# LBIC
Nikon confocal A1RHD
Standard operating procedure

Version 1.0 June 2019

Turning on/off the system

1. Turn on computer

2. Power on A1 controller

3. Power on lasers by turning the key.

   - Check that laser box is powered on (back of laser bed, right side above power cable there is a switch)

4. Turn on piezo stage controller

5. Turn on stage power supply and DIA lamp if bright field is needed

6. Power on microscope, wait until startup sequence is finished.

7. Log in with your LBIC account and start NIS-Elements AR

8. Turn off system in reverse order
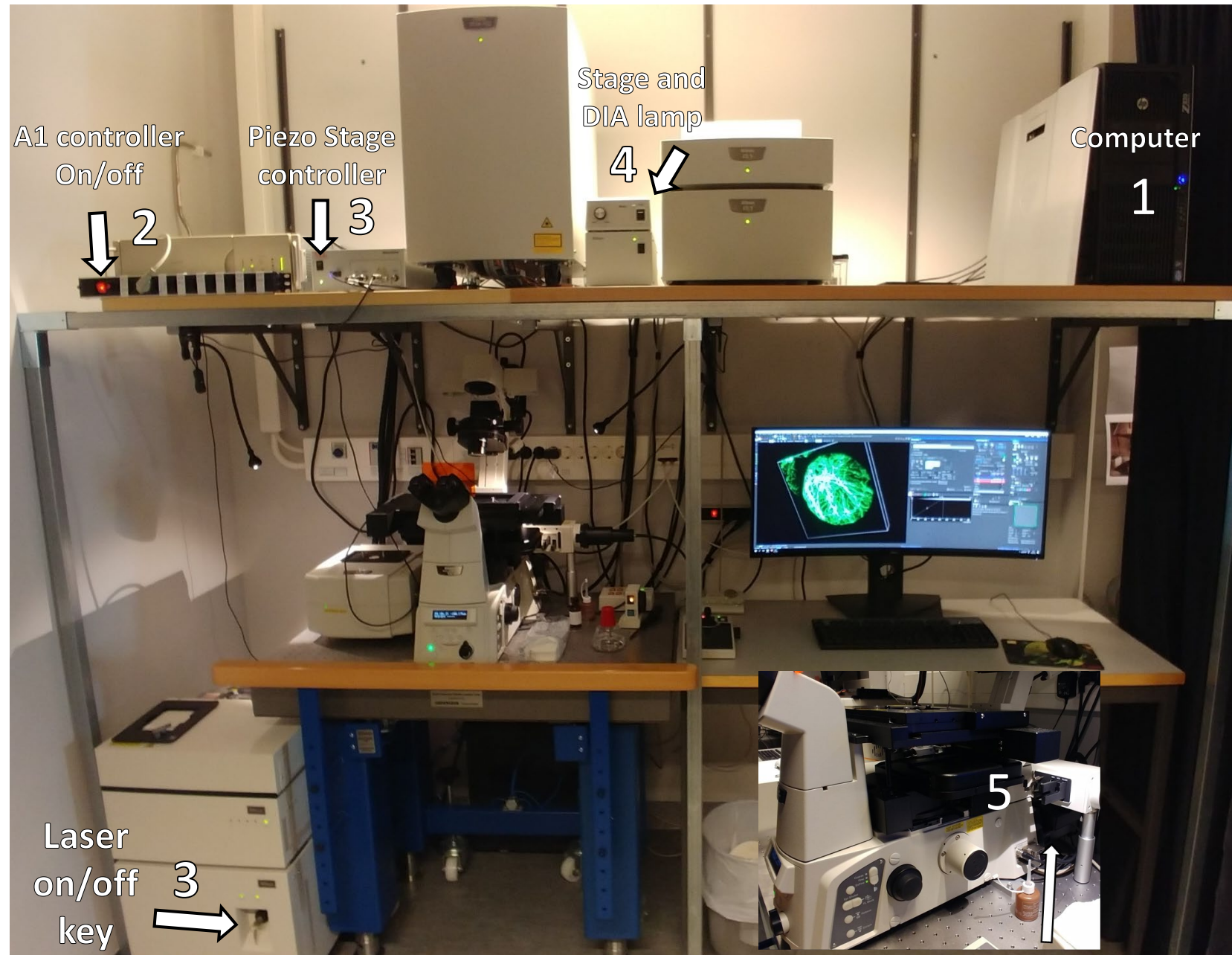

(2)

Finding your sample

1. Put your sample in the appropriate holder, if you have slides make sure the coverslip is facing down.

2. Start with either 10x or 20x objective

3. Navigate to a suitable place in your sample with the joystick.

4. Press "Eye port" to change the light path to the eye piece, if needed press the Sola button (on Sola pad) to activate the LED lights. Make sure the light path is set to L100.

5. Use the coarse focusing mode and find focus (around Z= 2000-2100), **careful not to crash the objective** into to sample or holder.

6. Press "Eye port" again to switch to confocal mode.
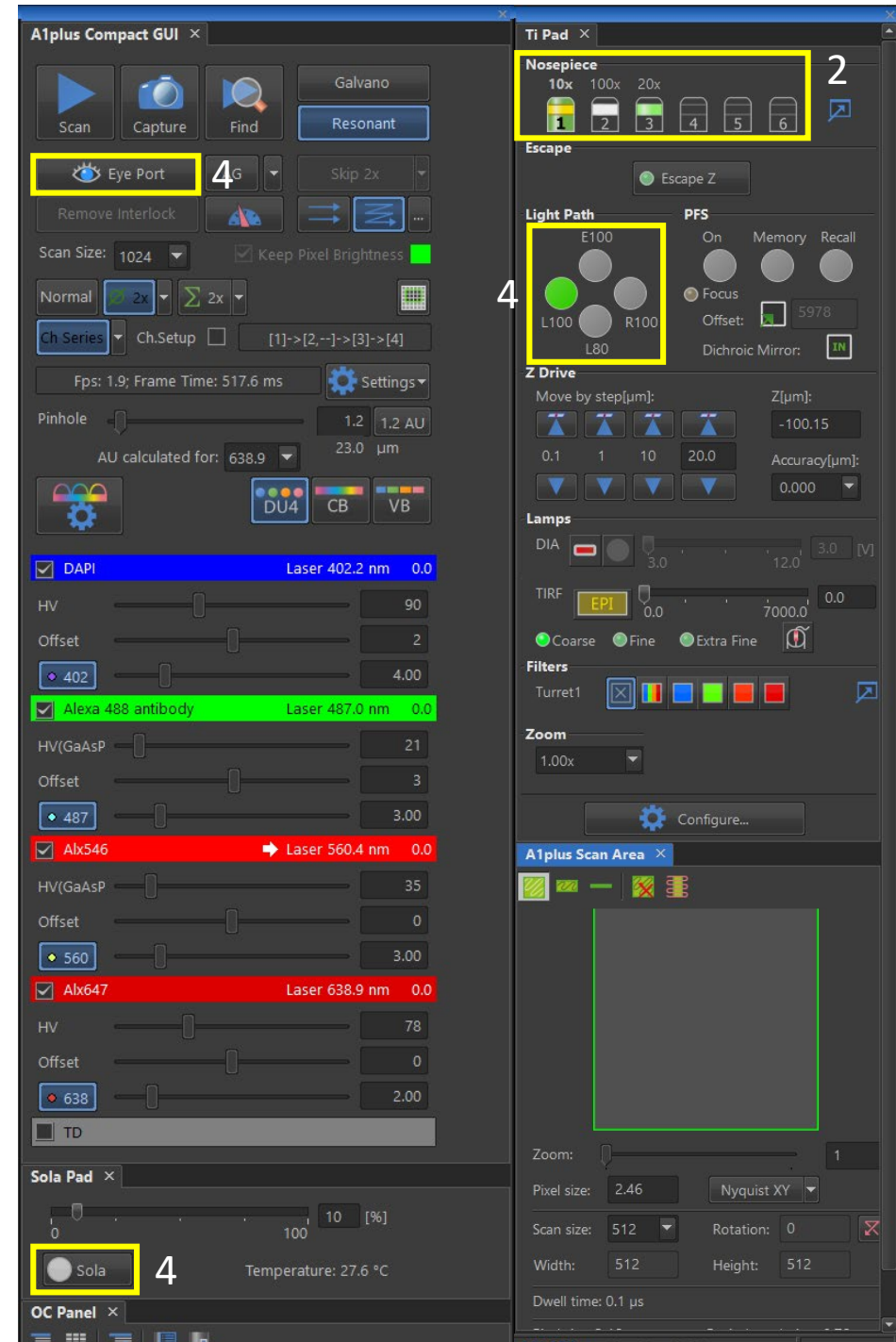
Confocal Acquisition: _Resonant_ scanner quick Start

