NIKON SIM

Start up:

- Turn on LED (button on green box to the right of the lasers)
- Turn on 4 laser keys.
- Connect to server (Note: DO NOT PLUG IN HARD DRIVES/FLASH DRIVES OR DOWNLOAD ANYTHING TO THE COMPUTER)
- Log into software

Shut down:

- Log off software.
- Click on disconnect CAM button to disconnect from server.
- Clean off objective using lens paper and isopropyl alcohol (IPA)
- Manual lower the objective by using the focus know and center it.
- Turn off LED (button on green box to the right of the lasers)
- Turn off 4 laser keys.

Acquisition Guidelines:

- In general we use the following acquisition conditions: 10Mhz, 14 bit; EM Gain <300; Exposure time and laser power balanced, as low as possible; LUT values <~5,000; conversion gain 1x, no binning

SIM Reconstruction Guidelines:

There are three different parameters for reconstruction with 3D SIM, ignore the third for 2D SIM, which generally have to be determined empirically: Illumination Modulation Contrast, High Resolution Noise Suppression (XY), Out of Focus Blur Suppression (Z)

- Illumination Modulation Contrast should usually be between 0.5-1
    o I have never had good results which I click "Auto" for this
- High Resolution Noise Suppression should generally be <1 – the higher it is the fewer artefacts you will have but also the less resolution
- Out of Focus Blur Suppression is usually 0.1-0.2 – the higher it is the less blur, and resolution, you will have
    o This is only used in 3D SIM, not 2D or TIRF SIM
- Tick the box to use separate settings for each channel
- Do not tick the box "Deconvolve when Illumination Contrast is Low"
- After reconstruction, look at FT, which should be a high contrast six lobed shape without lots of dots/points evident, and then adjust LUTs on reconstructed image, find a small spot and use the FWHM function in the Intensity Profile tool to estimate resolution