

Supplementary MATLAB toolbox structured illumination microscopy based on principal component analysis

Jiaming Qian^{1,2,3}, Yu Cao^{1,2,3}, Ying Bi^{1,2,3}, Hongjun Wu^{1,2,3}, Yongtao Liu^{1,2,3}, Qian Chen^{3,*}, and Chao Zuo^{1,2,3,*}**

¹Smart Computational Imaging (SCI) Laboratory, Nanjing University of Science and Technology, Nanjing, Jiangsu Province 210094, China

²Smart Computational Imaging Research Institute (SCIRI) of Nanjing University of Science and Technology, Nanjing, Jiangsu Province 210094, China

³Jiangsu Key Laboratory of Spectral Imaging & Intelligent Sense, Nanjing University of Science and Technology, Nanjing, Jiangsu Province 210094, China

*zuochao@njust.edu.cn

**chenqian@njust.edu.cn

ABSTRACT

This document provides an introduction to the open source MATLAB toolbox—PCASIMtoolbox and associated dataset, as well as the corresponding user guide and demo results.

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- A. Overview of PCA-SIM MATLAB toolbox**
- B. Installing PCA-SIM MATLAB toolbox**
- C. User guide for the first test demo**
- D. More simulation and experimental demos**

A. Overview of PCA-SIM MATLAB toolbox

Supplementary codes and dataset contain two folds (the “Test” folder, the “Simulation” folder) and one TEXT file (*.txt). The “Test” folder includes two subfolders (the “RawImage” folder, the “PCASIMtoolbox” folder) and 1 MATLAB files (*.m):

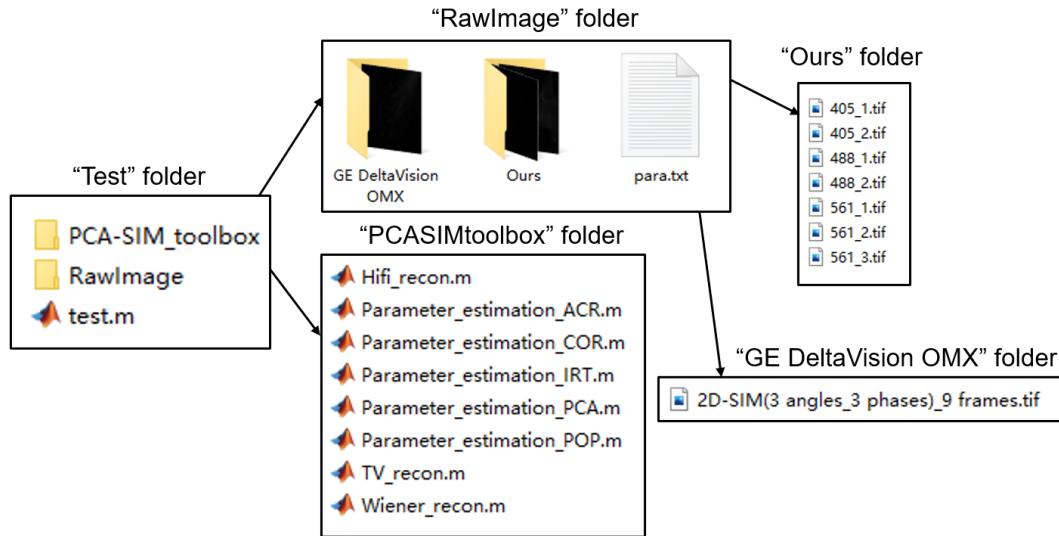


Figure S1. The “Test” directory.

- The folder “PCASIMtoolbox” contains an open-source, modular set of MATLAB functions for structured illumination microscopy (SIM), which provides five different parameter estimation methods (phase-of-peak (POP)¹, non-iterative auto-correlation (ACR)², image recombination transform (IRT)³, the cross-correlation approach (COR)⁴, and the proposed parameter estimation approach based on principal component analysis (PCA)), and three image reconstruction methods (Wiener reconstruction⁵, TV-SIM⁶ and HiFi-SIM⁷). The main function files include:
 - **Para_estimation_POP.m**: codes for the parameter estimation based on POP, which was implemented with reference to the literature¹.
 - **Para_estimation_ACR.m**: codes for the parameter estimation based on ACR, which was implemented with reference to the literature².
 - **Para_estimation_IRT.m**: codes for the parameter estimation based on IRT, which was implemented with reference to the literature³.
 - **Para_estimation_COR.m**: codes for the parameter estimation based on COR, which followed the procedure in fairSIM⁸.
 - **Para_estimation_PCA.m**: codes for the proposed parameter estimation approach based on PCA.

