

User guide of Lock-in-SIM software

We provide three open-access Lock-in-SIM software platforms, MATLAB GUI, Fiji/ImageJ plugin, and executable software. All standard nine-image raw 2D-SIM datasets are suitable for reconstruction. The core of software development is to facilitate common users to use our Lock-in-SIM method without adding too much additional time and effort in hardware building, data acquisition and parameter finetuning, similar to standard 2D-SIM. Therefore, all software were developed as user-friendly as possible. For example, all GUIs were designed with the same style, all software have the same running logic, and parameter settings are made as few as possible. For more professional uses, users could refer to the source codes.

Software 1. MATLAB GUI

1. System and environment requirements: Windows 10/11, MATLAB (R2018 or newer, with Image Processing Toolbox).

2. Installation steps:

- (1) Download and install MATLAB (Version: R2018 or newer, with Image Processing Toolbox).

- (2) Download Lock-in-SIM MATLAB code from our GitHub (<https://github.com/WenjieLab/Lock-in-SIM>).

3. Running steps:

- (1) Open MATLAB and "Lock_in_SIM.m". Change the current fold of MATLAB to the path of "Lock-in-SIM.m" file. Add "functions" fold to path. Run "Lock_in_SIM.m" to open the GUI (Guide Fig. 1).

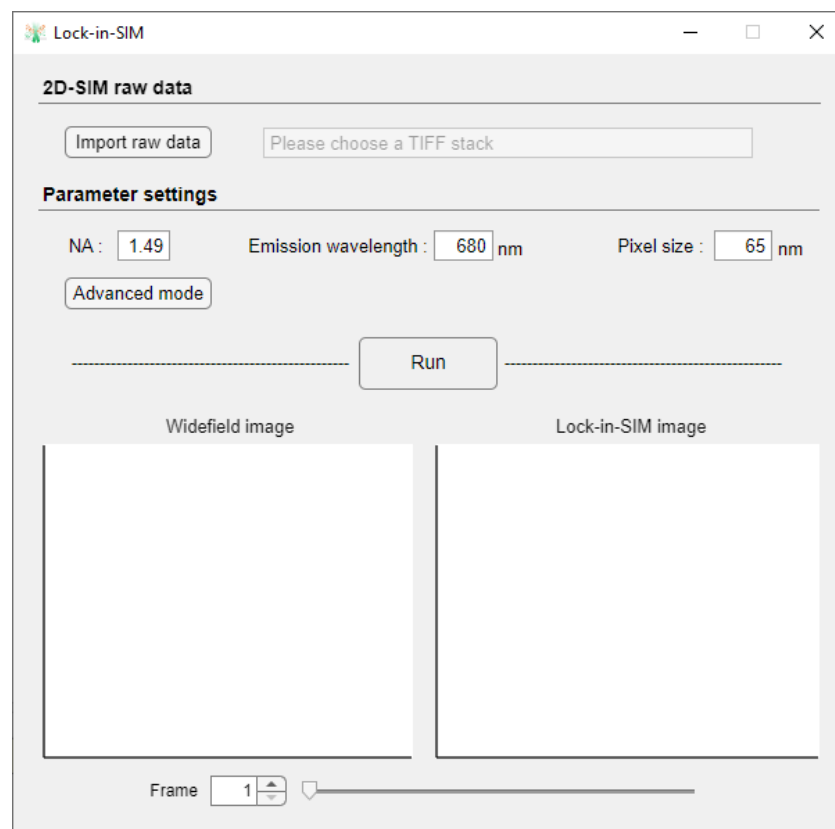
- (2) Press "Import raw data" to read raw data for reconstruction.

The file type should be a single Tiff stack (rather than image sequences). The stack sequence should be phase-angle-time (for time-lapse live-cell data)/depth (for z-sectioning data). Therefore, the total image number of the inputted stack should be $9 \times n$ ($n \geq 1$).

- (3) Set the reconstruction parameters, including NA of objective, emission wavelength of fluorophore, and pixel size of raw image.

Optional: We provide an optional advanced mode for users to adjust the value of α in equation (8), Supplementary Note 1 of our paper (defined as “Lock-in parameter”). If the advanced mode is turned off, Lock-in parameter of 0.8 will be used for reconstruction by default. If the advanced mode is turned off, users can adjust Lock-in parameter from 0 to 1, with higher value meaning higher background filtering. The default value of 0.8 should be suitable for the most cases.

