User manual for MRA & SecMRA deconvolution

This document helps users master the utilization of MRA and SecMRA deconvolution software, which is provided both in MATLAB file (.m) and executive file (.exe).

1. Software and source code download

MRA and SecMRA software can be downloaded as a Supplementary Code of the publication *Multi-resolution analysis enables fidelity-ensured deconvolution for fluorescence microscopy.*

It contains the following two .rar files:

MRA_exe.rar contains the MRA & SecMRA deconvolution software in the executable file format.

MRA_master.rar contains the MATLAB source code and GUI of the MRA & SecMRA deconvolution software.

2. Installation

First unzip the files, the master files in MRA_exe.rar are MRA.exe and SecMRA.exe, in MRA_master.rar are MRA.m and SecMRA.m.

To run the .exe files, the MATLAB software is not necessary but one should download and install the MATLAB runtime version 2020b for compiling from: https://ww2.mathworks.cn/products/compiler/matlab-runtime.html. After the installation, the MRA & SecMRA deconvolution software can be readily used.

To run the .m GUI, users need to install MATLAB at any version (this code is written based on MATLAB 2020b, different MATLAB version may have different function rules that influence the functioning). Open the MRA.m or SecMRA.m file, then click run to activate the software.

3. Functional part and parameter

The software interface of MRA and SecMRA deconvolution is shown below.

The functional part and parameter in *MRA deconvolution* are described as follows:

PSF parameter

- Wavelength: The emission wavelength used for fluorescence imaging
- Pixel size: The actual pixel size of the image
- NA: The numerical aperture used for fluorescence imaging
- Scaling factor: The resolution improvement offered by super-resolution microscopy

Spatiotemporal continuity denoising

- Activation: Decide whether to activate this functional part.
- 3D Framelet thresholding: The thresholding value for 3D framelet soft-thresholding, usually chosen in the range of 0.01-0.3.
- Maximum consecutive frame: The maximum consecutive frame used for spatiotemporal continuity denoising, usually chosen in the range of 10-100.

2D MRA deconvolution

- Activation: Decide whether to activate this functional part.
- Framelet thresholding: The thresholding value for 3D framelet soft-thresholding, usually chosen in the range of 0.0001-0.1.
- Curvelet thresholding: The thresholding value for 3D curvelet soft-thresholding, usually chosen in the range of 0.0001-0.1.
- RL times: The iteration time of Richardson-Lucy iteration, usually chosen in the range of 1-5.

The functional part and parameter in *SecMRA deconvolution* are described as follows:

PSF parameter

- Wavelength: The emission wavelength used for fluorescence imaging
- Pixel size: The actual pixel size of the image
- NA: The numerical aperture used for fluorescence imaging
- Scaling factor: The resolution improvement offered by super-resolution microscopy

Pre-debackground

- Activation: Decide whether to activate this functional part.
- Bias parameter: Measure the degree of discrimination with high background value, usually chosen in the range of 0.5-8.

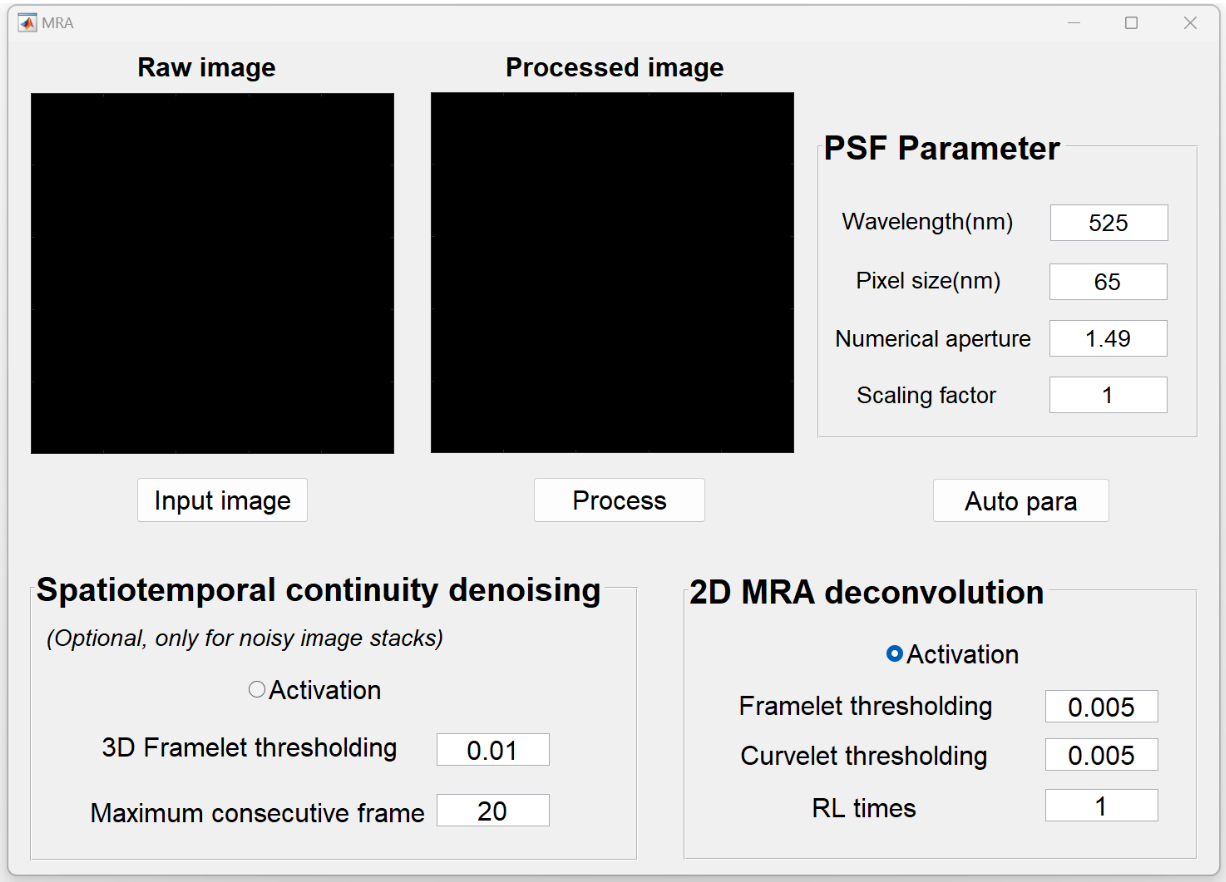
- Background thresholding: The global thresholding value, usually chosen in the range of 0.01-0.5.

