

Observing Life As It Happens

QUICKSTART GUIDE:
WIDEFIELD WF4
Nikon Ti-Eclipse
High Content Imaging System
(SAF, ROOM 410)



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Startup procedure

- The switches on wall should be on, but if they are not, switch them on: **1 to 5**.
- These control all the microscope, you do not have to touch any other unit, everything should be on by now.
- Start PC and login
- Run NIS Elements



The Microscope



- 1: Lasers for the Confocal
- 2: CoolLED pE-4000 LED unit
- 3: CoolLED pE-4000 LED control pad
- 4: CoolLED pE-100 brightfield LED
- 5: Nikon C2 confocal scanner
- 6: Colour camera
- 7: Four buttons that indicate the lightpath, active when green.
 - Top: eyepiece
 - Right: Hamamatsu ORCA-Flash4.0 CMOS widefield fluorescence camera
 - Bottom: Colour camera
 - Left: Nikon confocal scanner
- 8: 1.5x Optovar (controlled manually)
- 9: Hamamatsu Flash ORCA-Flash4.0 CMOS widefield fluorescence camera
- 10: Perfect Focus wheel to set up the PFS
- 11: Stage joystick controller

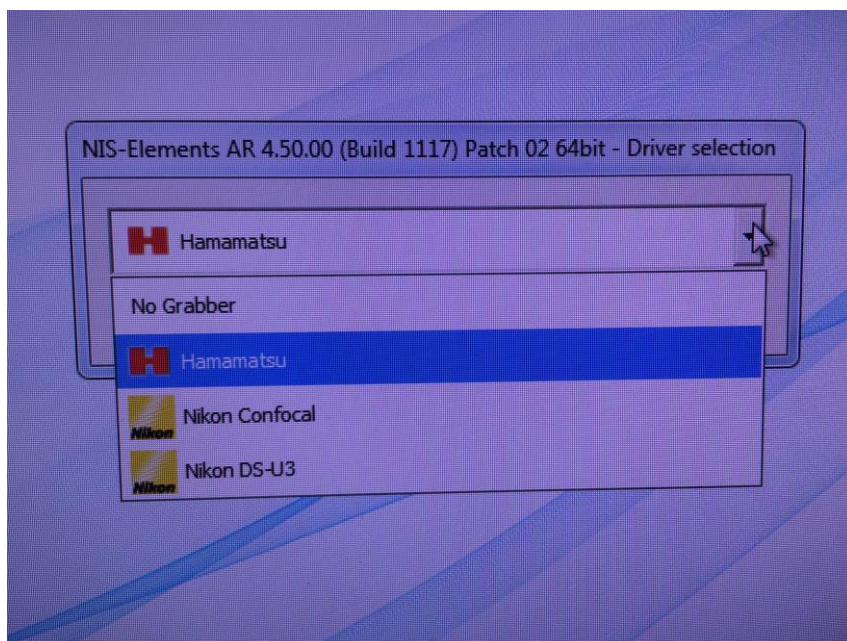
The Software

sStart NIS-Elements.

For Widefield mode, select Hamamatsu

For Confocal mode, select Nikon Confocal

For Colour Camera mode, select Nikon DS-U3



Adjusting brightfield Köhler illumination

- Select 10x objective in the software
- Move your specimen in xy-direction with the joystick, and focus on your sample.
- Adjust brightness with the brightfield lamp.
- Make sure condenser iris is not completely closed (**3**)
- completely close field iris (top black wheel, **1**) (if the image turns completely black, reopen until you see some light, proceed to next step and close again after you have focussed the iris)
- focus iris (black condenser focus wheel, **2**)
- centre iris (two silver Köhler screws, **4**)
- reopen field iris (**1**) until the edge is just not visible anymore