- `Wiener_recon.m`: codes for the Wiener reconstruction, which was adapted from HiFi-SIM⁷.
- `HiFi_recon.m`: codes for the reconstruction algorithm based on HiFi-SIM, which was adapted from HiFi-SIM⁷.
- `TV_recon.m`: codes for the reconstruction algorithm based on TV-SIM, which was adapted from Hessian-SIM⁹.
- The file “`test.m`”: demo codes for implementing the entire SIM super-resolution process, including the pre-processing of raw SIM images, experimental parameter estimation, and image reconstruction.
- The folder “`RawImage`” provides the raw SIM images captured by our home-made SIM microscope and commercial SIM microscope, and a text file (“`para.txt`”) describing the system parameters of these data:
 - “**Ours**” folder contains the raw SIM images collected by our home-made microscope with a resolution of 1024×1024 :
 - `405_1.tif`, `488_1.tif` and `561_1.tif`: the raw SIM images of a CV-1 in Origin Simian-7 (COS-7) cell sample with DAPI-labeled nucleus, Alexa FluorTM 568-labeled actin and MitoTrackerTM Green FM-labeled mitochondria. `405_1.tif` are the raw SIM images of the nucleus excited by 405 nm laser, `488_1.tif` are the images of mitochondria excited by 488 nm laser, `561_1.tif` are those of actin excited by 561 nm laser. These images were captured through a $100\times$ objective (UPlanSApo $100\times/1.40$ Oil, Olympus, Japan), and are the raw data for Fig. 3 in the main text.
 - `405_2.tif`, `488_2.tif` and `561_2.tif`: another set of raw SIM images of the COS-7 cell sample captured through the $100\times$ objective (UPlanSApo $100\times/1.40$ Oil, Olympus, Japan).
 - `561_3.tif`: the raw SIM images of actin of the COS-7 cell excited by 561 nm laser. These data were acquired by a $60\times$ objective (UPlanXApo $60\times/1.42$ Oil, Olympus, Japan), and are the raw data for Supplementary Fig. 8.
 - “**GE DeltaVision OMX**” folder contains the raw SIM images collected by GE DeltaVision OMX under conventional 2D-SIM mode with a resolution of 512×512 :
 - `2D-SIM(3 angles_3 phases)_9 frames.tif`: the raw SIM images of the microtubules in a COS-7 cell excited by 488 nm laser, which were provided by the literature⁷, and are the raw data for Supplementary Fig. 11.

The “**Simulation**” folder contains the codes and data for simulating the performance of different SIM algorithms at different signal-to-noise ratios (SNRs). The file “`License.txt`” is our copyright notice for the data codes.

B. Installing PCA-SIM MATLAB toolbox

First, unzip the downloaded toolbox file and copy it to the ‘toolbox’ folder under the MATLAB installation directory, for example, “D:\Pogarm File\matlab\toolbox”. Then click the “Set Path” button in MATLAB, find PCASIMtoolbox and add its path, as shown in Fig. S2. After adding the path, follow the steps shown in Fig. S3 to update the toolbox file path. Finally, enter “type para_estimation_pop” in the command window; if the source codes of the function is displayed in the command window, the toolbox is installed successfully.

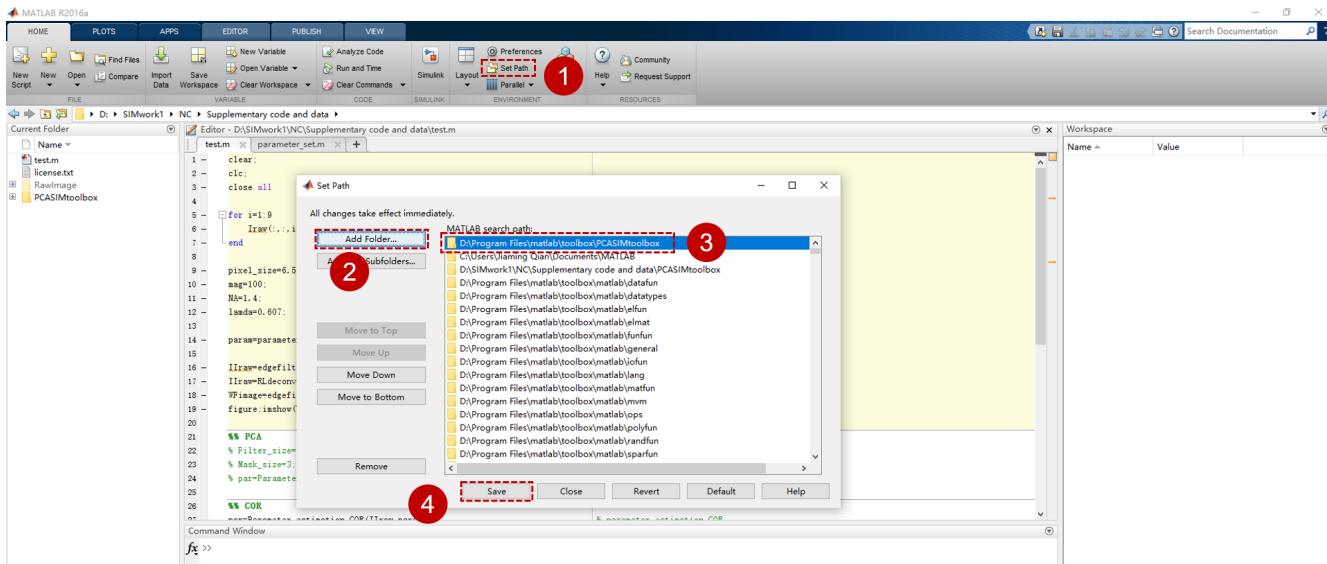


Figure S2. Steps to add PCASIMtoolbox’s path to Matlab.

