

Supplementary Information for

Multi-resolution analysis enables fidelity-ensured deconvolution for fluorescence microscopy

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Supplementary Notes

Supplementary Note 1: Deconvolution algorithms for fluorescence images

In this note, we briefly review the deconvolution algorithms that are developed for optical microscopy. In biomedical research, fluorescence images are inevitably disturbed by noise and optical blurring, which damage their fidelity and further utilization. Like what researchers do for macro-scale image processing, recovering real images from noise and blurring is usually the first step for further analysis. This process is termed as deconvolution. The deconvolution algorithm aims to recover the clean image from its degraded form based on the linear, position-invariant degradation model¹:

$$g = Af + w \quad (1-1)$$

where g denotes the observed image under the influence of blurring and noise, A is the blurring matrix, f is the clean image to be recovered, and w is the additive noise. Usually, the removal of the noise w is called denoising, and recovering the sharp image f from its blurred form Af is called deblurring. The deconvolution problem is ill-conditioned due to its high sensitivity to noise. If without constraint, the inverse problem (1-1) has infinitely many solutions.

The least squares approach is one simple classical solver to (1-1), which aims to find an x that minimizes the distance between Ax and g :

$$\arg \min_x \|Af - g\|^2 \quad (1-2)$$

This leads to the naïve solution: $x=A^{-1}g$, which is also called direct inverse filtering. In the frequency-domain, this solution means that the Fourier spectrum of the clean image f can be acquired by multiplying the Fourier spectrum of the blurred image b with the reciprocal of the optical transfer function (OTF). However, all optical systems have a compactly supported OTF, meaning that OTF gradually attenuates close to zero with the increase of frequency. While noise located in high frequency region in the image, would be greatly amplified if above multiplication with the reciprocal of OTF, leading to devastating results. For fluorescence images especially, the noise is considerable, thus the direct inverse filtering does not work at all.

Wiener deconvolution¹, also known as the minimum mean square error filtering, is a good alternative for the naïve inverse filtering, which incorporates the design to handle noise. It aims to find an estimation of the clean image with the least square error:

$$\min e^2 = E \left\{ \left(f - \hat{f} \right)^2 \right\} \quad (1-3)$$

where $\hat{f}$ is the estimation of the clean image, E denotes the expected value.

With the assumption that the image and noise are uncorrelated, and that the intensity level of the unregenerate image has a linear dependency with its degenerated form, (1-3) has the following solution in the

frequency-domain:

$$\hat{F}(u, v) = \left[\frac{1}{H(u, v) \left[|H(u, v)|^2 + S_w(u, v) / S_f(u, v) \right]} \right] G(u, v) \quad (1-4)$$

where $\hat{F}(u, v)$ is the Fourier spectrum of the estimated clean image, $H(u, v)$ is the OTF of the imaging process, $G(u, v)$ is the Fourier spectrum of the observed image, $S_w(u, v)$ and $S_f(u, v)$ are the power spectrum of the noise and unregenerate image, respectively.

It is usually assumed that a white noise is added, which simplifies (1-4) as:

$$\hat{F}(u, v) = \left[\frac{1}{H(u, v) \left[|H(u, v)|^2 + K \right]} \right] G(u, v) \quad (1-5)$$

where K is a constant characterized by the ratio of the power spectrum of the noise and unregenerate image.

Wiener deconvolution has been widely applied in fluorescence image processing, such as structured illumination microscopy (SIM) which is sensitive to noise due to its reconstruction process. Many SIM reconstruction algorithms²⁻⁴ use the Wiener deconvolution to denoise the original modulated raw images and merge different frequency components using the generalized Wiener filtering.

The Wiener filtering essentially is a frequency-domain optimization method, which consequently results in weakened details. Another widely used deconvolution algorithm is the Richardson-Lucy (RL) iteration^{5, 6} that derives in the spatial domain, which is categorized into the maximum likelihood estimation (MLE) method. It is derived based on the assumption of an addy Poisson noise, which was first applied in astronomical imaging for its photon-limited detection feature. The RL iteration works in the following way to estimate the most likely unregenerate image that produces the observed image:

$$f^{n+1}(x, y) = f^n(x, y) \left[\frac{g(x, y)}{h(x, y) \otimes f^n(x, y)} \cdot h(x, y) \right] \quad (1-6)$$

where $f^n(x, y)$ denotes the recovered unregenerate image after n iterations.

However, the RL iteration has some serious drawbacks⁷. As a MLE method, RL essentially is not a rigorous solution to the inverse problem and may over fit the Poisson noise type. It tends to generate artifacts rather than real high-frequency information (**Fig. S9**) and also encounters the problem of noise amplification. Another question lies in the iteration times, for usually, the RL does not converge to the solution due to the noise amplification, which leads to the phenomenon that the resolved image may be quite different with a different number of iterations.

To amend the shortcomings of the RL iteration, in astronomical imaging researchers usually reduce the number of RL iteration or damp the RL iteration⁷. For the fluorescence imaging, the RL deconvolution was firstly applied in widefield⁸ and confocal laser scanning microscopy (CLSM)⁹, which also encounters the

problem of a limited photon detection. However, as demonstrated by Dey et al.⁹, the standard RL iteration creates many false structures in the image of CLSM. To remedy this problem, they add a total variation (TV) constraint to damp the effect of RL iteration, which can control the solution acquired by RL iteration. Later, a few numbers (~5) of RL iterations is applied to enhance the image in some SIM reconstruction algorithms^{4, 10}.

Besides these simple deconvolution algorithms, the regularization-based inverse problem solver is more effective for fluorescence image deconvolution which usually is polluted by substantial noise. The idea is to add some constraints to the inverse problem solution using some prior knowledge of the unregenerate image. However, in the earlies the development of the regularization-based deconvolution algorithm is limited for solving such a problem needs an enormous computation. Recently, some efficient inverse problem solvers such as the fast iterative soft-thresholding algorithm (FISTA) and split-up Bregman iteration¹¹ have been proposed, which greatly benefited the regularization-based deconvolution algorithm. Another incompatibility of this method with the fluorescence images is the regularization applied, which is usually chosen as Tikhov-Miller or orthogonal wavelet.

