

NIS Elements – SIM manual

Important You must be trained by ESRIC staff before using this instrument.

Turn on the system according to the written instructions on the wall

3 hours before start (NB or leave system on overnight)

- Laserbed (switches on LHS wall)
- Lasers (turn keys on desired lasers)

Immediately prior to system use (do not leave overnight)

- PC

DO NOT SWITCH OFF: The Nikon Microscope controller, the Piezo controller

Initialise the Nis Elements

The only option to select is: Andor with N-SIM

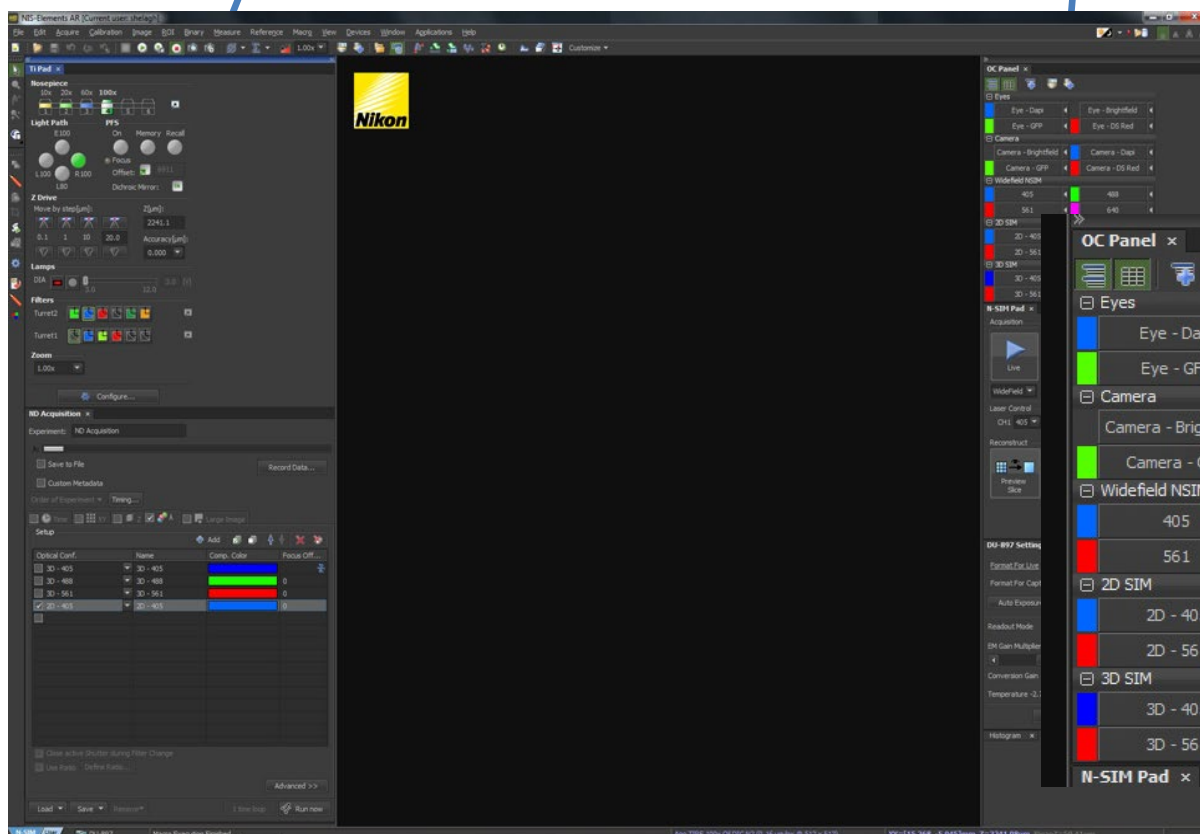


For user accounts select the generic 'User' account from the dropdown

Nikon user interface:

Drives the microscope stand

Controls the light path



Set up SIM Imaging (3D SIM)

Find the

Controls 3D and multicolour image acquisition

Controls SIM data reconstruction

ight).

