

# User manual for 3I-SIM reconstruction software

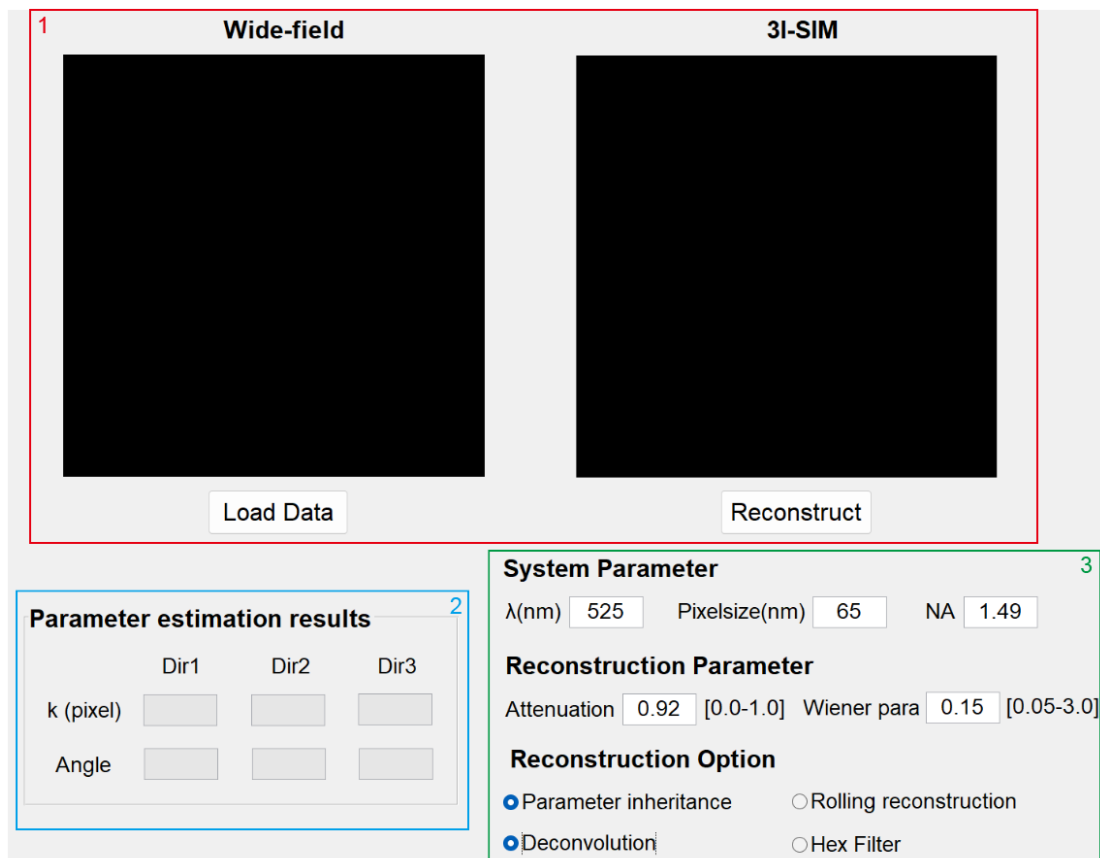
## 1. Abstract

This manual provides guidance for using the 3I-SIM reconstruction software, as part of the manuscript “*Triangle-beam interference structured illumination microscopy*”. It is a software used to reconstruct SR-SIM image with hexagonal modulation through lattice sheet.

## 2. Installation

We provided both the MATLAB source code in the .m format and a .exe executable file format. The code was developed and tested in the MATLAB 2020b version. To run the MATLAB source code, it is required to install a complete MATLAB software, which is available at <https://www.mathworks.com/products/matlab.html>. To run the .exe file, it is only required to install a MATLAB Runtime 2020b compiler, which is freely available at <https://www.mathworks.com/products/compiler/matlab-runtime.html>.