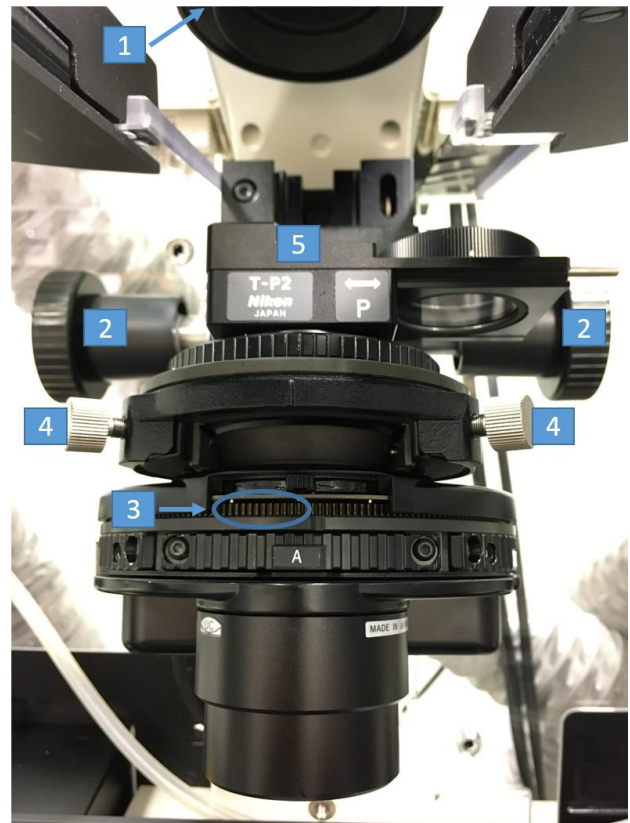
1: Field Iris

2: Condenser focus

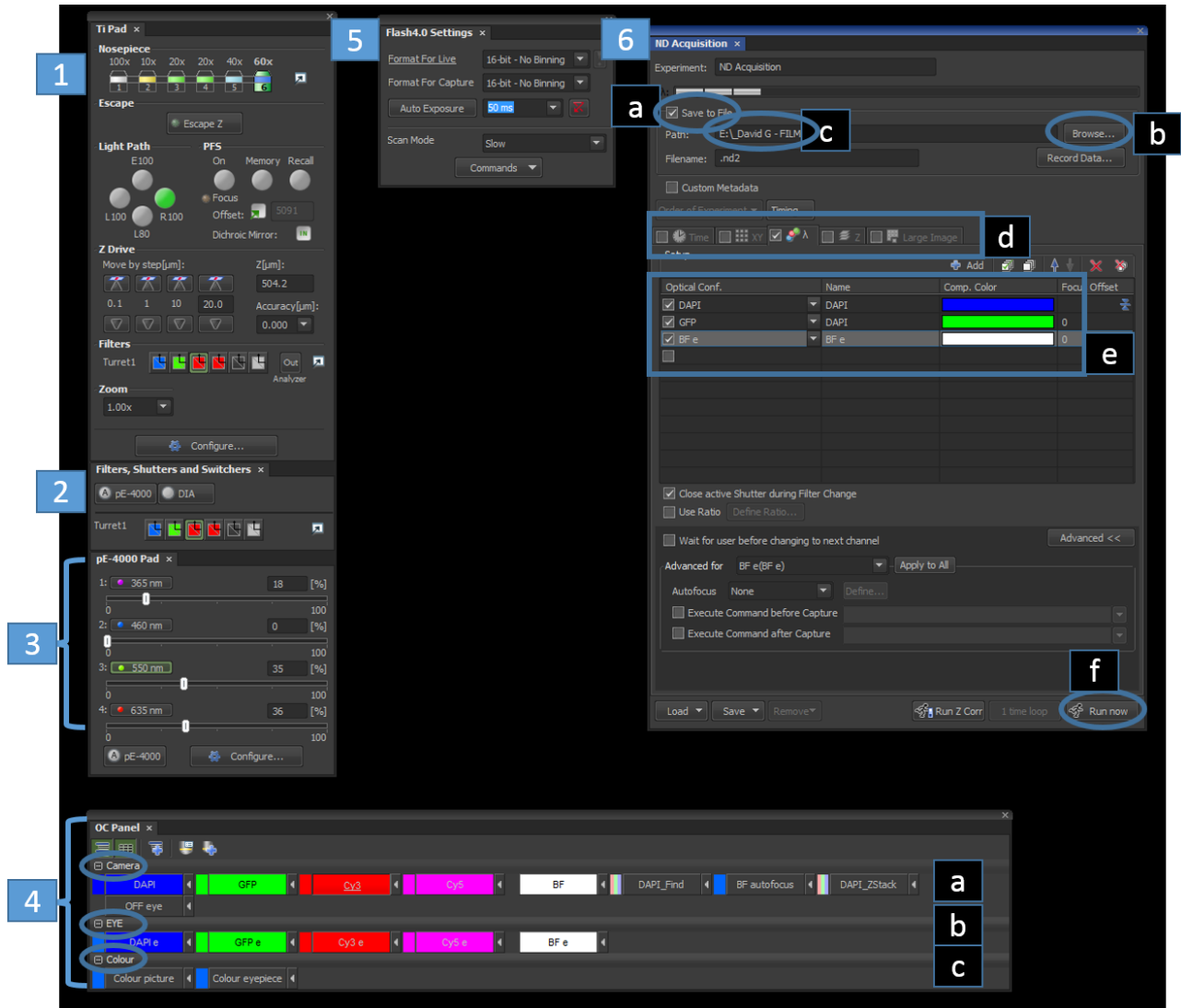
3: Condenser Iris

4: Kohler screws

5: Condenser turret



NIS-Elements



These boxes should be here on the desktop of the left hand side computer screen when you log in. If not, right click on the software space on the right hand side computer screen and select them in the Acquisition drop down menu.

They will re-appear, and you can drag them on the left computer screen.

They are:

- 1: Ti Pad
- 2: Filters, Shutters and Switchers
- 3: pE-4000 pad
- 4: OC panel
- 5: Flash4.0 settings
- 6: ND acquisition

Viewing your sample

- Select the objective (1)
- Select the OC you want in the **EYE set (4)**
- Turn on the pE-4000 for fluorescence, or DIA for brightfield (2)
- Adjust LED power (3)

Image Acquisition

1: Select the objective you would like to use

2: This box show which light source is ON or OFF

- pE-4000 is the LED source for all fluorescence channels
- DIA is the LED source for brightfield (transmitted light)

3: This box controls the pE-4000:

- Adjust the % power of each LED you are using

4: The OC (Optical Configuration) box has three sets of configurations:

- A. Camera: these are the configurations you use when you want to send the signal to the camera, see your sample on the screen and take an image.
- B. EYE: these are the configurations you use when you want to look at your sample on the eyepiece
- C. Colour: these are two configurations for the use of the Colour Camera. One for the eyepiece, one for the camera.

1- Select which channel you would like to set up in the **Camera set**

2- Adjust the corresponding LED

5: Adjust the exposure time for the channel you are setting up. In this box you can also adjust the binning.

After adjusting the LED and the exposure time, there will be a red exclamation mark in the OC you selected in the OC box. This is because these parameters have been changed. Click on the arrow on the right hand side of the OC you want to update.

Repeat this for all the channels you want to image.

6: In the Ti-Pad box, you configure your imaging experiment:

- A. Make sure to click Save to File, so that each image is saved automatically.
- B. Use the browser to select which folder you want to save your images to.
- C. Check that the folder selected is the correct one.
- D. Select which type of acquisition you want:
 - a. Time for a time series
 - b. XY for multi-position
 - c. λ for the channels you want to acquire
 - d. Z for a Z-stack
 - e. Large Image for a tile