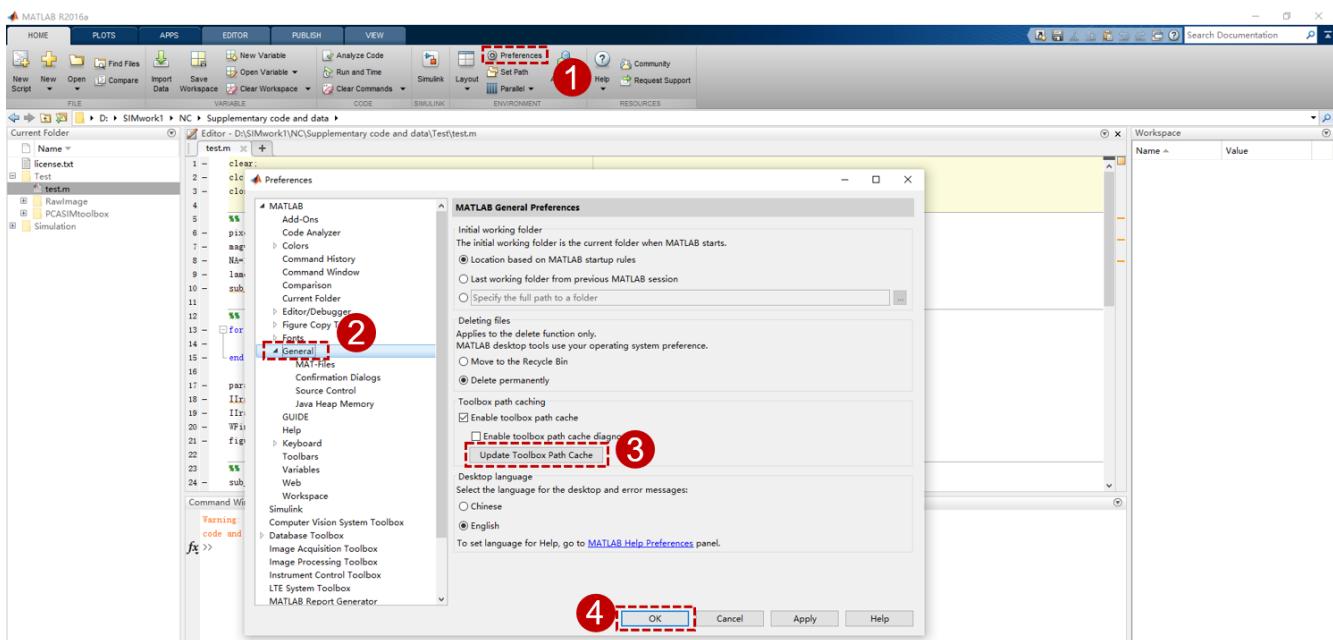


Figure S3. Steps to update the toolbox file path.

C. User guide for the first test demo

c1. System and sample parameter setting

Before running the demo codes (“[Test.m](#)”), it is necessary to set the system parameters, which include [Fig. [S4](#)]:

- Objective magnification
- Objective numerical aperture (NA)
- Camera pixel size
- Wavelength of the excited fluorescence

```
%% Set internal parameter
pixel_size=6.5;
mag=100;
NA=1.4;
lambda=0.607;
```

% Pixel size
% Magnification
% NA
% Fluorescent wavelength

Figure S4. Code for setting the system parameters.

The system parameters for the provided experimental data are listed on the text file “[para.txt](#)”. Through these parameters, an approximate point spread function (PSF) or optical transfer function (OTF) of the microscope can be calculated with the function “[parameter_set](#)”, as shown in Fig. [S5](#). Users can change these parameters to suit their system and samples.

c2. Experimental parameter estimation

When running the demo codes “[test.m](#)”, the raw SIM images are first read. Take “[RawImage/Ours/561_1.tif](#)” (the actin images of the COS-7 cell) as an example, the read nine raw structured illumination patterns are illustrated in Fig. [S6](#). From the magnified region in Fig. [S6](#) we can see the dense interference fringes. As a precaution against edge-related artifacts, the raw images are slightly edge-attenuated with the function “[edgefilter](#)”⁴. The Richardson-Lucy deconvolution¹⁰ is applied to remove certain out-of-focus background and noise with the function “[RLdeconv](#)”. Then the Fourier spectrums of the preprocessed SIM images, where the high frequency information of the sample is modulated to the passband, can be obtained through Fourier transform [Fig. [S7](#)].

To demodulate the high-frequency information in the spectrums for high-quality super-resolution reconstruction, it is essential to estimate reliable experimental parameters, which include:

- Wave vector
- Initial phase

```

function [out]=parameter_set(Iraw,Pixelsize,NA,lambda,mag)
    NPixel=size(Iraw,1);
    param.imgSize = NPixel;                                % Image size
    param.micronsPerPixel = Pixelsize/mag;                % Pixel size
    param.cyclesPerMicron = 1/(NPixel*param.micronsPerPixel); % Objective NA
    param.NA = NA;                                       % Wavelength (nm)
    param.lambda = lambda*1000;                           % Cutoff frequency 2NA
    param.cutoff = 1000/ (0.5*param.lambda/param.NA);     % Cutoff frequency radius
    param.sampleLateral = ceil(param.cutoff/param.cyclesPerMicron)+1; % Bands
    param.nrBands = 2;                                    % Phase offset initial value
    param.phaOff=0;                                     % Modulation initial value
    param.fac=ones(1,param.nrBands);
    param.attStrength = 0;
    param.OtfProvider = SimOtfProvider(param, param.NA, param.lambda, 1); % Generate approximate OTF
    PSF = abs(otf2psf((param.OtfProvider.otf)));          % Generate approximate PSF
    param.OTF=param.OtfProvider.otf;
    param.psf=abs(otf2psf((param.OtfProvider.otf)));
    out=param;
end

```

Figure S5. Code for calculating approximate PSF or OTF (function “parameter_set”).