Chu et al.¹² first introduced this method to optimize the image quality of SIM images using a well-known total-variation (TV) regularization to characterize the distinct continuity feature of fluorescence images:

$$\min \frac{\mu}{2} \cdot \|f - g\|_2^2 + \|\nabla f\|_1 \quad (1-7)$$

where f is the clean image to be recovered, g is the observed noisy image, and μ is the regularization parameter, which denotes the variation of a point.

The TV norm is proposed based on the idea that a clean image has a smaller TV value than its noisy form. For fluorescence imaging, the continuity of the cell structure has a good agreement with this norm. Due to the computational nature, SIM image usually suffers from a server noisy influence, which can be compensated by the post-processing TV deconvolution.

Hessian-SIM¹³ is a further development of the regularization-based deconvolution algorithm for SIM images. Different from TV norm, Hessian matrix considers the second partial derivative of three axes:

$$R_{Hessian}(f) = \int_{\Omega} \left\| \begin{pmatrix} f_{xx} & f_{xy} & \sqrt{\sigma} f_{xt} \\ f_{yx} & f_{yy} & \sqrt{\sigma} f_{yt} \\ \sqrt{\sigma} f_{tx} & \sqrt{\sigma} f_{ty} & \sigma f_{tt} \end{pmatrix} \right\|_1 dr \quad (1-8)$$

Using the Hessian penalty, the inverse problem is formulated as follow:

$$\min \frac{\mu}{2} \cdot \|f - g\|_2^2 + R_{Hessian}(f) \quad (1-9)$$

A huge improvement of Hessian-SIM is that it considers the connection between the x , y and t axis, which therefore brings a better image quality improvement. More importantly, they pointed out the application of the post-processing deconvolution algorithm in fast, long-term live-cell imaging. The algorithm guarantees good image quality with a lower photon dose (i.e., low phototoxicity, or short exposure), which allows live-cell

imaging with a longer duration and faster imaging speed.

Essentially, TV-SIM and Hessian-SIM are regularization-based denoising algorithms with no resolution improvement. This is presumably due to the incompatibility of variation-based regularization with the iterative inverse filtering (**Fig. S10**). Sparse-SIM¹⁴ introduces a new way to improve the resolution of fluorescence images based on the RL iteration. The idea of Sparse-SIM is to denoise the image first, then improve its resolution through a large number of RL iterations. The first denoising problem is arranged as follows:

$$\min \frac{\mu_1}{2} \cdot \|f - g\|_2^2 + R_{Hessian}(f) + \mu_2 \|x\|_1 \quad (1-10)$$

The denoising of the Sparse deconvolution is accomplished by the Hessian matrix, which is the same as Hessian-SIM. The addition of the l_1 norm of the sparsity, as stated by the authors uses the prior knowledge that an image with a higher resolution has a smaller number of pixels with a significant gray value. After the first denoising iteration, it executes a large number of RL iteration to improve the resolution. Sparse-SIM claims a ~60 nm resolution for live-cell imaging, which increases the upper limit of physical SIM imaging by 1.5 times.

In physical model based deblurring algorithms, such as FISTA¹⁵, a gradient step is usually used in the iteration to minimize the fidelity term (1-2), which is a straightforward and physical way to separate Ax . One major difference between this fidelity deblurring and RL deblurring is that the first one ensures the minimization of the fidelity term and the latter one seeks fine fitting based on the assumption and does not necessarily minimize the fidelity term. The fidelity-ensured deconvolution ensures that the recovered image f can be blurred back to its original form $g-w$ with the same (or shrinked due to insufficient deblurring) PSF used for the deblurring. While the RL-like MLE method deblurring only forces the data to confess its pre-assumed distribution, which does not necessarily minimize the fidelity cost (1-2). However, for biomedical research, the reliability of the images is the most important issue for image processing. Therefore, we believe that fidelity-ensured deconvolution is the suitable method for fluorescence image post-processing. However, it is fragile to noise and is incompatible with the variance-based denoising regularization, which is solved by the MRA regularization proposed in this paper.

Supplementary Note 2: Introduction to the multiresolution analysis

Supplementary Note 2.1: Orthogonal wavelet bases

The well-known Fourier transform has been one of the most important tools for signal analysis and processing in the past decades, which decomposes the signal with a set of sinusoidal functions:

$$\hat{f}(\omega) = \int_{-\infty}^{+\infty} f(t) e^{-i\omega t} dt \quad (2-1)$$

$$f(t) = \frac{1}{2\pi} \int_{-\infty}^{+\infty} \hat{f}(\omega) e^{i\omega t} d\omega \quad (2-2)$$

where $\hat{f}(\omega)$ is called the Fourier spectrum or frequency spectrum of the signal $f(t)$. This transform provides a good measurement of the variance in the signal.

However, deficits exist in the Fourier transform. For the most important one, the Fourier transform uses sinusoidal functions to approximate the signal space, which has an infinite support in the spatial domain. This leads to the absolute frequency localization and fails to offer time/spatial localization ability¹⁶.

To overcome such problem, the windowed Fourier transform (also named as short-time Fourier transform) has been proposed, which at once only transform a short range of the signal to the Fourier domain¹⁶:

$$\hat{f}(\omega, \tau_0) = \int_{-\infty}^{+\infty} f(t) g(t - \tau_0) e^{-i\omega t} dt \quad (2-3)$$

where $g(t)$ is the temporal (or spatial) window function that confines the range to be Fourier transformed, τ_0 is the center of the window. Through the moving of the window, the windowed Fourier transform provides some sort of spontaneous localization in the temporal (spatial) domain and the frequency domain.

