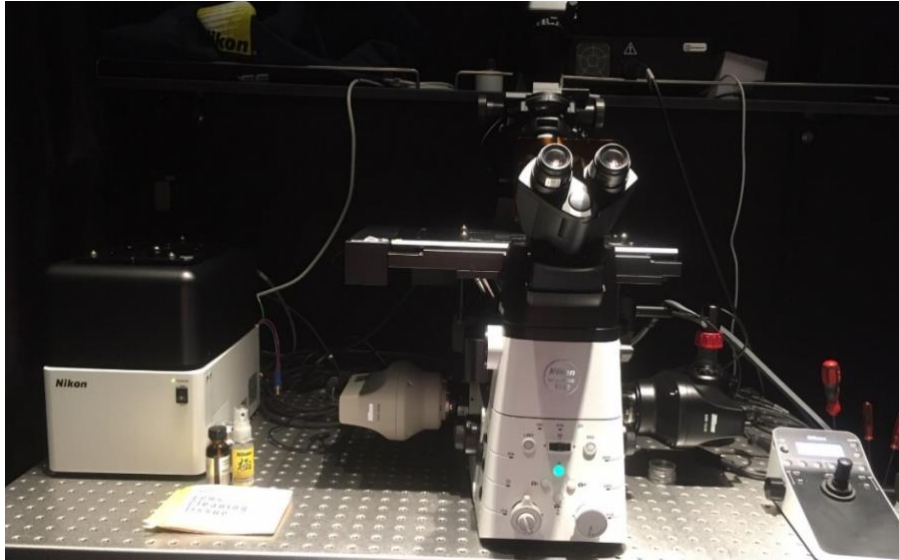


User guide Cyril

Widefield system with triggered switching of excitation light from a Lumencor SpectraX, DIC optics, monochrome Qi2 and color Ri2 large field of view camera. Okolab incubator (temperature 25 – 50 °C, CO₂, and humidifier). Room 470.



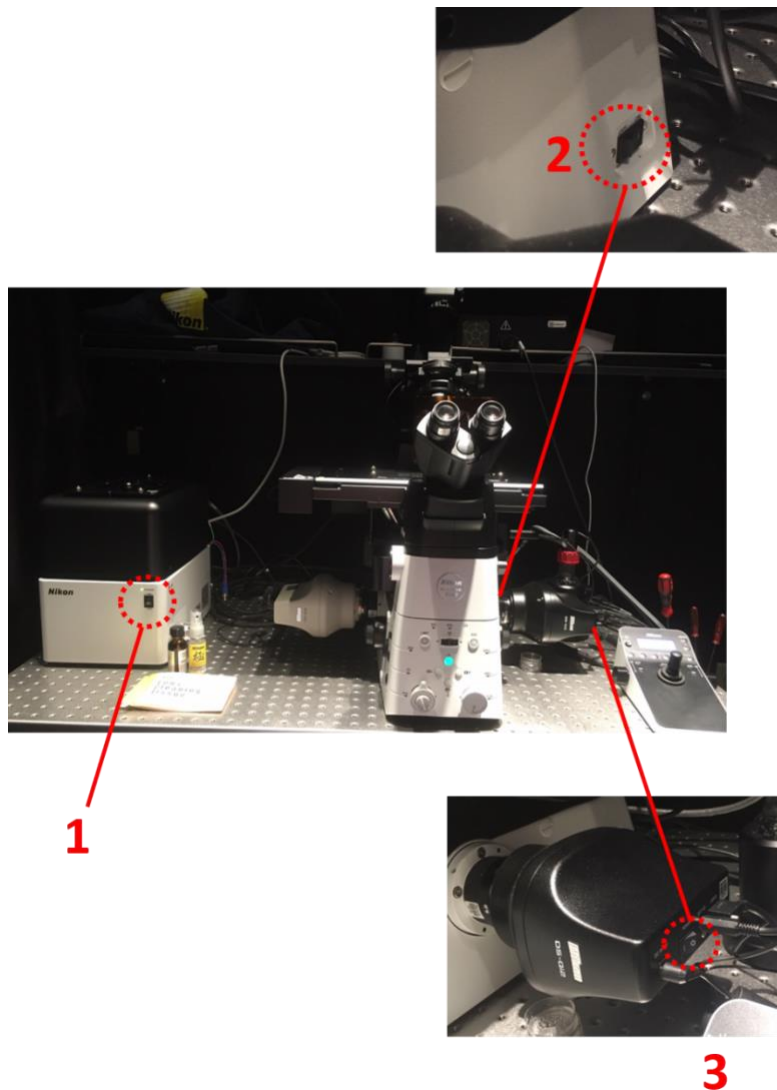
Contents

User guide Cyril	1
Start up with monochrome Qi2	2
Start up with color Ri2	4
Excitation and filter options	6
Excitation LED light from SpectraX	6
Filter cubes in the turret	6
Make new Optical Configuration (OC) and save changes	7
For fluorescence imaging:.....	7
For DIC imaging and bright field imaging:	8
ND acquisition	10
DIC settings and alignment	11
Setup Köhler illumination	11
Setup polarizers	13
Setup DIC.....	13
Perfect focus system (PFS).....	15
Okolab incubator (T: 25 – 50 °C, CO ₂ and Humidifier)	16
Start logging	18
Setting a temperature program.....	19
Important notes!.....	19
Upload to OMERO.....	21
Shutting down.....	21