- Modulation depth

Users can choose to call these functions “Para_estimation_POP.m”, “Para_estimation_ACR.m”, “Para_estimation_IRT.m”, “Para_estimation_COR.m”, and “Para_estimation_PCA.m” to perform the parameter estimation based on the corresponding algorithm. In particular, COR and PCA are capable of estimating wave vectors with sub-pixel accuracy and, on this basis, initial phase and modulation depth:

- The key to the COR-based approach is the sub-pixel optimization in the form of real-space phase gradient, and the step size of the subpixel optimization can be controlled by the variable “search”.
- The key to the PCA-SIM is the principal component analysis of the center of the 1-order spectrum using singular value decomposition (SVD). We also introduce a masking operator, which comprises a signal window and a padding window, to further optimize PCA results. The sizes of the signal window and the padding window are adjusted by the variables ‘Mask_size’ and ‘Filter_size’, respectively.

After running “Para_estimation_COR.m” or “Para_estimation_PCA.m”, the command window of MATLAB will output the running time of the corresponding algorithm.

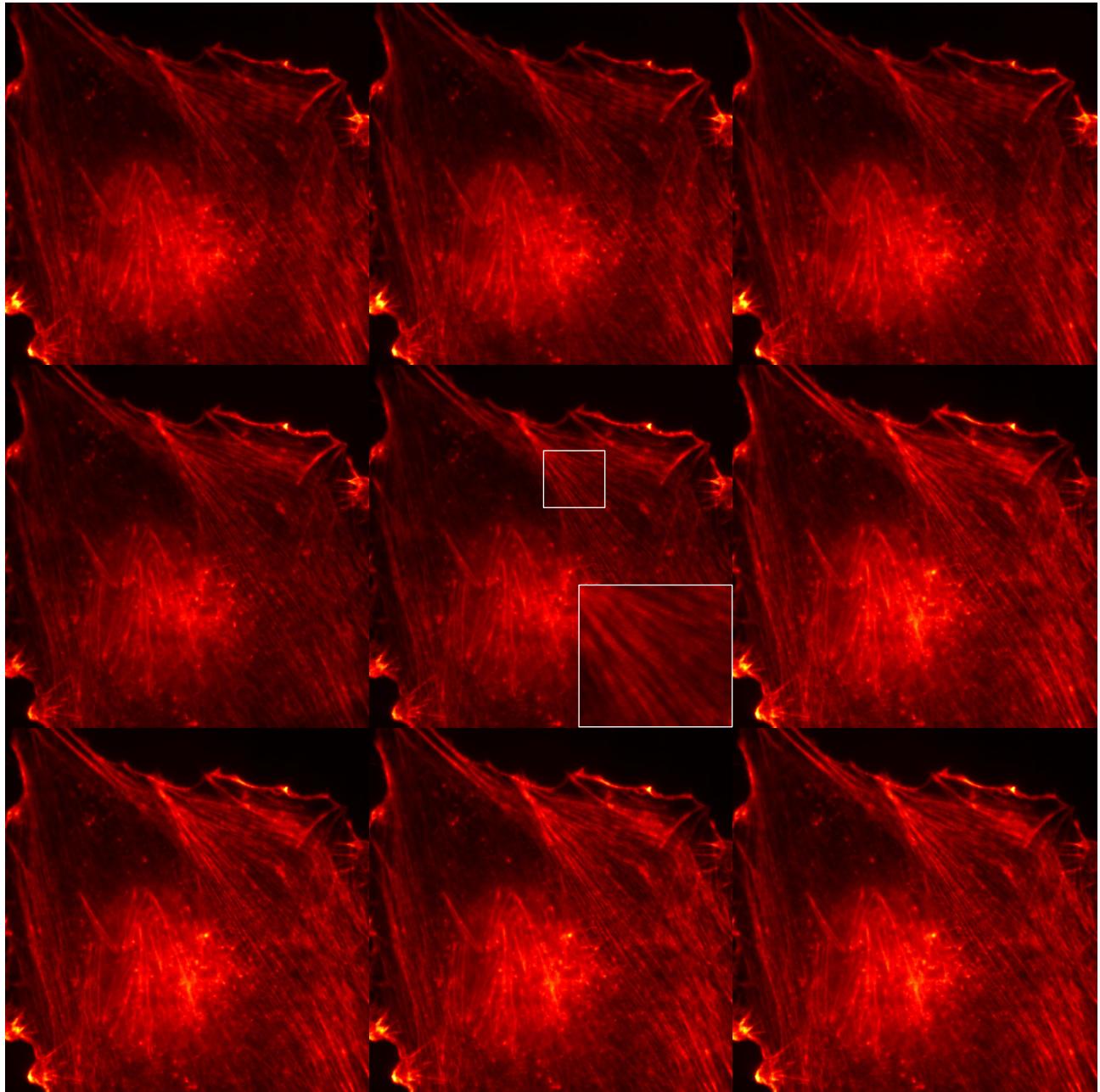


Figure S6. The read nine raw structured illumination patterns.