1. Setup your channel requirements

2. HV (Gain), start at 100 for 405/640 and 25 for 488/561 PMTs

3. Offset adjustment, depends on your averaging factor

    1. Usually between 2-40

4. Laser Power, start at 4%

5. No reason to go below 1024 x 1024

6. Select "Ch Series" if using more than one laser

7. Select 1.2 AU for Pinhole

8. Start "SCAN"

9. Adjust detector and lasers settings if needed (See 2-4)

10. Change pixel size by manually changing zoom factor in A1+ Scan Area" dialog (**View>Acquisition Controls>A1 Scan Area**) or pressing Nyquist for optimal pixel size for the selected objective and pinhole size.

11. Quickly Capture an image with the current settings. Image must be saved manually afterwards. Save as .nd2.

*If you need to go back to the eyepieces to check your slides, click "Eye Port", click it again to return to the confocal!

(4)
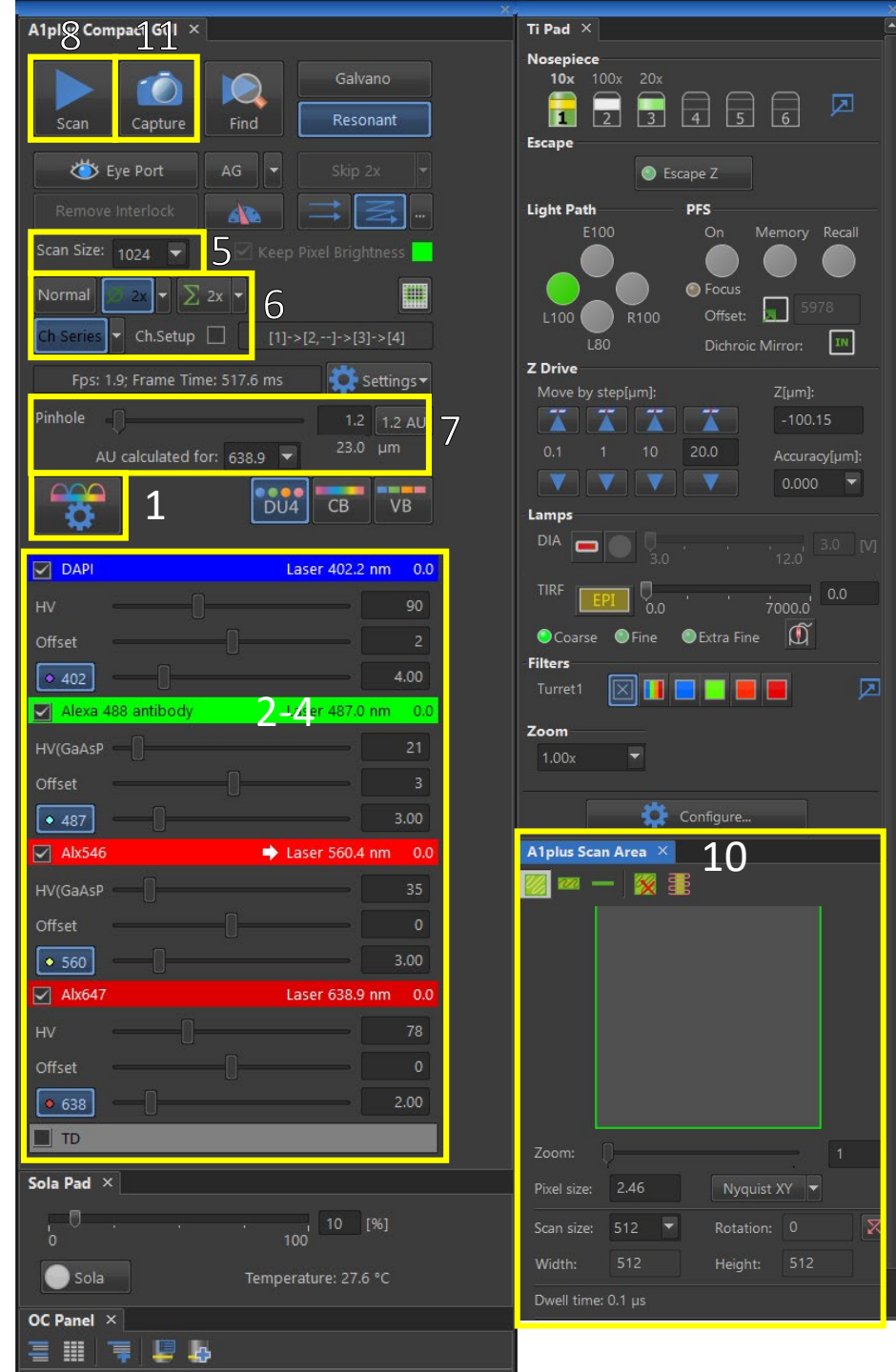
Confocal Acquisition: ND Acquisition

Very useful for setting up advanced imaging protocols

1. Enter file save path (O:\ is recommended)
2. Combine function tabs as needed by clicking the boxes
   - Time is for time lapse videos
   - XY saves coordinates (Z can be included with the include Z box)
     - XY points carry over with objective change (as long as the sample is not moved in the holder
   - Large image captures multiple fields of view and stitches them to one image
     - Very useful for creating maps of your sample with lower resolution (10x or 20x) that can be used to find points of interest and adding to XY (right click on the point and select "add this point to ND acquisition".
     - needs at least one XY point to work (this point will be the center point of the large image).
   - Z is used for capturing Z-stacks
     - Can be setup by either manually selecting top and bottom Z-values or in relative mode where the center Z-value is taken from XY (include Z needs to be selected). The latter is used when you want to capture Z-stacks in more than 1 XY point.
3. Contextual GUI for each function tab
4. Select Nikon A1 Piezo Z Drive when running Z-stacks (Max depth 100µm)

*XY points can be added also when viewing in the eye port. Useful for quickly adding points of interest before starting scanning. Pressing space bar adds a coordinate to the XY list.