The uncertainty principle that widely exists in quantum mechanics analysis also holds for signal measurement, which says that the uncertainty in the temporal (spatial) and frequency domain cannot be enough small simultaneously. The mean value and variance of the signal is defined as follow:

$$E[f(t)] = \int_{-\infty}^{+\infty} t |f(t)|^2 dt / \int_{-\infty}^{+\infty} |f(t)|^2 dt \quad (2-4)$$

$$\Delta[f(t)] = \left\{ \int_{-\infty}^{+\infty} \{t - E[f(t)]\}^2 |f(t)|^2 dt / \int_{-\infty}^{+\infty} |f(t)|^2 dt \right\}^{1/2} \quad (2-5)$$

Then the uncertainty principle holds¹⁷:

$$\Delta[f(t)] \cdot \Delta[\vec{f}(\omega)] \geq \frac{1}{2} \quad (2-6)$$

This formula indicates that the simultaneous determination of the time (spatial) and frequency range through any form of windowed Fourier transform is finite. It can be proved that when the maximum time frequency comprehensive analysis capability is reached (i.e., the inequality (6) takes equal sign), the window

function shall be a Gaussian function:

$$g(t) = \frac{1}{\sqrt{2\pi}a} e^{-\frac{1}{2}\left(\frac{t}{a}\right)^2} \quad (2-7)$$

where the parameter a can be adjusted to regulate the temporal (spatial) and frequency resolution. The windowed Fourier transform with a Gaussian window is also called the Gabor transform¹⁶.

In the regime of the time frequency comprehensive analysis capability, the Gabor transform is the best windowed Fourier transform. However, this method also has its limitations. Once the window function is selected, the time resolution and frequency resolution of the Gabor transform are consistent in all positions of the signal. However, for a natural signal, the signal will inevitably change faster in some positions and slower in some positions. We expect to measure the fast-changing time through a narrow window to obtain more abundant spectrum information, and measure the slow-changing part through a wide window to obtain more time information. However, due to the fixed time-frequency resolution of Gabor transform, this expectation cannot be achieved.

The multi-resolution analysis (MRA) tool¹⁸⁻²⁰ can offer a good solution to the problem mentioned above. The orthogonal wavelet^{21, 22} bases are the first MRA tool that successfully applied to image processing. It begins with a set of orthogonal bases with different resolution called the scale functions and wavelet functions:

$$\phi_{j,n}(t) = 2^{j/2} \phi(2^j t - n) \quad (2-8)$$

$$\psi_{j,n}(t) = 2^{j/2} \psi(2^j t - n) \quad (2-9)$$

The linear subspaces composed by $\phi_{j,n}$ and $\psi_{j,n}$ are symbolled by V_j and W_j , respectively. It is clear that the higher the j value, the higher the spatial (temporal) resolution is. The relation between each subspace is $V_{j-1} \subset V_j$, $V_j \perp W_j$, $V_{j-1} \oplus W_{j-1} = V_j$. It indicates that the scale/wavelet functions in a lower level can be represented by the higher ones:

$$\phi(t) = \sqrt{2} \sum_n h_n \phi(2t - n) \quad (2-10)$$

$$\psi(t) = \sqrt{2} \sum_n g_n \phi(2t - n) \quad (2-11)$$

where $\{h_n\}$ is called the low-pass filter of the wavelet transform, $\{g_n\}$ is called the high-pass filter of the wavelet transform. Once the low-pass filter and high-pass filter are determined, the orthogonal wavelet transform is determined.

It is obvious that as $j \rightarrow +\infty$, $\phi_{j,n}$ and $\psi_{j,n}$ can offer a precise representation of the $L_2(\mathbb{R})$ space. To avoid the evolution to the trivial base, the wavelet transform approximates the signal space in the following way:

$$L^2(\mathbb{R}) = \bigoplus_{i=-\infty}^{+\infty} W_i \quad (2-12)$$

In this case, any $L_2(R)$ signal can be decomposed as follow:

$$f(t) = f_j(t) + g_j(t) = \sum_m a_{j,m} \phi_{j,m}(t) + \sum_n d_{j,n} \psi_{j,m}(t) \quad (2-13)$$

where $a_{j,m}$ and $d_{j,n}$ is called the wavelet coefficient. The decomposition to the higher level can be implemented by the fast Fourier transform (FFT), which is first proposed by Mallat²⁰.

Supplementary Note 2.2: Framelet and curvelet system

Orthogonal wavelet bases have achieved great success in a wide range of image processing tasks. However, it is not capable of representing images in a very sparse way, the importance of which has been clarified later²³. It motivates people to construct redundant wavelet bases that can provide a sparser representation of image. As for image denoising, coefficient shrinkage is one of the most important ways that wavelet transform accomplishes this task. The importance for finding a multiscale base that can provide a sparser representation of image is that key features of the image (such as the sharp edge) is incorporated in some significantly higher transform coefficients, while the noise and some unimportant part is contained in some lower-value coefficients. By coefficient shrinkage the noise can be subtracted and the important part of the image can be preserved.

The frame wavelet (framelet)²⁴ is one of the most popular and effective tools to represent the image in a sparse way. Similar with the orthogonal wavelet bases, the framelet transform accompanies with a set of filters $\{u_0, u_1, \dots, u_s\}$, in which u_0 is the low-pass filter, u_1-u_s are the high pass filters. When $s = 1$, the framelet transform degenerates to the orthogonal wavelet transform. When the filter bank stratifies the following perfection reconstruction condition, the framelet is called a tight framelet²⁴:

$$\begin{bmatrix} \widehat{u}_0(\xi) & \cdots & \widehat{u}_s(\xi) \\ \widehat{u}_0(\xi + \pi) & \cdots & \widehat{u}_s(\xi + \pi) \end{bmatrix} \begin{bmatrix} \widehat{u}_0(\xi) & \cdots & \widehat{u}_s(\xi) \\ \widehat{u}_0(\xi + \pi) & \cdots & \widehat{u}_s(\xi + \pi) \end{bmatrix}^{\star} = I \quad (2-14)$$

where $\star$ denotes of the transpose and complex conjugate operation.