- (4) Press “Run” to run reconstruction. The widefield results and Lock-in-SIM results will be outputted as two stacks. The total image number of each stack should be n ($n \geq 1$). These two stacks will be shown in the bottom of MALAB GUI. Users can drag “Frame” button to look through different frames. At the same time, they will be also automatically saved into the “Lock-in-SIM” folder under the path of the imported raw data, named “(the file name of your imported raw data)_Lock-in-SIM.tif” and “(the file name of your imported raw data)_Widefield.tif”.



Guide Fig. 1. GUI of the open-access Lock-in-SIM software (MATLAB version)

Software 2. Fiji/ImageJ plugin

1. System and environment requirements: Windows 10/11, MATLAB Runtime (version 9.13), and Fiji/ImageJ.

2. Installation steps:

(1) Download and install Fiji/ImageJ.

(2) Download and install MATLAB Runtime (Version: R2022b (9.13)) from:
mathworks.com/products/compiler/matlab-runtime.html.

(3) Download Lock-in-SIM Fiji/ImageJ plugin from our GitHub
(<https://github.com/WenjieLab/Lock-in-SIM>).

(4) Paste the “Lock_in_SIM.jar” file into the “plugins” folder under the directory of Fiji/ImageJ software or pull “Lock_in_SIM.jar” file directly into the opened Fiji, then restart Fiji.

3. Running steps:

(1) Open raw data in Fiji.

The file type should be a single Tiff stack (rather than image sequences). The stack sequence should be phase-angle-time (for time-lapse live-cell data)/depth (for z-sectioning data). Therefore, the total image number of the inputted stack should be $9 \times n$ ($n \geq 1$).

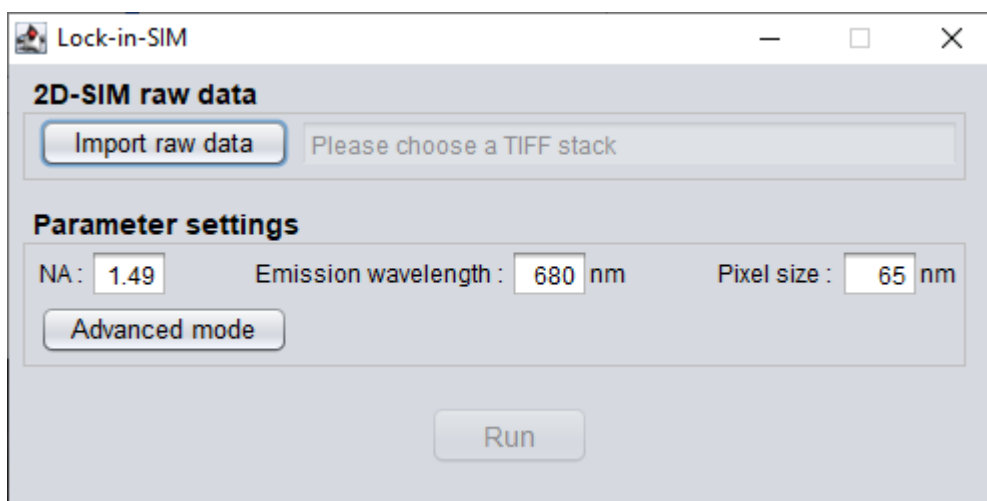
(2) Open Fiji. Press “Process→Lock In SIM” to open the GUI (Guide Fig. 2).

(3) Press “Import raw data” to choose raw data from the opened windows of Fiji for reconstruction.

(4) Set the reconstruction parameters, including NA of objective, emission wavelength of fluorophore, and pixel size of raw image.

Optional: We provide an optional advanced mode for users to adjust the value of α in equation (8), Supplementary Note 1 of our paper (defined as “Lock-in parameter”). If the advanced mode is turned off, Lock-in parameter of 0.8 will be used for reconstruction by default. If the advanced mode is turned off, users can adjust Lock-in parameter from 0 to 1, with higher value meaning higher background filtering. The default value of 0.8 should be suitable for the most cases.

