General issues:

- 1.5 thickness coverslips are best for SIM and Prolong Gold or Slowfade Gold (not Vectashield) give best results. You may also use homemade polyvinyl alcohol mounting media such as Gelvatol or Mowiol. Of course TIRF SIM has to be conducted in aqueous media (e.g. PBS).

- Here is the guidance from Nikon on which objective and type of SIM to do for different thickness samples: 100x FOV = 36x36μm; 60x FOV = 32x32μm

   100X 2D-SIM: ≤ 3 µm

   100X 3D-SIM: ≤ 7 µm

   100X TIRF-SIM ≤ 200 nm

   60X 3D-SIM ≤ 20 µm

      n.b. 60x is water immersion

- 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

Image reconstruction:

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
   - 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
   - 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