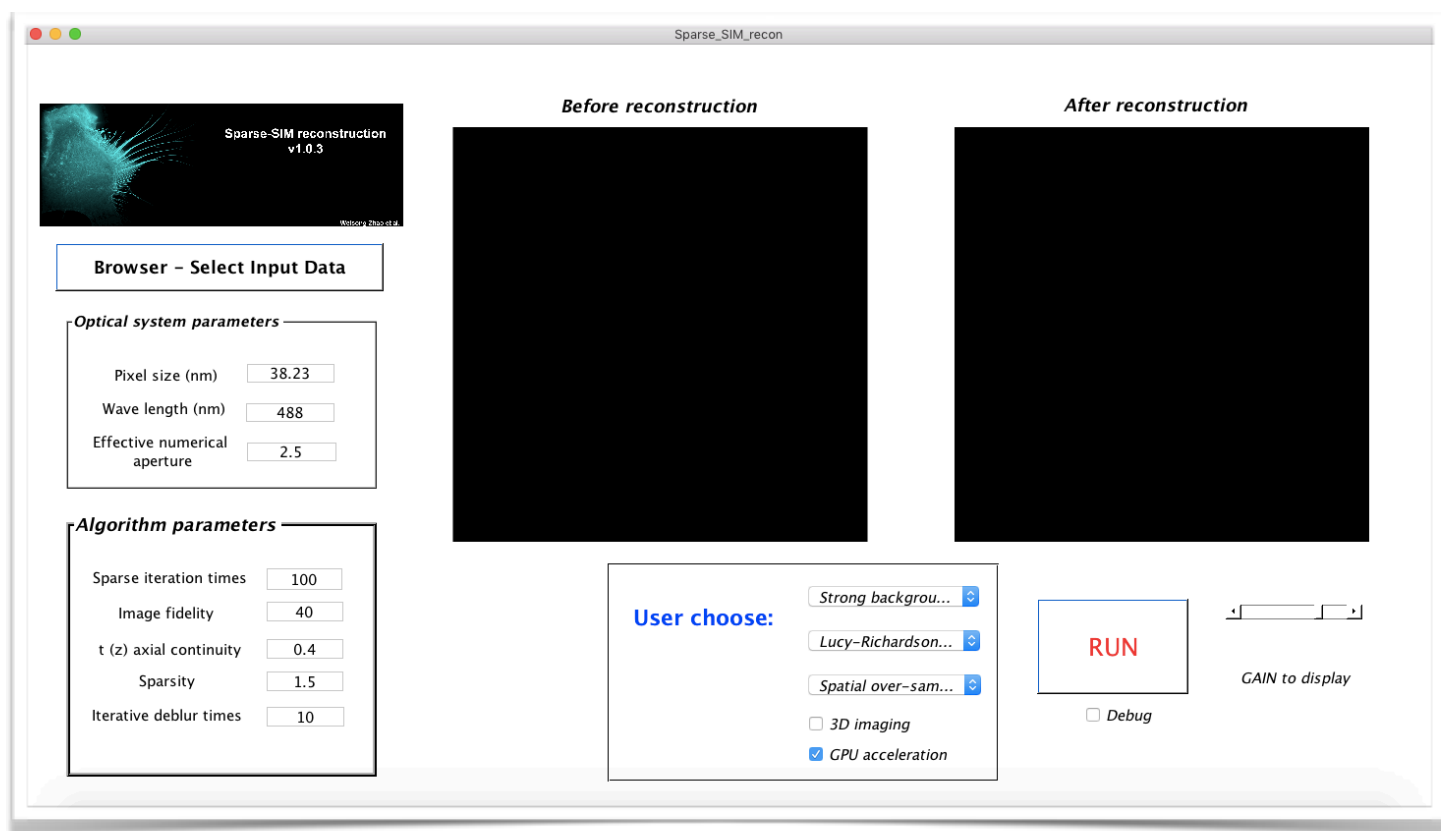


Sparse-SIM

User Manual: Version(1.0.3)

Weisong Zhao - 2021/2/21



Extending resolution of structured illumination microscopy with sparse deconvolution