The linearized piece-wise framelet is one of the most well-developed tight framelet, which can sparsely represent a wide range of images. The filter bank of the linearized piece-wise framelet is as follow:

$$u_0 = \left[\frac{1}{4}, \frac{1}{2}, \frac{1}{4} \right]; u_1 = \left[\frac{\sqrt{2}}{4}, 0, -\frac{\sqrt{2}}{4} \right]; u_2 = \left[-\frac{1}{4}, \frac{1}{2}, -\frac{1}{4} \right] \quad (2-15)$$

This filter design makes them very sensitive to the edges of the image. When the edges of the image are convolved with these filters, a large transformation coefficient value is generated. However, if the texture is uniform, it will not.

Besides, the geometrical multiscale bases are also studied, which show unique power in the image

processing of some geometrics (i.e., structures with high along-edge continuity). Curvelet is one representative geometrical multiscale^{25, 26}.

The starting point of the curvelet transform is to segment the Fourier space into a set of subspaces with unique direction. Each curvelet base locates a certain region in the Fourier domain with different frequency and direction. When performing a curvelet transform to an image, the feature that is similar to that expressed by a certain curvelet base would produce higher coefficients.

To achieve the perfect reconstruction, some mathematical efforts are made. A radial window $W(r)$ and an angular window $V(t)$ are established following the admissibility condition:

$$\sum_{j=-\infty}^{\infty} W^2(2^j r) = 1, \quad r \in (3/4, 3/2) \quad (2-16)$$

$$\sum_{j=-\infty}^{\infty} V^2(t - l) = 1, \quad t \in (-1/2, 1/2) \quad (2-17)$$

Then the curvelet is defined in the frequency domain:

$$U_j(r, \theta) = 2^{-3j/4} W(2^{-j} r) V\left(\frac{2^{\lfloor j/2 \rfloor} \theta}{2\pi}\right) \quad (2-18)$$

where $\lfloor j/2 \rfloor$ denotes the integer part of $j/2$. Under this definition, the support of U_j is a wedge-shape region in the frequency-domain. Define φ_j as the inverse Fourier transform of U_j , the curvelets at scale 2^j , orientation θ_l and position $x_k^{(j,l)} = R_{\theta_l}^{-1}(k_1 \cdot 2^{-j}, k_2 \cdot 2^{-j/2})$ is given by

$$\varphi_{j,l,k}(x) = \varphi_j(R_{\theta_l}(x - x_k^{(j,l)})) \quad (2-19)$$

where $\theta_l = 2\pi \cdot 2^{-\lfloor j/2 \rfloor} \cdot l$ is the rotation angles, (k_1, k_2) is the sequence of translation parameters, R_{θ} is the rotation matrix:

$$R_{\theta} = \begin{pmatrix} \cos \theta & \sin \theta \\ -\sin \theta & \cos \theta \end{pmatrix} \quad (2-20)$$

The curvelet coefficients of a function can be given by its inner product with the curvelets:

$$c(j, l, k) = \langle f, \varphi_{j,l,k} \rangle = \int_{\mathbb{R}^2} f(x) \overline{\varphi_{j,l,k}(x)} dx \quad (2-21)$$

Candes et al.²⁵ gives a detailed digital implementation of the curvelet transform.

Supplementary Note 3: Fluorescence image in the multiscale base domain

As stated in the main text, there are two cardinal attributes of fluorescence images: (1) high contrast across the edge, and (2) high continuity along the edge. The sharp-edge originates from the heightened specificity of fluorophores, which contrasts biological structures against background. High along-edge continuity originates from the connectivity of the biological specimen, and spatial sampling nature of fluorescence microscopes. “High along-edge continuity” can also be understood as if we collect grayscale values along the direction of the edge of the biological structure, then the continuity of the measured envelope is the highest.

The two features can be effectively detected by framelet and curvelet mentioned in **Supplementary Note 2**. The reason why framelet can sensitively detect edges is due to its unique filter design (equation 2-15). The edges of the image will generate a large framelet coefficient. The reason why curvelet can detect continuity along the edges is that it specifically locates the frequency range in a specific direction in the Fourier spectrum. The scaling of the framelet and curvelet bases ensures that they can detect these features with different scales. If fluorescent images possess these features, they could be sparsely expressed by frameworks and curves. And with the addition of noise (i.e., the destruction of sharp edge and along-edge continuity), the sparsity in framelet and curvelet domain would decrease.

To verify our assumption of the sparsity, we do a simulation using the raw fluorescence images of actin, mitochondrion, tubulin, and lysosome with relatively high SNR. Gaussian noise with various levels was added to the clean image. The framelet and curvelet transform is conducted for these groups of images, and the sparsity of these coefficients is measured by the Gini index²⁷:

$$S(\vec{c}) = 1 - 2 \sum_{k=1}^N \frac{c_k}{\|\vec{c}\|_1} \left(\frac{N - k + \frac{1}{2}}{N} \right) \quad (3-1)$$

where S denotes the Gini sparsity value, $\vec{c}$ is a coefficient array, and N is the number of the coefficient.

The results in **Fig. S2** show that both the sparsity of the fluorescence image in the framelet domain and the curvelet domain decreases with the increase of the Gaussian noise power, which is caused by the contamination of sharp-edge and along-edge continuity. These results enlighten us that by increasing the sparsity of the fluorescence image in the multiscale base domain, the clean signal component can be recovered from the noise.

The increase of the sparsity in the multiscale base domain means the reduction of the number of the multiscale base coefficient that is significantly non-zero. One of the simplest ways to increase the sparsity is hard-thresholding of the MRA coefficient, which assign 0 to the coefficient less than a thresholding value. We then conduct hard thresholding to the noisy image using a threshold value of 0.02, 0.05, 0.10, and 0.15. The results in **Fig. S3** show the effect of applying framelet and curvelet hard-thresholding to noisy input images. One can see that with the increase of the threshold value, the sparsity value increases, and the noise in the image has been gradually removed. With a threshold value of 0.15, the framelet and curvelet domain sparsity

of each image all exceed 0.95. While the framelet and curvelet-domain thresholding affect the image quality differently. The framelet domain hard thresholding can effectively remove the noise from the original image, highlighting the high-contrast sharp edge. Through the enlargement of specific detail, it can be observed that the background noises are gradually removed with the increased threshold value. However, some fine details and signal continuity are also disrupted. Curvelet domain hard thresholding can subtract the fine details and enhance the signal continuity. The curvelet approach achieves this outcome for the random distribution of noise and strong geometrical feature of the fluorescence signal. The noise is usually randomly spaced in a fluorescence image, which does not possess a strong directional feature and can be successfully removed by curvelet thresholding. The biological structure instead has a regular distribution, which forms strong fluorescence signal geometrical features and thus is enhanced by the curvelet thresholding. However, compared with framelet, curvelet is less effective to remove background noise and highlight the high contrast sharp edge. Based on the above observation, combining the feature of framelet and curvelet may be quite effective way to enhance the fluorescence images.