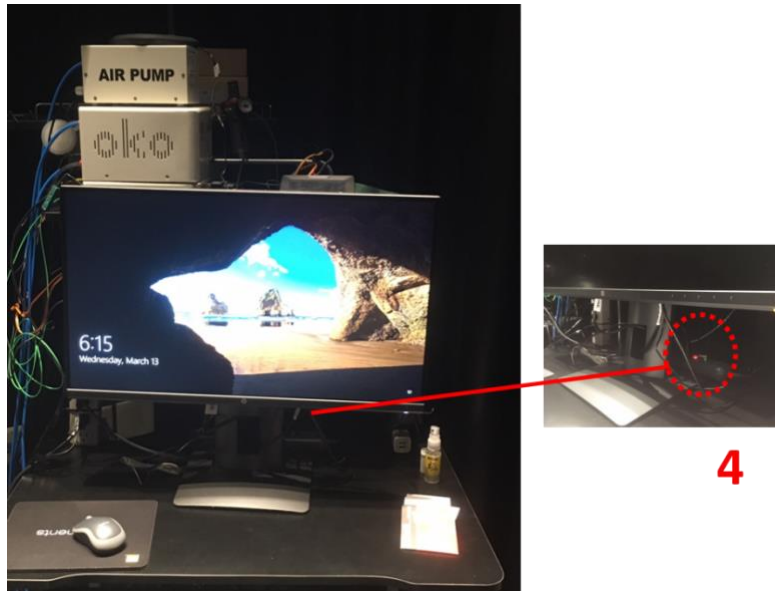
Start up with monochrome Qi2

Start PC (if it's not on).

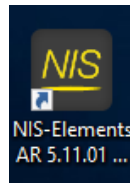
1. Switch on the Nikon control box on the left.
2. Within 15 seconds after step 1, switch on the Nikon Ti2 stand, bottom right side. The microscope will initialize itself, this process shouldn't take too long. The 4 buttons on the front will light up in a pattern and the display of the joystick will show its initializing routine. When the initializing is done, only one of the 4 buttons on the front of the microscope should be on and the display of the joystick should say "PARTS/ PFS ". Restart the microscope if has not initialized properly.
3. Switch on the monochrome Qi2 camera, black camera on the right.



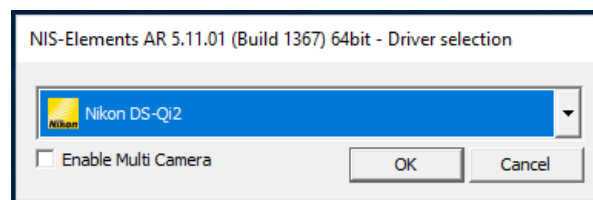
4. If you want to use the incubator, switch on the Okolab incubator, by switching on the power strip behind the computer screen.



5. Log on the computer with your UCSD Active Directory credentials.
6. Start NIS- elements software (NOT the analysis option).



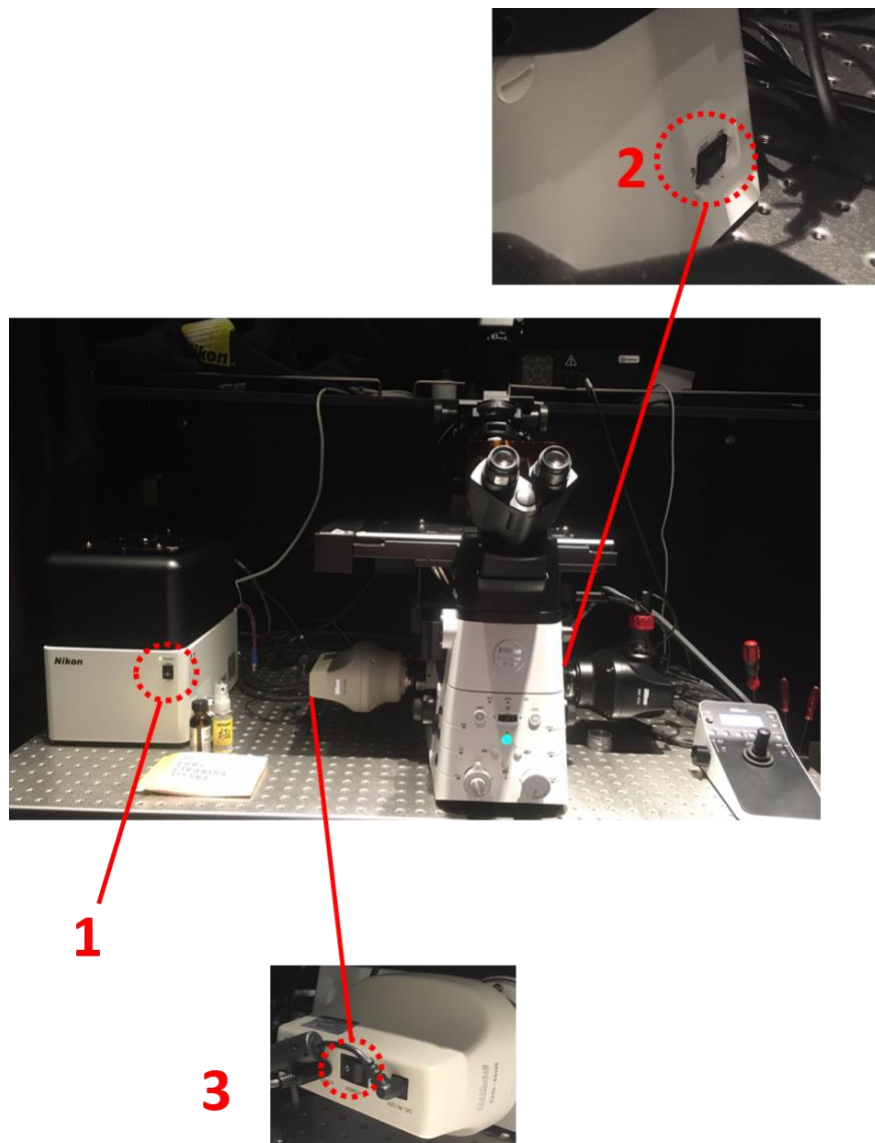
7. Select the “Nikon DS-Qi2” camera from the drop-down menu.



