Nikon W1 spinning disk last updated 20190815

Sign up online. <http://signups.med.nyu.edu/facilities/equipment/signups/skirball-spinning-disk>

All users must be trained by Nikon (Phuson or Kevin) 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.

**Shutdown**

Dropping the lens manually before shutdown is critical to prevent lenses from crashing into the bottom of the stage.

The lens should be focused to lowest position manually with the focus knob, not with the ESC button, when:

\* end of every session
\* changing stages

Do Not use ESC button for the end of session lens drop. Focus manually with the focus knob.

**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 lab name.

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.

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 lab or user may have account(s) to run the software. This means that most of each account’s settings will be protected.

Within each account, each user may save the screen layout the way they like it in a tab at the lower left.

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, will have to be manually set in the OC window when the new account is first used. More granular settings may be made, for instance as explained here <http://microscopynotes.com/spindisk/setupnewuseracct.html>

**Equipment startup**

To turn on:

Power strip "STEP 1".

Computer on.

Nikon Elements.

On software startup, choose Twin Cam or Single Cam. (*troubleshooting below*)

 (Single Cam requires removing filter block. Filter block must be stored in dedicated box.)

ND Acquistion window, immediately unclick Move To Position and also delete all old XYZ positions using the little red x on the far right of the window.

Recommend checking camera alignment with quad beads for pixel registration.

**Dynamic range**

For practical use, recommend setting the LUTs to peak around 6,000.

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 to 65,000 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. *The HiLo LUT may not serve as a warning for this as saturation may occur in the 55000 range and the LUT doesn’t work until 65535.*

**Lenses**

10X air (not recommended for spinning disk)

20X Water

40X Water

40X Oil

60X Oil

100X Silicon Oil

*Note that the oil for the 100X lens is different than the oil for the 20, 40, and 60X.*

**Z series**

Three things to consider when setting up Z series:

1. Method of setting boundaries and range.

2. Mechanical focus in microscope, piezo by serial port (faster), piezo by triggering (fastest).

3. Perfect focus on or off (only useful when doing time series). This is recommended for multi position time lapse. PFS has to be on while setting up positions. Best Z mode is asymmetric with 0 steps below and only go up.

If using the piezo, make sure you Home the Piezo before starting an acquisition (this function is under ND acquisition). It should read 100.00 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.

**Updating settings**

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.

**Dual Cam vs Single Cam**

Beam splitter is 565SP.

(As of 20190815) The cameras are as follows:
12209 straight shot (blue-greens)
12261 reflected (reds)

With the beam splitter pulled out and stored in its box, camera 12209 may be chosen in the Acquire menu > Select Andor camera.

As of August 2019, triggered piezo does not work in single cam mode.