Weisong Zhao[#], Shiqun Zhao[#], Liuju Li[#], Haoyu Li^{*}, and Liangyi Chen^{*}

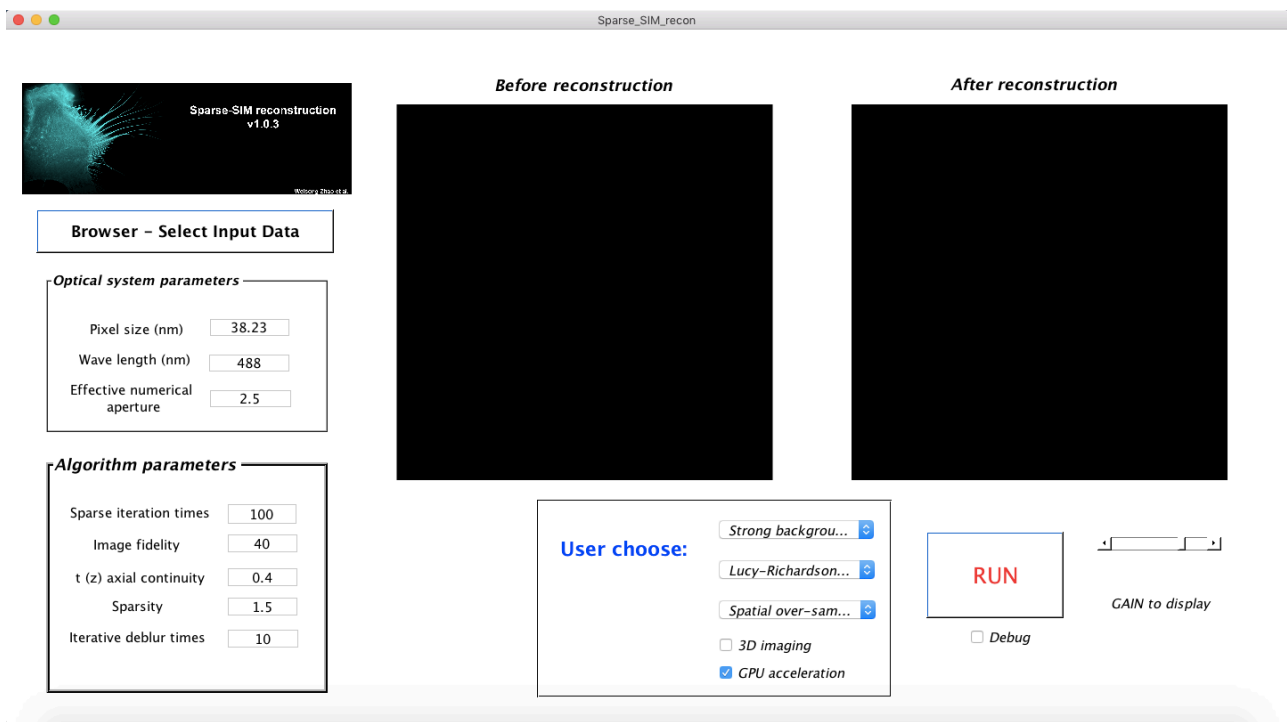
^{*}Correspondence should be addressed to Haoyu Li (lihaoyu@hit.edu.cn) or Liangyi Chen(lychen@pku.edu.cn).

[#]These authors contributed equally to this work.

This software package is a tool for SIM images reconstruction, including xy (2D), xy-t (2D along t axis), and xy-z (3D) images. It is based on the natural transcendental knowledge of fluorescent biological images: sparsity and continuity along xy-t(z) axes.

Details

- ❖ **Input data structure:** It should be the .tif data.
- ❖ This software is not designed to be compatible with various data structures for now, the update version of source code in [GitHub](https://weisongzhao.github.io/Sparse-SIM/) e.g. <https://weisongzhao.github.io/Sparse-SIM/> maybe compatible with the various data.
- ❖ **Easy to use:**
- ❖ If you are using Win10-64bit or MacOS-64bit systems, the [SparseSIM_web_installer.exe](#), and [SparseSIM_web_installer.app](#) are provided in the link <https://github.com/WeisongZhao/Sparse-SIM/releases/tag/v1.0.3>. Execute the [SparseSIM_web_installer.exe](#) or [SparseSIM_web_installer.app](#) and follow the instructions. The APP will be installed at [/Sparse_SIM/application/Sparse_SIM.exe \(.app\)](#) as default. Or you can download the Matlab runtime v9.3 (for 2017b, [Win10](#), or [MacOS](#)) at first, and execute the [for Matlab users/Sparse_SIM.exe \(.app\)](#). Additionally, if you are a Matlab 2017b user, (great!) you can execute the [for Matlab users/Sparse_SIM.exe](#) or [Sparse_SIM.app](#) directly (without downloading these things through internet).
- ❖ Of course, you can use the source code directly: for Win10 is [src_win/install.m](#) and for MacOS or Unix-like users, please just run the [src_unix/install.m](#) and the GUI will pop up:



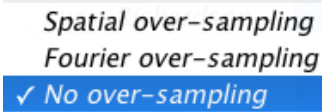
Parameters of GUI

- **Browser:** Select your data to reconstruct;
- **Pixel size:** The pixel size of input images;
- **Wave length:** The emission wavelength of the fluorescence probes.
- **Effective numerical aperture:** Effective NA of images after sparse reconstruction;
- **Sparse iteration times:** The iteration times of Sparsity reconstruction;
- **Image fidelity:** The parameter for images fidelity (distance between the images after and before reconstruction);
- **t (z)-axial continuity:** The t or z axial continuity of input video or volume;
- **Sparsity:** The sparsity of the data;
- **Iterative deconvolution times:** The iteration times of post deconvolution;
- **GPU acceleration:** Whether or not to compute on CUDA-GPU;
- **3D imaging:** The input images are whether or not 3D volume;
- **GAIN:** The brightness of the image can be altered via this control bar;
- **Iterative deconvolution:** The iterative deconvolution methods chosen by user.

☒ **Lucy-Richardson deconvolution (LR)**
☐ LandWeber deconvolution (LW)
☐ No deconvolution

Three options: accelerated **LR**, **LW** methods, and no deconvolution. Interestingly, even with Nesterov momentum acceleration, the **LW** method may be much slower than the vector extrapolation version **LR** method. So, the common iteration times of **LR** is 5~15, and **LW** is 30~50;

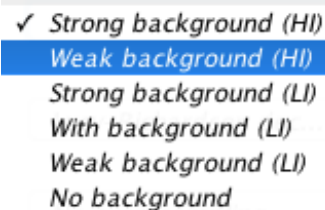
- **Over/up sampling:** The over-sampling methods (written as up-sampling in main text) chosen by user to achieve better image quality.



Spatial over-sampling
Fourier over-sampling
✓ No over-sampling

Three options: **spatial**, and **Fourier** over-sampling, and no over-sampling. As the **Fourier** over-sampling is sensitive to noise, the low SNR data is recommended to be processed with **spatial** over-sampling method;

- **Background:** The background estimation level chosen by user:



✓ Strong background (HI)
Weak background (HI)
Strong background (LI)
With background (LI)
Weak background (LI)
No background

Six options: **Strong background (HI)**, **Weak background (HI)**, **Strong background (LI)**, **With background (LI)**, **Weak background (LI)** and **No background** are provided. **HI** is the High-dose illumination, and **LI** means low-dose illumination, which denote to two different types of background. For the strong fluorescence background originated from out-of-focus emission and cellular auto-fluorescence (usually contained in high SNR data) is regarded as **HI**. The low, and stable noise-like background (commonly exhibited in low SNR data) is seen as **LI**.

Furthermore:

- The maximum intensity projection (MIP) is used to display the 3D volumetric data in the Sparse-SIM UI. Users can adjust parameter values of the **Image fidelity**, **t (z)-axial continuity**, **Sparsity**, **Iterative deconvolution times**, **Background (including 6 options)**, and **Over sampling (including 3 options)** to enhance the image quality.
- The **debug** mode (choose first 5 frames from the input image stacks) is offered to help users to select the parameters more quickly.

Parameters

	2D-SIM Actin Fig4a	2D-SIM LysoView Fig4m	TIRF-SIM Caveolae Fig4j	SD-SIM sCMOS Actin Ex. Fig15	SD-SIM sCMOS CCP Ex. Fig15	SD-SIM EMCCD Peroxisome Fig6e	SD-SIM EMCCD Tubulin Fig6e	SD-SIM EMCCD Lysosome Fig6e
Pixel size	32.5	32.5	32.5	38.23	38.23	94	94	94
Wave-length	488	488	488	488	561	405	488	561
Effective NA	3	3	5	2.5	2.5	2.5	2.5	2.5
Sparse iteration	100	100	100	100	100	300	300	300
Image fidelity	150	30	1000	40	60	40	25	25
$t(z)$ axial continuity	1	0.1	0.1	0.4	0.6	0.1	0.1	0.1
Sparsity	10	2.5	90	2	5	0.5	0.45	0.4
Deblur times	30	5	50	10	15	10	7	7
Background	No	Strong(HI)	No	Weak(HI)	Strong(HI)	No	No	Strong(LI)
Deblur type	LR	LR	LW	LR	LR	LR	LR	LR
Over-sample	No	No	Fourier	No	No	Spatial	Spatial	Spatial
If 3D imaging	No	No	No	No	No	No	No	No

We offered 8 datasets in the [Supplementary Data](#), and used the SD-SIM/ Ex.Fig15_sCMOS/2colorActin.tif as an example in this manual.