(5)
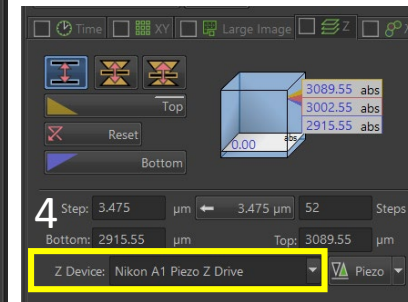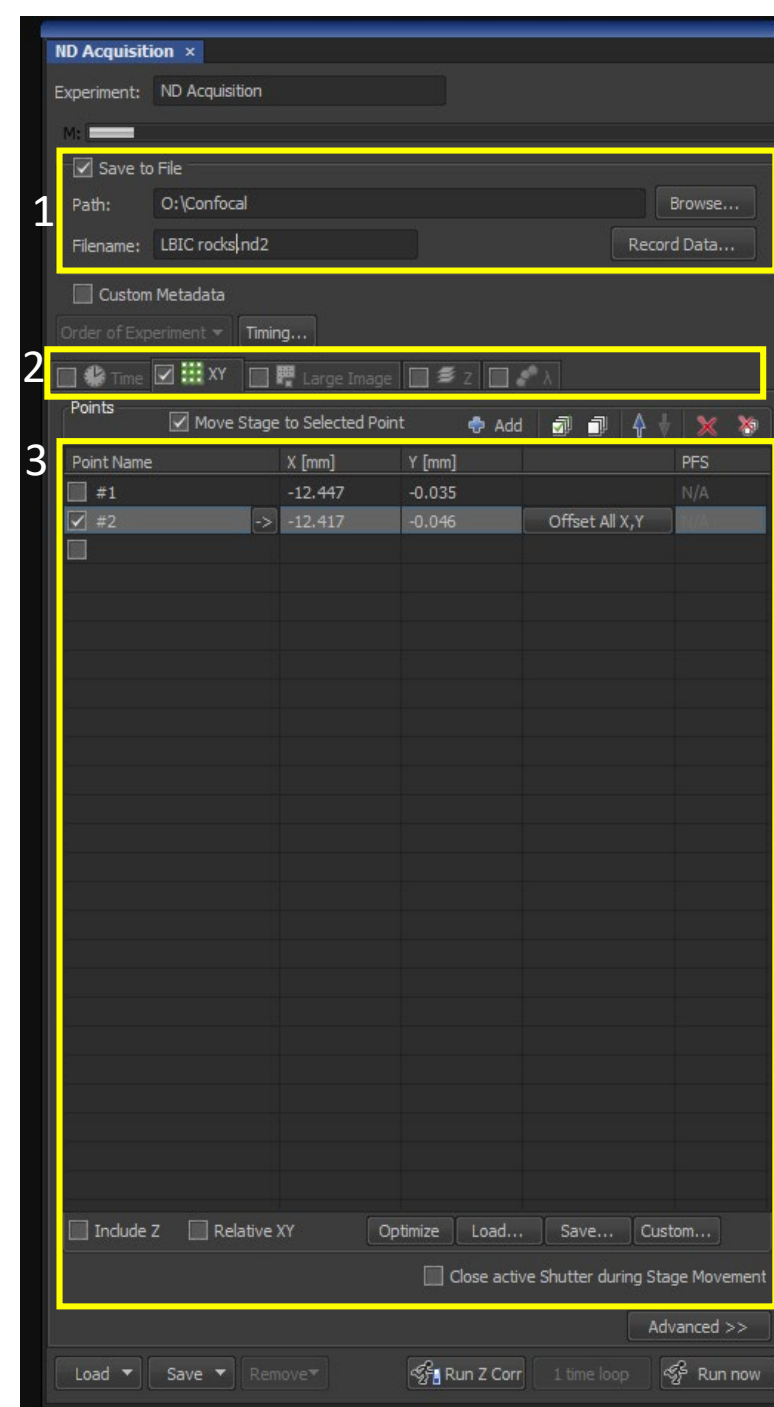
New useful features in NIS v. 5.X

1. Live-denoise & deconvolution

- **Runs on GPU and does not slow acquisition**

- **Activate by clicking live denoising button (1) while scanning**

- **Deconvolution can be turned off if the results are unsatisfactory**
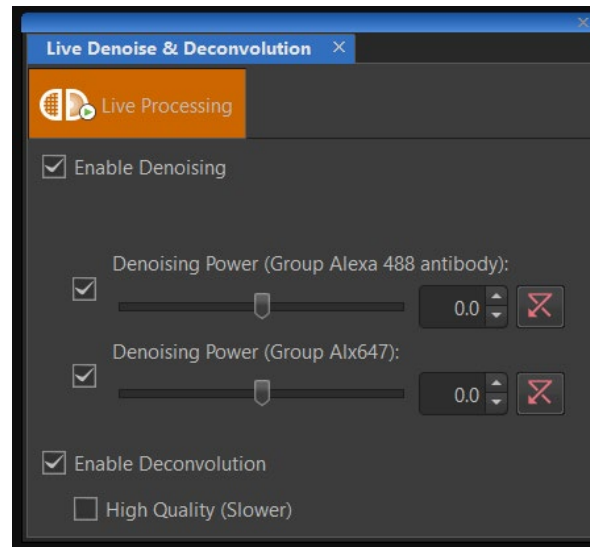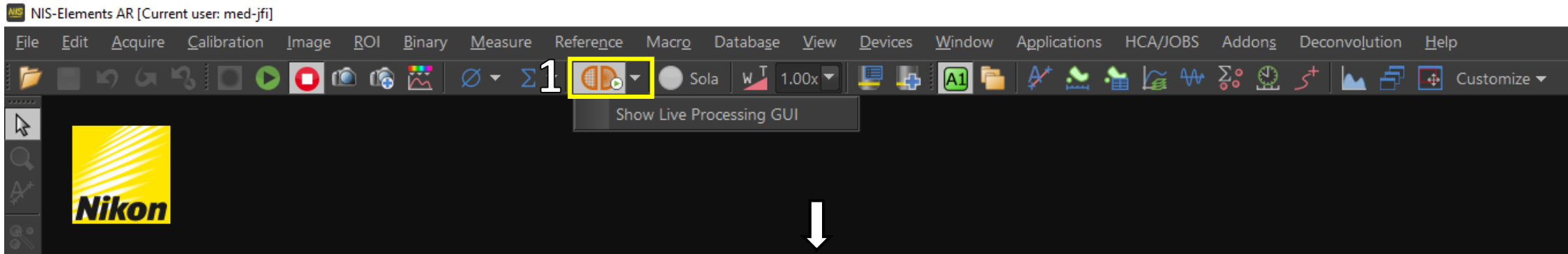
New useful features in NIS v. 5.X