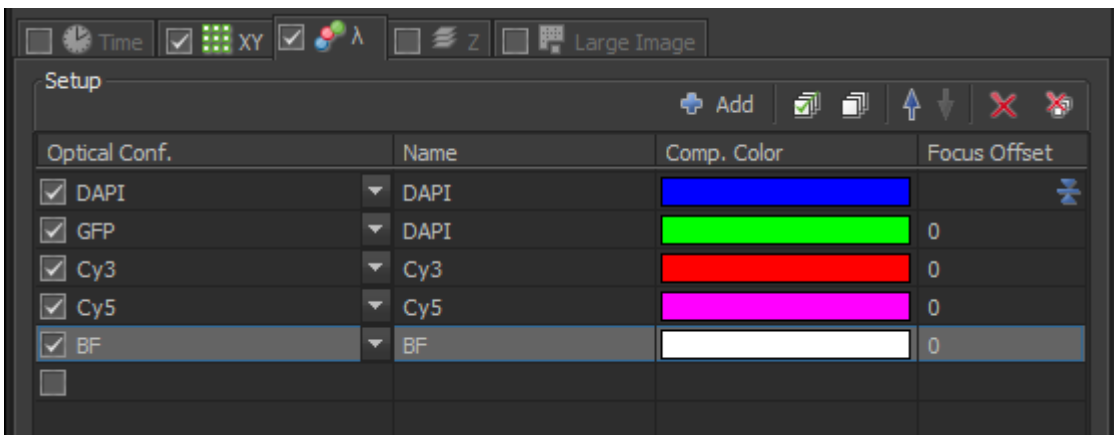
You can combine any of those together.

E. Here you add the channels you want to image by using the drop-down menu to select the corresponding OC.

F. Once this is done, press **RUN NOW** to start the acquisition.

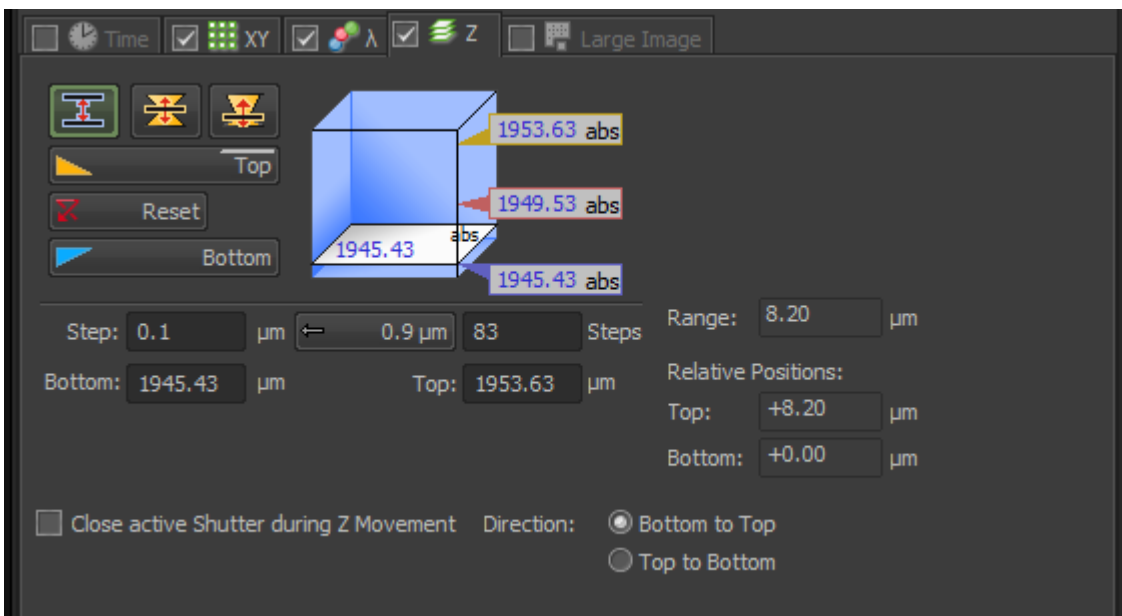
Channels

- In the drop down menu, select the channels you want to image by adding the corresponding OC.