Histogram and camera settings

sample by

1) N.B. The only objective which can be used for SIM is the 100x 1.49NA objective (*). A trick to focus is to turn on the perfect focus and move the stage up until it bleeps and stops flashing to indicate it is almost in focus. Turn off the perfect focus

(* we have a 60x water immersion objective for live imaging but no environmental control at present, the grating would also need to be changed for this)

2) Visualise the sample on the camera, select 2D or 3D acquisition mode for this depending on the SIM type used

3) Press live to visualise the sample,

Ensure the default settings under the live tab are correct

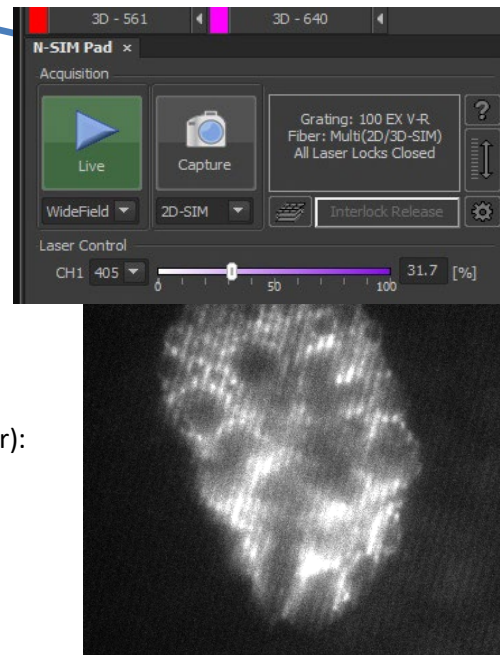
(The sample will need to be refocused on the camera. NB: camera is a 512 x 512 so only a small field of view is visible)

4) The live image needs to be focussed so that the grating is visible and can be seen moving.

The image (right) is a good example of this.

5) To ensure good contrast optimise the following (in this order):

- Camera sensitivity
- Exposure time
- Laser Power



The
clearly

The Andor xION897 camera is only linear to gain 300 this is the maximum it should be set to.

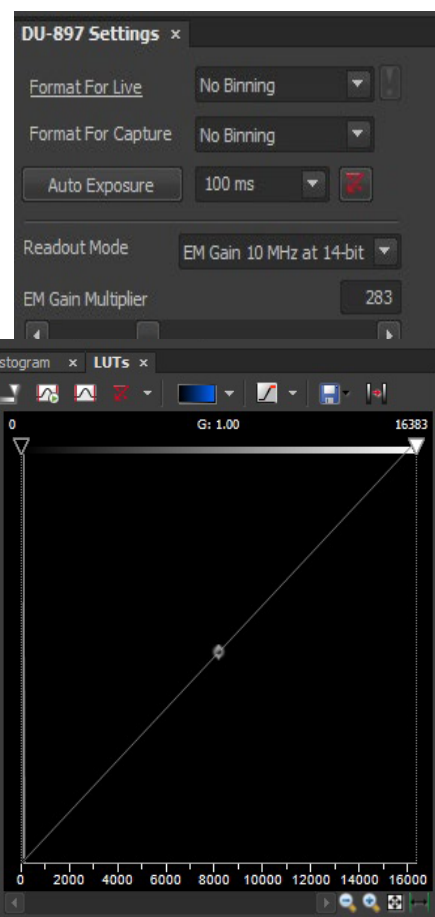
Press record in each channel (right click) to record the acquisition parameters (gain, exposure, laser power) for each channel to be image acquisition. 3D is shown here but 2D is the

NB: N-SIM will work when the collects data in the 12 bit range. Use the histogram to ensure no overexposure

Press Histogram

By adding autoscaling the image display will appear brighter (although the actual data will not change), this can help

The dashed blue line indicates where the histogram should to



used for
same.

only
camera
there is

focussing
extend

Setting up the volume sampled for 3D SIM

For 3D SIM at least a micron volume needs to be collected for reconstruction (see Gustafson 2008)

To set up the z stack

Ensure:

- The step size is set to $0.120\mu\text{m}$
- The Z device is the MCL NanoDrive
- Piezo setting is as shown
- Direction Bottom to top is selected

On the Z-menu in the MDA tab (bottom right hand corner) ensuring it is reset

- Go to the bottom of sample and press bottom
- Go to top of sample and press top

The grating should just about still be in focus at the top
the bottom for the best reconstruction.