2. Scanning wizard (large images)

- More intuitive way of quickly doing large images (stitching)

- Under "Addons" in the drop down toolbar

- Different shapes can be chosen

- Example (rectangle by 2 corners): While scanning, move to top left corner of sample, click button in yellow box > move to lower right corner and click button in blue box > decide if you want to save to file or not > click Run
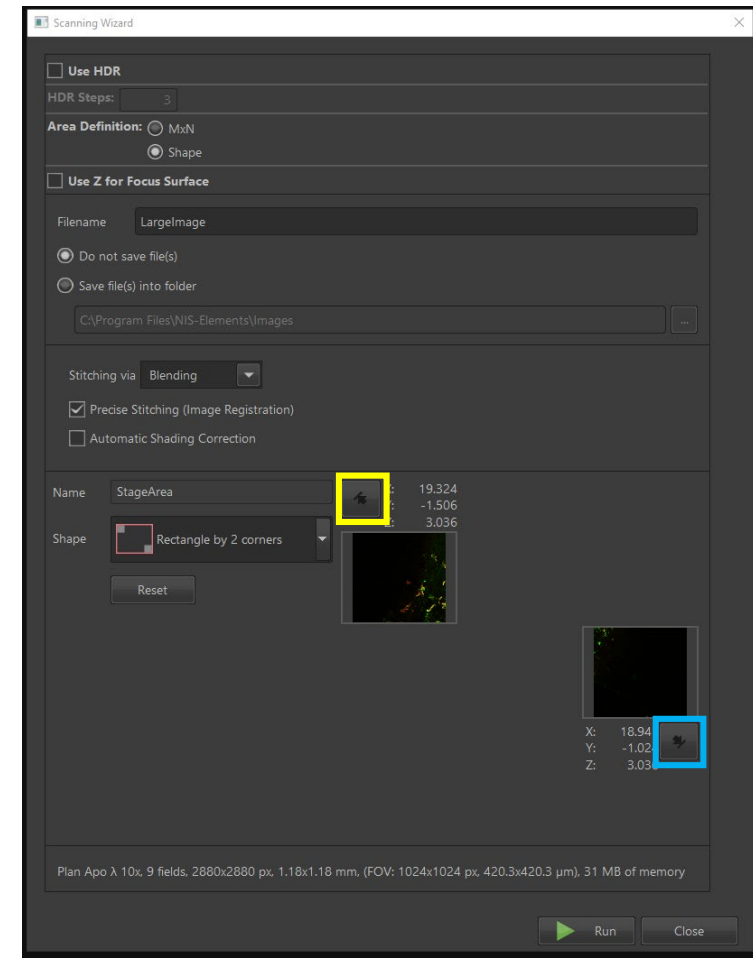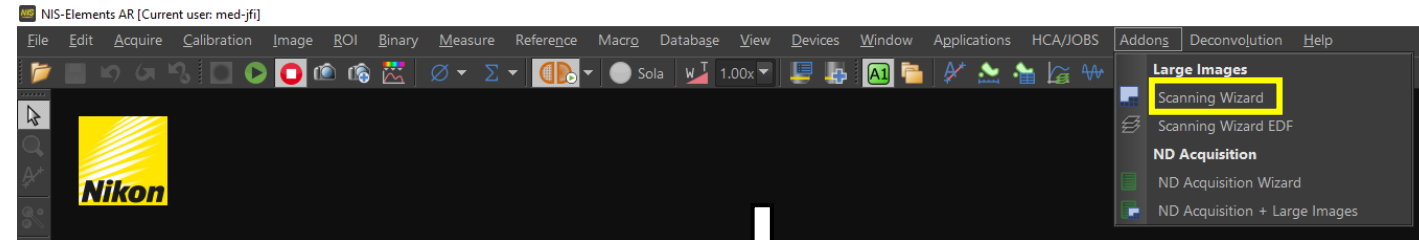
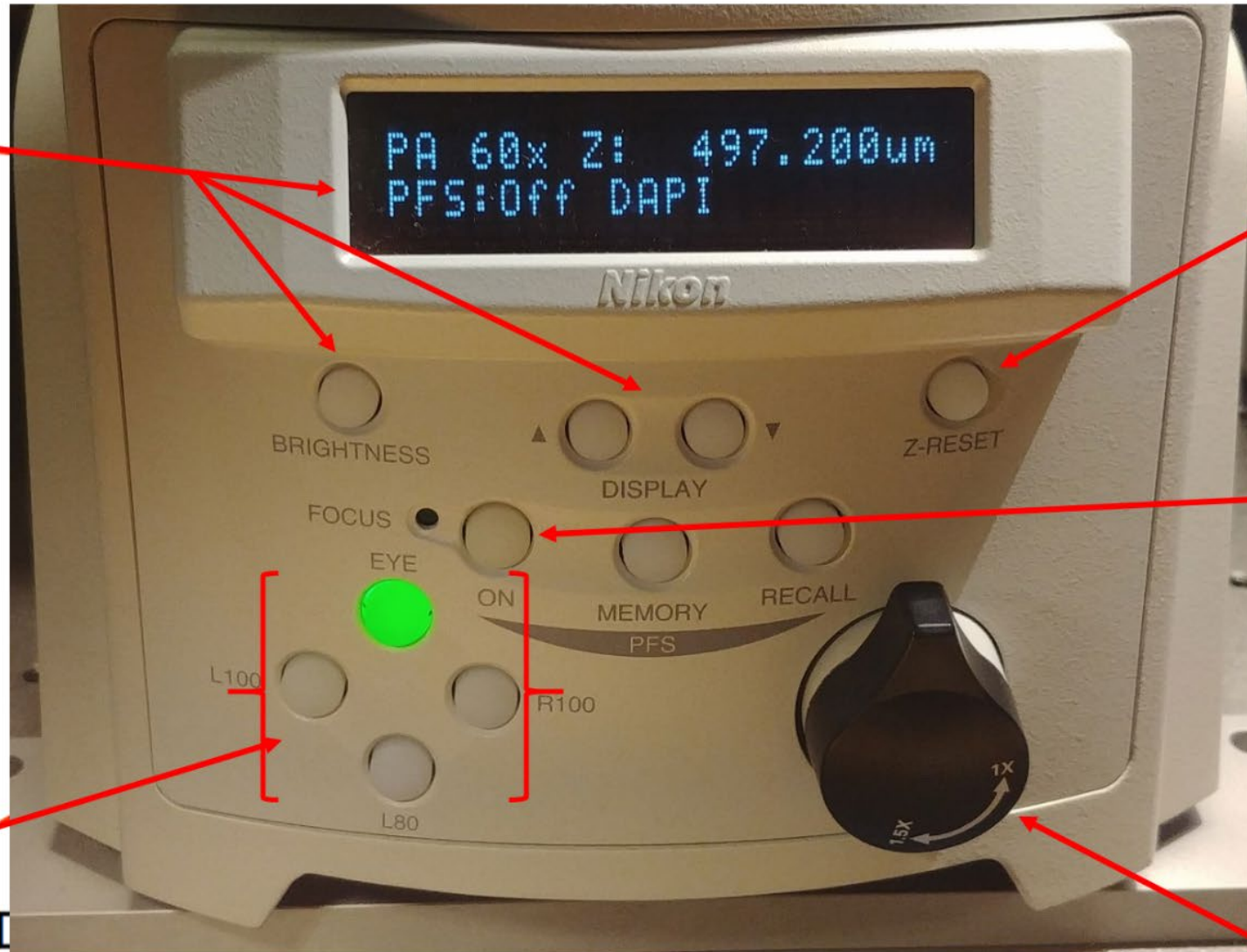**Display:**