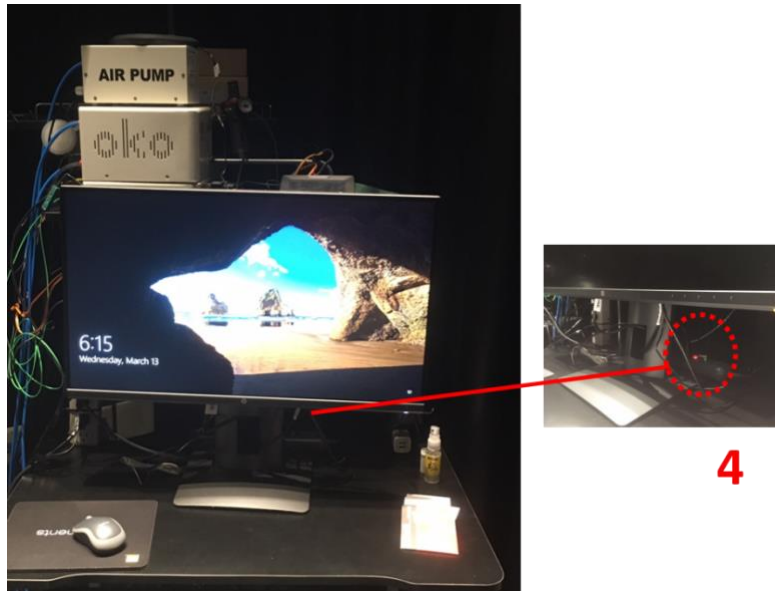
Start up with color Ri2

Start PC (if it's not on).

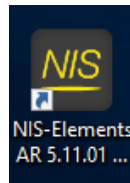
1. Switch on the Nikon control box on the left.
2. Within 15 seconds after step 1, switch on the Nikon Ti2 stand, bottom right side. The microscope will initialize itself, this process shouldn't take too long. The 4 buttons on the front will light up in a pattern and the display of the joystick will show its initializing routine. When the initializing is done, only one of the 4 buttons on the front of the microscope should be one and the display of the joystick should say "PARTS/ PFS ". Restart the microscope if has not initialized properly.
3. Switch on the color Ri2, white camera on the left.



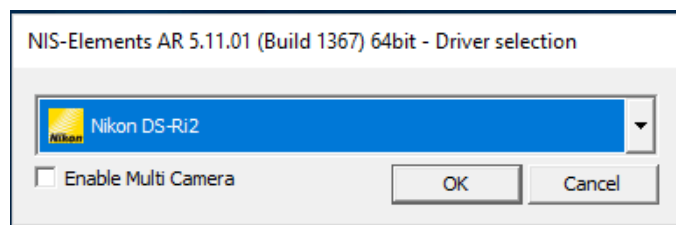
4. If you want to use the incubator, switch on the Okolab incubator by switching on the power strip behind the computer screen.



5. Log on the computer with your UCSD Active Domain credentials.
6. Start the NIS-elements software (NOT the analysis option).



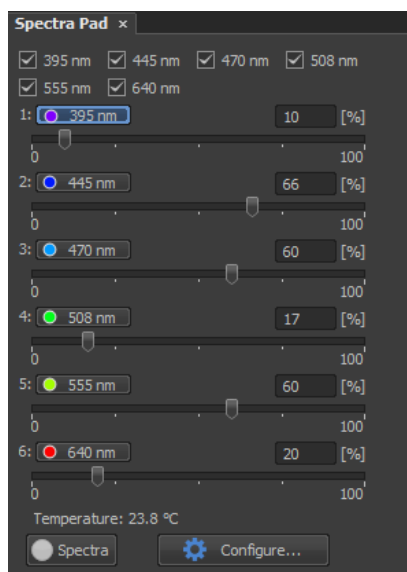
7. Select the “Nikon DS-Ri2” camera from the drop-down menu.



Excitation and filter options

Excitation LED light from SpectraX

The SpectraX contains 6 LEDs which can be controlled independently by NIS elements.



1. 395 nm, violet light of 383 – 407 nm
2. 440 nm, blue light of 430 – 450 nm
3. 470 nm, cyan light of 458 – 482 nm
4. 508 nm, green light of 498 – 523 nm
5. 555 nm, yellow light of 543 – 558 nm (or optional 563 – 588 nm)
6. 640 nm, red light of 625 – 655 nm

Filter cubes in the turret

There are 6 positions in the turret.

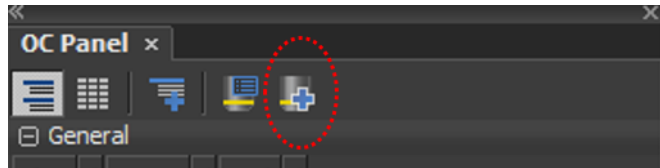


1. DIC Analyzer
2. LED-DAPI-A (96369); Excitation 379 – 405 nm; Dichroic mirror 409 nm; Emission 414 – 480 nm
3. GFP 4050B (96372); Excitation 443 – 489 nm; Dichroic mirror 495 nm; Emission 497 – 551 nm
4. LED-TRITC-A (96374); Excitation 540 – 568 nm; Dichroic mirror 573 nm; Emission 579 – 640 nm
5. LED-Cy5-5070A (96376); Excitation 590 – 645 nm; Dichroic mirror 652 nm; Emission 659 – 736 nm
6. Quad band (77015809);
Excitation 352 – 404, 461 – 488, 543 – 566, and 626 – 644 nm;
Dichroic mirror 414 – 450, 500 – 530, 580 – 611, and 660 – 800 nm;
Emission 414 – 450, 500 – 530, 580 – 611, 661 – 800 nm