Setup colour acquisition (2+3D SIM)

In the lambda (colour) menu

Select the wavelengths to be used for Sim (either check the boxes or press the dropdown menu)

Acquire the SIM Image (2+3D SIM)

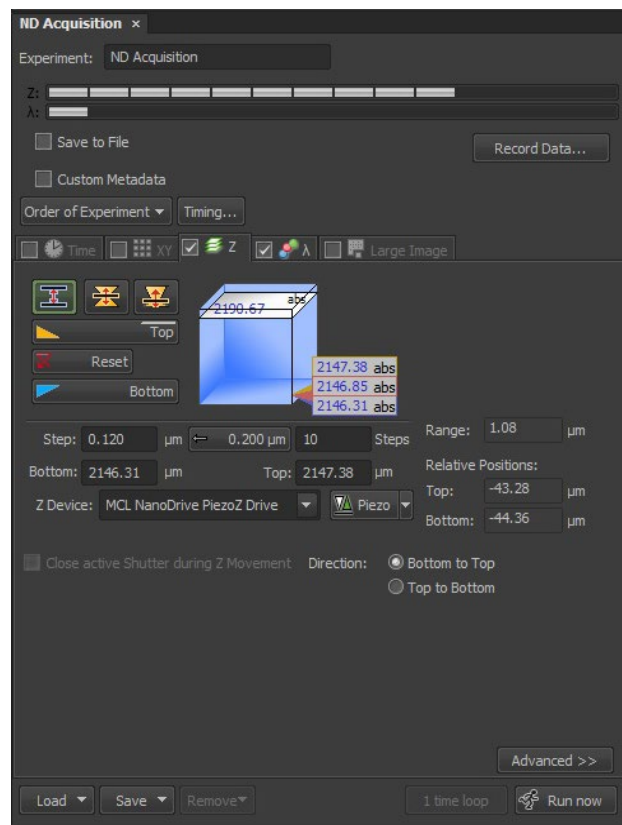
Press Run in the MDA

Watch the data acquisition and make sure that your sample is coming into focus.

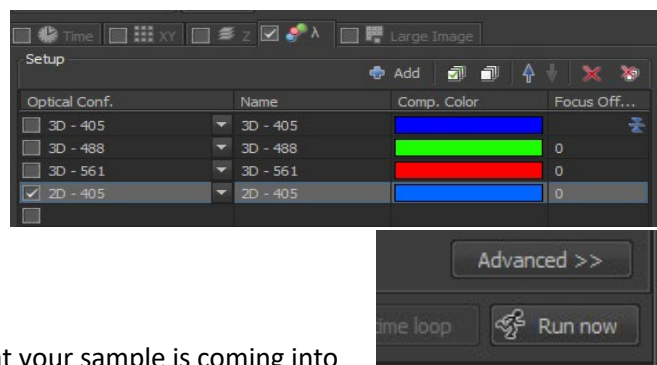
Sim data acquisition and reconstruction.