Spatiotemporal continuity denoising

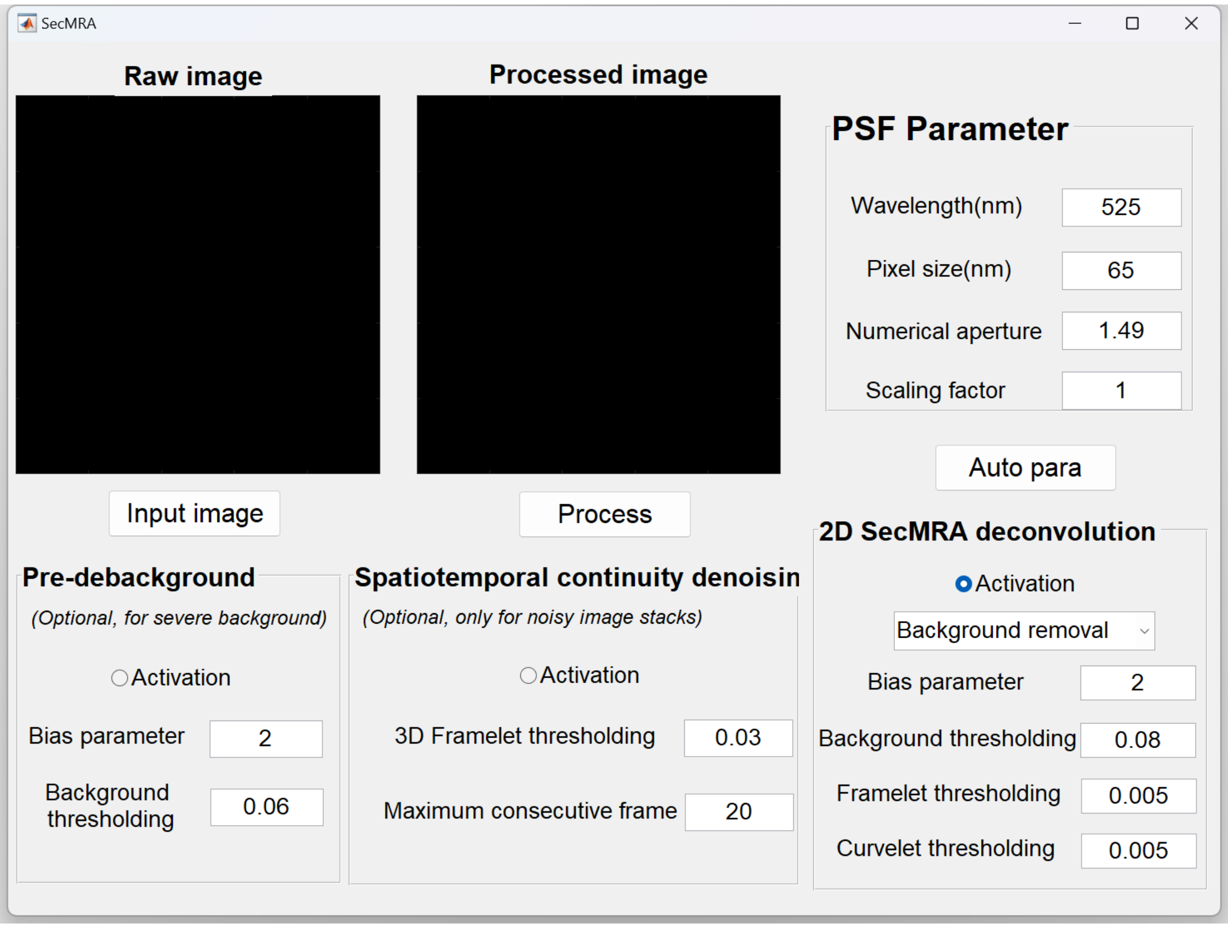
- 3D Framelet thresholding: The thresholding value for 3D framelet soft-thresholding, usually chosen in the range of 0.01-0.3.
- Maximum consecutive frame: The maximum consecutive frame used for spatiotemporal continuity denoising, usually chosen in the range of 10-100.

2D SecMRA deconvolution

- Activation: Decide whether to activate this functional part.
- Background removal & Intensity correction: The mode of 2D SecMRA deconvolution. For fluorescence background removal, choose background removal. For correcting undesired intensity distribution, choose intensity correction.
- Bias parameter: Measure the degree of discrimination with high background value, usually chosen in the range of 0.5-8.
- Background thresholding: The global thresholding value, usually chosen in the range of 0.01-0.5.
- Framelet thresholding: The thresholding value for 3D framelet soft-thresholding, usually chosen in the range of 0.0001-0.1.
- Curvelet thresholding: The thresholding value for 3D curvelet soft-thresholding, usually chosen in the range of 0.0001-0.1.



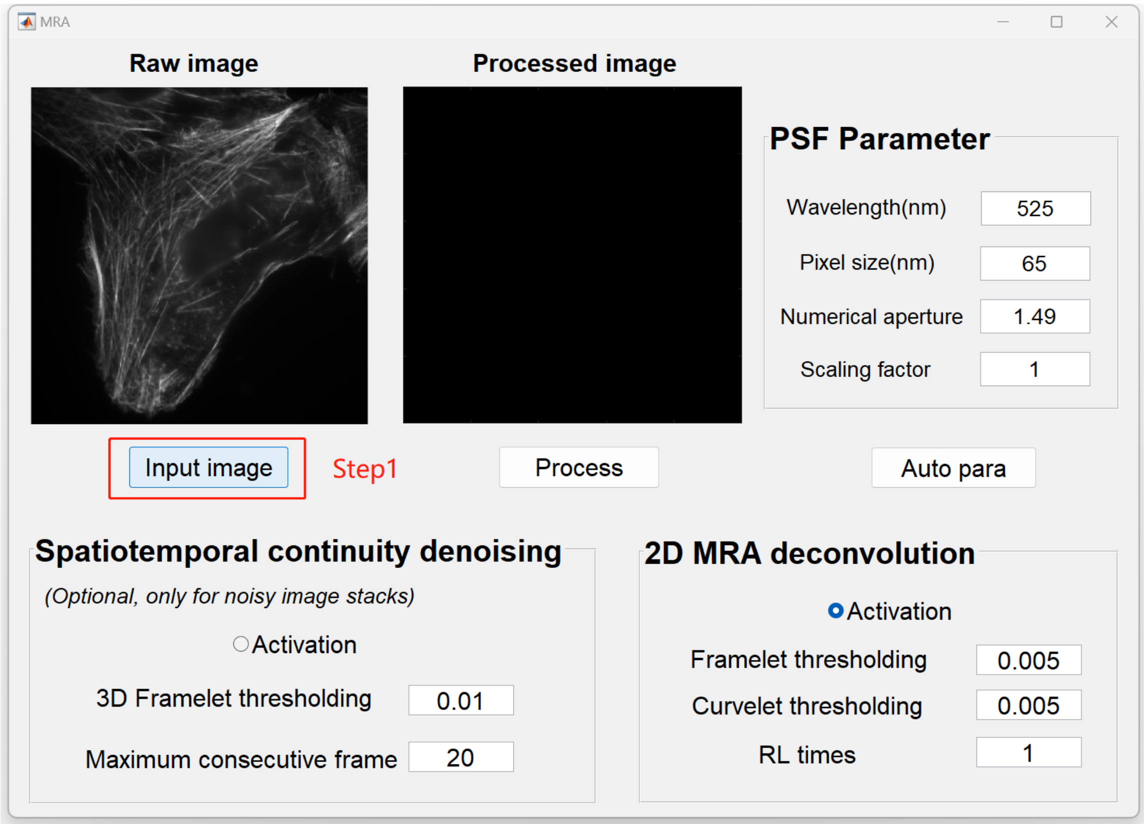
The software interface of MRA deconvolution.