The reconstructed video is then saved in the folder Data/SHReconstructed/ ****_**_**_2colorActin_Video/2colorActin_reconstructed.tif.

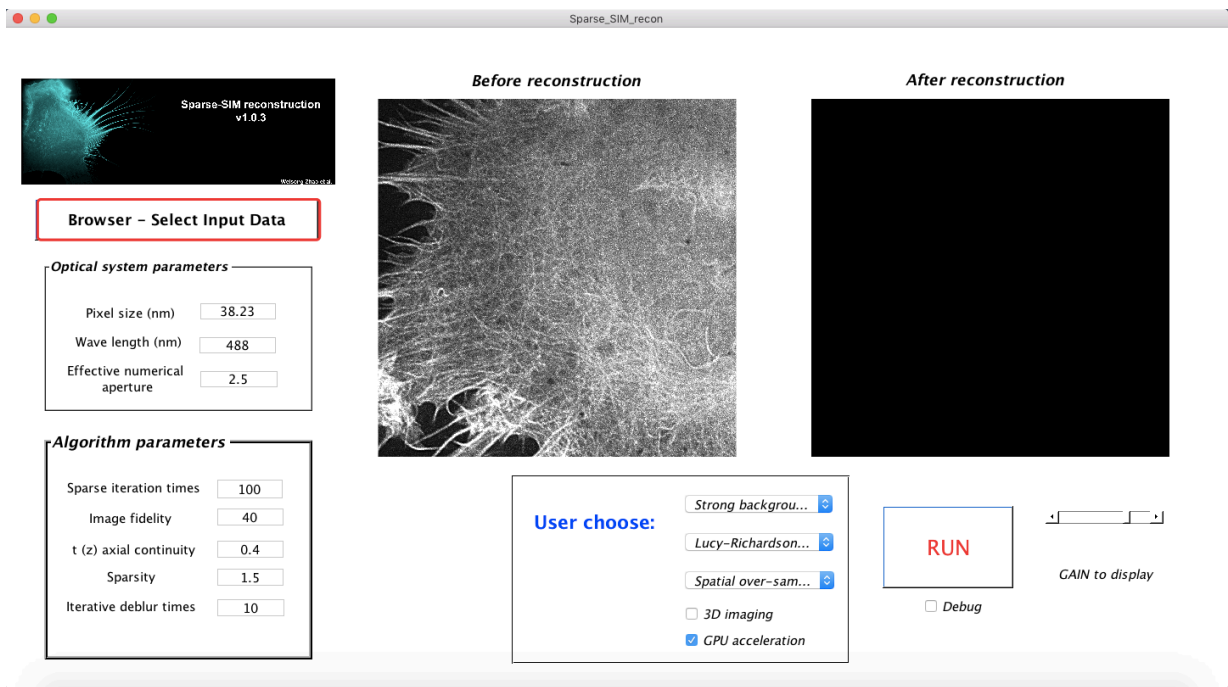
The ****_**_**_ is determined by the date you analyze the data eg. (2019_01_19_19: 2019 January 19th 19 o'clock).