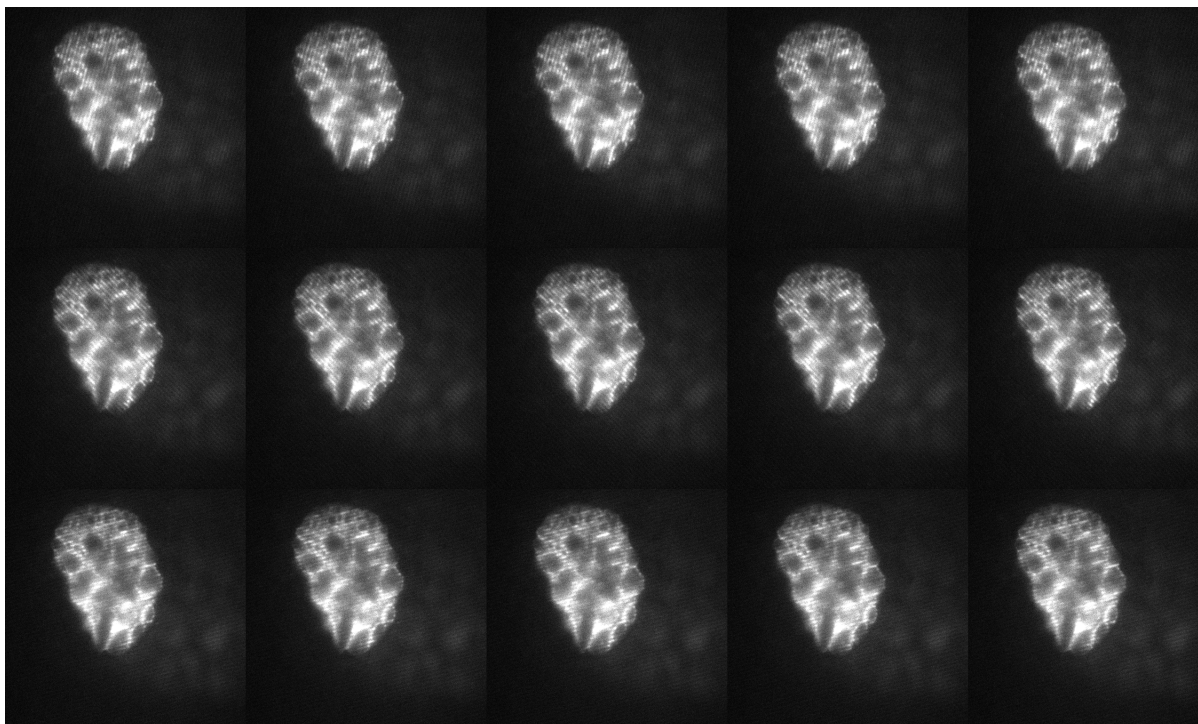
To be able to adequately reconstruct SIM data it is essential that the grating and intensity is evenly matched in all phases and rotations of the SIM system.

Good SIM Illumination:

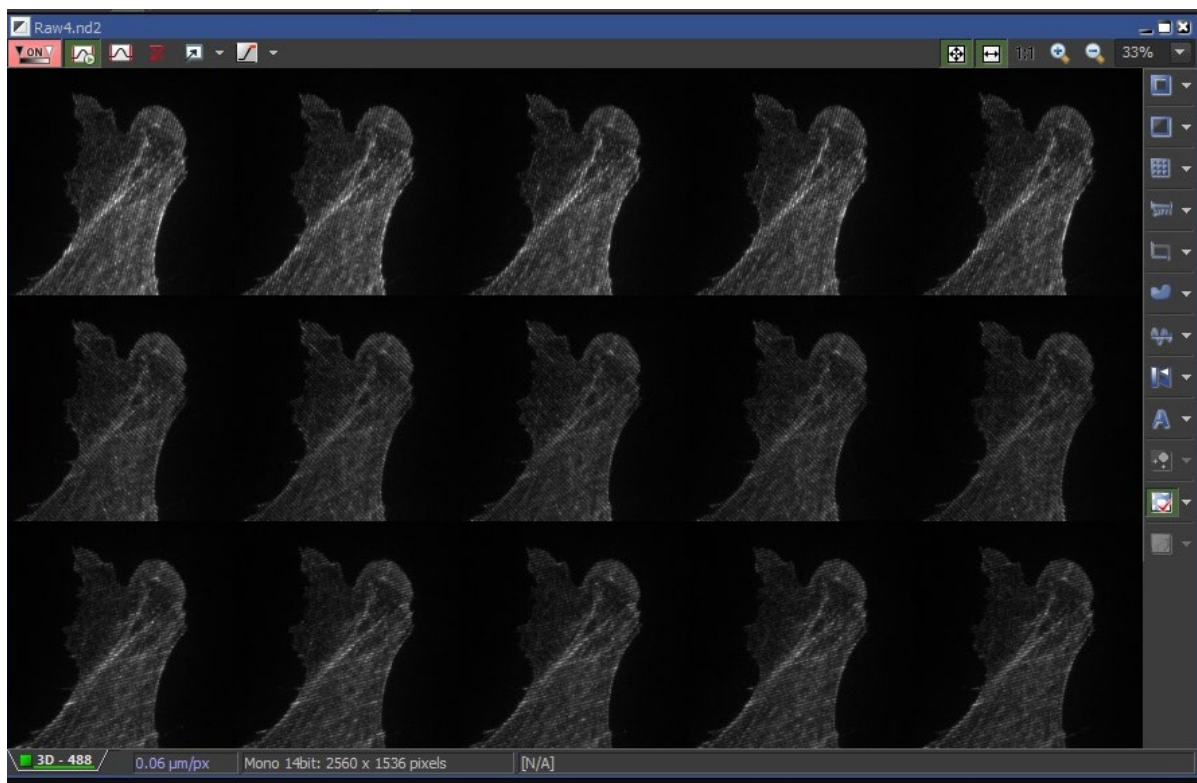


and





Bad SIM illumination:



The only way to be 100% sure you have resolution enhancement is to look at the Fourier transforms and look at the data quality using **SIM Check**. www.micron.ox.ac.uk/software/SIMCheck.shtml

This plugin is installed in Fiji and is on the SIM computer. SIM check can tell you If you have enough brightness to reconstruct well and how much drift you have as well as looking at the Fourier transforms for you.

To check out different parameters use the **preview reconstruction** button in N-SIM software.

To test which parameters are best for reconstruction take an and reconstruct the images with different settings and then do

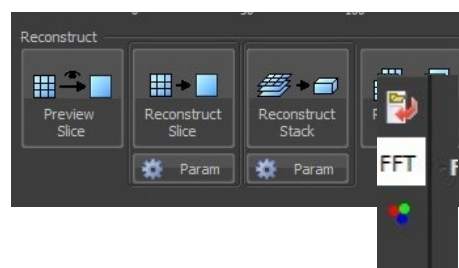


image
Fourier

transforms of your data. You will see with higher values that the diameter of your FT will be smaller indicating less high frequency/resolution data within the image. A FT with 6 petals and fuzzy edges is ideal.

In the data reconstruction box:

Illumination modulation contrast = This adjusts the strength of the different Moire fringe components in reconstruction. For good data (ie. where you have minimal spherical aberration, good signal to noise and good contrast in your stripey patterns – minimal out of focus light) the optimum range should be 0.5-1.0. This give better high resolution information. Auto should only be used for 2D SIM. Not recommended at all for SIM.

High resolution noise suppression = This is the Wiener filter. A low number means minimal suppression of high frequencies. A high number means strong suppression of high frequencies ie. you are actually reducing the resolution of image. For a good sample (see above – high SNR etc) you should somewhere between 0.1 and 1.0. Because SIM boosts high frequency information this can include noise so it is always advisable to have a little bit of suppression. If your signal to noise is poor you can use this feature to suppress artefacts (the fold patterning artefact in particular) with this setting. But you of course pay a resolution price for doing it.

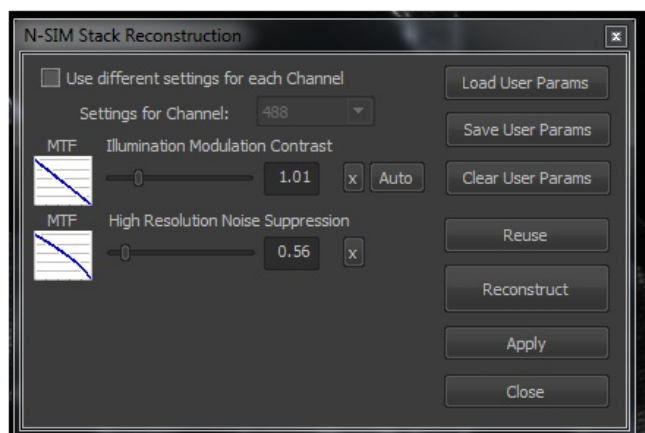
2/3D SIM on single slice, Out of focus blur suppression* = This

parameter is available in the 3D slice reconstruction mode. In this case the raw data should be acquired with one of the 3D-SIM gratings but you do not need a Z-stack. This reconstruction parameter is an optical sectioning type implementation of SIM as far as we can tell. You will see with high values of this parameter that holes get punched in the Fourier transform. In practise start with a low value (<0.1). If you see patterned 6 fold symmetry artefacts then increase this value and repeat until you are happy with the results. Basically the more out of focus light you have (the thicker the sample) the higher this value needs to be to compensate for it. In practise you will probably then also need to play around with the illumination modulation contrast setting a bit as well.

*if you take a Z-stack and reconstruction with the 'stack' algorithm this option is not available. The algorithm then takes into account information in neighbouring slices and should do a better job of reconstruction the data since it knows where the out of focus information has come from. I believe the minimum number of slices for this to work is 6 but imaging but 11 would recommend setting a top and bottom Z-stack and then making sure you have a few extra slices beyond where you would normally set the range.