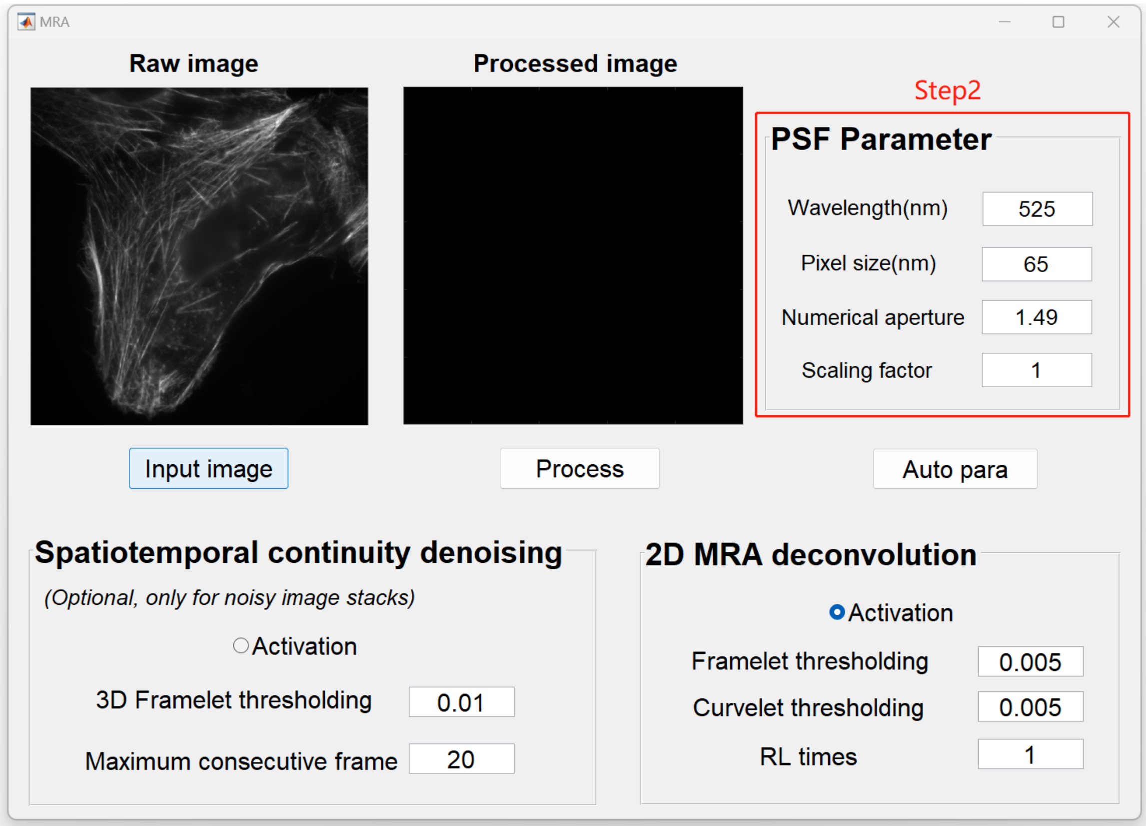
The software interface of SecMRA deconvolution.

4. Utilization

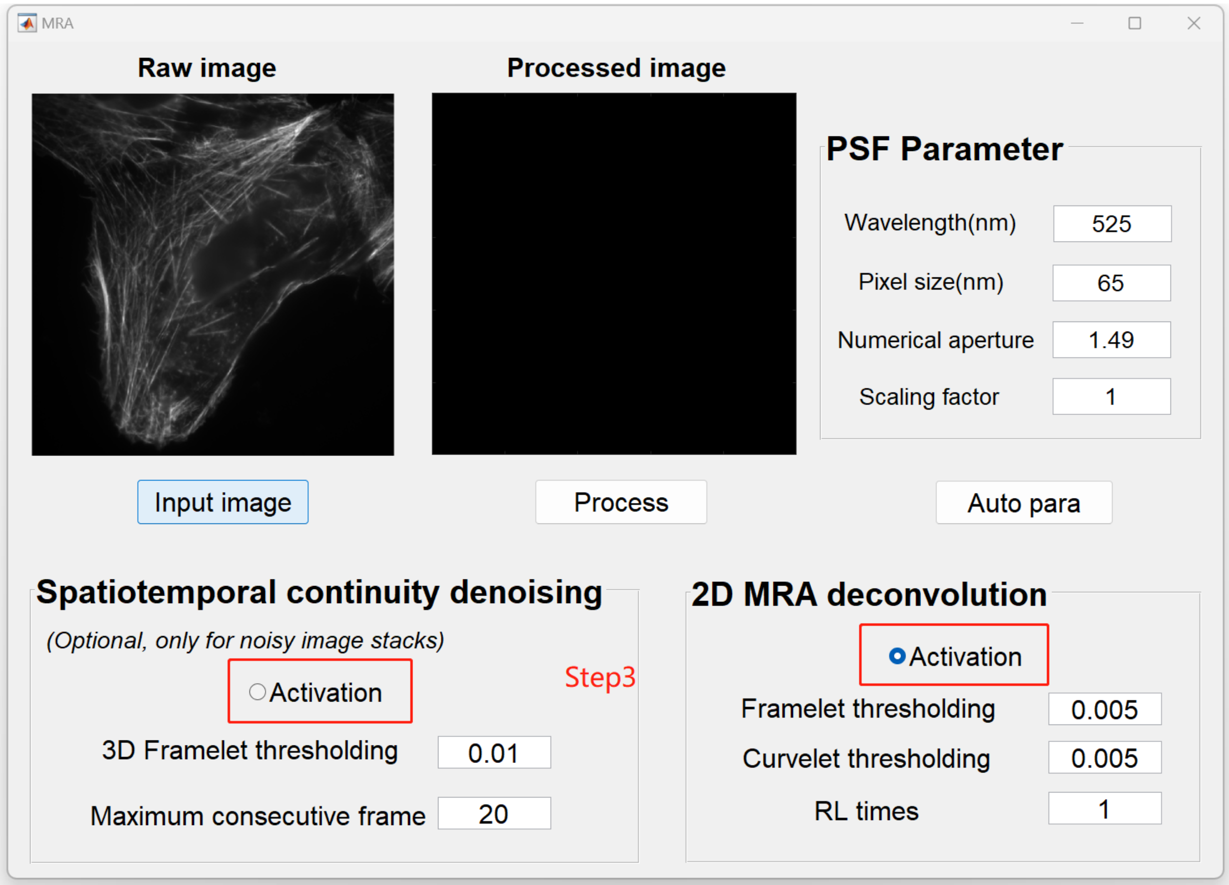
Step 1. Open the MRA or SecMRA software, then click the input image button to choose the image to be processed, the format must be .tif:



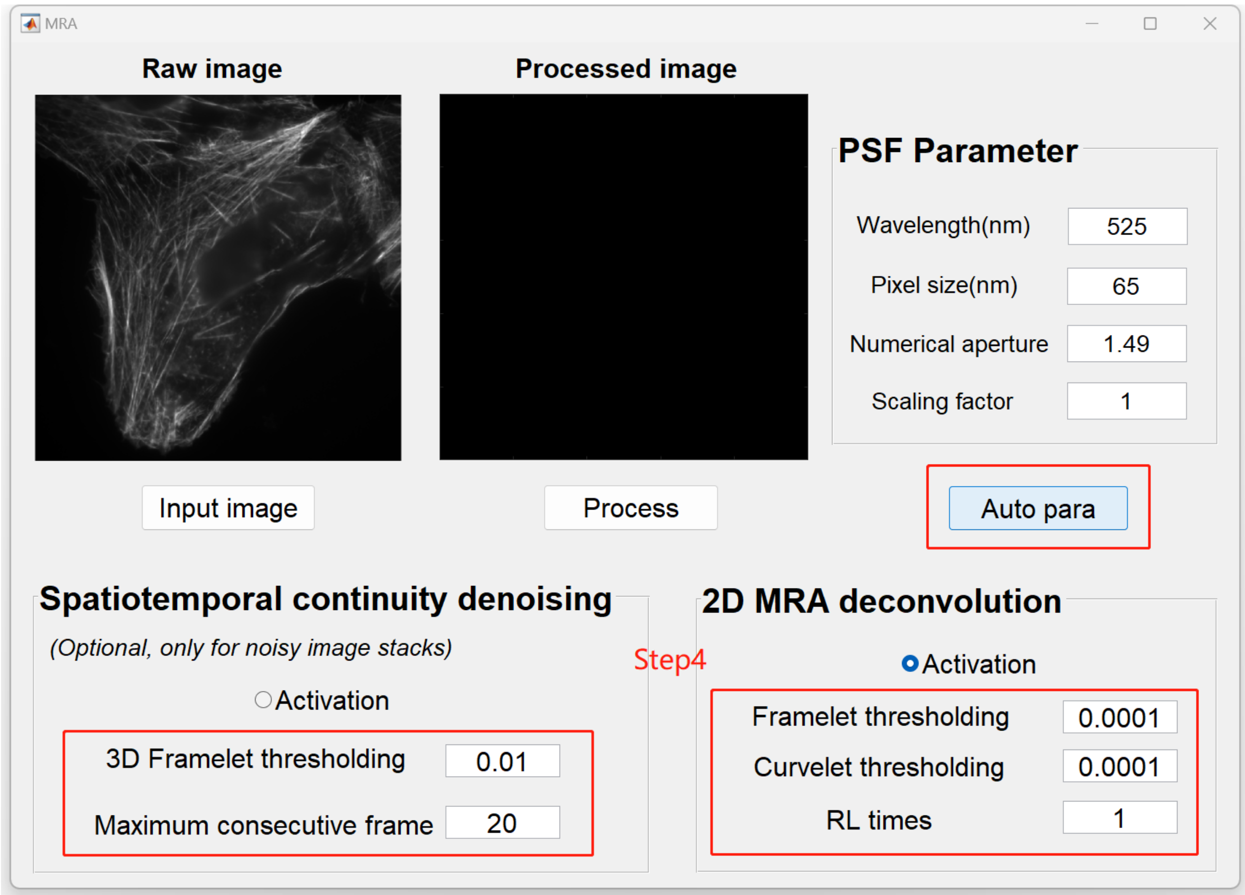
Step 2. Check the imaging parameter for PSF generation:



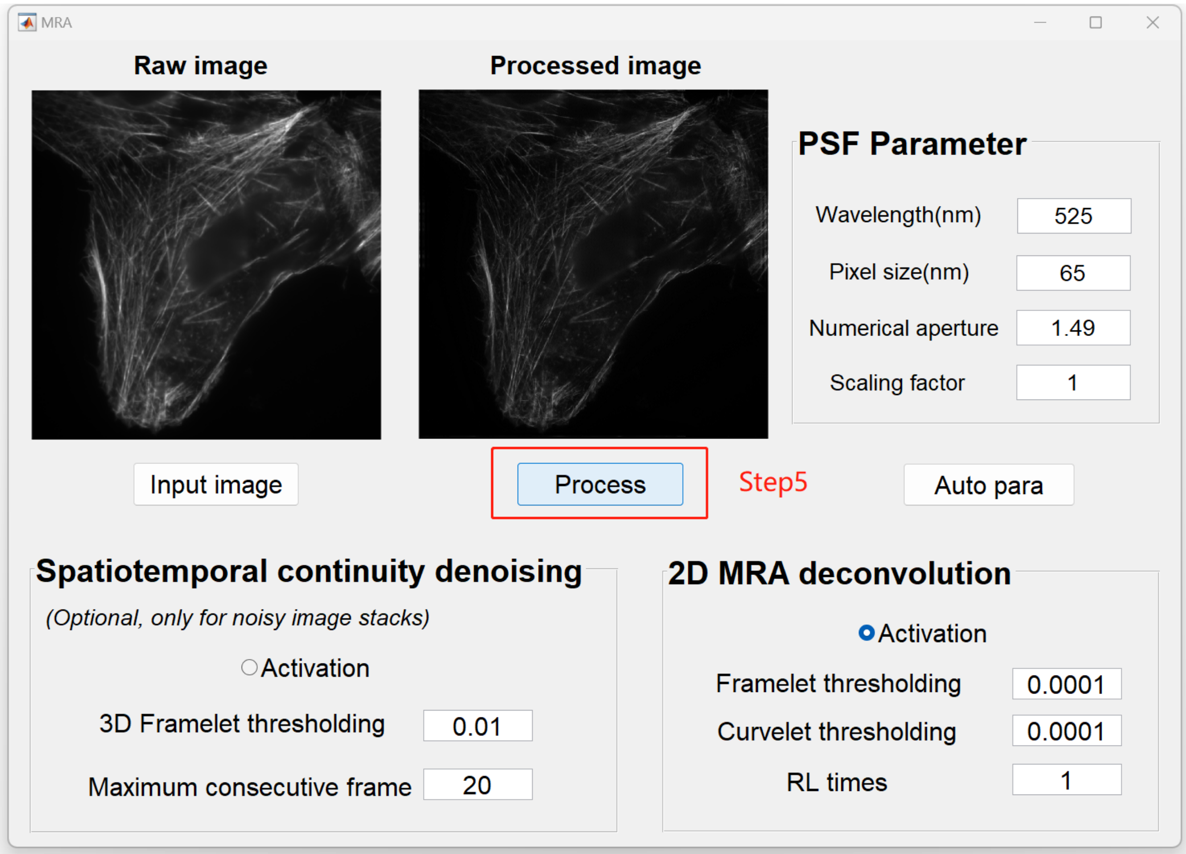
Step3. Decide the functional parts to be activated:

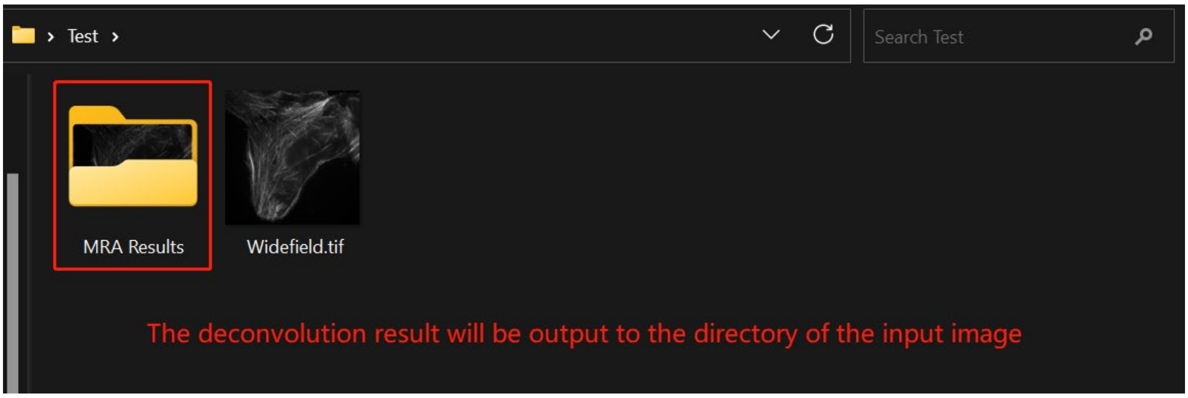


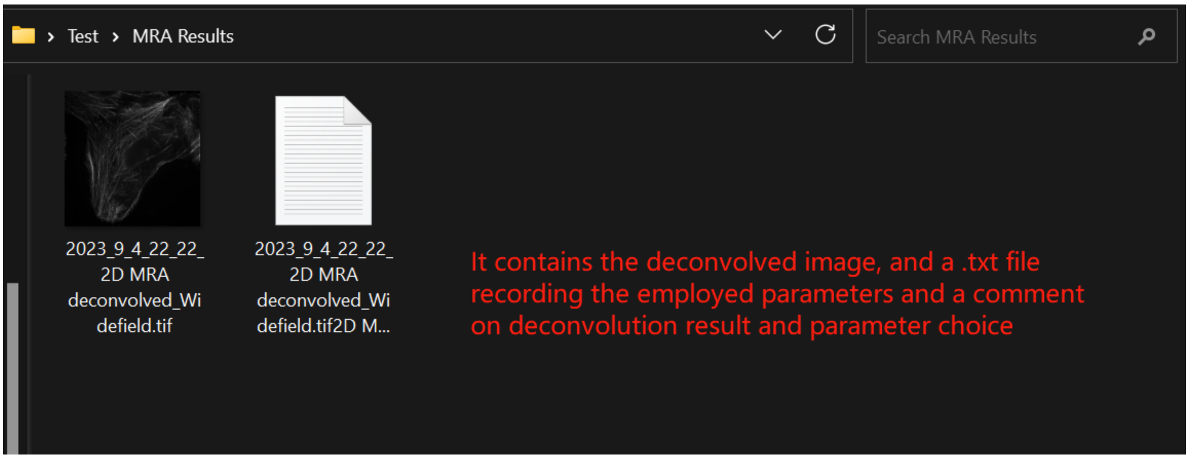
Step 4. Input the parameters used for deconvolution. For a quick deconvolution, click the "Auto para" button to automatically decide the parameters used for deconvolution



Step 5. Click the process button. The processed image will output in the directory of the input image.


MRA
Raw image
Processed image
PSF Parameter
Wavelength(nm) 525
Pixel size(nm) 65
Numerical aperture 1.49
Scaling factor 1
Input image
Process
Step5
Auto para
Spatiotemporal continuity denoising
(Optional, only for noisy image stacks)
Activation
3D Framelet thresholding 0.01
Maximum consecutive frame 20
2D MRA deconvolution
Activation
Framelet thresholding 0.0001
Curvelet thresholding 0.0001
RL times 1


Test
Search Test
MRA Results
Widefield.tif
The deconvolution result will be output to the directory of the input image


Test › MRA Results
Search MRA Results
2023_9_4_22_22_2D MRA deconvolved_Widefield.tif
2023_9_4_22_22_2D MRA deconvolved_Widefield.tif2D M...
It contains the deconvolved image, and a .txt file recording the employed parameters and a comment on deconvolution result and parameter choice

5. Interaction

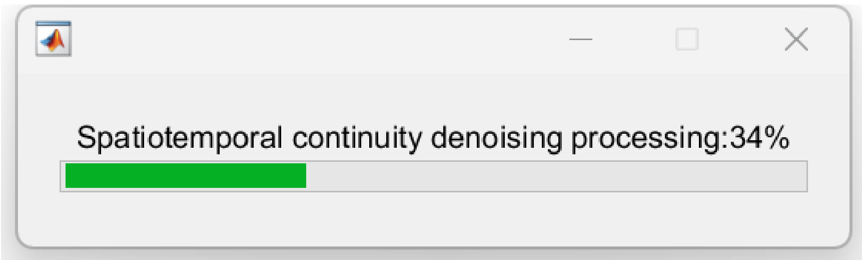
The software integrates the following interaction to help users understand the deconvolution process

(1) Reminder of progress

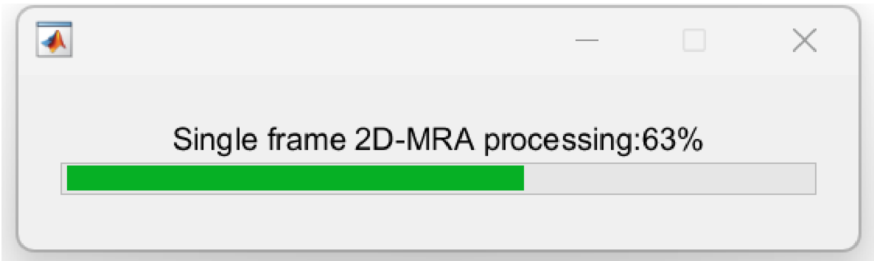
During the calculation, the processing progress is both shown in the command window of MATLAB and progress bar.

The progress printed in the MATLAB command window

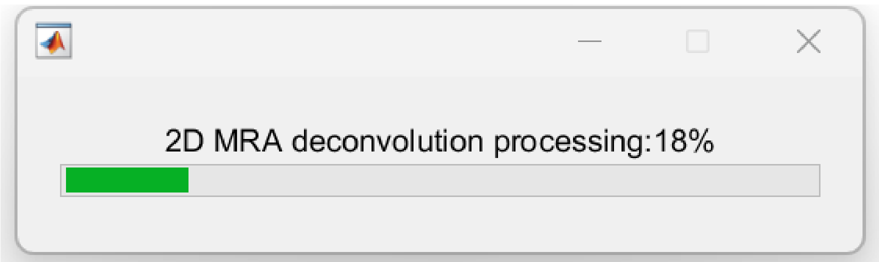
The progress bar of the spatiotemporal continuity denoising



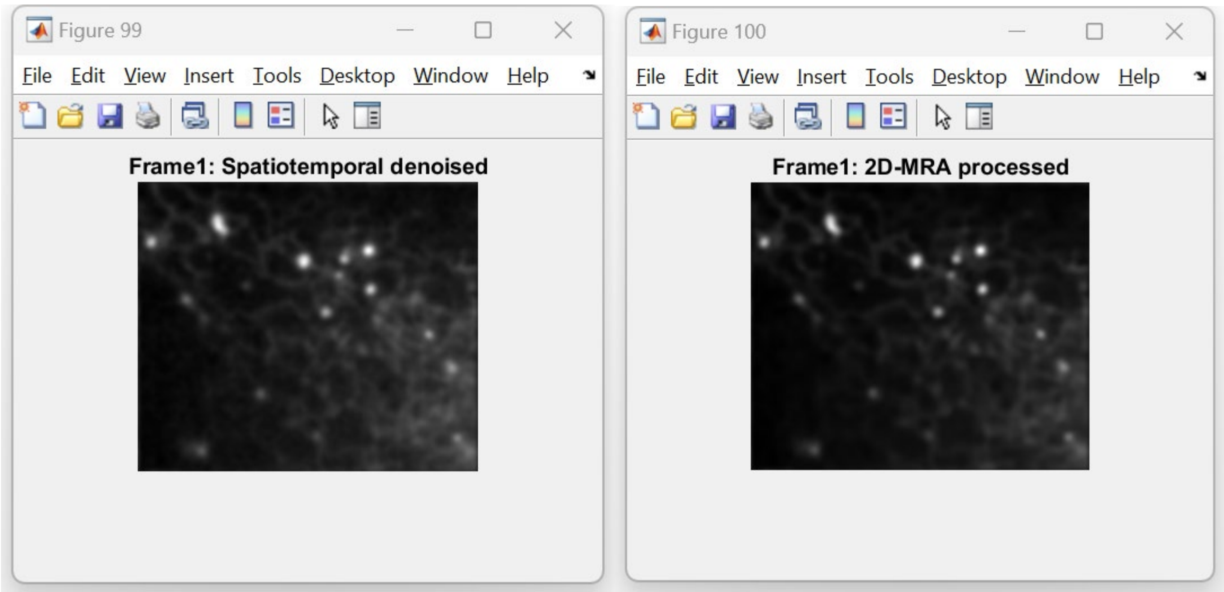
The progress bar of the 2D deconvolution for a single frame