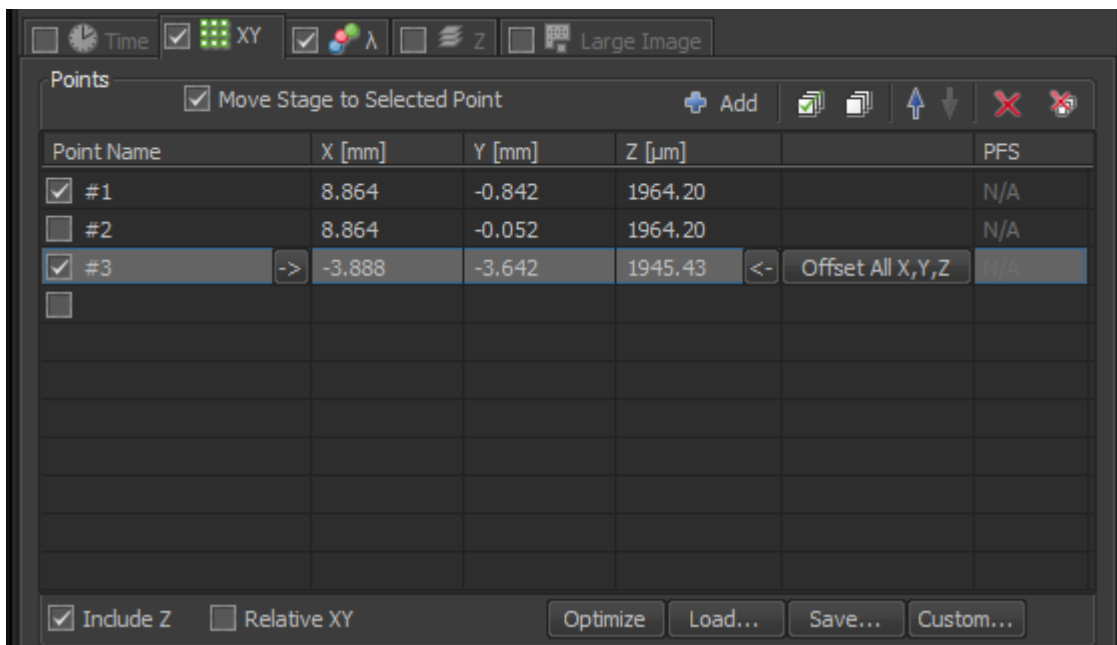
Z stack

- Set Bottom and Top positions.
- Set Step (Z increment).
- You can use the step suggested by the software.
- You can also use a stack centred on a certain Z position (set range). This can be symmetrical (ie +10um and -10um for a range of 20um) or asymmetrical (ie +15um and -5um).



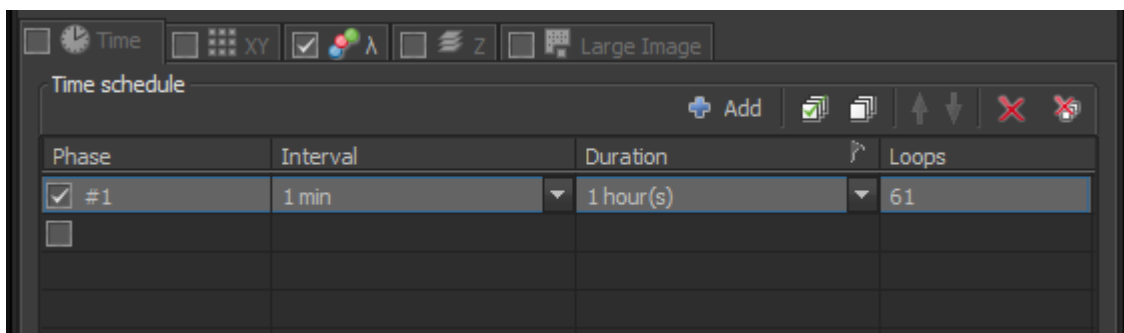
Positions

- While in live mode, move stage with joystick, focus and add position by clicking where #1 is.
- Add as many positions as you want.
- **Click on 'Include Z' to also add the Z position so your positions are in focus, and if you are doing a Z stack.**