Usually,

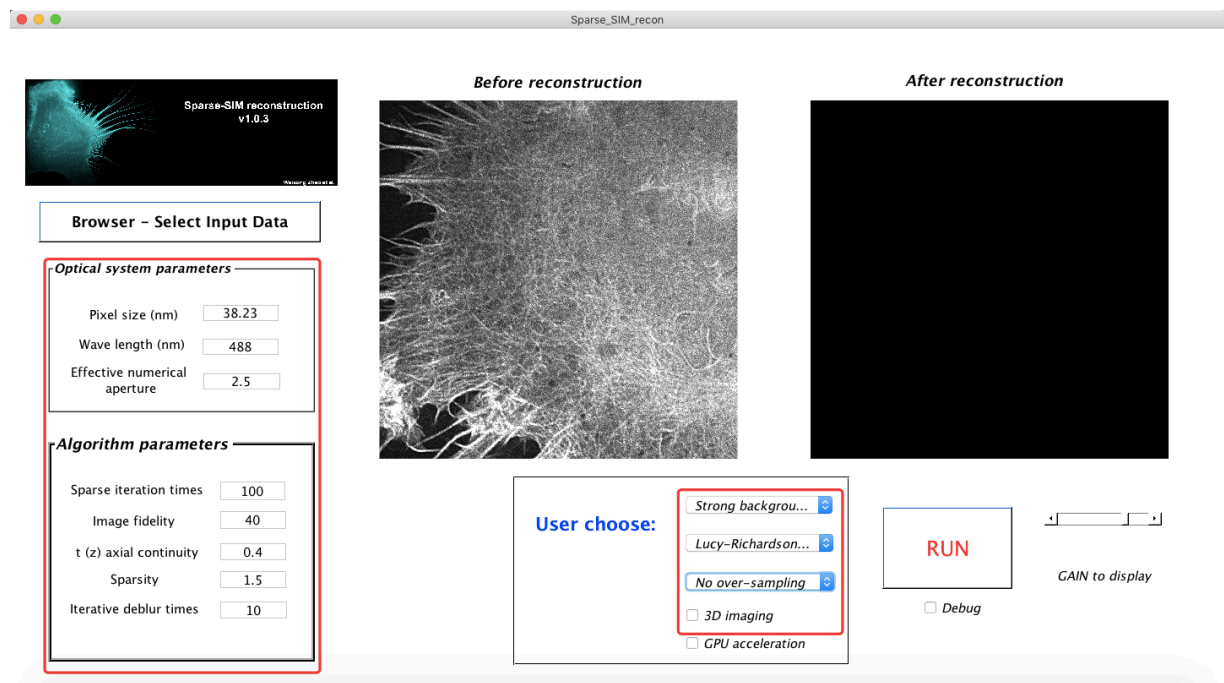
- **Iterative deconvolution times** for **LW**, and **LR** should be 5-15, and 30-50, respectively. **Sparse iteration times** should be 100. If the **Spatial over-sampling** operation is selected, the **Sparse iteration times** should be 200 or 300 and the **Image fidelity**, and **Sparsity** should be small enough to avoid 'zero artifacts'.
- **Sparsity** and **t(z)-axial continuity** should be smaller than one tenth of **Image fidelity**.
- **Image fidelity** should be larger than 10.

Example: SD-SIM/Ex.Fig15_sCMOS/2colorActin.tif

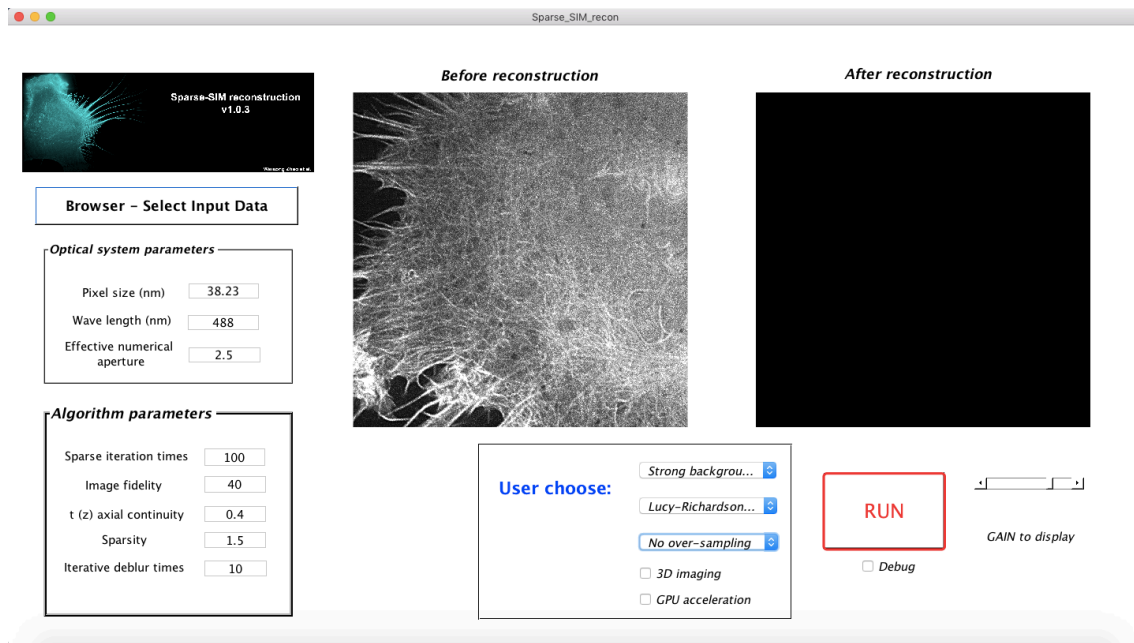
Step1. Choose the offered data.