## 3. Software interface



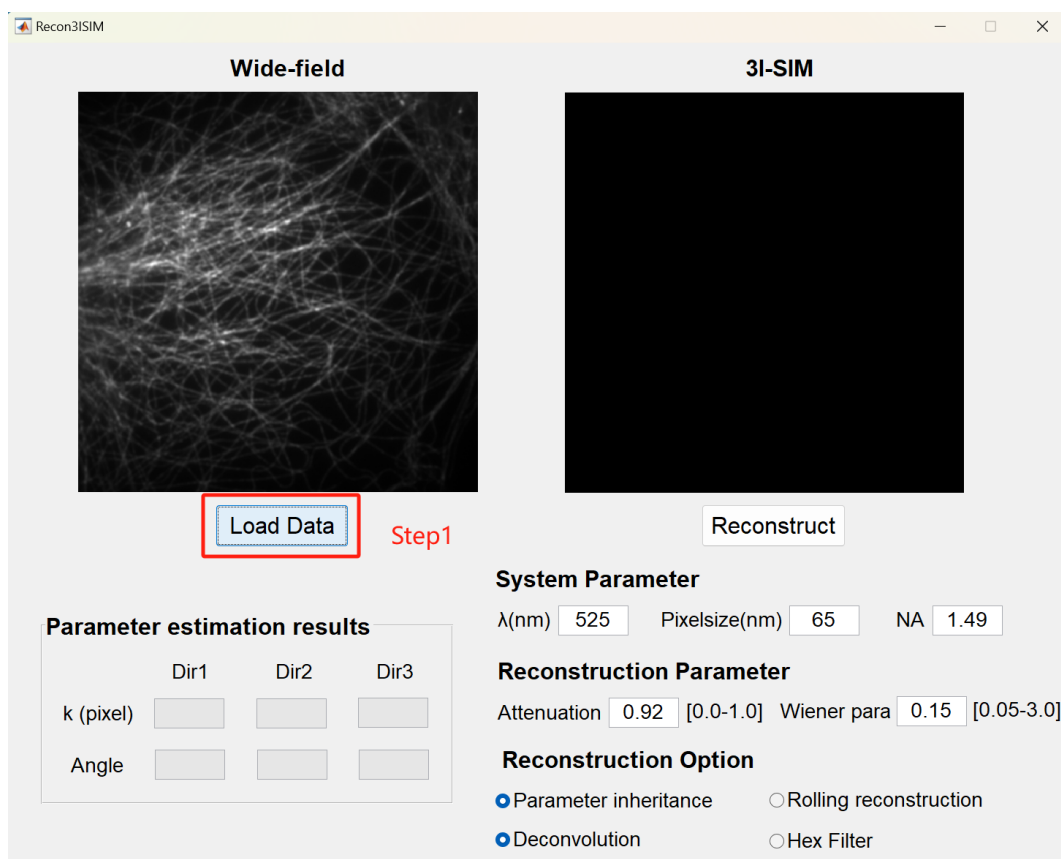
The software contains three regions:

- **Region 1:** The data loading and display region, which are used to load data and display the results.
- **Region 2:** The region displays the parameter estimation results.
- **Region 3:** The region for typing reconstruction parameters and choosing options.  
Details regarding parameters and options are as follows:
  - **$\lambda$  (nm):** The emission wavelength (typical value: 525-685 nm)
  - **Pixelsize (nm):** The real pixel size of the image (typical value: 65 nm)
  - **NA:** The numerical aperture of the objective lens (typical value: 1.49)
  - **Attenuation:** The OTF attenuation parameter (typical value: 0.9-0.99)
  - **Wiener para:** The Wiener parameter used for Wiener deconvolution (typical value: 0.05-3.0)
  - **Parameter inheritance:** Decide whether to determine the illumination pattern using the superimposed time-lapse images.
  - **Rolling reconstruction:** Decide whether to apply rolling reconstruction to time-lapse image stack
  - **Deconvolution:** Decide whether to apply deconvolution to each reconstructed image
  - **Hex Filter:** Decided whether to apply attenuation the shifted frequency point