Brightness Adjustment and Display Info

Light Path:

Eye-Eyepiece Viewing

L100-Confocal

R100-NOT USED

L80-NOT USED

Resets Current Z position to Zero
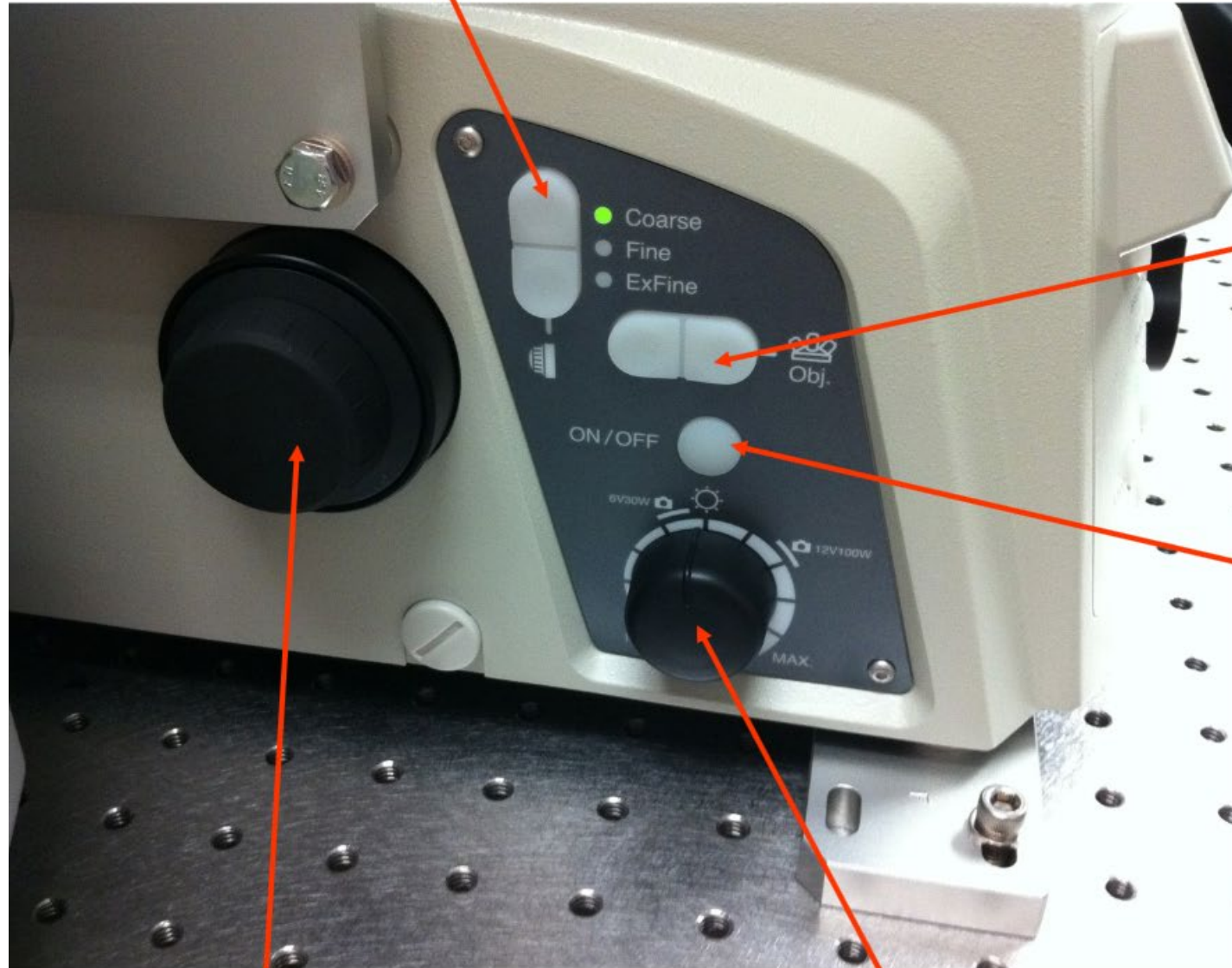
Perfect Focus Adjustments

Magnification Changer: 1x or 1.5x
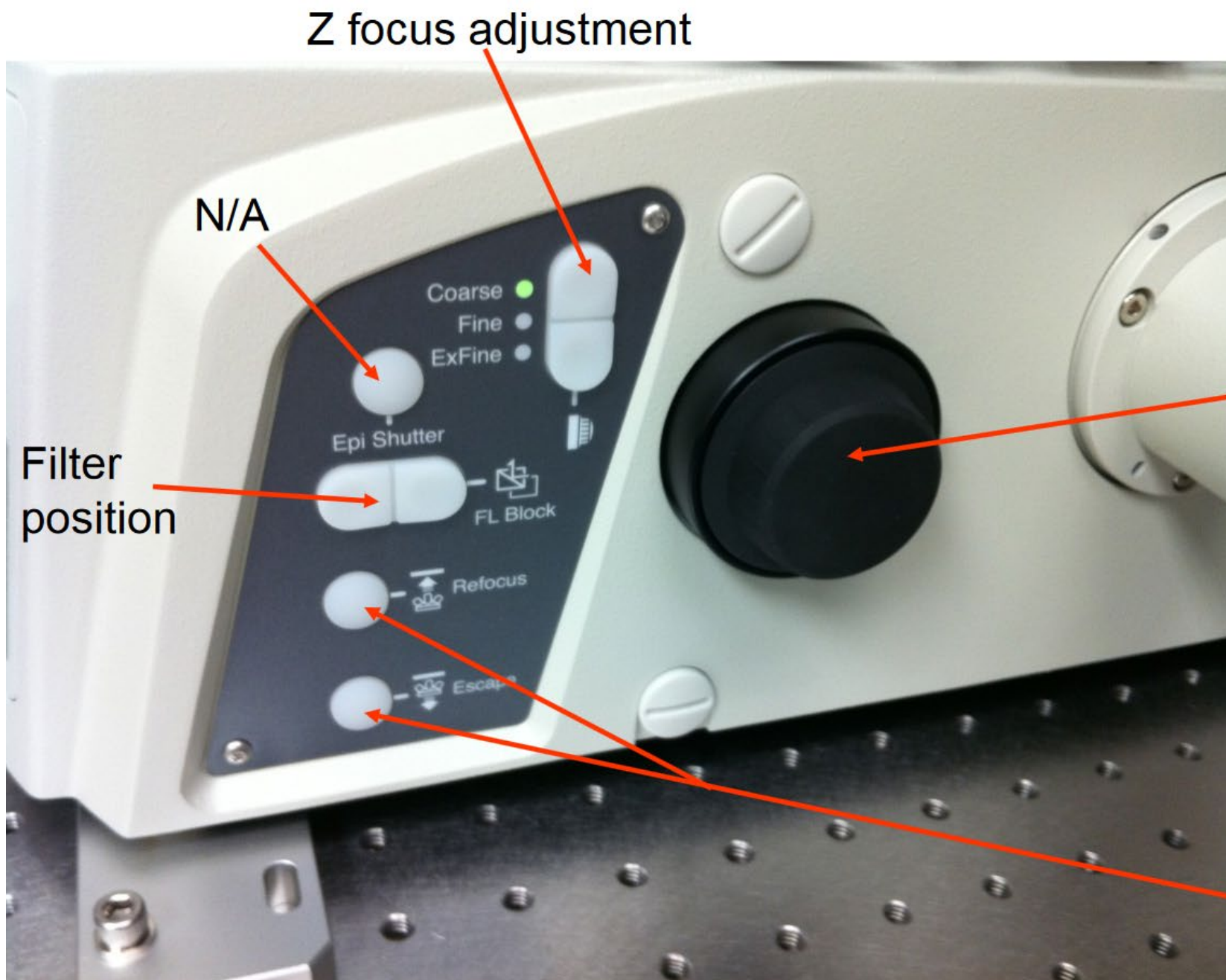
Please default to 1x