The progress bar of the 2D deconvolution for an image stack

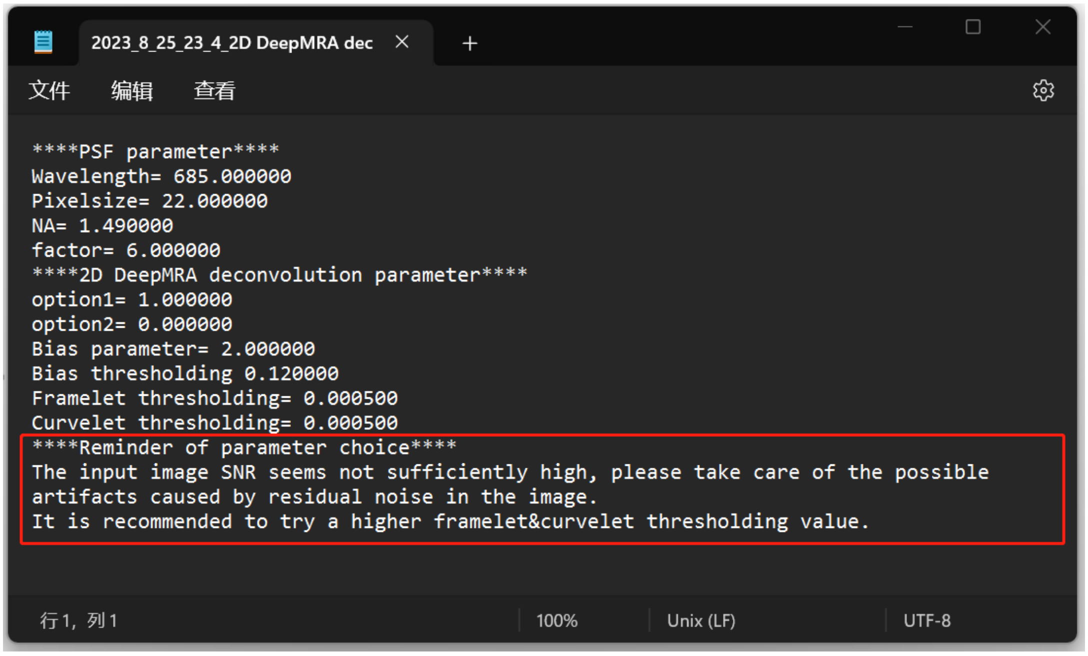


The first image after each processing procedure would display in a figure window. In case that improper parameters are chosen, one can detect through the figure window and shut down the processing to avoid wasting of time.

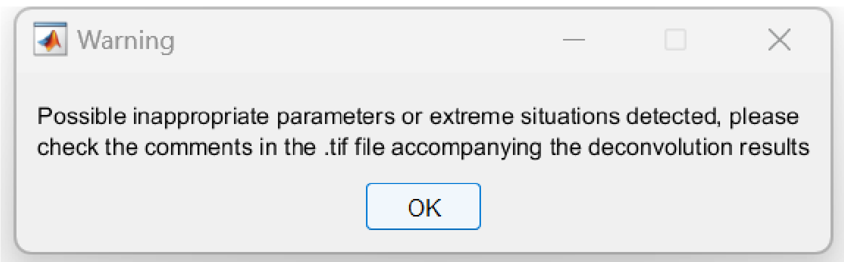


(2) Comment on parameter choice

After each deconvolution, a. tif file is generated to record the parameters used for this deconvolution and the evaluation of the parameters used. Users should view this file to grasp the deconvolution results:



If the parameters are not used properly, a messenger box will also pop up in the software to remind the user after the deconvolution is completed:

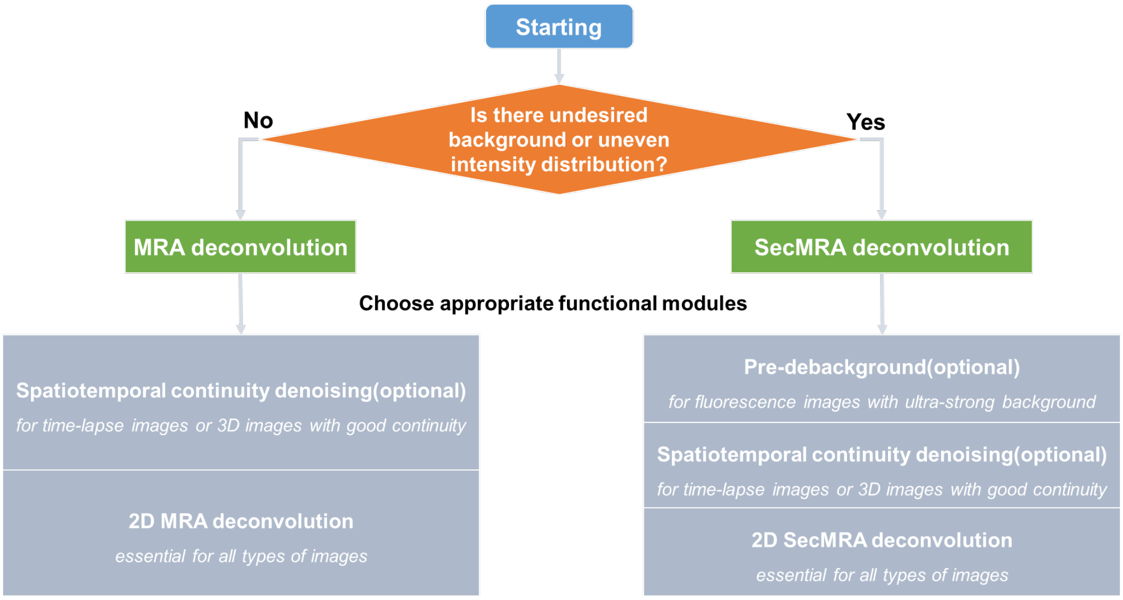


6. Parameter tuning guidance

Though the software has provided auto parameter function, it may not always give the optimal solution since the imaging and sample conditions are very complex. Here we also provide a guideline for manual parameter adjustment.

Step 1: Choosing deconvolution model

The first step for fluorescence image optimization is choosing the proper deconvolution model for it. If the fluorescence image contains undesired background information or uneven intensity distribution, choose SecMRA deconvolution, else choose MRA deconvolution.



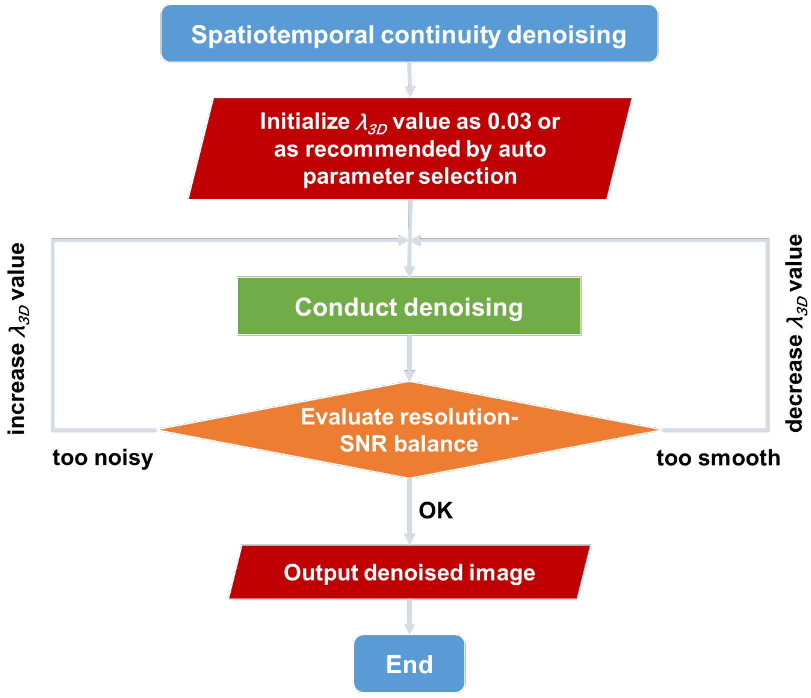
Procedure of choosing MRA and SecMRA deconvolution functional module