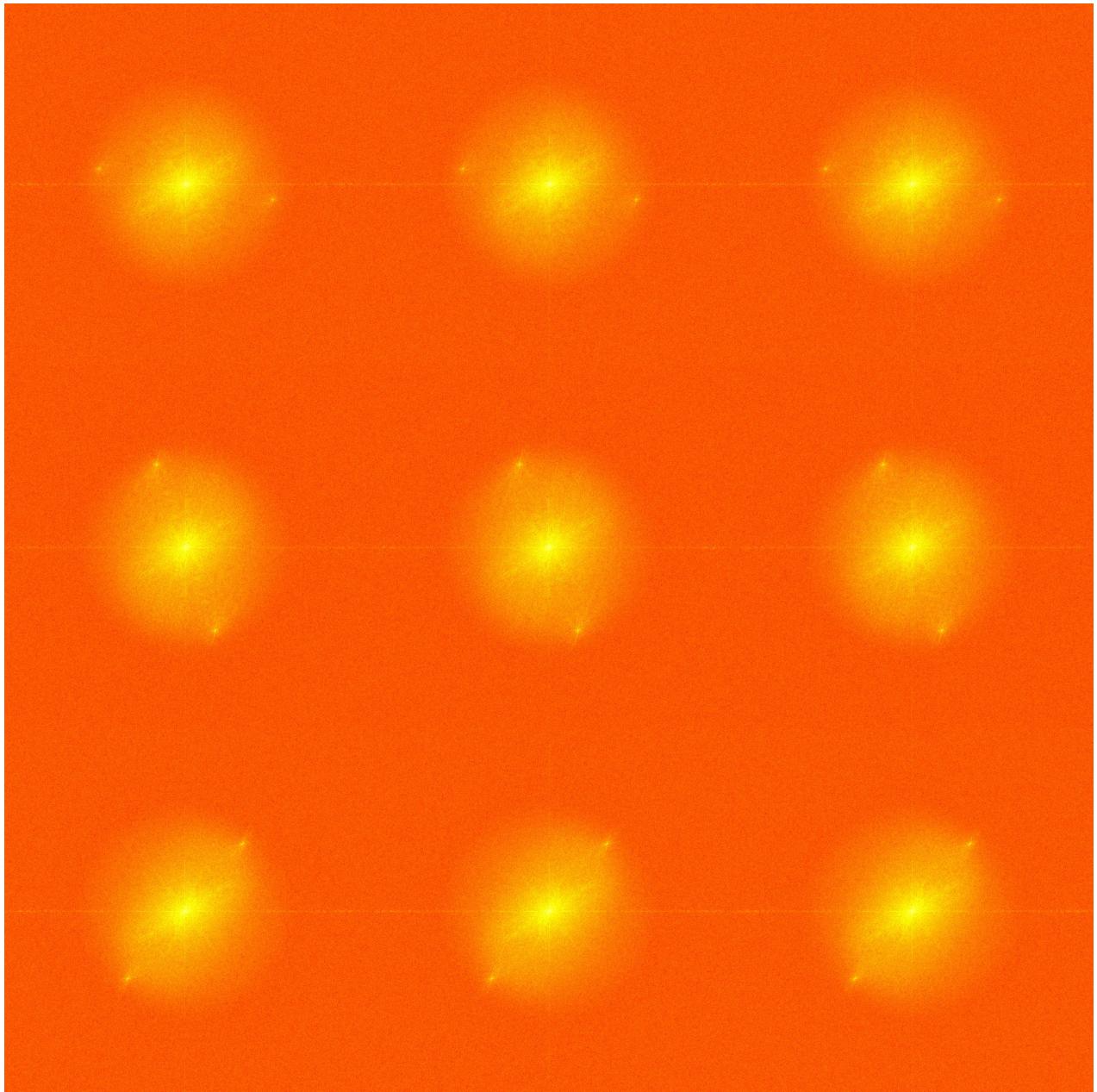


Figure S7. The Fourier spectrums of the preprocessed SIM images.

c3. Image reconstruction

With the estimated experimental parameters (wave vector, initial phase, and modulation depth), the 0- and ± 1 -order spectrums can be accurately separated. Then the high-frequency spectrums can be carried to where they should be, and merged [Fig. S8]. Figure S9 shows the separated spectrums, and Fig. S10 shows the spectrum of the wide-field image and the merged spectrum with high-frequency information.

Separate=separateBands(IIrawFFT(:,:, (I-1)*3+... 1:I*3), par(I).phaOff, param.nrBands, param.fac);	% Spectrum separation
Shifted=zeros(NPixel*2,NPixel*2,3); Shifted(:,:,1)=placeFreq(Separate(:,:,1)); Shifted(:,:,2)=placeFreq(Separate(:,:,2)); Shifted(:,:,3)=placeFreq(Separate(:,:,3)); Shifted(:,:,2)=NfourierShift(Shifted(:,:,2), -(2-1)*par(I).px,... -(2-1)*par(I).py); Shifted(:,:,3)=NfourierShift(Shifted(:,:,3), (2-1)*par(I).px,... (2-1)*par(I).py); Coarse_frequency=Coarse_frequency+Shifted(:,:,1)+Shifted(:,:,2)+... +Shifted(:,:,3);	% Separated spectrum with double size % Spectrum shift % Spectrum shift % Spectrum merging
Shifted(:,:,1)=otfToVector(Shifted(:,:,1), param.OtfProvider, 1, 0, 0, 1, 0); Shifted(:,:,2)=otfToVector(Shifted(:,:,2), param.OtfProvider, 2,... -(2-1)*par(I).px, -(2-1)*par(I).py, 1, 0); Shifted(:,:,3)=otfToVector(Shifted(:,:,3), param.OtfProvider, 2,... (2-1)*par(I).px, (2-1)*par(I).py, 1, 0); Fine_frequency=Fine_frequency+Shifted(:,:,1)+Shifted(:,:,2)+... Shifted(:,:,3);	% Deconvolution % Deconvolution % Deconvolution % Deconvolved spectrum merging

Figure S8. Code for spectrum separation and merging.