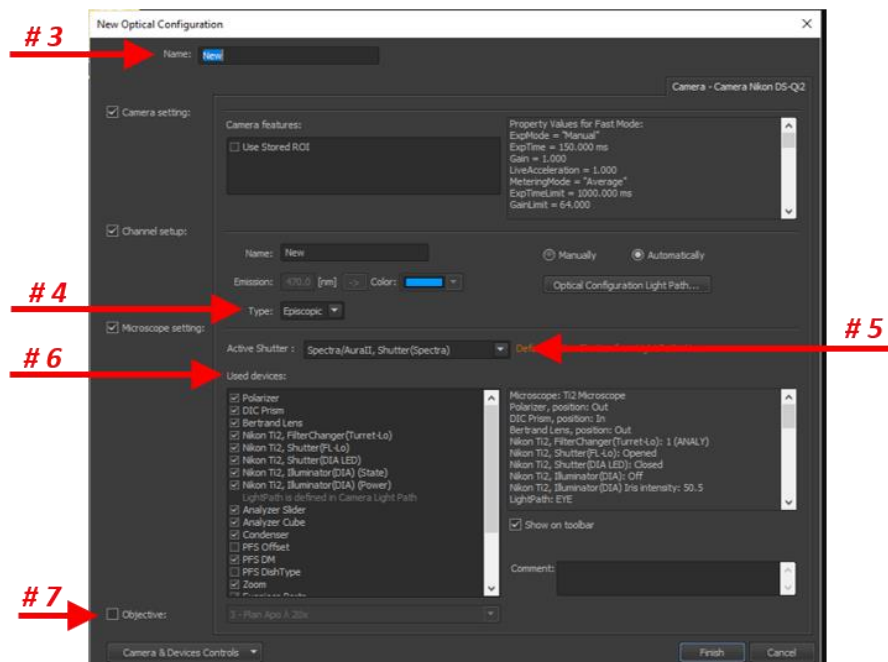
Optional cube: CFP/YFP/mChXt (C195428, 77074656).

Make new Optical Configuration (OC) and save changes

1. In the OC panel, click the “+ new configuration” icon.



2. A new window “New optical configuration” will pop up.
3. Name the new OC.



For fluorescence imaging:

4. At channel setup, manually select the color (or leave it automatically), select “Episcopic” Type
5. Select “Spectra/Aurall, shutter (Spectra)” as active shutter.
6. Deselect “Used devices” that are not necessary to prevent cluttering. Minimum devices required for fluorescence imaging:
 - a. Nikon Ti2, filterchanger (Turret-Lo)
 - b. Nikon Ti2, (DIA LED)
 - c. Nikon Ti2, (DIA) (State)
 - d. Analyzer cube
 - e. Condenser
 - f. PFS DM
 - g. Spectra/Aurall, Multilaser(Spectra) line used
 - h. Spectra/Aurall, Shutter(Spectra) power

Continue at step 7

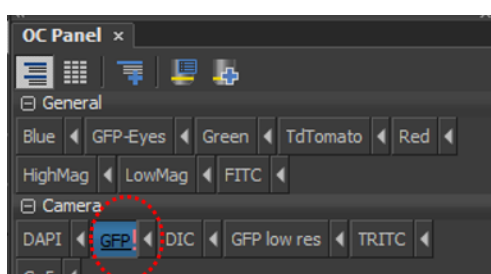
For DIC imaging and bright field imaging:

4. At channel setup, manually select the color “Brightfield” and type “Diascopic”.
5. Select “Nikon Ti2, shutter (DIA LED)” as active shutter.
6. Deselect “Used devices” that are not necessary to prevent cluttering. Minimum devices required for DIC imaging:
 - a. Polarizer
 - b. DIC prism
 - c. Bertrand lens
 - d. Nikon Ti2, filterchanger (Turret-Lo)
 - e. Nikon Ti2, Illuminator (DIA) (Power)
 - f. Analyzer slider
 - g. Analyzer cube
 - h. Condenser
 - i. PFS DM
 - j. Spectra/Aurall, Shutter(Spectra)

Continue at step 7

7. Deselect “Objective”.
8. Click “Finish”.
9. Set all parameters as desired, like: Camera settings (exposure time, binning), Spectra pad settings (LED and intensity), Position of the filter turret and condenser.
 - a. For fluorescence:
 - i. set the condenser turret at position 1: Shutter.
 - ii. Choose the appropriate SpectraX LED and power
 - iii. For best fluorescence signal, check if there is a prism underneath the objective, if so, remove the prism from underneath the objective. (place it in a holder in the transparent drawers)
 - iv. Choose the appropriate filter cube (position 2 – 6)
 - v. To acquire images light path should be to the right, to look at your cells through the eyepiece light path should be at middle up.
 - vi. Set the exposure time of the camera
 - b. For DIC:
 - i. Choose the DIA LED and power
 - ii. Set the condenser at position 5: N1 or 6: N2 (See DIC section)
 - iii. Polarizer at condenser turret should be in place
 - iv. Normaski prims underneath the objective should be in place (See DIC section)
 - v. Choose the analyzer cube (position 1)
 - vi. To acquire images light path should be to the right, to look at your cells through the eyepiece light path should be at middle up.
 - vii. Set the exposure time of the camera
 - c. For brightfield:
 - i. Choose the DIA LED and power
 - ii. Set the condenser at an open position 2, 4 or 7.
 - iii. Polarizer at condenser turret should be OUT