(8)

Z focus adjustment

Objective Adjustment- will rotate to next objective lens

On/Off for transmitted light (brightfield)

Focus Knob

Rheostat for transmitted light

(9)

Z focus adjustment

N/A

Filter position

Coarse
Fine
ExFine

Epi Shutter

FL Block

Refocus

Escape

Focus Knob
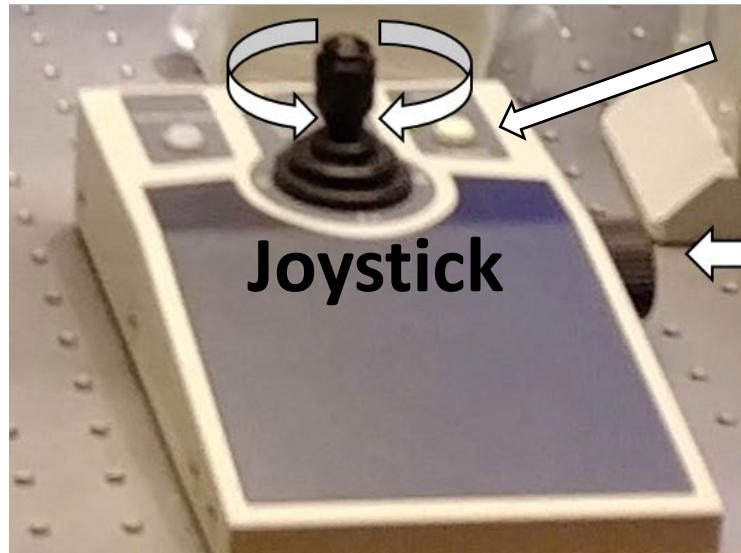
Escape-lowers objective

Refocus-raises objective back up

When "escaped" focus is disabled (10)

Stage movement sensitivity

Z-focus adjustment

Joystick

Focus knob

Changing sample holder

The Resonant confocal sample holders for running with Piezo stage:

1. **35mm Petri dish holder**
2. **Regular slides (75mm x 25mm)**

Exchanging between them is simple

1. **<u>Escape the objectives</u>**
2. Remove screws with the small allen key (3) located on top of shelf. (please don´t loose the screws!!)
3. Remove holder and put on shelf.
4. Change sample holder (gently)
5. Fasten screws gently. DO NOT OVER TIGHTEN!
6. Put back allen key back on shelf.
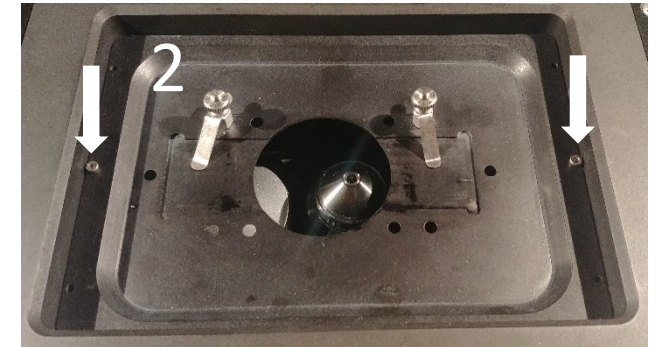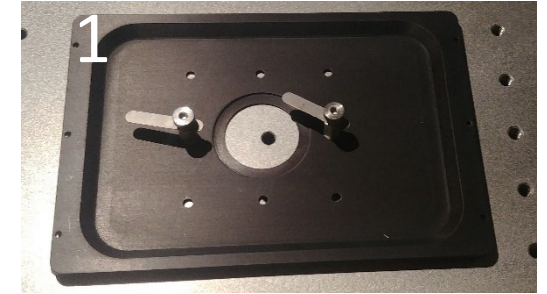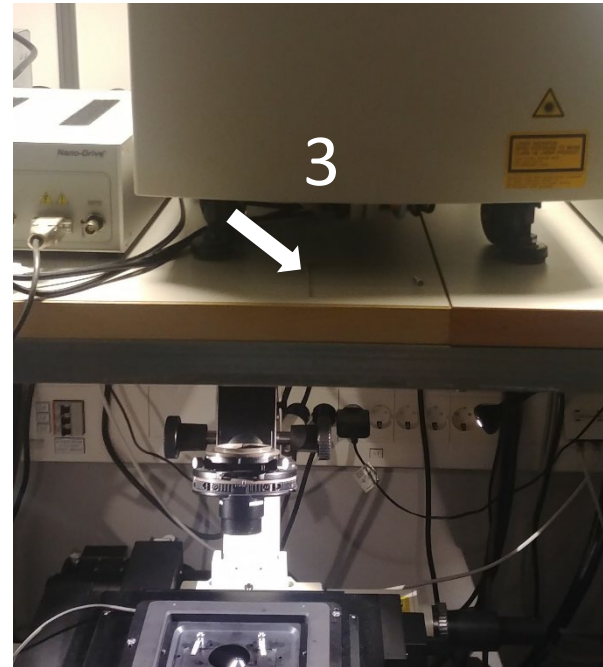
Changing sample holder

**The confocal sample holders for use _without the piezo stage_:**

1. **Multi-holder - slides and small petri dishes**
2. **Multiwell plate holder - for all standard multiwell plates**
3. **Oko-labs stage top incubator (see next page) with fittings for two 35mm petri dishes and one 75mm x 25mm slide - standard slides and chamber slides**

Exchanging between them is simple

1. Remove Piezo stage by loosening the three metal screws. The screws can be left in their sockets when holder is lifted out
2. Remove plastic screws with screwdriver located on top of shelf.
3. Remove holder and put on shelf.
4. Change to the desired sample holder (they only fit one way)
5. Fasten plastic screws gently. DO NOT OVER TIGHTEN! The screws break quite easily.
6. Put back screwdriver on shelf.