In order to suppress noise interference and achieve higher-quality super-resolution reconstruction, Wiener reconstruction [[Wiener_recon.m](#)] is performed to achieve the final image reconstruction. We also provide TV-SIM and HiFi-SIM reconstructions, and users can use them by calling “[TV_recon](#)” or “[Hifi_recon](#)” functions. Figure S11 illustrates the wide-field image and the super-resolution image acquired by PCA-SIM.

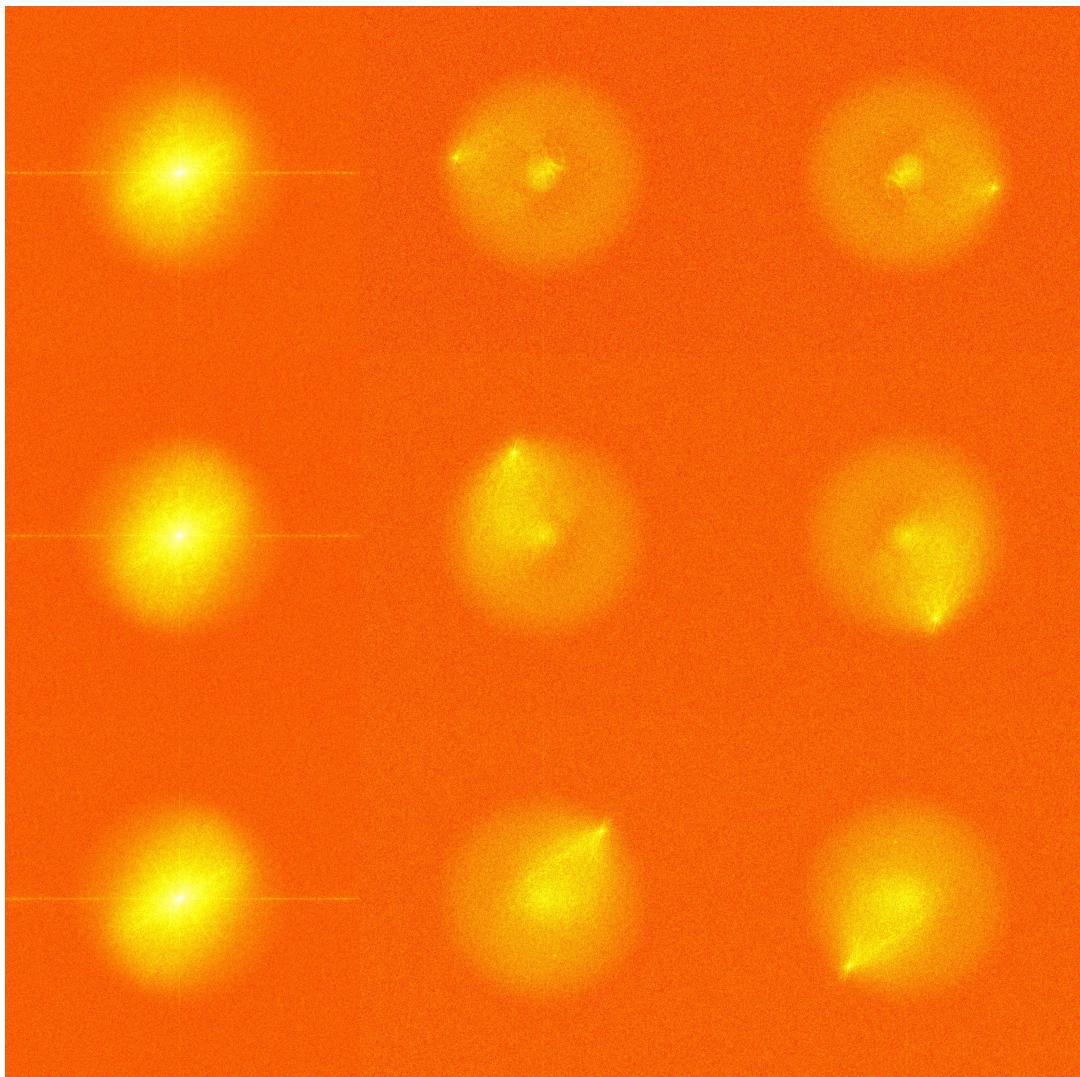


Figure S9. The separated 0- and ± 1 -order Fourier spectrums.

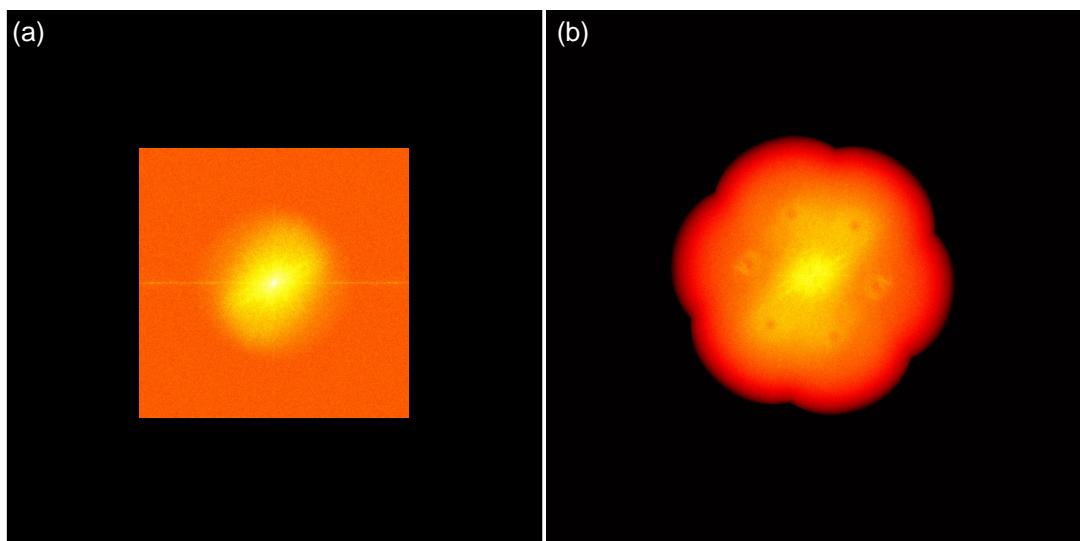


Figure S10. The spectrum of the wide-field image (a) and the merged spectrum (b) with high-frequency information. Note that the size of the spectrum map is twice the size of the raw image.