<http://www.opticsinfobase.org/boe/abstract.cfm?uri=boe-5-8-2580>

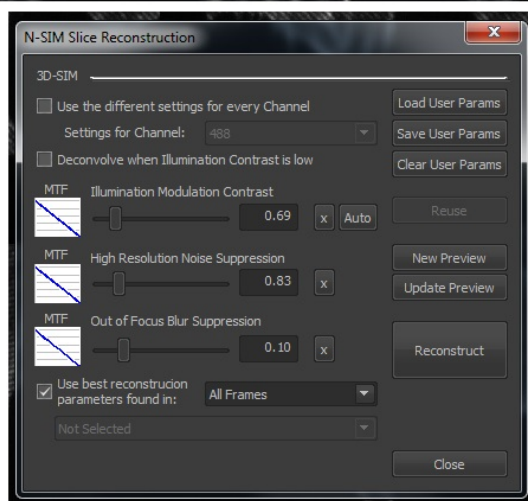
3D SIM on a single slice: The principal advantage of the 3D SIM with the slice reconstruction (ie. no Z-stack) is that you are not forced to take a Z-stack and therefore if you are doing live imaging your resulting effective frame rate will be better. The slice reconstruction uses information from the optical section to reconstruct although the mathematical methodology for this is not available.



the

will

3D



the

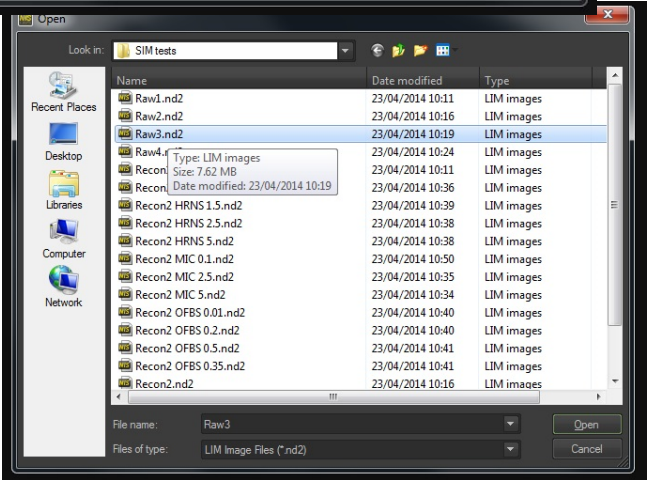
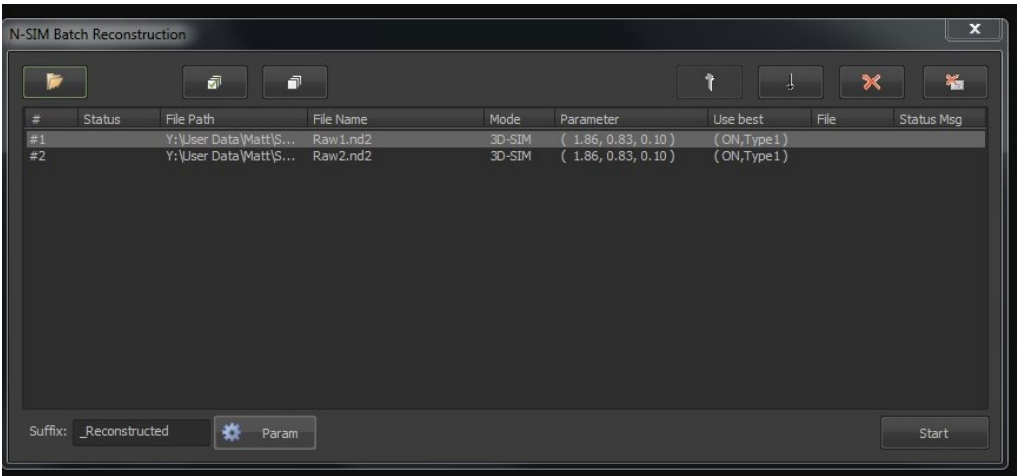
be

6-

will

Batch reconstruction: Batch reconstruction means it is possible for SIM data from several images to be reconstructed by the computer while you go and do something else (our recommendation is have a cup of tea).

