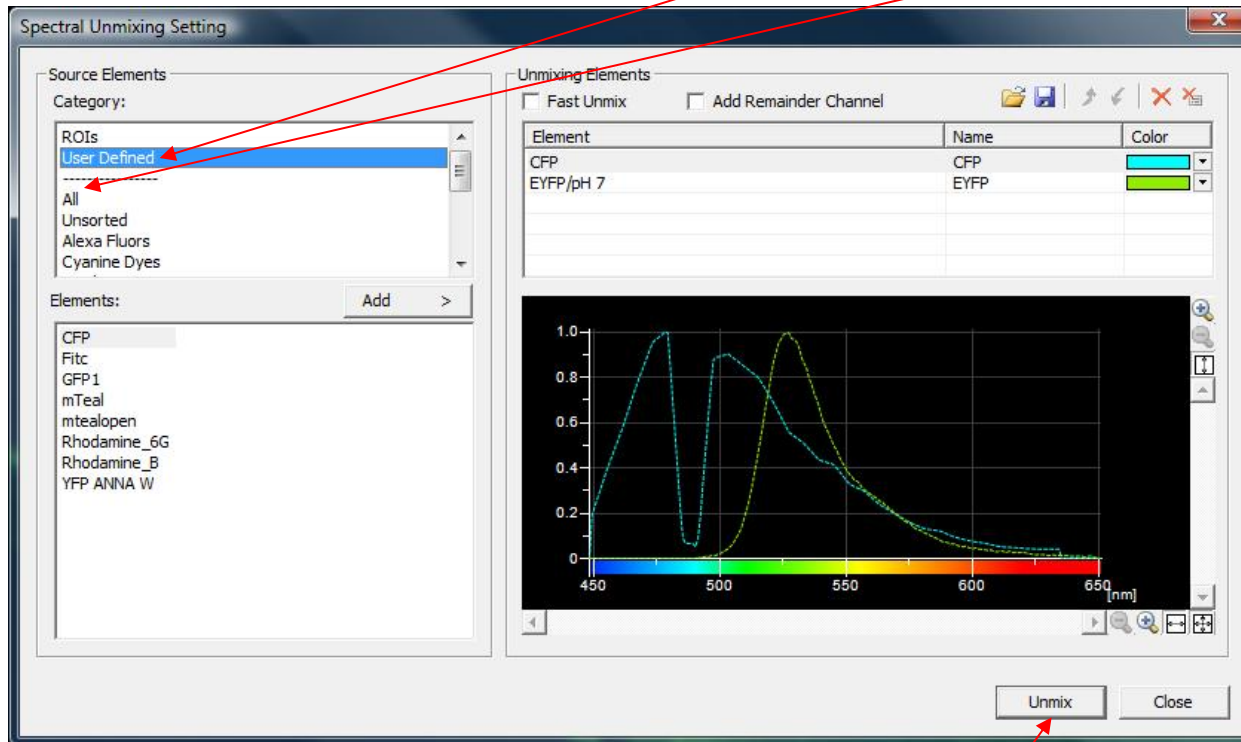
When an image is captured the spectrum of a ROI can be stored and recalled later for spectral unmixing.

# Unmixing



Select unmixing settings from the image menu

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



To start the unmixing select unmix