Supplementary Note 4: Formulation of MRA and SecMRA deconvolution

Supplementary Note 4.1: 2D-MRA deconvolution

The above discussions enlightened that the co-effect of framelet and curvelet might be an approach that can remove the noise and background but simultaneously preserve the fine structure of a fluorescence image. Based on the prior knowledge that a clean fluorescence image has a co-sparsity in framelet and curvelet system, we introduce the fidelity-ensured, MRA-based deconvolution model for the image processing of fluorescence images:

$$\arg \min_x \|b - Ax\|_2^2 + \lambda_1 \|Wx\|_1 + \lambda_2 \|Cx\|_1 \quad (4-1)$$

where W denotes the linearized piece-wise framelet transform, C denotes the curvelet transform, the first term ensures the fidelity in the deconvolution process, the second term is the sparse term in the framelet domain, the third term is the sparse term in the curvelet domain, λ_1 and λ_2 are the regularization parameters that balance the two sparsity terms and the fidelity term, $\|_1$ and $\|_2$ denotes the l_1 and l_2 norm, respectively.

The gradient-based method is optimal for solving this large convex optimization problem. Here we develop an iterative scheme to solve the minimization problem (3-1) based on the fast iterative shrinkage-thresholding algorithm (FISTA)¹⁵, which is one of the most effective gradient-based methods with fast convergence speed. FISTA aims to solve the following generalized optimization problem:

$$\min_x F(x) \equiv f(x) + g(x) \quad (4-2)$$

where $F(x)$ is the total cost function, $f(x)$ is a convex and smooth function, and $g(x)$ is a convex but possibly unsmooth function. In the MRA deconvolution model, we have:

$$\begin{aligned} f(x) &= \|b - Ax\|_2^2 \\ g(x) &= \lambda_1 \|Wx\|_1 + \lambda_2 \|Cx\|_1 \end{aligned}$$

The gradient method that can minimize $f(x)$ is:

$$x_k = x_{k-1} - t_k \nabla f(x_{k-1}) \quad (4-3)$$

The proximal regulation of the gradient iteration can be equivalently written as:

$$x_k = \arg \min_x \left\{ f(x_{k-1}) + \langle x - x_{k-1}, \nabla f(x_{k-1}) \rangle + \frac{1}{2t_k} \|x - x_{k-1}\|^2 \right\} \quad (4-4)$$

Using the approximation (3-4), the iteration scheme of $F(x)$ is:

$$x_k = \arg \min_x \left\{ f(x_{k-1}) + \langle x - x_{k-1}, \nabla f(x_{k-1}) \rangle + \frac{1}{2t_k} \|x - x_{k-1}\|^2 + \lambda_1 \|Wx\|_1 + \lambda_2 \|Cx\|_1 \right\} \quad (4-5)$$

Ignoring the constant terms, we have:

$$x_k = \arg \min_x \left\{ \frac{1}{2t_k} \left\| x - (x_{k-1} - t_k \nabla f(x_{k-1})) \right\|^2 + \lambda_1 \|Wx\|_1 + \lambda_2 \|Cx\|_1 \right\} \quad (4-6)$$

The l_1 norm in the iteration scheme is separable, thus soft thresholding can be applied after the gradient iteration of $f(x)$ to minimize the function $g(x)$:

$$y_k' = W^T \mathcal{T}_{\lambda_1/L} (W y_k) \quad (4-7)$$

$$y_k'' = C^T \mathcal{T}_{\lambda_2/L} (C y_k') \quad (4-8)$$

where L is the Lipchitz constant of the initial image, W^T and C^T denote the inverse framelet and curvelet transform respectively, $\mathcal{T}_\lambda$ is the shrinkage operator defined as:

$$\mathcal{T}_\lambda(x) = (|x| - \lambda) \text{sgn}(x) \quad (4-9)$$

FISTA adopts a similar approach as above with a smarter choice of y_k to accelerate the iteration of this process. Then the iteration process of the MRA deconvolution model is formulated as follows:

Iteration scheme for 2D-MRA deconvolution

Input: Noisy image x , blurring matrix A , regularization parameter λ_1 and λ_2

Initialization: Let $x_0=x$, $t_0=1$

Iteration:

The k th iteration ($k \geq 1$):

$$x_k = x_{k-1} - t_k \nabla f(x_{k-1})$$

$$x_k' = W^T \mathcal{T}_{\lambda_1/L} (W x_k)$$

$$x_k'' = C^T \mathcal{T}_{\lambda_2/L} (C x_k')$$

$$x_k = x_k''$$

$$t_{k+1} = \frac{1 + \sqrt{1 + 4t_k^2}}{2}$$

$$x_{k+1} = x_k + \left(\frac{t_k - 1}{t_{k+1}} \right) (x_k - x_{k-1})$$

Until cost function $\|b - Ax\|_2^2 + \lambda_1 \|Wx\|_1 + \lambda_2 \|Cx\|_1$ converges

In the algorithm execution, we stop the iteration when the variance of the consecutive cost function is relatively low:

$$\frac{F(x_{k-1}) - F(x_k)}{F(x_{k-1})} < 0.01\% \quad (4-10)$$

Though our proposed problem can be effectively minimized by FISTA, another issue is by how far can

MRA deconvolution approaches the ideal non-diffraction. In the absence of noise, there is no need to constrain the regularization term of the noise. Reducing the fidelity term can improve the contrast of the image to almost diffraction free (as shown in **Fig. S9**). In fact, since images always contain noise, the latter two items need to be weighted (i.e., λ_1 and λ_2 values). This results that in most cases where although MRA can remove most diffraction blurring, there is still some distance from the ideal non-diffraction situation. Though the artifact-prone RL cannot be count on to further infer the unsolved high-frequency information, several times of RL can improve the contrast of the image, making it closer to the ideal non-diffraction case.