## 4. Running procedure

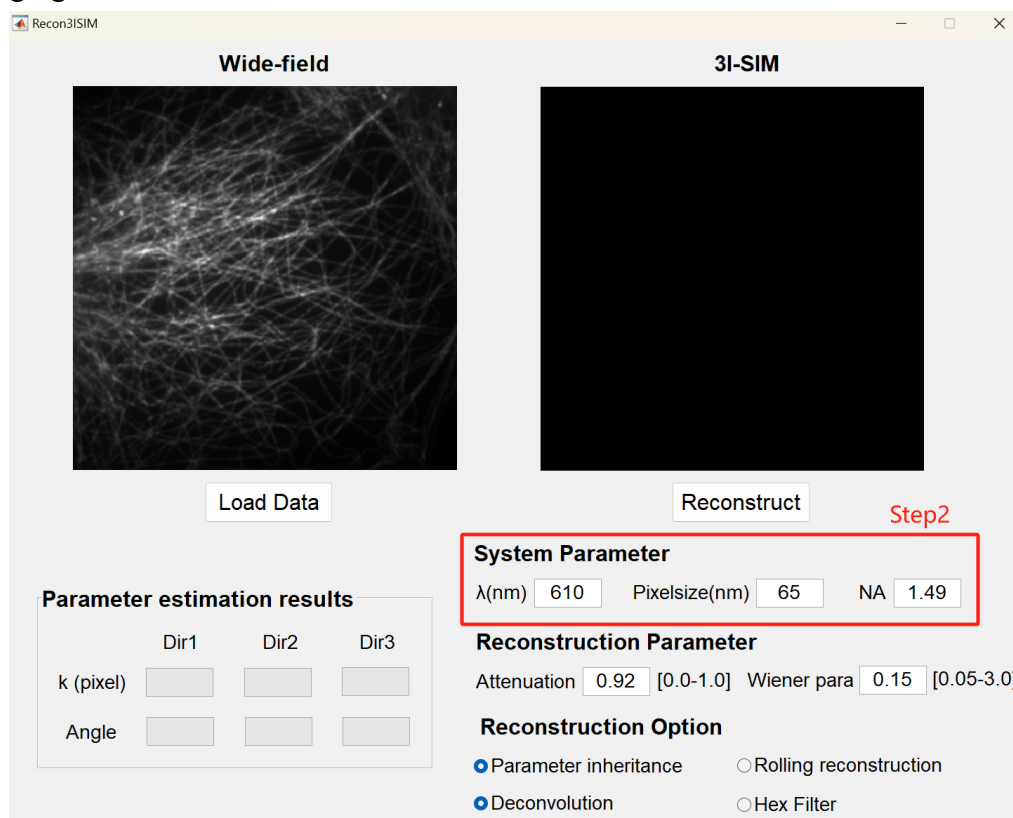
### Step 1. Load the raw data

Click the load data button and select the raw 3I-SIM images to be reconstructed. The software supports typical image format such as .tif, .jpg and .png. For a single-frame 3I-SIM reconstruction, it requires 7 raw modulated images.



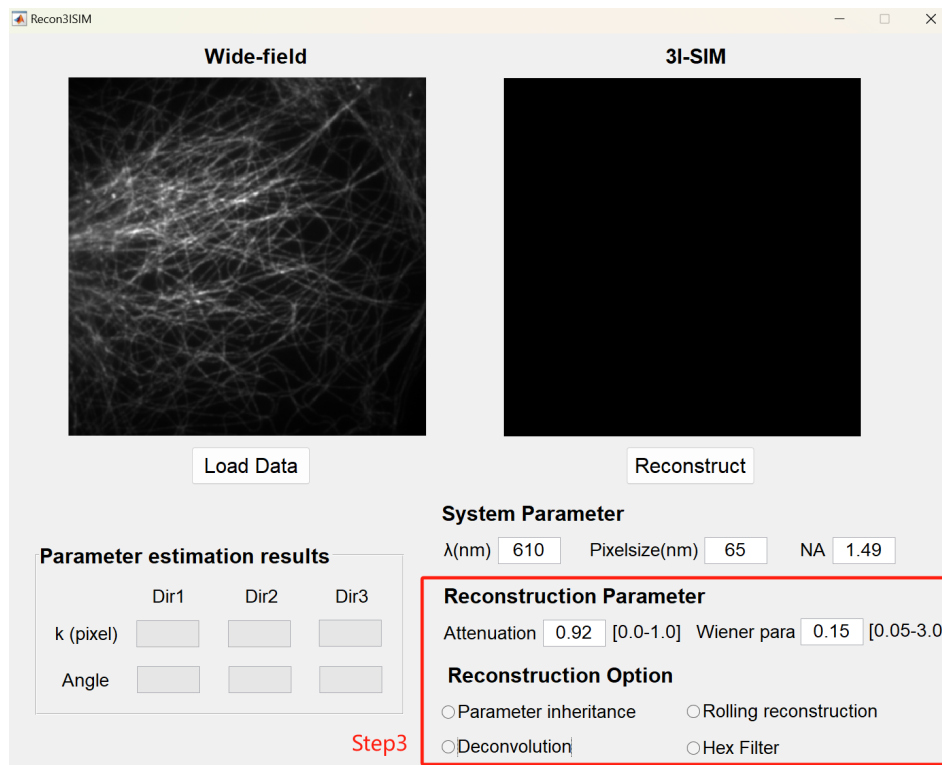
## Step 2. Check the system parameter

Properly modify the parameters listed in the System Parameter column according to the imaging condition.