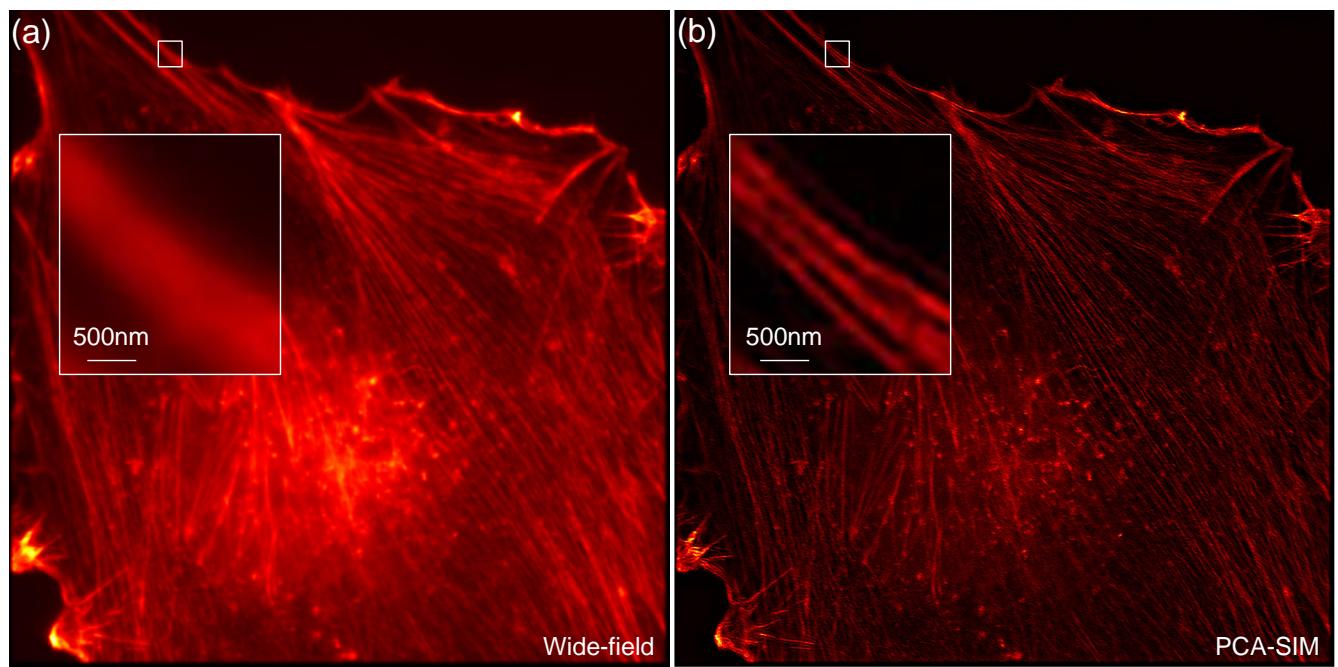


Figure S11. The wide-field image (a) and the super-resolution image acquired by PCA-SIM via running the main program (b). Scale bars: 500 nm.

D. More simulation and experimental demos

d1. demo for reproducing the simulation result shown in Fig. 1 of the main text

We provide simulation codes and data to reproduce Fig. 1 in the main text (performance comparison of PCA-SIM and COR under different noise conditions). The related codes and data are in the folder “**Simulation**”. The provided data are images of bovine pulmonary artery endothelial (BPAE) cells, which were artificially multiplied by the structured illumination image and convolved with a simulated point spread function of a low cutoff frequency. In the simulation codes “[simulation.m](#)”, we use the function “`wgn`” to add Gaussian noises of different powers to the simulated SIM data, and use PCA-SIM and COR for parameter estimation, respectively. After running the simulation codes, the results shown in Fig. [S12](#) can be obtained. Since the noises are randomly added, the single-run results may differ slightly from Fig. 1a in the main text.