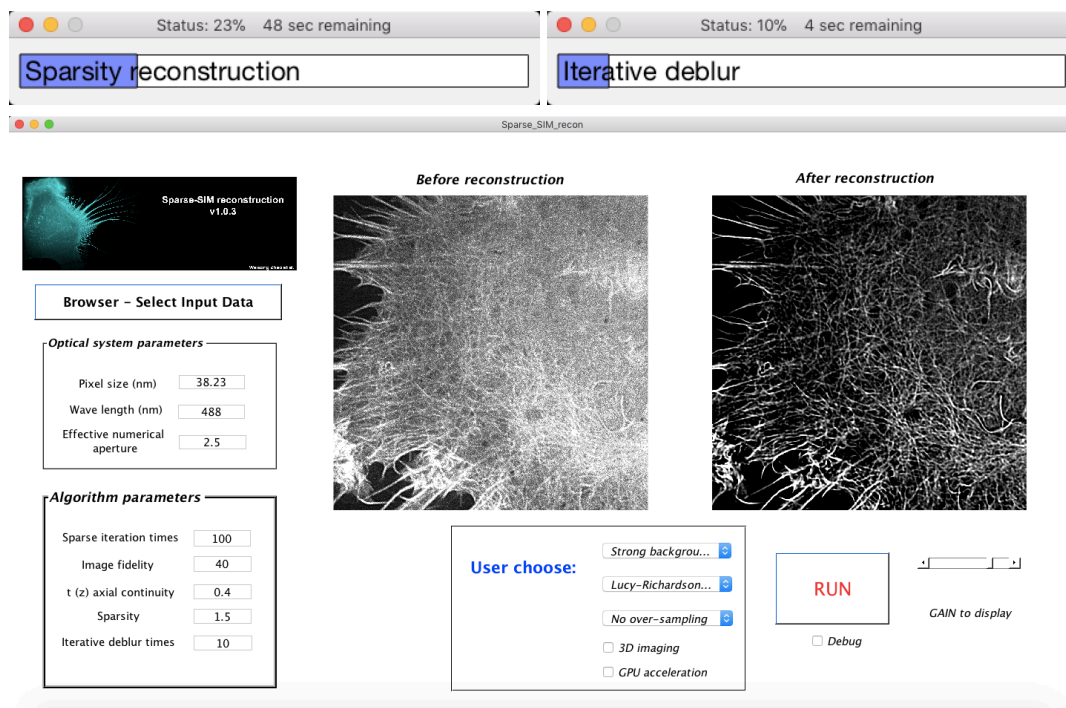
Step2. Set the parameters.



Step3. RUN



Step4. END



If the GPU memory is large enough to hold all variables, GPU processing is recommended for 60~100 fold improvement of execution speed. To achieve more effective GPU acceleration, the CUDA C code (.cu) for GPU acceleration is generated, and compiled to the binary form .mex. Specifically, the program that consumes the most computing resources are converted into CUDA mex:

back_diff_cuda.mexw64, and forward_diff_cuda.mexw64

These CUDA mex files are compiled in our local working stations. As they are related to the type of GPU (e.g. TITIAN RTX), the form of operating system (e.g. Windows 10), and the version of CUDA (e.g. 10.0), we did not apply these mex files in the released version of Sparse-SIM UI.

This software has been tested with Matlab R2017b on (Win 10: 128 GB and NVIDIA Titan Xp: 12GB; CUDA 9.1), Matlab R2019b on (Win 10: 128 GB and NVIDIA Titan RTX: 24GB; CUDA 10.0) and (MacOS 10: 8GB without GPU acceleration). One or more GPUs with large memory is recommended for fast execution (see Matlab documentation for supported GPU models).

/src_unix is the source code for Unix-like systems (including MacOS).

/src_win is the source code for Windows systems.

<https://github.com/WeisongZhao/Sparse-SIM/releases/download/v1.0.3/DATA.zip> holds all the provided example data.

<https://github.com/WeisongZhao/Sparse-SIM/releases/tag/v1.0.3> holds the binary executable files for MacOS, and Win10 systems.

License:

This software and corresponding methods can only be used for **non-commercial** use, and they are under GNU General Public License as published by the Free Software Foundation, version 3 of the license.