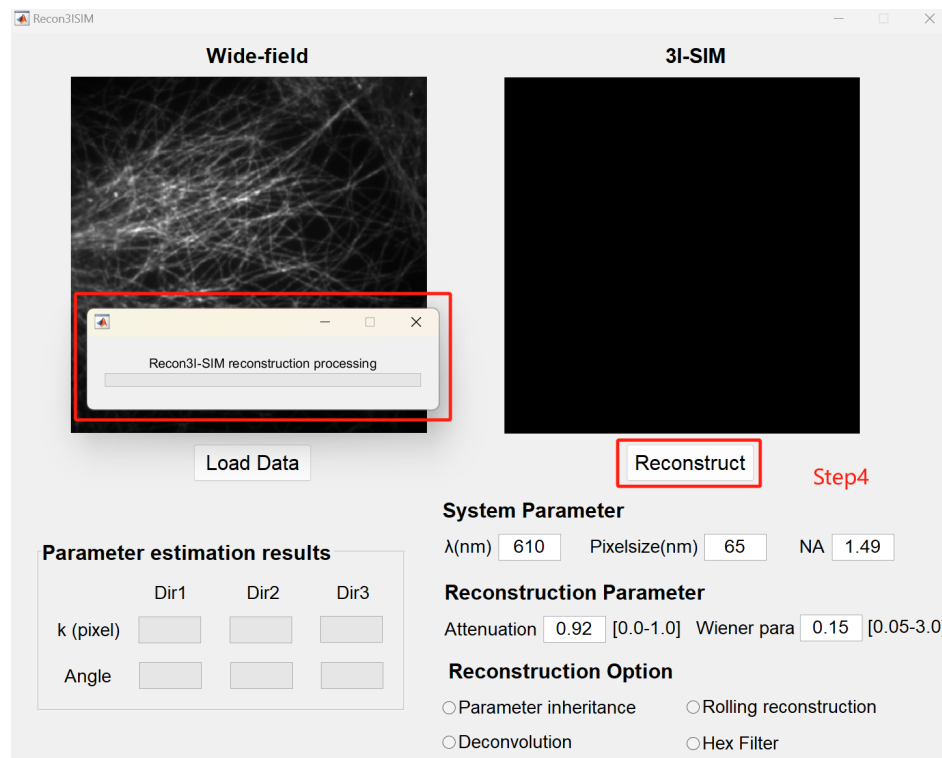
### Step 3. Check the Reconstruction Parameter and Option

Check the Reconstruction Parameter and Option column according to reconstruction demands.



### Step 4. Process data

Click the Reconstruct button and wait for the reconstruction.

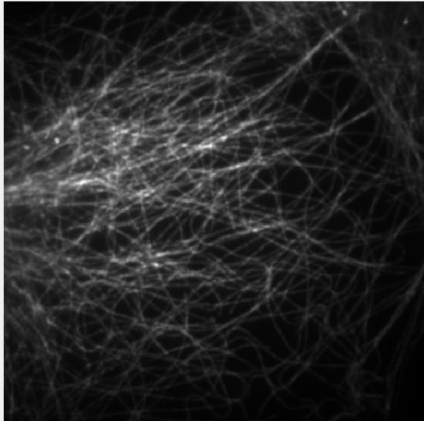


## Step 5. Check the results

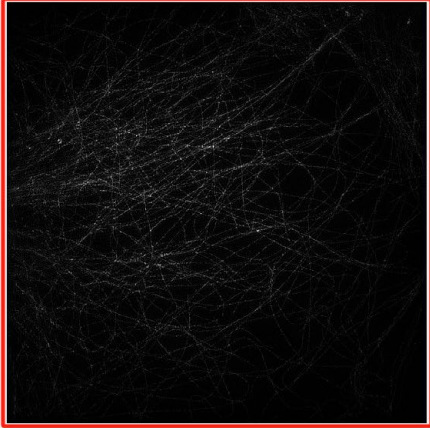
Check and evaluate the results.

Recon3ISIM

**Wide-field**



**3I-SIM**



Load Data

Reconstruct

Step5

**Parameter estimation results**

|           | Dir1   | Dir2    | Dir3   |
|-----------|--------|---------|--------|
| k (pixel) | 136.54 | 136.27  | 136.35 |
| Angle     | 68.670 | -51.351 | 8.766  |

**System Parameter**

$\lambda$ (nm) 610    Pixelsize(nm) 65    NA 1.49

**Reconstruction Parameter**

Attenuation 0.92 [0.0-1.0]    Wiener para 0.15 [0.05-3.0]

**Reconstruction Option**

☐ Parameter inheritance    ☐ Rolling reconstruction

☐ Deconvolution    ☐ Hex Filter