We have demonstrated the effect of additional RL iterations after MRA in several data (**Fig. S16**). The results show that additional RL would significantly improve the contrast of MRA image especially under low-SNR cases. However, it is also important to note that the number of RL should not be high (should be less than 5 times), otherwise it will significantly increase artifacts and errors. Another consideration for opening this operation is that users may have the demands to further sharpen the edge to facilitate visual perception (which though may not be real).

Supplementary Note 4.2: 2D-SecMRA deconvolution

The performance of 2D MRA deconvolution algorithm is limited by the fluorescence background. Adding a spatial sparsity regularization $\|x\|_1$ may help remove some uniform background information. However, in many cases because of out-focus interference and uneven fluorophore accumulation, the background is largely uneven. In this case, the uniform thresholding to minimize $\|x\|_1$ does not work. One commonly used method is to subtract the background first and then conduct deconvolution. However, we found that this scheme would lose high-frequency and low-intensity details because the preliminary background subtraction inevitably amplifies the noise. Moreover, this method also cannot deal with the severe background. It is essential to overcome this condition. Here we develop the SecMRA deconvolution algorithm, which aims to mitigate the influence of fluorescence background and uneven intensity distribution.

The idea is to introduce a bias soft-thresholding mechanism of the image to alleviate the influence of background and some ultra-bright parts. To achieve this outcome, the first step is to estimate the background. We do so by performing a 5-level framelet transform of the original image and subtracting the coarse approximation of the image on the 5th-level scale coefficients. Then we formulate the weighting matrix as follows:

$$M = p \cdot f_{coarse} + (1 - p) \cdot f \quad (4-12)$$

where f_{coarse} is the coarsened image obtained by only using the fifth level scaling function information of the framelet transform, f is the original image, p is a coefficient representing the contribution of the background.

Given a weighting matrix, we soft-threshold the original image with a uniform threshold value, and impose a higher threshold value to the estimated background or ultra-bright point and a lower threshold value to the other part:

$$T = mask_1 \cdot \left[\frac{(1-c)}{g_{critical}} \cdot M + g_{critical} \right] \cdot T_0 + mask_2 \cdot \left[(1 + M - g_{critical})^k \cdot T_0 \right] \quad (4-13)$$

where T_0 is the uniform thresholding value, $g_{critical}$ is the critical grey value of the background, c is the minimal threshold weighting, k is the bias coefficient, $mask_1$ and $mask_2$ operation are defined as follow:

$$mask_1(e_{ij}) = \begin{cases} 1, & e_{ij} \leq g_{critical} \\ 0, & e_{ij} > g_{critical} \end{cases} \quad (4-14)$$

$$mask_2(e_{ij}) = \begin{cases} 0, & e_{ij} \leq g_{critical} \\ 1, & e_{ij} > g_{critical} \end{cases} \quad (4-15)$$

In this formulation, the thresholding matrix T is bias to different pixel. For the pixel value higher than the critical background value, we use a polynomial function to increase its threshold value, which is referred from

the conventional Gamma transform. For the pixel with a grey value lower than the critical background value, we use a linear function to reduce its threshold value, which is beneficial for preserving low-intensity signals. In this case, we penalize the high-background component and enhance the low-background component, which alleviates the influence of fluorescence background.

The procedure of SecMRA deconvolution is as follows:

Iteration scheme for 2D-SecMRA deconvolution

Input: Noisy image x , blurring matrix A , regularization parameter λ_1, λ_2 and background thresholding value λ_3 , critical gray value $critical$, bias parameter k , background contribution coefficient p

Initialization: Let $x_0=x, t_0=1$

Generate bias thresholding matrix:

$$M = p \cdot b_{coarse} + (1 - p) \cdot b$$

$$\lambda_{3bias} = mask_1 \cdot \left[\frac{(1-c)}{g_{critical}} \cdot M + g_{critical} \right] \cdot \lambda_3 + mask_2 \cdot \left[(1 + M - g_{critical})^k \right] \cdot \lambda_3$$

Iteration:

The k th iteration ($k \geq 1$):

$$\begin{aligned} x_k &= x_{k-1} - t_k \nabla f(x_{k-1}) \\ x_k' &= W^T \mathcal{T}_{\lambda_1/L}(Wx_k) \\ x_k'' &= C^T \mathcal{T}_{\lambda_2/L}(Cx_k') \\ x_k''' &= \mathcal{T}_{\lambda_{3bias}/L}(x_k'') \\ x_k &= x_k''' \\ t_{k+1} &= \frac{1 + \sqrt{1 + 4t_k^2}}{2} \\ x_{k+1} &= x_k + \left(\frac{t_k - 1}{t_{k+1}} \right) (x_k - x_{k-1}) \end{aligned}$$

Until both $\|b - Ax\|_2^2 + \lambda_1 \|Wx\|_1 + \lambda_2 \|Cx\|_1$ and $\lambda_{3bias} \|x\|_1$ converge

For simplicity of use, we empirically introduce two modes for 2D SecMRA deconvolution: background removal and uneven intensity correction, which takes background contribution coefficient p value in Eq. (3-12) as 0.8 and 0.3, respectively. The critical grey value of the background $g_{critical}$ is chosen as 0.2. In this case we only need to tune the bias parameter k and background thresholding value λ .

The SecMRA deconvolution with bias threshold overcomes the barrier of traditional deconvolution algorithm when processing fluorescence images with the uneven background. However, the trade-off between MRA contribution and bias thresholding contribution is still inherent. When dealing with some fluorescence images with ultra-strong background, we introduce a preliminary background subtraction before the 2D

Iteration scheme for preliminary background subtraction

Input: Image with background x , bias parameter k , background contribution coefficient p , background thresholding value λ , and iteration time n .

