

N-SIM Quick User Guide

Principle: N-SIM extends spatial resolution via the virtual extension of the microscope's Fourier Space (frequency space). Striped excitation illumination adds a set of new, high frequency (high resolution) components to the original (standard resolution) frequency components. The N-SIM reconstruction software places these new high frequency components in their true position in Fourier space.

Sample Guidelines: High spatial frequency components must be detected and computationally restored. Best results are achieved by a bright fluorescence signal, low background, and high density of label. A #1.5 coverslip is required. Sample properties will ultimately determine the depth of imaging. Guidelines for approximate depth of imaging for N-SIM modes and objectives are as follows:

100X 2D-SIM: $\leq 3 \mu\text{m}$

100X 3D-SIM: $\leq 7 \mu\text{m}$

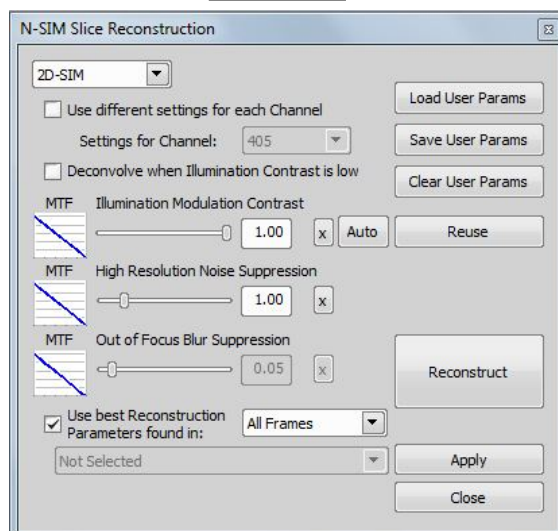
100X TIRF-SIM $\leq 200 \text{ nm}$

60X 3D-SIM $\leq 20 \mu\text{m}$

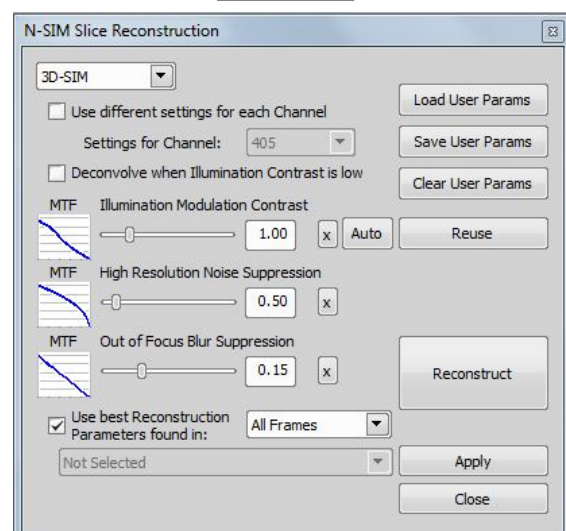
Reconstruction Parameters:

The settings shown below are a good starting point for samples that are appropriately labeled (see above). However, optimization may be necessary.

2D-SIM



3D-SIM



Deconvolve when Illumination Contrast is Low: Compares high frequency, moiré diffraction components against image noise

- When the box is checked and a warning appears only a Weiner deconvolution is performed for those z planes noted.
- If the check box is unchecked, or a value other than "Auto" is entered for Structured Illumination Contrast, full SIM processing occurs.

Illumination Modulation Contrast: Balances the contributions of the original and high frequency, moiré diffraction components

- Auto setting automatically adjusts weight of frequency components.
- Setting of 1 results in no adjustment to the weight of frequency components.
- For 2D-SIM, larger values decrease weight of the xy high spatial frequency components.
- For 3D-SIM, larger values will decrease the weight of xy high frequency components and increase the weight of z high frequency components.

High Resolution Noise Suppression: De-emphasizes or emphasizes the contrast of high spatial frequencies in a non-linear fashion. Can be used to suppress image noise.

- Setting of 1 is linear suppression
- Settings below one will increase the contrast of highest spatial frequencies
- Settings above one will lower the contrast of highest spatial frequencies

Out of Focus Blur Suppression: This option is active for data collected in 3D-SIM mode. The filter adjusts the strength of out of focus light removal to provide an optical sectioning effect.

- Larger values remove more out of focus light, yielding a thinner optical section. Values > 0.2 should be avoided to prevent possible removal of high frequency information and loss of resolution.

Use Best Reconstruction Parameter Found In: The software identifies the best reconstruction calculation from all planes in a data set and applies it to the entire z stack or time lapse.