(13)
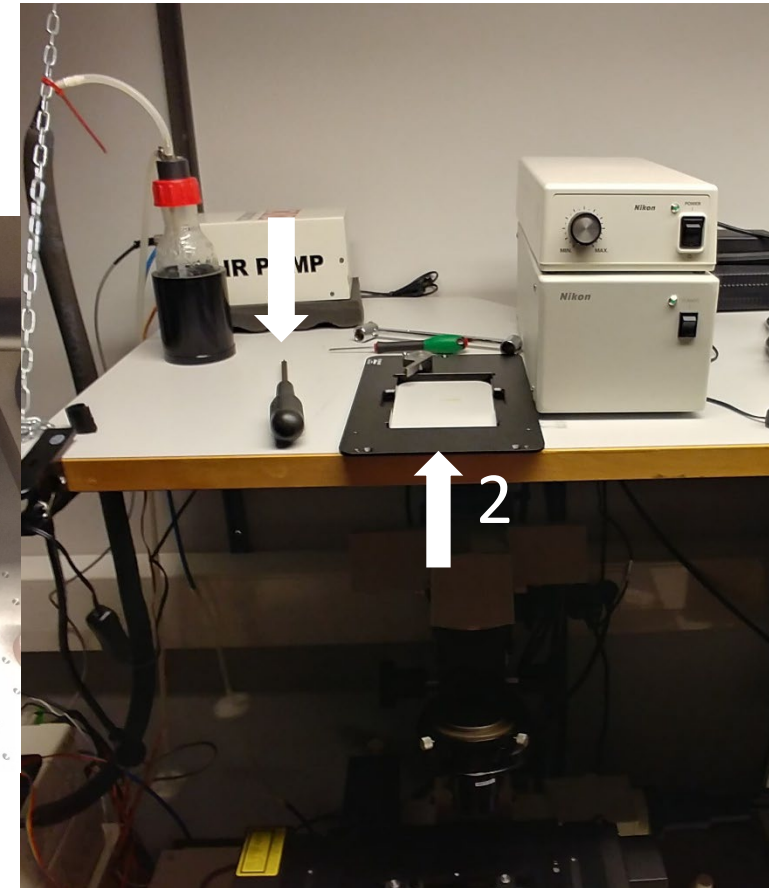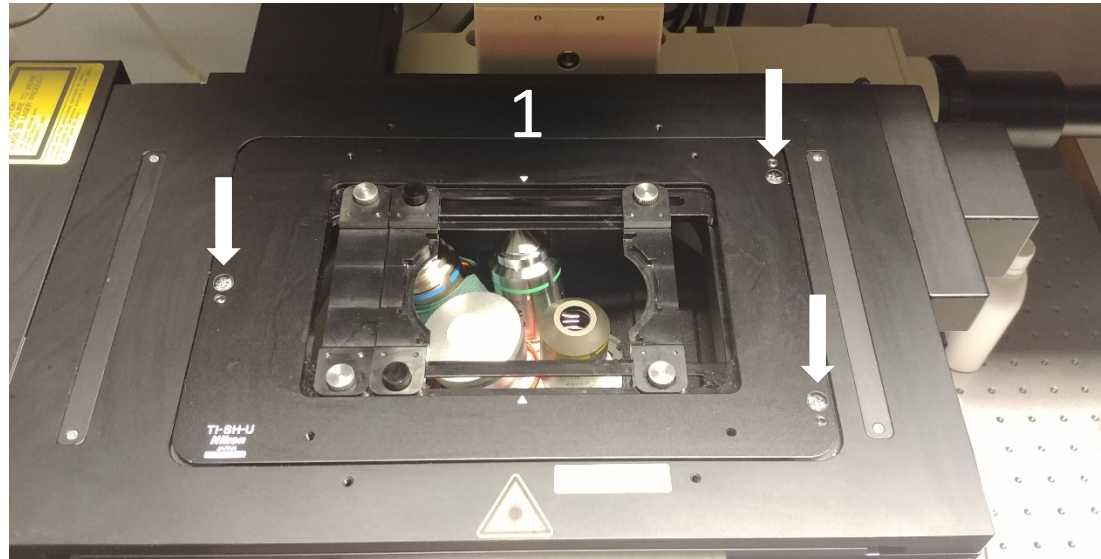
Using the Perfect Focus System (PFS)
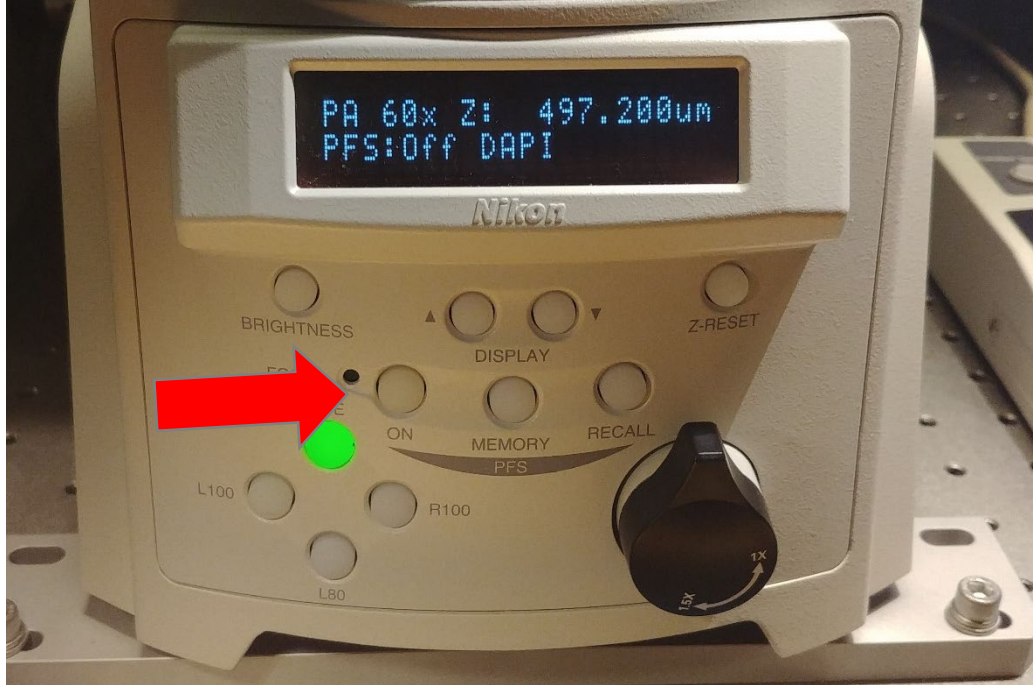
1. Focus your sample to your eyes or monitor.

2. Turn on the PFS by pressing the "ON" button located on the front of the microscope (red arrow) or on the TiE pad in the NIS Elements software.

3. You will hear the PFS system engage and the "ON" button will become a solid green color.
   *If blinking, adjust the focus knob until it locks on to your sample*

4. The image may go out of focus when the PFS system engages-this is normal, as it finds your coverslip top/bottom. Use the external PFS offset controller to refocus the image (blue arrow).

