Instruction for Nikon A1R-HD25 laser scanning confocal microscope

Content: **Check-in and Start up** • **Check out** • **Appendix**

Check-in and Start up

There are 1 remote switch and a power strip that supply power to all the components.

1. remove the cover
2. remote switch: power up to turn on the microscope, piezo stage, laser launch, scanner, and epifluorescence LED light source
3. laser launch: turn the key to ON, press the button(s) to activate the laser(s) you will need
4. turn on the power strip for the computer and monitor
5. log into Windows and then launch Nikon Elements
6. click OK to select A1 for Acquisition

Check out

1. lower the nosepiece (objective turret) e.g., set the focus (Ti2 ZDrive, Z1) to 500 μm
2. remove your prep
3. if applicable, remove oil/immersion fluid on immersion objectives and clean (see **Appendix**)
4. switch to the 10× objective
5. save your experiments and quit Nikon Elements
6. copy your data to external storage media or network file servers, as needed
7. submit your usage using the google form
8. shutdown Windows
9. after the computer has shutdown, switch off the power stripe
10. laser launch: push the button(s) to disable the laser(s), turn the key to OFF
11. power down the remote switch
12. put the cover back on the microscope
13. clean up the work area

A simple imaging protocol

- Ti2 Pad
  - Zoom: 1×
  - Filters: Turret-Lo should be in an empty position (#1 or #6)
  - Condenser: 1 OPEN, if you plan to use the TD
- A1 LFOV Scan Area
  - 512
  - Zoom Size: 1
- A1 plus Pad
  - Averaging — 1 (galvo), 4 or 8 (resonant)
  - Dwell time — use default
  - Channel mode — select either simultaneous or sequential, also see **Ch Setup**
  - Scanning — select either Resonant or Galvano (galvo)
  - click to activate as many channels as needed, each channel has 3 sliders
    1. laser power — 2 to 5
    2. detector Offset, see **offset** setup
    3. detector Gain, see **gain** setup
  - Pinhole — set the desirable size in AU, e.g., 1 or 1.2; for multi-channels, select the channel you want to optimize and set the desirable size in AU, all the other channels will then share that same physical pinhole size

Note:

- It is a good practice to verify all the parameters before acquire.
- All channels will share a common value for each acquisition parameter e.g., averaging, dwell time, physical pinhole size, etc. If you need to have specific values for different channels, you can setup an optical configuration (OC) for each channel to use the specific values and then apply these OC in the λ tab of ND Acquisition.

Ch. Setup

This minimize unintentional exposure during adjustment of acquisition parameters by activating only one channel at a time in a multi-channels experiment. It is only available in sequential mode.

1. Channel mode: select one of the sequential modes to enable Ch. Setup

   Note: If you use simultaneous mode, you can switch to sequential mode temporaily to take advantage of Ch. Setup.

2. click on Ch. Setup
3. click on one of the channel buttons to adjust that particular channel alone

Detector setup: gain and offset

- gain: adjusts the sensitivity of the detector, higher gain will result in a narrower dynamic range
  - detectors 2 and 3, for 488 and 561, are GaAsP PMT
    - good starting point: 40

      Caution: GaAsP PMT can be easily damaged by over exposure at high voltage or gain. ***DO NOT set the Gain above 60***.
  - detectors 1 and 4, for 405 and 640, are multialkaline PMT
    - good starting point: 100
- offset: determines where zero intensity is, a common setting is about 4 (galvo) or 10 (resonant with 8 line average)
  1. select the channel(s) you need, enable **Ch. Setup** in case of multi-channels
  2. go live
  3. set gain
  4. laser power: click on the box to the left of the slider to blank the laser
  5. enable and configure the Pixel Saturation Indication e.g., set green for undersaturation
  6. LUT: expand the X-axis to show a full scale of 100 or so intensity unit
  7. LUT: expand the Y-axis, as needed
  8. adjust offset until the intensity value of the pixels with minimum intensity is above 0 e.g., about 10 intensity unit, the image should have no understaturated pixels
  9. laser power: please remember to click the box again to enable the laser after the setup

Optimize

1. select the channel(s) you need, enable **Ch. Setup** in case of multi-channels
2. go live
3. if image is all dark, do the following until you can see some signal to perform the next step
   i. check with Eye-Dia or Epi to make sure that the object of interest is centered and in focus
   ii. check the laser launch to see if the key is ON and the lasers are activated
   iii. check all the parameters to make sure they are in a reasonable range
   iv. in the LUT panel, reduce the display range by dragging the high limit (4095) to the left
   v. fully open the pinhole
   vi. raise the gain: ***NO more than 60*** for the GaAsP PMT in detector 2 or 3, up to 255 for the multialkaline PMT in detector 1 or 4
   vii. slowly raise the laser power
4. focus and locate the object of interest
5. set the pinhole for acquire e.g., 1.0 or 1.2 AU
6. lower the gain to reduce noise and improve dynamic range
7. reset the LUT to full range whenever possible

Note: The Pixel Saturation Indication enables easy detection of oversaturated and undersaturated pixels. It can help in detector **gain** and **offset** setup.

