User guide of Lock-in-SIM software

The Lock-in-SIM algorithm was developed primarily to **eliminate background** and enhance effective resolution in 2D-SIM imaging. We offer three open-access Lock-in-SIM software platforms: **MATLAB GUI, Fiji/ImageJ plugin, and an executable software** version. This is the first open-access algorithm compatible across these three platforms and the second Fiji/ImageJ plugin in 2D-SIM field. All **standard raw 2D-SIM datasets (3 angles×3 phases×n frames)** are supported for reconstruction. To facilitate users of commercial SIM systems, we **additionally provide a Fiji/ImageJ plugin for converting various SIM data sequences**, enabling seamless integration of our algorithm into commercial 2D-SIM data processing workflows. Note that commercial SIM systems not based on standard SIM theory (3 angles×3 phases)—such as Zeiss Lattice-SIM, CrestOptics DeepSIM, and Visitech iSIM—are incompatible with our algorithm.

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.

Note:

1. The software is related to our Lock-in-SIM publication:

   Liu W., Zhang M., Zhu W. et al. Visualizing intraorganellar ultrastructures, dynamics, and interactions with open-access background-free Lock-in-SIM. Nature Communications 16, 10765 (2025).

   https://doi.org/10.1038/s41467-025-65805-w

2. Open-access Lock-in-SIM raw data can be downloaded from our Figshare (https://figshare.com/articles/figure/Open-access_raw_datasets_for_Lock-in-SIM/26130994).
3. The current user guide is the first released version (v1.1). For the future updated version of both software and user guide, please refer to our GitHub (https://github.com/WenjieLab/Lock-in-SIM).
4. For any feedback of the paper and the software, please contact wenjieliu11@gmail.com.

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By Wenjie Liu

Software 1. 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: https://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*n (n≥1).

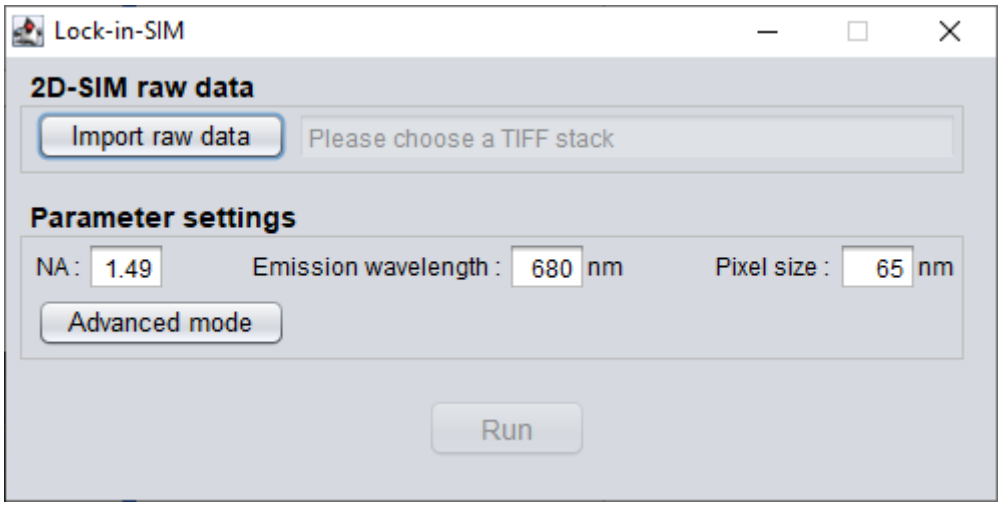
(2) Open Fiji. Press "Plugins→Lock-in-SIM" to open the GUI (Guide Fig. 2).

(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 on, 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≥1). These two stacks will be shown as separate windows. Users can then save them manually through Fiji/ImageJ.



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

4. **Raw SIM Converter**:

We additionally provide a “Raw SIM Converter” plugin alongside the "Lock-in-SIM" plugin in the Fiji/ImageJ software package. This Converter can be used to convert different raw SIM sequences. The steps for using the plugin are as follows:

(1) Choose “Data type”: “Single” denotes processing a single stack. “Batch” denotes processing multiple stacks simultaneously. For batch processing, please ensure that all stacks have the same acquisition parameters and are located within the same file directory.