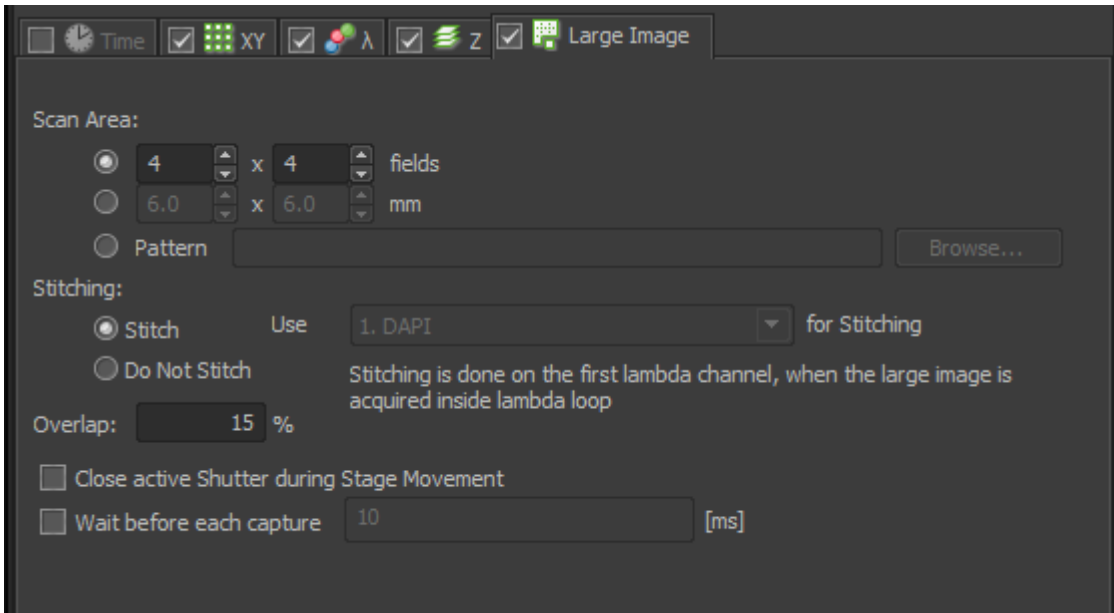
Time series

- Select the interval you want between your acquisitions, in msec, sec, min or hours.
- Select the duration of the whole experiment.



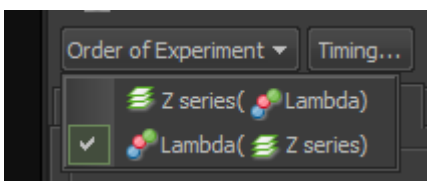
Large Image

- Select the number of fields of view you want to acquire, or the size in mm of the area.
- Select Stitch, or Do Not Stitch. Overlap is 15% by default.



Order of experiment

- Select the order in which the acquisition will be performed
 - **Z series (Lambda)**: this will acquire all channels for each slide of the Z stack (slow as the cube will change for each channel for each slice)
 - **Lambda (Zseries)**: this will acquire all slices of the Z stack, per channel (**faster**)
- You can also check how long the acquisition will take by clicking on 'Timing'.s



Temperature and CO₂

- If needed, ask to insert the small controlled sample holder for temp and CO₂.
- Turn on the OKO Touch temperature and CO₂ controller
- Set temperature
- You can insert the fine green temperature probe through the small hole in the controlled sample holder and put it in a well with medium only.
- You can then set the temperature control to be controlled by the whole microscope chamber (white cable temperature probe, on the side of the condenser) or by the actual medium in the multiwell plate (with the fine green cable probe).
- Turn on CO₂.

At the end of your session

- Update time on PPMS.
- Remove your samples.
- Clean objective lenses with fresh lens tissue and close incubation chamber.
- Put back the 10X objective for the next user.
- Clear up the desk.
- Secure your data (e.g. copy them to the server).
- Log off.