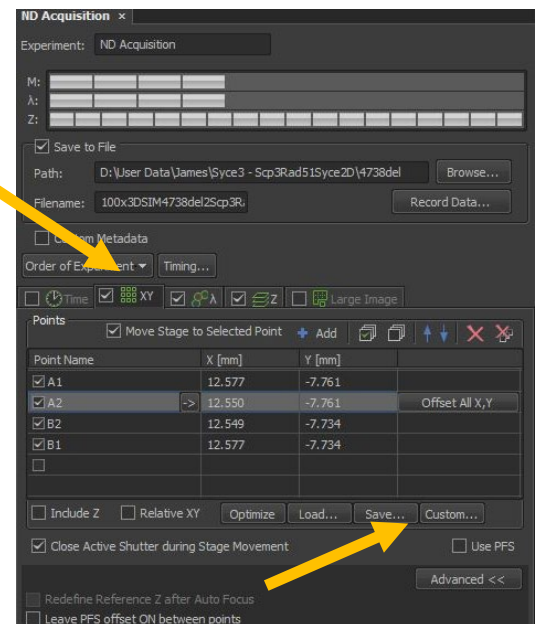
In the batch reconstruction tab go to the folder icon. The open dialogue box pops out. Click on each image you want batch reconstructed. You can alter the parameters for reconstruction by pressing the Settings button for Parameters if different reconstruction parameters are required for each image.



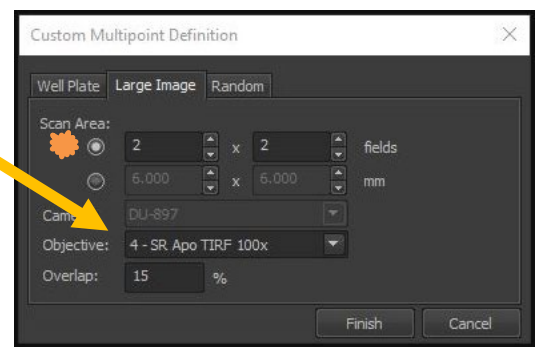
Stitching and Tiling:

It is possible to stitch together a Reconstructed image on the SIM system.

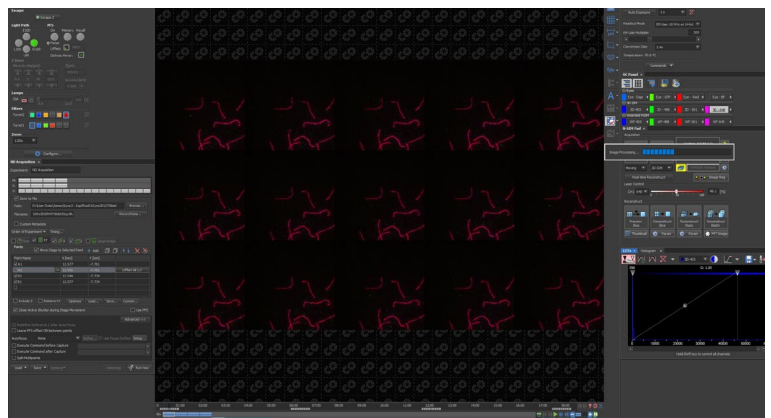
1. **Set up the multipoints.** At date of writing (Jan 2020) it is still not possible to use large image Acquisition for SIM data. Instead a 'Custom' Multipoint acquisition must be used.
 - a. Set up and optimise the acquisition parameters. It is recommended to us as high a laser power and as short a exposure time as possible, particularly as the laser will bleach the surrounding areas in tiling.
2. **Set up multipoint acquisition** by checking the 'XY' box
 - a. Press Custom



- b. The Custom Multipoint Definition GUI will appear.
 - i. Ensure Large Image is selected
 - ii. Ensure the correct **Objective** is selected
 - iii. Use 15% Overlap
 - iv. The image starts in the top left hand corner. Move the stage to here.
 - v. Set Scan area:  You need to guess how many fields you need. I recommend using 1 z slice and Widefield NSIM acquisition reconstruction