(2) Choose “Mode”: Choose between “2D-SIM” or “3D-SIM” depending on the type of raw data being processed.

(3) Set the number of time points and/or Z section for time-lapse and/or Z-sectioning data conversion. If the data does not include multiple dimensions, set the corresponding dimension value to 1. For example, if the data is a single-frame

2D-SIM data (9 images), both the number of time and Z section should be set to 1.

(4) Choose “Input”: “General” denotes the sequence used in Lock-in-SIM reconstruction, that is, phase-angle-time (for time-lapse data)/depth (for z-sectioning data). The data sequence from Multi-SIM (NanoInsights-Tec) is the same as the “General” sequence here. “GE” denotes the data sequence from GE OMX-SIM system. “Nikon” denotes the data sequence from Nikon N-SIM system. “CSR” denotes the data sequence from CSR Biotech HIS-SIM system.

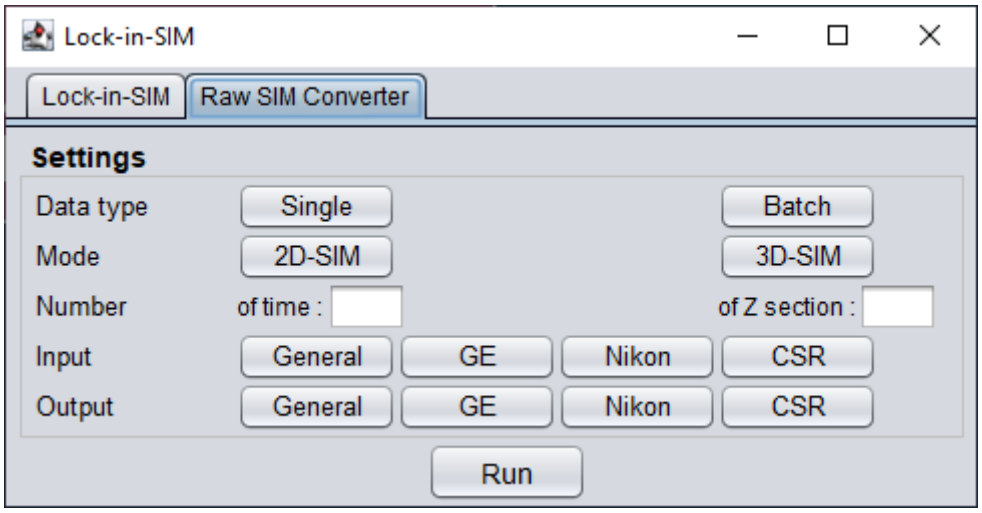
(5) Choose “Output”.

(6) Press “Run”: A pop-up window will appear, allowing users to select the input data. For the "Single" processing, choose a single stack file. For the "Batch" processing, select a folder containing multiple stacks of the same acquisition parameters. The converted results will be automatically saved into the same file path, named “(the file name of your input data)_XX sequence.tif”.

Note:

1. The input data needs to be translated and saved into Tiff format first to enable reading by the plugin.

2. For multi-color data conversion, it needs to be translated and saved into single-color stack first to enable reading by the plugin.



Guide Fig. 3. GUI of the Raw SIM Converter (only Fiji/ImageJ plugin version)

Software 2. 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.