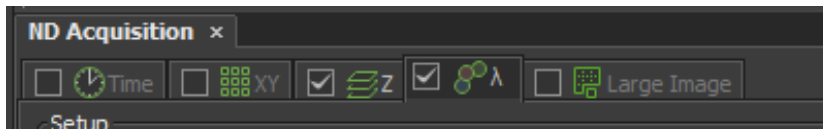
- iv. Normaski prisms underneath the objective should be OUT
 - v. Choose the analyzer cube (position 1), or better remove a cube to create an empty position and use that empty position.
 - vi. To image, light path should be to the right for the monochrome camera, to look at your cells through the eyepiece light path should be at middle up, or light path should be to the left for RGB camera.
 - vii. Set the exposure time of the camera
10. A red exclamation mark will appear next to the “OC name” in the “OC panel”. Click on the white arrow next to the red exclamation mark to save/assign the current settings to this OC. Any time you make changes to an OC, such as adjusting the LED power or changing the filter cube, you should see this red exclamation mark. If you fail to select it, the OC will revert to the last saved state when you return to it.



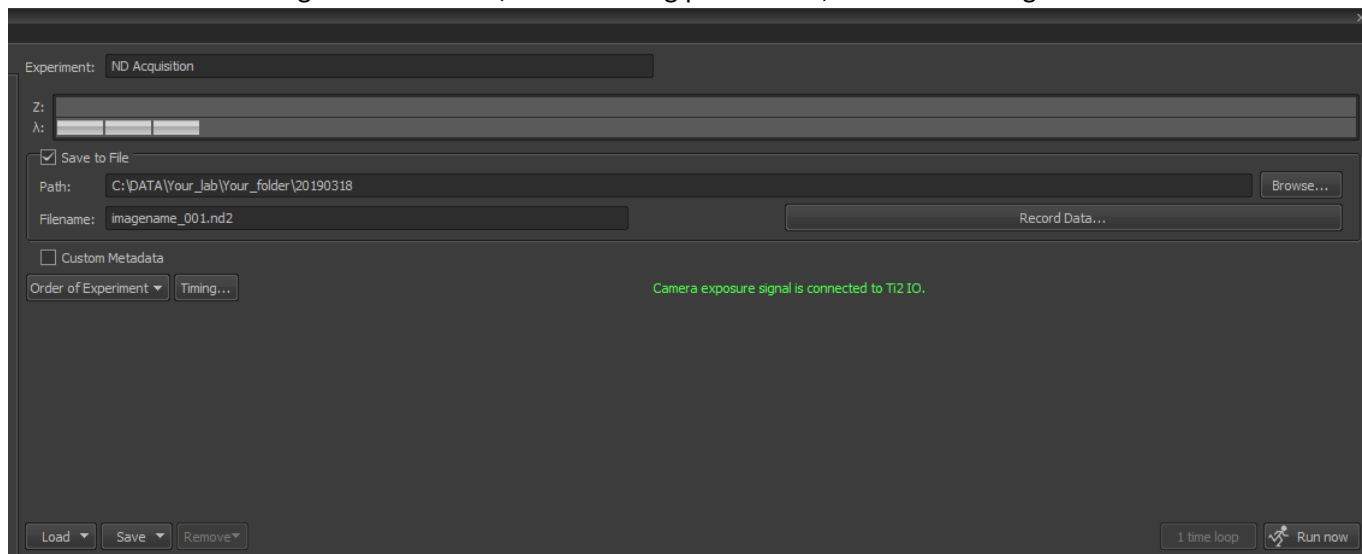
ND acquisition

ND = Number of Dimensions.

1. Check the boxes of the dimensions you want to use for your acquisition: Time, XY position, Z-stack, lambda (OC configurations), large image (tilescan).



2. Click on the box in each tab to activate the dimension. The above example would be for doing a Z-stack with multiple wavelengths (OCs).
3. After setting the dimensions, set the saving parameters, shown on the right.



4. Select save to file
5. Save it in “Data” folder on this computer (C-drive), in your personal folder. For example: Save C:\DATA\Your_lab\Your_folder\20190318
6. The order of the experiment is very important!! In general, the fastest and best for the system is the option with least physical moving parts, which would be: 1 Lambda, 2 Z-stack, 3 Large image, 4 XY.
7. Click “Run now” to start the acquisition.

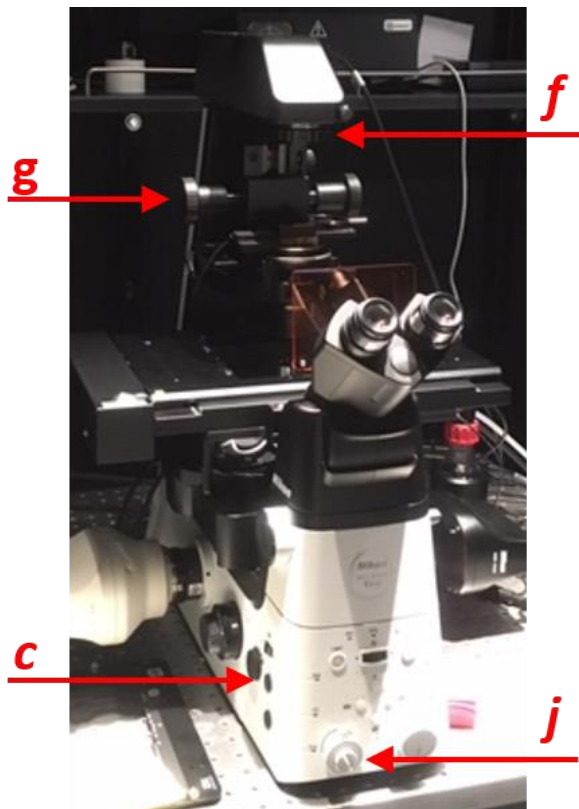
DIC settings and alignment

It is important to align all components in order to obtain good quality DIC images. This alignment is objective specific, if you change objectives you need to follow the procedure again. This process contains 3 parts:

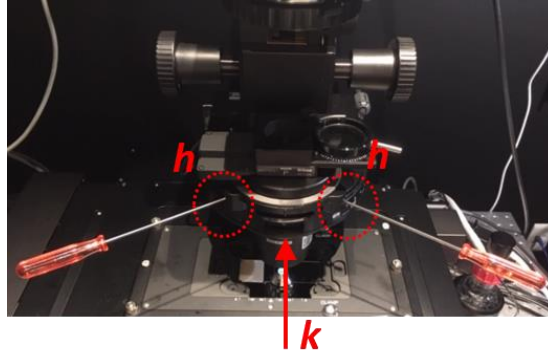
1. Setup Köhler illumination – Make sure that the condenser lens is in focus and centered.
2. Setup polarizers – Insert polarizers, check max extinction.
3. Setup DIC – insert Nomarski prisms (2x) and set the right angle of the condenser polarizer.

Setup Köhler illumination

1. Set Köhler illumination – Make sure that the condenser lens is in focus and centered. See images for the knobs at the corresponding alphabetic numbering. The exact position of the condenser is objective specific, you should adjust this when you change objectives.
 - a. Put your sample in the stage holder.
 - b. Select an open position in the condenser turret (position 2, 4 or 7).
 - c. Switch on the DIA LED (c or in the software), adjust power if desired.
 - d. Select the analyzer cube in the filter turret-lo.
 - e. Find/focus on your sample. Move to a position with minimal objects.
 - f. Close the field diaphragm almost completely (f)
 - g. Look through the eye piece and move the condenser lens up/down by turning the wheel (g) to focus on the edges of the field diaphragm (you'll see the field diaphragm as an octagonal structure).



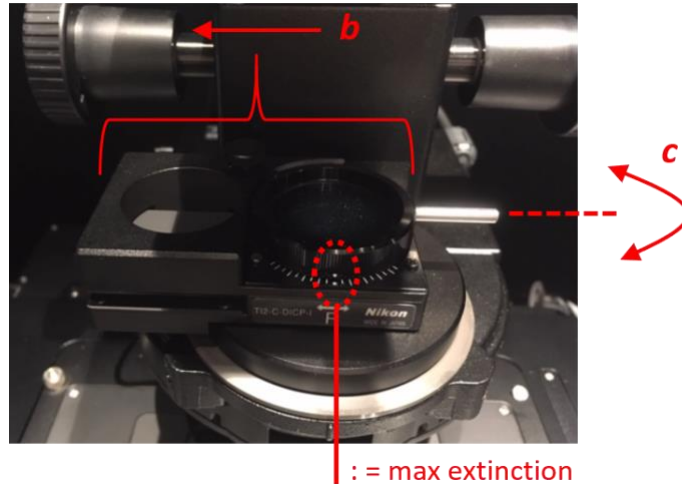
- h. Center the condenser in the middle by push/pull method using two #2 hex keys (see image below). The octagonal structure should be in the middle of your field of view, while looking through the eye piece. Tip: open the field diaphragm further to see if the edges of the octagonal structure are at an even distance from the outskirt of the round field of view.



- i. Open the field diaphragm (*f*) just enough to illuminate your field of view, e.g. just so that you just don't see the octagonal structure anymore. If you open it completely you would have more background due to stray light.
- j. Place the Bertrand lens (*j*) in place, look through the eyepiece, focus if required (the small turning knob within the big Bertrand lens knob).
- k. Now you see the front aperture plane. As a rule of thumb, set the condenser diaphragm at 80% – 70% open. This diaphragm sets the NA of the condenser lens. Opening it to the point where you no longer see it expanding in the Bertrand lens will give you the maximal NA, and therefore higher resolution. Closing it will increase your contrast but will reduce the resolution of your image. Once everything is set up, you could play a bit with this diaphragm to see what works best for your sample.
- l. You're Köhler illumination is now aligned.

Setup polarizers

2. Setup polarizers – Insert polarizers, check max extinction.
 - a. Make sure the analyzer cube is selected in the turret-lo (position 1).
 - b. Slide the condenser polarizer in the light path.



- c. Align the dots for maximum extinction (:) by turning on the metallic bar (c). (If you don't see max extinction you can adjust the fixed position of the entire slider by the hexagonal screw under the metallic bar.)
 - d. When you look through the eyepieces (DIA led open, condenser at open position, light path to middle/top) you should see a complete dark field of view.

Setup DIC

3. insert Nomarski prisms (2x) and adjust the angle of the condenser polarizer. The prisms are objective specific, make sure you have matching prims when you switch objectives.
 - a. Place the matching Nomarski prism underneath the objective. Note: we have 2 prisms for a 40x objective, prism "40x II" is for the 40x Oil objective NA 1.3, prism "40x I" is for the 40x Air objective NA 0.90.



