

## **Nikon W1 spinning disk instructions** last updated 20200615

All use must be reserved in advance online.

<https://nyulangonesignups.resourcescheduler.net/resourcescheduler/scheduleWeek.asp?Topic=RES&TopicID=84&View=week&>

All users must be trained by Nikon or Michael C before unassisted use allowed. We recommend official training first followed by or including expert user in lab if working on an ongoing project to assure continuity of protocols.

### **Equipment startup**

To turn on:

Computer on. (This may be done before or after steps below.)

If the computer is already on and logged in, log out.

Power strip the left of the microscope labeled "STEP 1".

Immediately follow with power switch on microscope base (right rear).

Log in to to Nikon account. There is no password.

Run software Nikon Elements.

In the drop down menu, choose your profile. Do not use someone else's profile.

Check whether filter block is in camera splitter. Put it in or remove it based on your need. When in single camera mode, the filter block must be stored in dedicated small white box.

In the ND Acquisition window, delete all old XYZ positions using the little red **x** on the far right of the window.

If you are using two cameras, we recommend checking camera alignment with quad beads for pixel registration. See <http://microscopynotes.com/spindisk/beadalignment/>

### **Shutdown**

Copy files to server (see section on Saving Data below).

Clean lens.

Switch to 10x lens.

Quit software.

Check that lens is at lowest focus position. If it isn't, manually turn focus knob to get it there.

Power switch at base of microscope. Wait for lights to finish blinking and touchscreen to turn off (approximately 5 seconds).

Power Strip #1.

Log out of computer account.

Clean up.

All loose parts in correct boxes, drawers, etc.

### **Dynamic range**

For practical use, we recommend setting the exposure to peak around 6,000. (There are methods to lock the LUT for easiest visualization [such as this one](#).)

You may want to go up to 16,000 (14 bits) for less noise or down lower for more light sensitive samples.

The cameras are 16 bits with the ability to image with values peaking in the 50,000 range depending on gain settings, but full range unnecessary except in rare situations where imaging order of magnitude intensity differences in a single field.

DO NOT SATURATE THE CAMERA as this could damage the electronics.

DO NOT have any pixel intensities above 50,000.

*The HiLo LUT does not serve as a warning for this as saturation may occur in the 55000 range and the LUT doesn't work until 65535.*

### **Camera Gain**

The sweet spot for camera sensitivity vs. noise appears to be in the EM gain range of 200 to 250.

However, for less noise, drop the gain and for more sensitivity, increase it. *Do not use gain above 200 if using brightfield or Nomarski and make sure no pixels are saturated; too much light can burn out the cameras.*

Use readout gain of 10 Hz for lower noise. 30 Hz is 3X faster, which may be necessary for live work, but is noisier.

Each camera's gain must be constant for all exposures in a sequence. For instance, in single camera mode, every lambda channel must have the same gain and readout speed unless a dummy image is collected in addition with the new gain setting.

### **Saving Data**

There are three places you may store data:

D: extra high speed, total space 1 TB

E: total space 5.45 TB

F: total space 5.45 TB

Please sort your data into a folder with an obvious name, your name or the lab name.

DO NOT store any files on the desktop, drive C:, Pictures, Documents, etc.

Files should be stored in Nikon's ND2 proprietary format to preserve information such as lens, spatial scale in XYZ, timing, and other technical details. Software such as ImageJ, Fiji, Imaris can read this format. Ask Michael for macro support if you want to automate ImageJ conversions to other formats, automate removing channels, processing all images automatically, etc.

Immediately move your data to a server, \\research-cifs.nyumc.org\research recommended. Shortcut on desktop and other key places. More information at <http://microscopynotes.com/how-to-connect-to-server.html>

To disconnect from server: Windows icon in lower left > person icon at top of list > sign out.

No USB disks or flash drives.

Data on Desktop or drive C: may be deleted without warning.

Data older than 1 week anywhere may be deleted without warning.

### **Accounts**

Each user may have account(s) within Nikon Elements to run the software. This means that most of each account's settings will be protected.

Recommend manually saving your favorite settings. Type Ctrl-n to open OC window for Exporting settings.

An easy way to set up a new account is to log into an account with settings you like and duplicate. Some settings, such as active shutters and using the mouse to control the focus in live mode, will have to be manually set in the OC window or Devices menu when the new account is first used. For instance, in the Devices menu the piezo should be set to the home position and mouse joystick control set to Ti focus motor. The AOTF as shutter may need to be set in the OCs (use Ctrl-n).