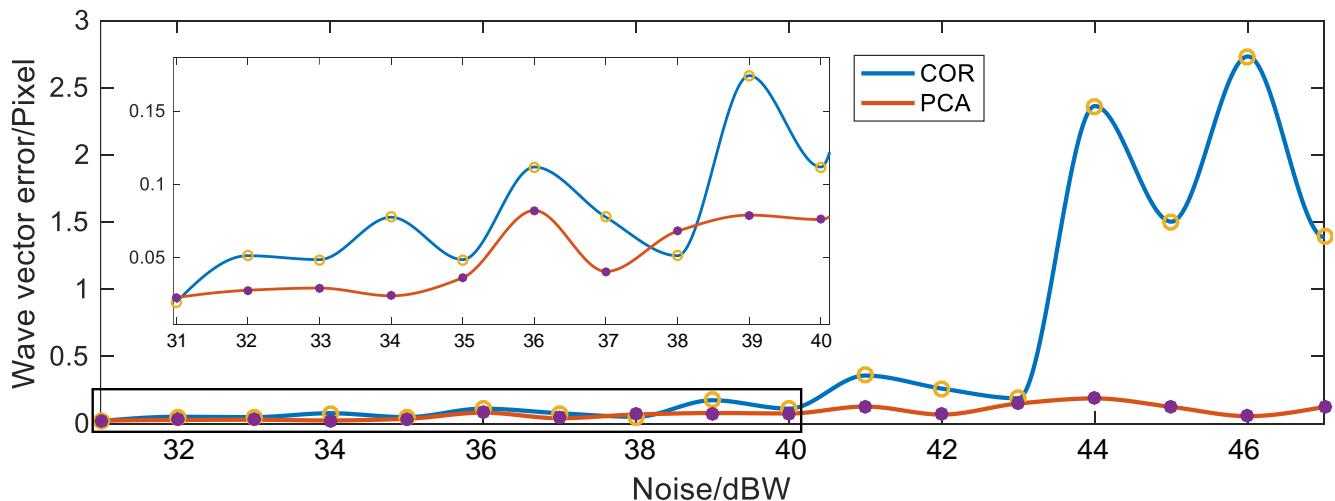


Figure S12. The error distributions of the wave vectors estimated by PCA-SIM and COR under different SNRs after running the simulation program.

d2. demo for reproducing the results shown in Fig. 3 of the main text and Supplementary Figs. S7 and S10

By selecting different algorithm functions and running the demo codes, the super-resolution results reconstructed by corresponding methods can be obtained. Figure [S13](#) presents a combination of results from different methods by processing data [“[405_2.tif](#)”, “[488_2.tif](#)” and “[561_2.tif](#)”]. In particular, processing data [“[405_1.tif](#)”, “[488_1.tif](#)” and “[561_1.tif](#)”], [“[561_2.tif](#)”] and [“[2D-SIM\(3 angles_3 phases\)_9 frames.tif](#)”] can reproduce Fig. 3 in the main text and Supplementary Figs. 7 and 10, respectively.

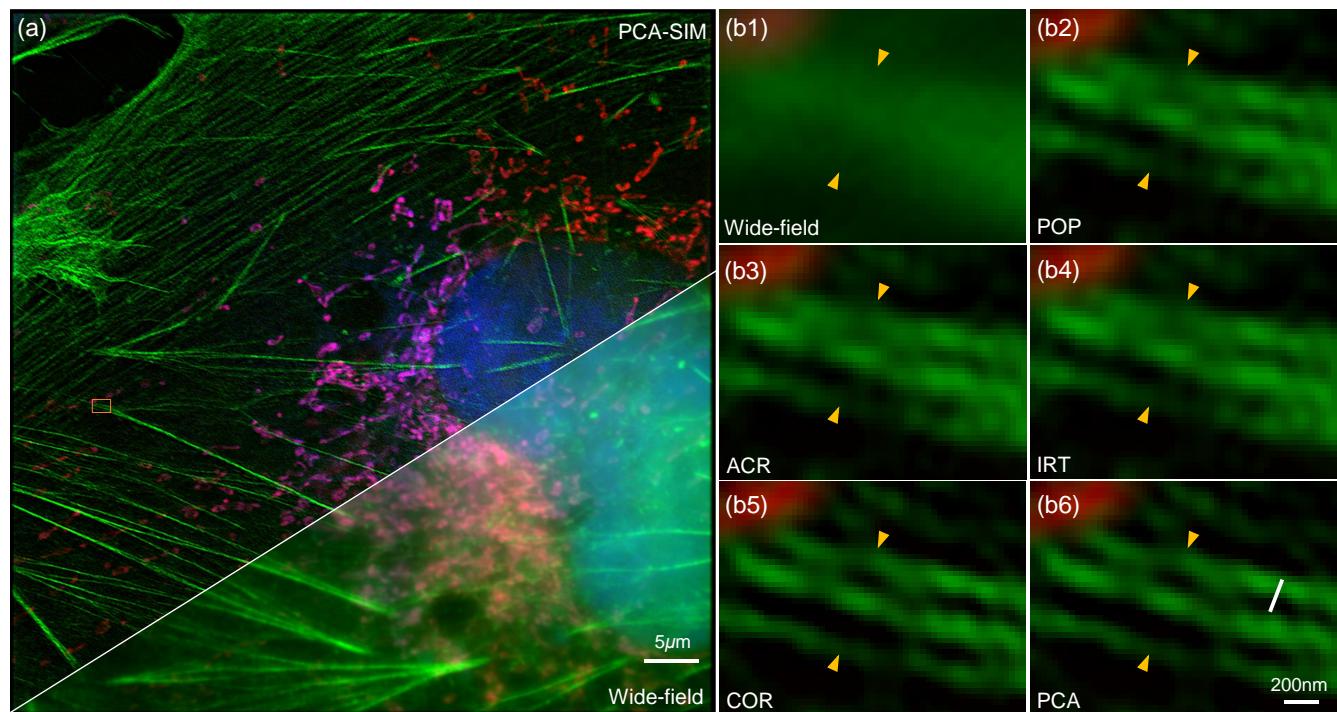


Figure S13. Comparative experiment on super-resolution results of a COS-7 cell sample by different approaches (100 \times /1.40 Oil). (a) The wide-field image and the super-resolution image acquired by PCA-SIM. (b) Magnified super-resolution images obtained by different methods. Scale bars: 5 μ m (a); 200 nm (b).

References

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