- b. Select the matching prism in the condenser turret. On the objective it will be written "...DIC N1..." or "...DIC N2...". For example, see image of 100x objective: the 60x Apo Plan λ = N2. High NA objectives usually have N2 written

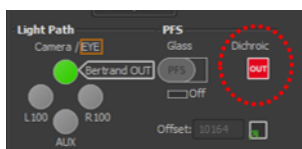


- c. Focus on your sample.
- d. While looking at your sample through the eyepiece (and moving through Z) determine the best angle of the polarizer for your sample (also called shear angle; move the metallic bar of step 2.c). This will be the angle in which you see the most 'depth' in your sample. Note: don't use a plastic lid on your sample because the birefringence of the plastic does not play well with polarized light! The okolab chamber lid is DIC compatible, and we have a plain glass lid for multi-well plates. The direction of the polarizer will determine the direction of the 'shadow'. Tip: you might see it better in live mode and zoom on the objects where you want to see 'depth'.
- e. Save all your settings in a dedicated DIC OC. OC summary: DIC prism is "In", light path to the right (camera), Analyzer "In", Condenser turret at N1 or N2. Important settings in your OC: (1) The shutter should be "Nikon Ti2, shutter (DIA LED)", (2) include the condenser as "active device".
- f. When all physical components are in the correct place for DIC, the DIC indication light on the microscope stand will be continuously on (instead of blinking).
- g. Optional: go back to step 1.j-k and see if the current setting of the condenser diaphragm is as desired.

Perfect focus system (PFS)

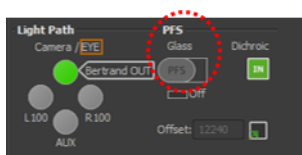
The perfect focus works by measuring the reflection from your coverslip, it works best with a glass cover slip and water based medium. Also, not all objectives are well-suited for PFS.

1. Place the PFS dichroic mirror in place by clicking it in NIS-elements, you find a “PFS” section in your “Ti2 pad” section. The red “out” button will become green. The PFS indication light on the front of the microscope stand will blink, if you’re close to your sample.



3 options to continue:

2. Click on the PFS button in the software.



3. Focus on your sample (usually the microscope moved a bit away from your original Z position).

Or

2. Switch the PFS on using the “PFS” button on the Z-wheel of the microscope stand or “PFS” button on the joystick device.
3. Focus on your sample (usually the microscope moved a bit away from your original Z position).

Or

2. Focus on your sample.
3. In the software click on “square-button-next-to-Offset” button. The microscope focuses on your sample using the PFS. Usually you will be at the same Z position as step 2.



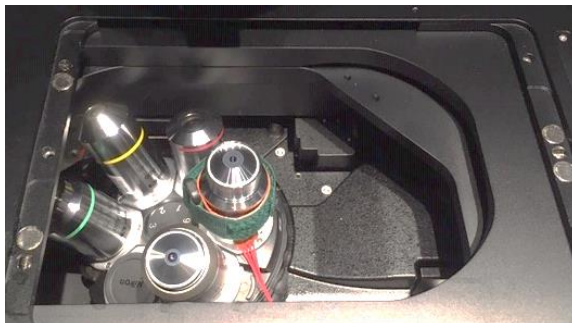
Continued

4. From now the microscope will stay at this distance relative to the coverslip until you actively change the focus. The indication light on the front of the microscope stand will be continuously on.
5. You can switch the PFS off by clicking the PFS button in the software (or on the microscope or joystick).



Okolab incubator (T: 25 – 50 °C, CO₂ and Humidifier)

1. Switch on the Okolab system by switching on the power strip behind the computer screen.
2. Put the objective heater on the objective you want to use. (Don't use the objective heater if you switch continuously between objectives).



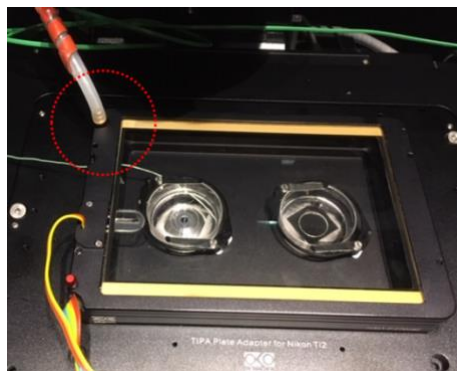
3. Switch **one objective back** and **one objective forward** to make sure that the objective is perfectly aligned in case you moved it while putting on the objective heater.
4. Place the Okolab chamber in the stage and place the appropriate sample holder in the chamber.
5. Place a dummy sample in the sample holder, so the inside of the chamber can equilibrate. Use the magnetic clamps to hold the 35 mm dishes in place.
6. If you want to use the sample/reference sensor. Slide it through the inlet in the side of the chamber and hold it in the sample.



7. If you will use the CO₂ and humidifier, check if there is still enough distilled water in the bottle (on microscope table, on the right of the microscope). If it is below 1/8th add ddH₂O until 3/4th full.



8. Connect the CO₂/humidifier inlet from the humidifier bottle to the chamber and place the lid on the incubator.



9. Make sure all the wires and tubes are not obstructing the movement of the microscope stage.
10. Set the temperature (or the start temperature, if you will run a program). Tap on the temperature box, set the desired temperature, tap OK.



11. Set the CO₂ percentage. Tap on the CO₂ box, set the desired percentage, tap OK. (The CO₂ outlet from the wall should always be open, but if you need to find it, it is behind the computer screen of Sterling.)



12. Select if you want to use chamber or sample sensor to control the temperature. Using the chamber sensor to control will allow faster heating and cooldown, but the temperature of your sample might be for a few minutes a degree hotter than you desire. Using the sample sensor to control, will make sure your sample is not too warm, but equilibrating exactly to the desired temperature will take longer (~ 1h). Select the sample sensor to control when the temperature is super critical for your experiment.
 - a. Tap on the gears.
 - b. Tap on temperature
 - c. Tap on control mode
 - d. Select sample or chamber

If you selected chamber: use current calibration. If you need to calibrate, set the parameters at: accuracy 0.10 and stdev 0.20 (Calibration might take a while!! Or use factory settings).