### **Lenses**

10X air (not recommended for spinning disk)

20X Water

40X Water

40X Oil

Use Cargille Labs Type LDF at room temperature.

User Cargille Labs Type 37 when imaging above 30 degrees C.

60X Oil

Use Cargille Labs Type LDF at room temperature.

User Cargille Labs Type 37 when imaging above 30 degrees C.

100X Silicon Oil

Oil for the 100X lens is different than the oil for the 20, 40, and 60X.

### Z series

**There are three ways to set up the range for Z series:**

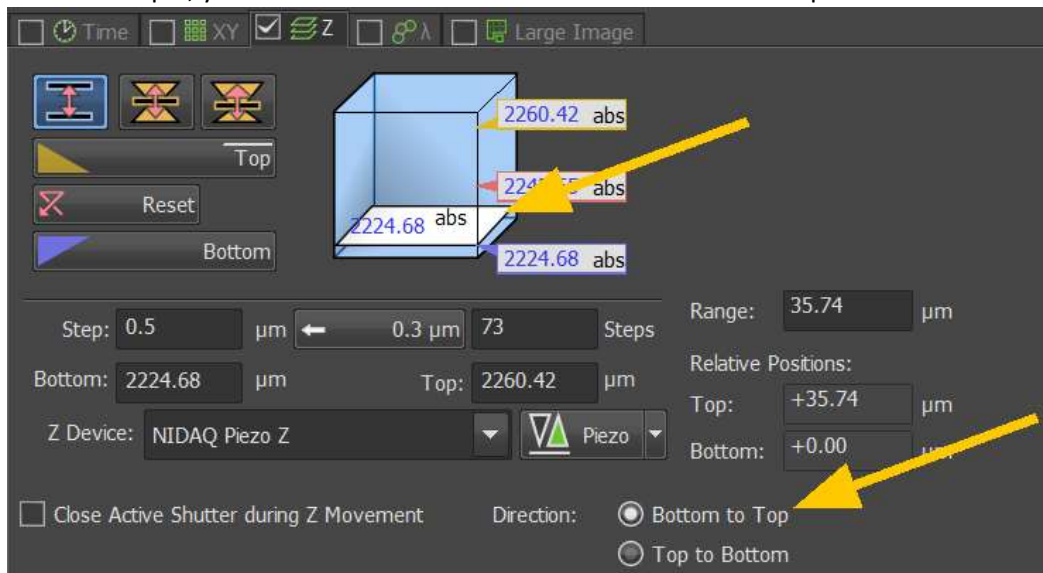
1. Set top boundary and bottom boundary.
2. Set middle position and range above and below this.
3. Set the bottom (zero) and a range above. Best for use with perfect focus when doing time series. Recommended for multi position time lapse. PFS has to be on while setting up positions.

When you set a top and bottom, before clicking Run make sure that the direction of travel is set to where the microscope focus is physically set each time.

Top to Bottom should be set when you are at the top.

Bottom to Top should be set when you are at the top.

In this example, you need to have the Direction set to Bottom to Top.



If the white plain were at the top of the cube, it would need to be set for Top to Bottom.

### **There are three ways to move the focal plane during the Z series:**

1. Ti1 Drive is the motor inside the microscope. This moves the lens up and down.
2. Nidaq Piezo is faster. It moves the stage up and down.
3. Triggered Piezo is the fastest. It moves the stage up and down faster than the screen can refresh the images, so you may not see images updating during image collection.

If using the piezo, make sure it begins from the home position. It should read 110.00 um at the bottom right of the screen.

Also make sure that the Ti2 drive is the one being selected if using the mouse wheel to scroll. You can check this under Devices > Mouse joystick > Ti2 drive.

### **FRAP/Photoactivation**

See <http://microscopynotes.com/spindisk/miniscanner/index.html>

### **Troubleshooting a few things**

To update OC (optical configuration) settings:

- \* In configurations import the most recent settings file, C:\Important Element Files - DO NOT DELETE\
- \* Manually check that the shutters are correctly set for each method (may hold down shift key to select multiple methods), then set shutter in pull down menu in center of window. DIA for brightfield, Aura for widefield, and AOTF for confocal.
- \* Select an OC and in camera window, Advanced Camera Settings, set temperature, shift speed, voltage as per <http://microscopynotes.com/spindisk/cameranotes/>
- \* If stage moving in wrong direction(s), recalibrate objective lens. This requires a high contrast sample be image and choose to use a single color for calibration.