Z-stack

There are 2 ways to setup the Z-stack in ND Acquisition.

- define the Top and Bottom
- define the range: symmetric or asymmetric around the center
    - center can be define by the Home position
    - select Relative to use the current Z position

Note:

- Step-by-step Nikon A1 Piezo Z: do not choose this option in ND Acquistion Z Device because it doesn't work well.
- It is a good practice to verify that the same Z device is selected for the focus controls,
  for example:
    - Devices > Mouse Joystick and Auto Focus Z: Ti2 ZDrive
    - ND Acquistion Z Device: Ti2 ZDrive
- If you will use the Piezo Z, check the current Z position and make sure the Z travel range will not be exceeded; you can center the Piezo Z as described next.
- In ND Acquisition and XYZ Navigation, the Piezo button provides 2 options.
    - Keeps Z position and centers Piezo Z: centers the Piezo Z (Z2 set to 0), but maintains the overall Z position (Z1 + Z2); this is a good option, especially if you use the Piezo Z for your Z-stack
    - Move Piezo Z to Home position

Appendix

Content: **Köhler illumination** • **DIC** • **immersion objective**

Köhler illumination

1. select EYE-DIA in the software for diascopic mode with the optical path set to 100% Eye
2. move the polarizer out of the light path
3. set condenser turret to postion 1 (Open)
4. open fully both field and aperture diaphragms
5. set the microscope focus (Ti2 ZDrive, Z1) to 500 μm
6. select the 10x objective
7. load a sample and bring an object of interest to focus
8. switch to the desirable objective and focus
9. close the field diaphragm, note the out of focus image of the edge of the diaphragm
10. use the condenser focus knobs, adjust the condenser height to bring the edge of the diaphragm into focus

    Note: You can adjust the tension of the condenser focus knob—hold the knob on the left side and turn the one on the right counterclockwise to loosen or clockwise to tighten.
11. use the 2 mm Allen wrench(es) to center the image of the diaphragm, open the field diaphragm to just smaller than the field of view might help
12. open the field diaphragm to just beyond the field of view
13. close down the aperture diaphragm until the intensity of the illumination just starts to dim—this is about 70% of the objective pupil plane
14. when switching to a different objective, you might need to repeat this procedure to optimize the illumination

DIC

essential components

- polarizer (above condenser): manual
- condenser prism (in condenser): motorized
- objective prism (in nosepiece): software detectable (Intelligent), please ask facility staff to install the prism for immersion objective lens as needed
  - 40× WI: 40× I
  - 60× O: 60× II
- analyzer (in Turret-Lo): motorized

setup

1. do Köhler illumination
2. slide in the polarizer and rotate to the home position
3. select the appropriate condenser prism for the objective used: N1D for 10×, N2D for the rest
4. fully open the aperture diaphragm
5. switch in the analyzer
6. rotate the polarizer to obtain the optimal effect

immersion objective

setup

1. locate the object of interest with the 10× or 20× and center it in the field of view
2. record the XYZ position
3. set the microscope focus (Ti2 ZDrive, Z1) to 500 µm
4. switch in the immersion objective
5. use the stage XY control to move the sample out of the way to expose the objective
6. apply an appropriate amount of immersion medium
7. recall the recorded XYZ position
8. check the focus of the object of interest

   Caution: When focusing deeper into the sample, move slowly and pay attention to any changes, if the image doesn't change or move sideway, stop immediately and evaluate. The objective is most likely pushing against the coverglass. You should reduce the Z right away. Your sample is not compatible with the immersion lens. *Do not move the stage in the XY direction or you will scratch the objective lens!*

clean up

1. set the microscope focus (Ti2 ZDrive, Z1) to 500 µm
2. remove the sample
3. blot to remove the immersion medium by gently placing the designated lens tissue on the objective lens
4. repeat a few times with a clean area of the lens tissue until most immersion medium are gone
5. apply the appropriate cleaning agent to the special cotton swab (Puritan 869-WC, cotton wool without glue or binder), the cotton wool should be damp but not dripping

immersion medium	**cleaning agent**
water	95% ethanol
GenTeal	water followed by 95% ethanol
Immersol W	95% ethanol
Nikon F oil	no need to clean

6. gently roll the cotton swab on the front lens from one side to the other, you might need to do it a few times to cover the whole surface of the lens
7. repeat the above step with a fresh cotton swab and cleaning agent, as needed