Step 2: MRA deconvolution parameter tuning

Step 2.1 Spatiotemporal continuity denoising (optional)

This step only needs to be activated when 2D deconvolution cannot effectively remove noise, and the input 3D data has good continuity in temporal or axial direction. Usually, we suggest skipping this step and performing 2D deconvolution first, as 3D thresholding generally leads to the loss of fine details. If the SNR of the input image is very low and a thresholding value >0.05 is used in 2D deconvolution but still cannot effectively control the noise, consider activating this step.

In this step, the parameter needed for tuning is the thresholding value of 3D framelet coefficient, which is symbolled by $\lambda_{3D\text{-}framelet}$. We can first initialize $\lambda_{3D\text{-}framelet}$ through automatic parameter selection, or put it as 0.01. If the image still contains noise after processing at current $\lambda_{3D\text{-}framelet}$ value, we just need to increase it and try again. On the contrary, if the image is over-smoothed at current $\lambda_{3D\text{-}framelet}$ value, we need to reduce its value. When finding a situation where most of the noise in an image has been removed, parameter tuning is accomplished.



Procedure of spatiotemporal continuity denoising parameter finetuning.

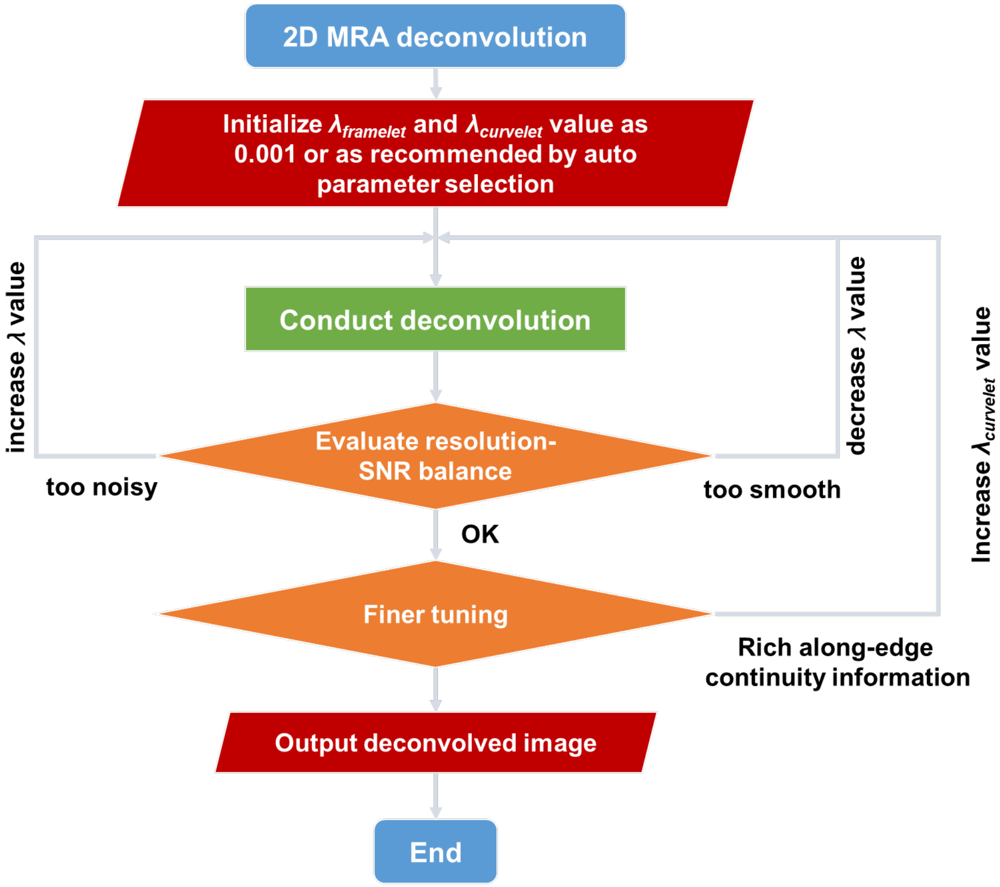
Step 2.2 2D MRA deconvolution

In 2D MRA deconvolution, firstly several parameters should be input to generate the theoretical PSF used for deconvolution, including emission wavelength, pixel size, and numerical aperture (NA). For super-resolution imaging, we add a PSF scaling factor to consider the PSF modulation by the SR technique, which denotes the estimated multiple of resolution improvement compared to a widefield microscope.

The parameters needed to be tuned in the 2D MRA deconvolution are the regularization parameter of framelet and curvelet, which are symbolled by $\lambda_{framelet}$ and $\lambda_{curvelet}$, respectively. We can initialize $\lambda_{framelet}$ and $\lambda_{curvelet}$ with the same value as suggested by the automatic parameter selection or at 0.001.

The logics of tuning $\lambda_{framelet}$ and $\lambda_{curvelet}$ is simple: If the image still contains noise after processing at current thresholding value, increase it. On the contrary, if the image is over-smoothed at current value, reduce it. Also remind checking the .txt file after deconvolution through our software, which gives comment on the parameter choice.

For most cases, just holding the same $\lambda_{framelet}$ and $\lambda_{curvelet}$ value can generate satisfactory results. If the imaging sample contains rich along-edge continuities, such as microtubule, actin or mitochondria with clear cristae, increasing the portion of $\lambda_{curvelet}/\lambda_{framelet}$ could yield a better result.

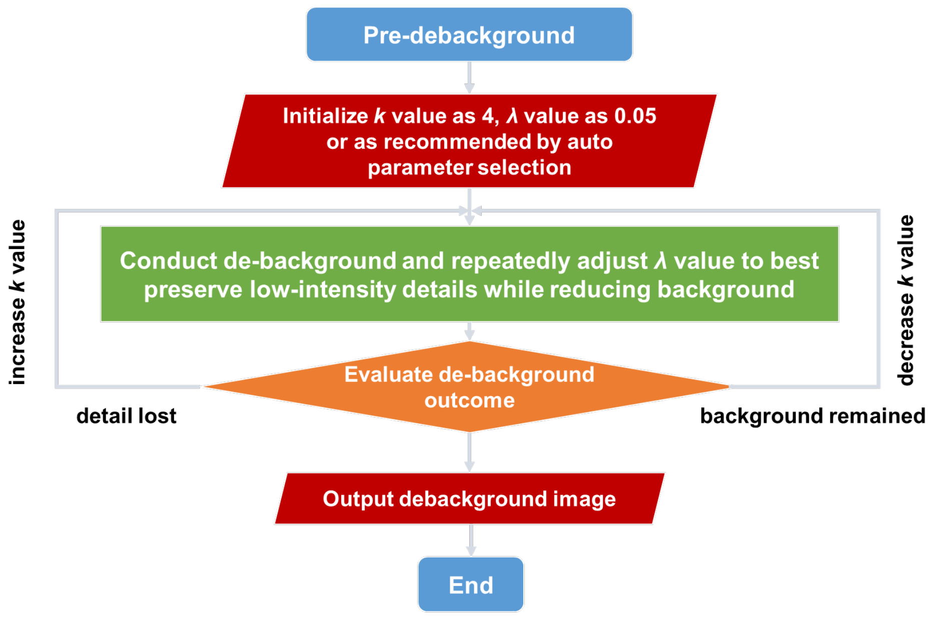


Procedure of 2D MRA deconvolution parameter finetuning.