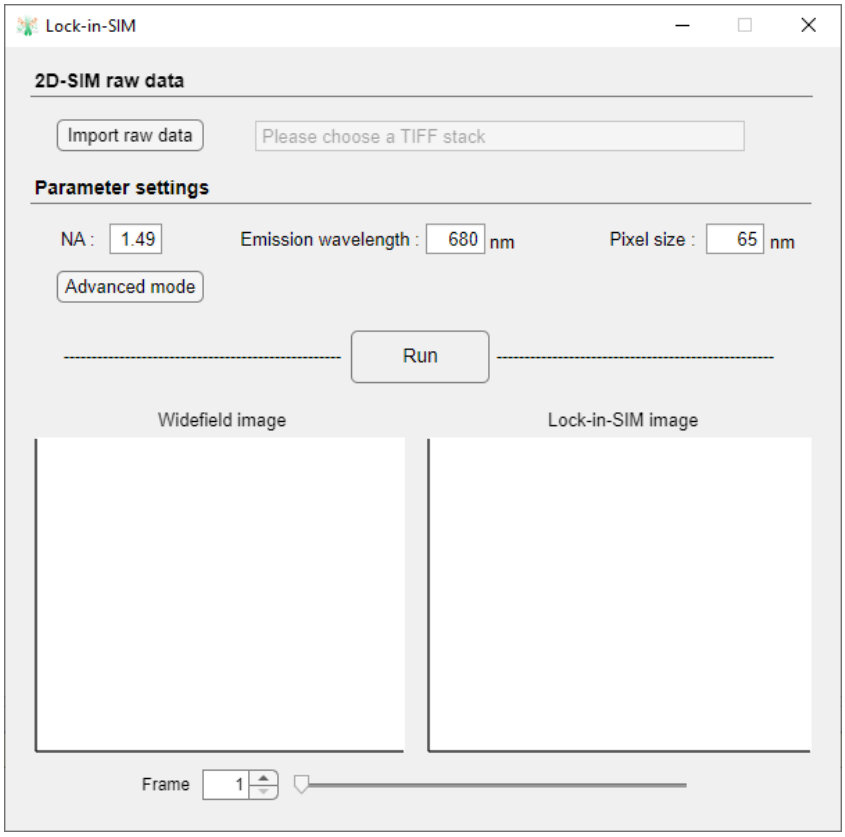
The file type should be a single Tiff stack (rather than image sequences). The stack sequence should be phase-angle-time (for time-lapse live-cell data)/depth (for z-sectioning data). Therefore, the total image number of the inputted stack should be 9*n (n≥1).

(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 on, 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≥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 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: https://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. 4).

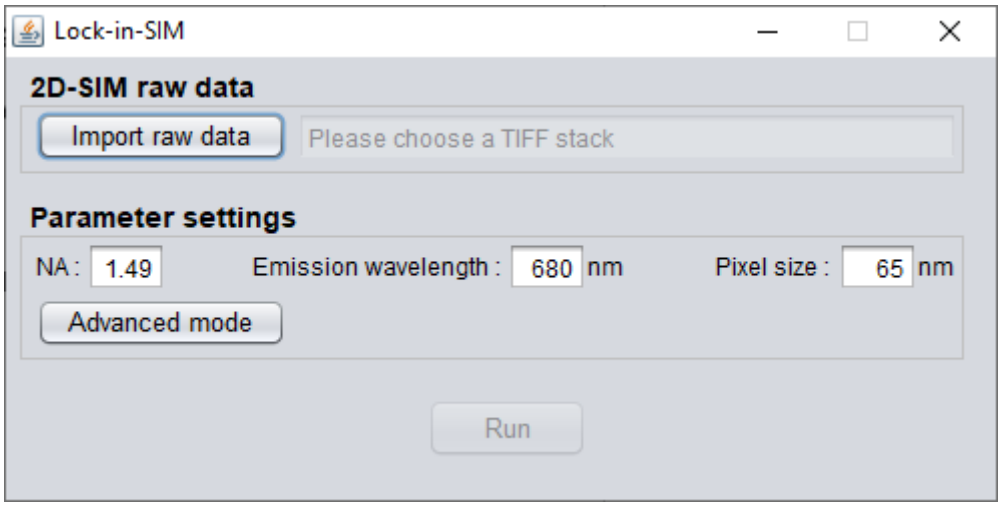
(2) Press “Import raw data” to read raw data for reconstruction.

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*n (n≥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 on, 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≥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. 4. GUI of the open-access Lock-in-SIM software (executable software version)