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

Check out

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

A simple imaging protocol

- 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
  - Scanning — select either Resonant or Galvano (galvo)
  - activate as many channels as needed, each channel has 3 sliders
    1. laser power — 2 to 5
    2. detector Offset, see **Detector setup** and **Offset setup**
    3. detector Gain, see **Detector setup**
  - Pinhole — select the channel you want to calculate the desirable size (AU)

Note:

- it is a good practice to verify all the parameters before acquire
- all channels will share the same parameters e.g., averging, physical pinhole size, etc.; otherwise, you can setup an optical configuration for each channel to use in the λ mode of ND Acquisition

Detector setup

also see **Offset setup**

- detectors 2 and 3 for 488 and 561 are GaAsP PMT
  - good starting point: Offset 4 (galvo) or 10 (resonant with 8 line average), Gain 40

    Caution: GaAsP PMT can be easily damaged by high voltage (Gain). **DO NOT set the Gain above 60**.
- detectors 1 and 4 for 405 and 640 are multialkaline PMT
  - good starting point: Offset 4 (galvo) or 10 (resonant with 8 line average), Gain 100

Ch. Setup

This allows adjustment of a single channel in a multi-channel experiment. It is only available in sequential mode.

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

   Note: If you use simultaneous mode, you can switch to sequential mode temporaily to use Ch. Setup.
2. click on Ch. Setup
3. click on one of the channel buttons to adjust that particular channel alone

Offset setup

This is for adjusting detector offset to keep the intensity value of minimum intensity pixels above 0. A common setting is about 4 (galvo) or 10 (resonant with 8 line average).

1. enable **Ch. Setup** and select the channel you need
2. laser power: click on the box to the left of the slider to disable/blank the laser
3. enable Pixel Saturation Indication e.g., set green for undersaturation
4. set gain
5. go live
6. LUT: expand the low end to show a full scale of 100 or so intensity unit
7. adjust offset until the intensity value of the minimum intensity pixels is above 0 e.g., about 10 intensity unit, the image should have no green pixels

Optimize

1. enable **Ch. Setup** and select the channel you need
2. go live
3. if image is all dark, do the following until you can see some signal
   i. check with Eye-Dia or Epi to make sure that the object of interest is centered and in focus
   ii. check all the parameters to make sure they are in a reasonable range
   iii. in the LUT panel, reduce the display range by dragging the high limit (4095) to the left
   iv. fully open the pinhole
   v. 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 and 4
   vi. slowly raise the laser power
4. focus and locate the object of interest
5. set the pinhole for acquire e.g., to 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.

- 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:

- it is a good practice to verify that the same Z device is selected in both locations.
  for example:
  - Devices > Mouse Joystic and Auto Focus Z: Nikon A1 Piezo Z
  - ND Acquistion Z Device: Nikon A1 Piezo Z Drive
- In ND Acquisition, the Piezo Z 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)
  - Move Piezo Z to Home position

Appendix

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

Köhler illumination

1. set microscope to diascopic mode and the optical path to 100% Eye e.g., select EYE-DIA in the software
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 (Z1) to 500 µm
6. select the 10x objective
7. load a sample and bring an object of interest to focus
8. close the field diaphragm, note the out of focus image of the edge of the diaphragm
9. 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.
10. 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
11. open the field diaphragm to just beyond the field of view
12. close down the aperture diaphragm until the intensity of the illumination just starts to dim—this is about 70% of the objective pupil plane
13. 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 epi filter turret): 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 (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

clean up

1. set the microscope focus (Z1) to 500 µm
2. remove the sample
3. blot to remove the immersion medium by gently pressing the designated lens tissue against the objective lens
4. apply the appropriate cleaning agent to the special cotton swab (Puritan 869-WC, no glue or binder), the cotton should be damp but not dripping
    - water: 95% ethanol
    - GenTeal: water followed by 95% ethanol
    - Immersol W, Nikon F, Cargille LDF: chloroform
5. gently roll the cotton swab on the front lens from one side to the other, you might need to do it twice to cover the whole surface of the lens
6. repeat the above step with a fresh cotton swab with cleaning agent