Step 3: SecMRA deconvolution parameter tuning

Step 3.1 Preliminary background subtraction (optional)

This step is only used for images with ultra-strong fluorescence background. The parameter needed for tuning in this step is the bias coefficient $\boldsymbol{k}$ and thresholding value $\boldsymbol{\lambda}$. The inhomogeneity of the background determines the choice of bias coefficient $\boldsymbol{k}$. For simplicity of parameter tuning, we recommend starting $\boldsymbol{k}$ value at 4. Then we start $\boldsymbol{\lambda}$ value at 0.05 and repeatedly adjust the $\boldsymbol{\lambda}$ value to subtract most of the backgrounds while preserving fine details. If under current bias coefficient $\boldsymbol{k}$, by adjusting $\boldsymbol{\lambda}$ value background reduction is achieved but some interested weak signals are removed, then we increase the $\boldsymbol{k}$ value and then continue adjusting the $\boldsymbol{\lambda}$ value. Else if the bias background is over-mitigated and the global background is not removed, then we increase the $\boldsymbol{k}$ value and then continue adjusting the $\boldsymbol{\lambda}$ value. We repeat this process until the optimal result is achieved. Usually, an integer adjustment of the $\boldsymbol{k}$ value is enough to generate satisfactory results. More subtle adjustments may result in better results.



Procedure of preliminary background subtraction parameter finetuning.

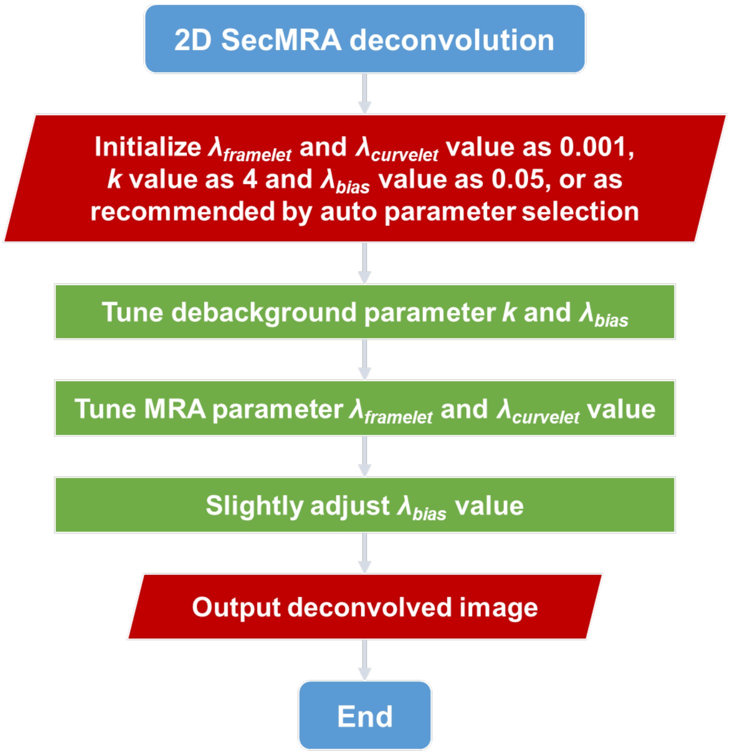
Step 3.2 Spatiotemporal continuity denoising (optional)

The principle for tuning this part is the same as that discussed for MRA deconvolution.

Step 3.3 2D SecMRA deconvolution

Similar to 2D MRA deconvolution, to execute 2D SecMRA deconvolution, emission wavelength, pixel size, and NA should be given to generate the theoretical PSF for deconvolution.

The parameters needed to be tuned in the 2D SecMRA deconvolution include: (1) Background mitigation parameter, including the bias coefficient $\boldsymbol{k}$ and background thresholding value $\boldsymbol{\lambda}$. (2) MRA parameter, including $\boldsymbol{\lambda_{framelet}}$ and $\boldsymbol{\lambda_{curvelet}}$. We start with the default parameter value, and first tune the background mitigation parameter according to the principle introduced in Step 3.1, then adjust the MRA parameter according to the principle introduced in Step 2.2. If the MRA parameter tuning influences the global contrast, we can then slightly tune the bias thresholding value $\boldsymbol{\lambda}$ to adjust the image. Supplementary Fig. 24 shows the flow chart of the parameter fine-tuning in 2D SecMRA deconvolution.



Procedure of 2D-SecMRA deconvolution parameter finetuning.

Key points for parameter tuning:

- If the image contains heavy background or uneven intensity distribution that is expected to alleviate, choose SecMRA software, else choose MRA software.
- The "*2D-MRA deconvolution*" and "*2D-SecMRA deconvolution*" functionalities are enough to deal with most cases. Only consider opening other parts when 2D functionality cannot meet the requirements well. For extremely noisy 3D image stacks with good continuity, consider activating "*Spatiotemporal continuity denoising*". When the background is too severe and cannot be eliminated, consider turning on "*Pre-debackground*".
- If the results of automatic parameter adjustment do not meet the requirements, the parameters can be quickly adjusted as follows:

  (1) Denoising-deblurring balance: If you want to improve the resolution of the obtained image, reduce "*Framelet thresholding*" and "*Curvelet thresholding*". If there is residual noise in the image that has not been removed, increase "*Framelet thresholding*" and "*Curvelet thresholding*". If spatiotemporal continuity denoising is activated, similarly adjust "*3D Framelet thresholding*".

  (2) Uniform-nonuniform debackground balance: If the uniform background is not well eliminated, increase "*Background thresholding*". Else if the heavy background in some areas has not been removed, increase "*Bias parameter*". If some low-intensity signal is removed, increase "*Bias parameter*" and reduce "*Background thresholding*". If the heavy background region is excessively attenuated, reduce "*Bias parameter*" and increase "*Background thresholding*".

"*RL times*" does not contribute to the improvement of real resolution, but serve as a functionality to sharpen images and enhance visual perception. It should be less than 5 times, otherwise it would result in significant artifacts.

7. References

The development of MRA and SecMRA software refers to the code in the following works:

[1] Beck, A. & Teboulle, M. A fast iterative shrinkage thresholding algorithm for linear inverse problems. SIAM Journal on Imaging Sciences **2**, 183-202 (2009).

[2] Candes, E., Demanet, L., Donoho, D., Ying, L. Fast discrete curvelet transforms. *Multiscale modeling & simulation* **5**, 861-899 (2006).

[3] Unser, M.A., Selesnick, I.W., Aldroubi, A., Li, K.Y. & Laine, A.F. in Wavelets: Applications in Signal and Image Processing X (2003).

[4] FrameLab in https://github.com/nhenscheid/FrameLab/blob/dcc96cb950d15d9d4c40e4d2c451d5c9ff737ad8/matlab/classes/%2BTransforms/%40FrameletSystem

[5] Hansen P C, Nagy J G, O'leary D P. Deblurring images: matrices, spectra, and filtering[M]. Society for Industrial and Applied Mathematics, 2006.