Initialization: Let $x_0 = x$

Iteration:

The k th iteration ($k \geq 1$):

$$\begin{aligned} M &= p \cdot x_{k_{coarse}} + (1-p) \cdot x_k \\ \lambda_{bias} &= mask_1 \cdot \left[\frac{(1-c)}{g_{critical}} \cdot M + g_{critical} \right] \cdot \lambda / n + mask_2 \cdot \left[(1 + M - g_{critical})^k \right] \cdot \lambda / n \\ x_{k+1} &= \mathcal{T}_{\lambda_{bias}}(x_k) \end{aligned}$$

Until reach maximum iteration times.

Supplementary Note 4.3: Accelerated iteration in high-SNR cases

Our cost function is composed of two parts: The fidelity term $\|b-Ax\|_2^2$ contributes to resolution improvement, and MRA sparsity term $\lambda_1\|Wx\|_1+\lambda_2\|Cx\|_1$ controls the noise. In high-SNR cases, the observed image b contains little noise and has sufficient reliability as a basis for improving resolution. In this case, a finely reduced fidelity penalty is highly desired to improve resolution as high as possible. Reflecting in parameter choice, a lower λ_1 and λ_2 value should be chosen to increase the relative weight of the fidelity term. Though FISTA is an effective approach to minimize the cost function of the inverse problem, one question is, does it mean that there is almost no change in the image when the overall loss function is almost convergent? Our simulation in Supplementary Fig. 10 shows that a large number (>1000) of the inverse filtering is needed to finely recover some high-frequency details in noiseless condition. While a common iteration times to satisfy the FISTA convergence criterion we set in (4-10) is <50 times. We also tried in simulation and real fluorescence data (**Fig. S11**), which shows that at typical iteration times there are still some high-frequency details cannot be resolved. When we tried to increase the number of iterations to the order of 10^3 , some high-frequency details started to be resolved, which can be verified in GT.

However, there are also some drawbacks to doing so: so many iterations result in a long calculation time. To solve this problem, we propose a solution to accelerate the algorithm speed under high signal-to-noise ratio. Due to the fact that the main time-consuming steps in the calculation are the framelet and curvelet transformations, we approximate the original iteration with the following iteration scheme: perform several times inverse filtering during the iteration process to reduce the fidelity term, and then perform one time framelet and curvelet thresholding. In simulation and experimental data, we have demonstrated that this improvement can better reduce the fidelity term and effectively control noise (**Fig. S11**). In the deconvolution model with given λ value, our improved scheme has lower fidelity cost and higher MRA sparsity cost compared to the original iterative scheme. The final iteration result of the acceleration iteration scheme with a given λ value is almost identical to the final iteration result with a smaller λ value before improvement, but the calculation time of the acceleration scheme is only 1/20 of it.

Our acceleration strategy is to better reduce the cost of fidelity items. When the SNR is low, this is not applicable because the observed image b has too much noise to support significant resolution improvement through inverse filtering. Therefore, we only activate the accelerated iteration strategy under high SNR conditions. The judgment of image signal-to-noise ratio can be defined by selecting regularization parameters. Based on the actual parameter adjustment experience in the article, we have set acceleration parameters in MRA based on the selected parameters:

$\max(\lambda_1, \lambda_2) \leq 1e-4$: interval of two MRA thresholding is set as 40

$1e-4 < \max(\lambda_1, \lambda_2) \leq 1e-3$: interval of two MRA thresholding is set as 20

$5e-3 < \max(\lambda_1, \lambda_2) \leq 8e-3$: interval of two MRA thresholding is set as 10

$8e-3 < \max(\lambda_1, \lambda_2)$: turn off acceleration mode

For SecMRA, considering that when using this deconvolution method, there is a lot of defocused information in the image. We have appropriately reduced the trust in the fidelity term and set the acceleration parameters as follows:

$\max(\lambda_1, \lambda_2) \leq 1e-3$: interval of two MRA thresholding is set as 20

$1e-3 < \max(\lambda_1, \lambda_2) \leq 6e-3$: interval of two MRA thresholding is set as 10

$6e-3 < \max(\lambda_1, \lambda_2)$: turn off acceleration mode

When spatiotemporal continuity denoising is enabled, it means there is more noise in the image, and we will also not enable acceleration mode.

The detailed accelerated iteration schemes for 2D-MRA and 2D-SecMRA deconvolution are shown below:

Accelerated iteration scheme for 2D-MRA deconvolution

Input: Noisy image x , blurring matrix A , regularization parameter λ_1 and λ_2 , and the round of iterative inverse filtering between two MRA sparsity thresholding *interval*

Initialization: Let $x_0=x$, $t_0=1$

Iteration:

The k th iteration ($k \geq 1$):

If $\text{mod}(k, \text{interval}) = 0$

$$x_k = x_{k-1} - t_k \nabla f(x_{k-1})$$

$$x_k' = W^T \mathcal{T}_{\lambda_1/L}(Wx_k)$$

$$x_k'' = C^T \mathcal{T}_{\lambda_2/L}(Cx_k')$$

$$x_k = x_k''$$

$$t_{k+1} = \frac{1 + \sqrt{1 + 4t_k^2}}{2}$$

$$x_{k+1} = x_k + \left(\frac{t_k - 1}{t_{k+1}} \right) (x_k - x_{k-1})$$

Else

$$x_k = x_{k-1} - t_k \nabla f(x_{k-1})$$

Until reach maximum iteration times.