Note: For safety, the focus knobs on the microscope stand will not function while the PFS is in operation.

Moves PFS prism in/out of light path

Coarse/Fine Focus

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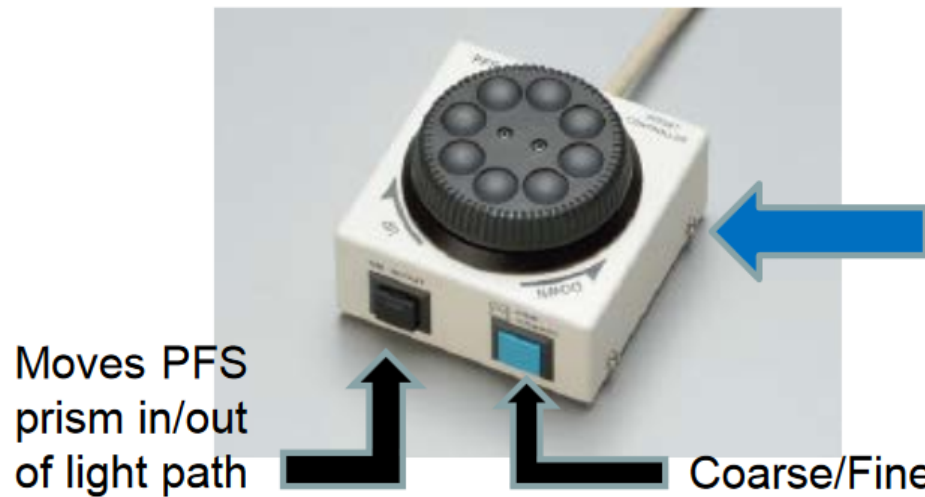
Troubleshooting

- No light when looking in eye port?
  - Check that the Sola is activated
  - Focus is wrong
    - Start at around Z: 2000 and go up slowly
  - Sample upside down?
    - Always put the sample with the coverslip facing the objective

- Black image when scanning?
  - Are the lasers on?
    - Check the laser box and key and start the lasers that are needed.
    - Remember to press the "Remove interlock"
    - Lasers can be turned on/off in NIS

    On
    Off

  - Lightpath is set to "Eye port"
    - Press "eye port" to switch to confocal mode (remember to do this twice when you have first started the system.)
  - Did you select the correct channels?
    - Click the settings button to set up the channels you need

(15)
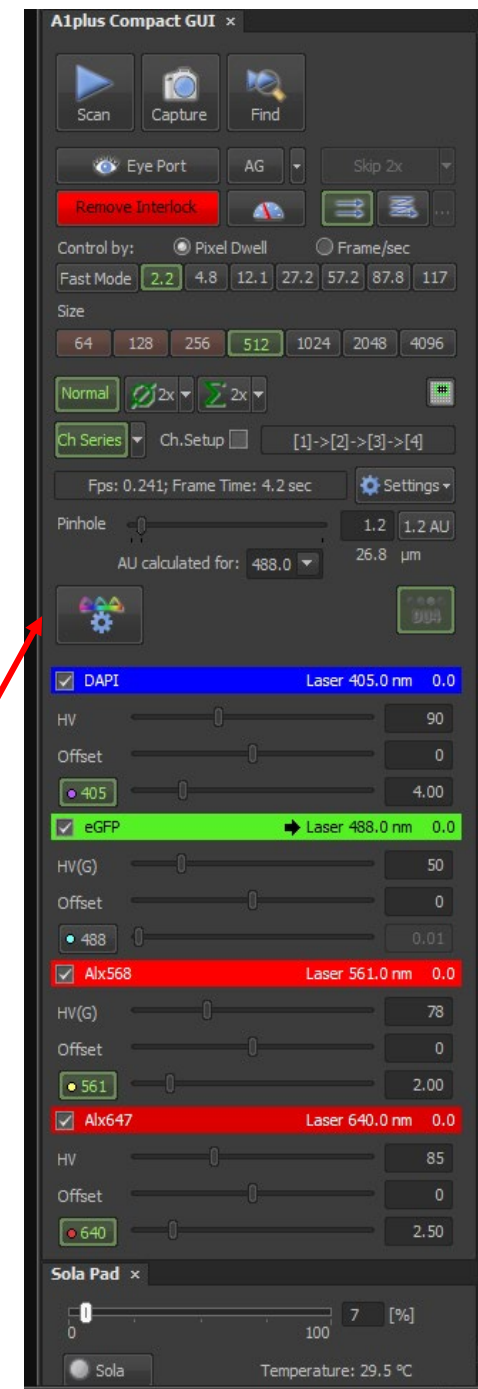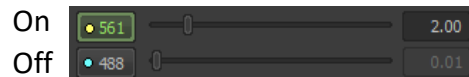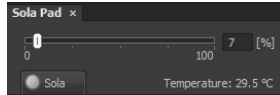
Tips and tricks

- Most dialogs in NIS-elements can be found under the **View tab -> Acquisition/Analysis/Visualization Controls**

    - As a shortcut these can also be found by right clicking in the back open space of elements

- NIS-Elements obeys most Windows-based functions, shift/control and Alt are used often; right-clicks are used to display options/properties

- NIS-Elements uses "drag and drop" for overlaying windows/datasets

- ND2 Files can be opened/viewed outside of NIS-Elements with a free download on: https://www.nikoninstruments.com/Products/Software/NIS-Elements-Advanced-Research/NIS-Elements-Viewer

- There is also a plugin (Bio-formats) for **ImageJ** that enables reading of most image formats.

    - https://docs.openmicroscopy.org/bio-formats/5.8.2/users/imagej/index.html

- You can also download Fiji (ImageJ with a lot of pre-installed plugins, including Bio-formats) from here:

    - https://fiji.sc/

- The easiest way to export images to .tiff files is by doing it through the **Edit -> Create montage (8bit RGB)** function and creating your desired montage. Remember that you **lose bit depth** so any form of analysis must not be done on the resulting files. Always keep a safe copy of the raw image files (.nd2) and save as new .nd2 files when you modify the original data, for example removing background or cropping.

(16)

Tips and tricks cont.

- Use the ND Acquisition GUI to simplify the imaging process

- Create Optical configurations (OC) to save clicks
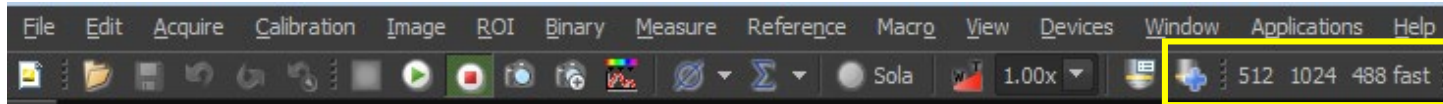
  - When creating new OC, uncheck the objective box in the bottom left margin to avoid unwanted objective changes

- Always use the Pixel Saturation indication tool to avoid saturation

- The help section is a good resource to search and covers almost everything in NIS-elements

- Ask Sebastian (0731536005) if you get stuck.