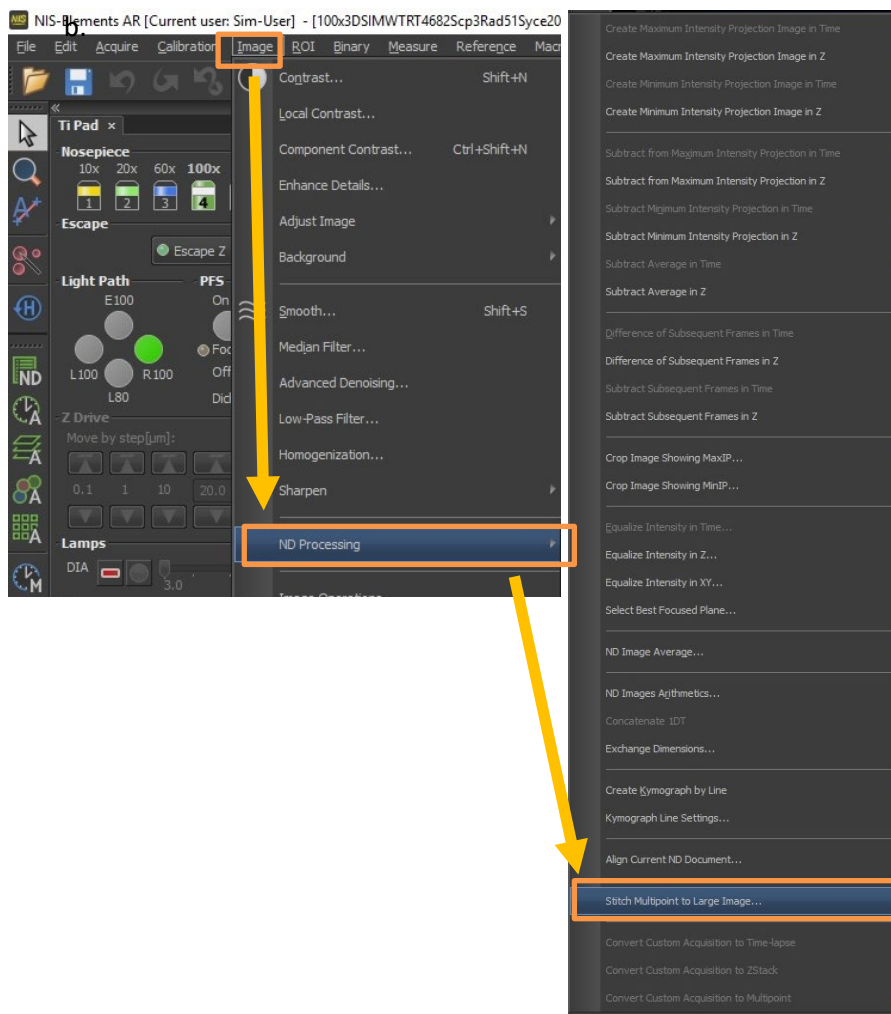


3. **Check the Positions** (usually labelled A1, A2, B1, B2 etc) are defined, z stack is defined and the Channels are defined (the XY, λ , Z check boxes are set and checked). Acquire the stack – Press **Run Now**. Reconstruct the stack using **Reconstruct Slice** or **Reconstruct Stack** .
 - a. In the final Image M is position and Z is stack.



4. Stitch the Reconstructed Image:

- a. Once the Image is reconstructed go to **Image** – in the main menu, choose the **ND Processing** submenu, then the **Stitch Multipoint to Large Image Option**

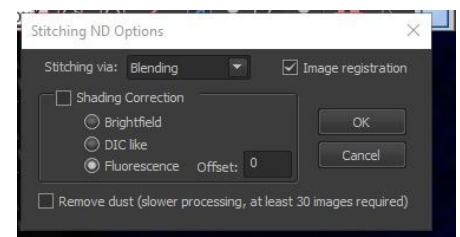


There are a lot of options! Keep looking down the list.

Here!

5. The **Stitching ND Options** Dialogue will pop out.

- a. Select Stitching via **Blending**. **Un Check Shading Correction** and **ensure Image registration** is checked. Make sure the images are in A1, A2, B1, B2 order as the software expects this. Please see the facility staff for help with this.
- b. Press OK



6. The Final stitched image will appear.

- a. Save this image
- b. There still may be stitching artefacts...

7.