If you use the sample sensor: place the sample sensor (thin green wire) in your sample or a reference well filled with water. During initial heating/equilibrating, place the sensor in the dummy sample and fill the dummy sample with water. (If you didn't already do this)

13. Check if the objective heater is enabled (or disable it, if you don't need it).
 - a. Tap on the gears.
 - b. Tap on temperature
 - c. Tap on objective heater
 - d. Choose enable. If you see "enabled" is selected, you can also go back to the home screen.
 - e. Use custom calibration (this should be at a temperature close to 19.3 °C).

Start logging

1. Go to the home screen, pay attention to the floppy disk and USB-stick icon in the bottom.



Note: if you selected chamber mode to control, you'll see the temperature of the chamber, not the temperature of your actual sample. If you selected: sample sensor to control you will see the temperature of your actual sample (or reference well, wherever you put the green sensor in).

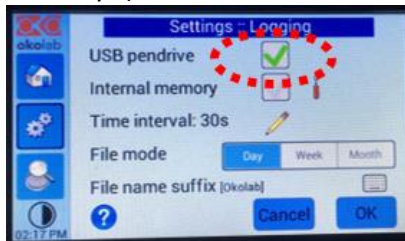
2. Connect the adapter (USB to micro-USB) to a USB stick
3. Plug the micro USB in the left side of the Okolab touch device. (The order of connecting is important!!).



4. Now the USB icon is shown in color.



5. Tap on the floppy disk to open the window to start data logging. Choose to save on USB pendrive. (Don't save on the internal memory!!)



6. Now you will see that the USB icon has changed to an USB + round red circle. Now it is saving.



7. When you're done data logging, tap on the floppy disk uncheck "saving to USB pendrive" click OK. The USB icon will change again to a colored USB icon (without the red circle).

Logging with date prefix will make 1 text file per day, all data points will be appended in one file. Also if you started data logging option multiple times that day.

Setting a temperature program

1. Tap on the gears
2. Tap on "Thermal cycles"
3. Choose "New"
4. Enter the two temperatures you want to cycle between (you can only cycle between two temperatures). Tap "next".
5. Enter the dwell time on each temperature. Note: the dwell time includes the heating or cooling time between the two temperatures. If you choose the dwell time too short, the incubator will never reach the other temperature.
6. Enter the number of cycles. This is counter intuitive!!! 1 cycle: only temperature 1. 2 cycles: temperature 1 and then temperature 2 (no repeat). 4 cycles: T1, T2, T1, T2.
7. You can save the program (configuration 1-4, doesn't matter which one, just overwrite each time, there is no way to tell which configurations belong to whom).
8. Tap start or adjust the start time and then press start.

Important notes!

If you use 35 mm dishes, use the magnetic holders to keep your dish in place.

When you open the chamber but leave your sample in (maybe you need to add something), tell the system that you open the chamber. Otherwise the system will try to compensate for the heat loss by cooking your sample.

1. Tap the gears
2. Tap temperature
3. Tap open incubator

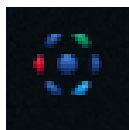
When you open the chamber and exchange your sample, don't leave the chamber open, not on the bottom (towards the objectives) and not the top (without a lid). Especially towards the bottom, you will lose a lot of heat and (worse!) water will condense on the objective(s). When you exchange your sample, leave your old sample in the chamber until you have your new sample ready to swap it out. Or replace your old sample with a dummy sample (take all the time you need) and then replace the dummy with your new sample.

When you use the incubator in chamber mode, you can still read the temperature measured by the external sensor (green thin wire), but this temperature will not be automatically recorded. In other words, you need to note this temperature yourself.

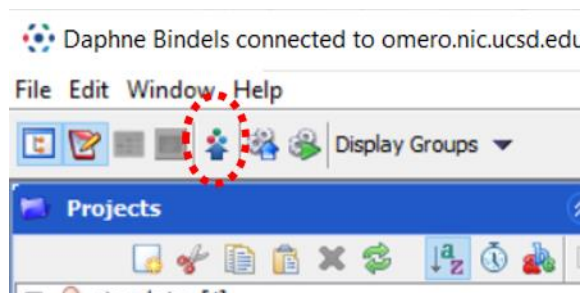
1. Tap on the gears
2. Temperature
3. Next page, the arrow right bottom
4. Tap on external sensor.

Upload to OMERO

1. Save and close all your images in NIS elements software.
2. Open OMERO (Should be pinned to your taskbar).



3. Log in with your OMERO credentials. Username: first part of your email address. Password: Default Password (You should change your initial password using the omero.nic.ucsd.edu interface).
4. Use the import function



See also our OMERO client (app) user guide for uploading and downloading and our OMERO web user guide for making figures.

Shutting down

1. Put the objective in "escape" mode, press "ESC" button on the front of the microscope body.
2. If you used the Okolab incubator.
 - a. Take out the chamber holder, place it next to the Okolab touch device.
 - b. Clean the sample sensor (green thin wire) with ethanol.
3. Clean the objective. If you used an oil immersion objective:
 - a. Wipe the oil with a dry lens paper
 - b. Spray lens cleaner (little Nikon spray bottles) on a new clean lens paper. Clean the objective with this.
 - c. Dry the objective with a clean dry lens paper.
4. Check if the sample holder is clean, clean it if it isn't clean.
5. Close NIS elements software
6. Close OMERO and other programs
7. Sign-out of windows.
8. Shut down the hardware in reverse order compared to starting up (See pictures at starting-up procedure).
 - a. Switch off Okolab on the power strip
 - b. Switch off the camera
 - c. Switch off microscope stand
 - d. Once the lights are out on the microscope stand, switch off Nikon control box.