- (5) Press “Run” to run reconstruction. The widefield results and Lock-in-SIM results will be outputted as two stacks, named “(the file name of your imported raw data)_Lock-in-SIM.tif” and “(the file name of your imported raw data)_Widefield.tif”. The total image number of each stack should be n ($n \geq 1$). These two stacks will be shown as separate windows.



Guide Fig. 2. GUI of the open-access Lock-in-SIM software (Fiji/ImageJ plugin version)

Software 3. Executable software

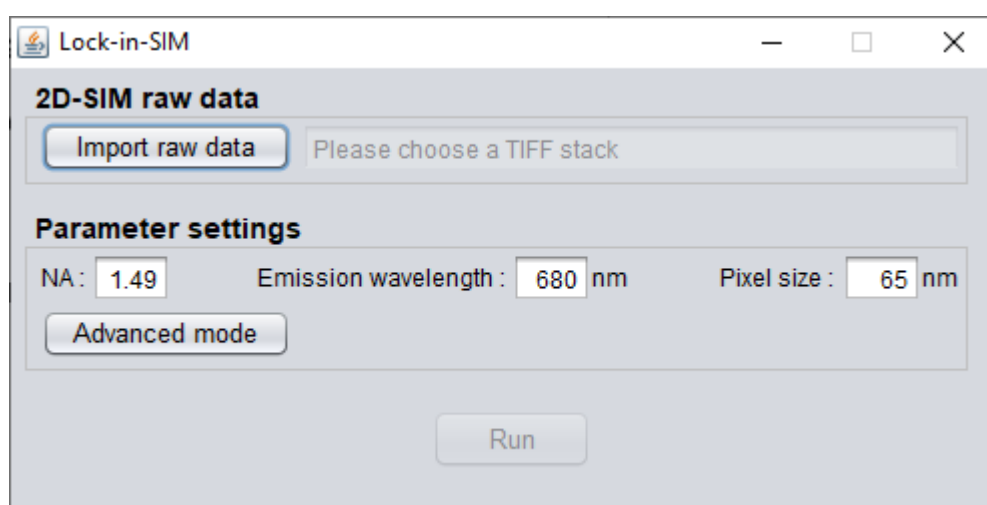
1. System and environment requirements: Window 10/11, MATLAB Runtime (version 9.13).
2. Installation steps:
 - (1) Download and install MATLAB Runtime (Version: R2022b (9.13)) from:
mathworks.com/products/compiler/matlab-runtime.html.
 - (2) Download Lock-in-SIM executable software from our GitHub
(<https://github.com/WenjieLab/Lock-in-SIM>).
3. Running steps:
 - (1) Run “LockinSIM.exe” to open the GUI (Guide Fig. 3).
 - (2) Press “Import raw data” to read raw data for reconstruction.

The file type should be a single Tiff stack (rather than image sequences). The stack sequence should be phase-angle-time (for time-lapse live-cell data)/depth (for z-sectioning data). Therefore, the total image number of the inputted stack should be $9 \times n$ ($n \geq 1$).

- (3) Set the reconstruction parameters, including NA of objective, emission wavelength of fluorophore, and pixel size of raw image.

Optional: We provide an optional advanced mode for users to adjust the value of α in equation (8), Supplementary Note 1 of our paper (defined as “Lock-in parameter”). If the advanced mode is turned off, Lock-in parameter of 0.8 will be used for reconstruction by default. If the advanced mode is turned off, users can adjust Lock-in parameter from 0 to 1, with higher value meaning higher background filtering. The default value of 0.8 should be suitable for the most cases.

- (4) Press “Run” to run reconstruction. The widefield results and Lock-in-SIM results will be outputted as two stacks. The total image number of each stack should be n ($n \geq 1$). These two stacks will be shown as separate windows. At the same time, they will be also automatically saved into the “Lock-in-SIM” folder under the path of the imported raw data, named “(the file name of your imported raw data)_Lock-in-SIM.tif” and “(the file name of your imported raw data)_Widefield.tif”.



Guide Fig. 3. GUI of the open-access Lock-in-SIM software (executable software version)

Note:

1. Related Lock-in-SIM paper will be released soon.
2. The current user guide is the first released version (v1.0). For the future updated version of both software and user guide, please refer to our GitHub (<https://github.com/WenjieLab/Lock-in-SIM>).

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