Accelerated iteration scheme for 2D-SecMRA deconvolution

Input: Noisy image x , blurring matrix A , regularization parameter λ_1, λ_2 and background thresholding value λ_3 , critical gray value $critical$, bias parameter k , background contribution coefficient p , and the round of iterative inverse filtering between two MRA sparsity thresholding $interval$

Initialization: Let $x_0=x, t_0=1$

Generate bias thresholding matrix:

$$M = p \cdot b_{coarse} + (1-p) \cdot b$$

$$\lambda_{3bias} = mask_1 \cdot \left[\frac{(1-c)}{g_{critical}} \cdot M + g_{critical} \right] \cdot \lambda_3 + mask_2 \cdot \left[(1+M - g_{critical})^k \right] \cdot \lambda_3$$

Iteration:

The k th iteration ($k \geq 1$):

If $mod(k, interval) = 0$

$$x_k = x_{k-1} - t_k \nabla f(x_{k-1})$$

$$x_k' = W^T \mathcal{T}_{\lambda_1/L}(W x_k)$$

$$x_k'' = C^T \mathcal{T}_{\lambda_2/L}(C x_k')$$

$$x_k''' = \mathcal{T}_{\lambda_{3bias}/L}(x_k'')$$

$$x_k'''' = x_k'''$$

$$t_{k+1} = \frac{1 + \sqrt{1 + 4t_k^2}}{2}$$

$$x_{k+1} = x_k + \left(\frac{t_k - 1}{t_{k+1}} \right) (x_k - x_{k-1})$$

Else

$$x_k = x_{k-1} - t_k \nabla f(x_{k-1})$$

Until reach maximum iteration times.

Supplementary Note 4.4: MRA-MLE deconvolution

To illustrate the advantages of the model-solution framework, we develop MRA-MLE in contrast with MRA-Model (abbreviated as MRA) framework applied in the article. The design of MRA-MLE utilizes the framelet and curvelet regularization, and an advanced MLE deconvolution framework enlightened by Sparse deconvolution. An additional $\|x\|_1$ regularization of the original MRA cost function to amplify the effect of the following RL iteration:

$$\min \|b - Ax\|_2^2 + \lambda_1 \|Wx\|_1 + \lambda_2 \|Cx\|_1 + \lambda_3 \|x\|_1 \quad (4-16)$$

The iteration scheme of MRA-MLE is first denoising, and then deblurring. In the first denoising iteration we treated Ax as whole with no resolution improvement using knowledge from the PSF. After the denoising process, we execute RL iteration many rounds to improve the resolution. The iteration scheme can be summarized as follow:

Iteration scheme for MRA-MLE deconvolution

Input: Noisy image x , blurring matrix A , regularization parameter λ_1 and λ_2

Initialization: Let $x_0=x$, $t_0 = 1$

Iteration for denoising:

The k th iteration ($k \geq 1$):

$$x_k = x_{k-1} - t_k \nabla f(x_{k-1})$$

$$x_k' = W^T \mathcal{T}_{\lambda_1/L}(Wx_k)$$

$$x_k'' = C^T \mathcal{T}_{\lambda_2/L}(Cx_k')$$

$$x_k''' = \mathcal{T}_{\lambda_3/L}(x_k'')$$

$$x_k = x_k'''$$

$$t_{k+1} = \frac{1 + \sqrt{1 + 4t_k^2}}{2}$$

$$x_{k+1} = x_k + \left(\frac{t_k - 1}{t_{k+1}} \right) (x_k - x_{k-1})$$

Until the cost function $\|b - Ax\|_2^2 + \lambda_1 \|Wx\|_1 + \lambda_2 \|Cx\|_1 + \lambda_3 \|x\|_1$ converges

Iteration for deblurring:

Execute RL iteration many times

Supplementary Note 5: Spatiotemporal continuity denoising

To achieve long-term live-cell imaging, it is usually essential to lower the photon dose so that lower phototoxicity allows the cell to live longer. In this case, the fluorescence image quality would inevitably worsen. Fast imaging also leads to poorer image quality due to the short exposure time. The 2D-MRA deconvolution algorithm that uses the co-sparsity of framelet and curvelet domain can help alleviate the poor image quality in the two cases. However, the pure 2D method would ignore some 3D data features such as continuity. The utilization of the 3D structural information expectedly would bring some sort of improvement.

The 3D Framelet transform is also sensitive to the 3D signal edge and capable of dealing with cross-edge denoising tasks. Denote the 1D framelet bases as ϕ , ψ_1 , and ψ_2 . The 3D framelet base can be generated by the tensor product of the 1D framelet base:

$$\Phi(x, y, z) = \phi(x) \otimes \phi(y) \otimes \phi(z) \quad (5-1)$$

$$\begin{aligned} \{\Psi(x, y, z)\} = \{ & \phi(x) \otimes \psi_{l_1}(y) \otimes \psi_{l_2}(z), \phi(x) \otimes \phi(y) \otimes \psi_{l_2}(z), \\ & \phi(x) \otimes \psi_{l_1}(x) \otimes \phi(z), \psi_{l_1}(x) \otimes \phi(y) \otimes \psi_{l_2}(z), \\ & \psi_{l_1}(x) \otimes \phi(y) \otimes \phi(z), \psi_{l_1}(x) \otimes \psi_{l_2}(y) \otimes \phi(z), 1 \leq l_1, l_2 \leq 2 \} \end{aligned} \quad (5-2)$$

where $\Phi(x, y, z)$ is the scaling function, $\Psi(x, y, z)$ is the wavelet function. The framelet coefficient can be obtained by the inner product of the 3D data with the 3D framelet bases.

The denoising of 3D framelet can be achieved by soft-threshold its decomposition coefficients:

$$f = F_{3D}^{-1} T_{\lambda} (F_{3D} g) \quad (5-3)$$

where F_{3D} denotes the 3D framelet transform, F_{3D}^{-1} denotes the inverse framelet transform.

One of the major drawbacks of the separable 3D transform such as the 3D framelet transform is that it lacks the direction detection ability. This is the reason why this separable 3D wavelet transforms alone do not apply widely in the video denoising task. The complex dual-tree wavelet transform is developed with the idea to separate the positive frequency and negative frequency part of the separable wavelet transform. This dealing grants the dual-tree wavelet the ability of a sharper direction detecting.

The dual-tree wavelet transform is oriented by adding an imagery part to the conventional wavelet transform:

$$\psi(x) = \psi_h(x) + j\psi_g(x) \quad (5-4)$$

where $\psi_h(x)$ is the conventional separable wavelet base, $\psi_g(x)$ is the Hilbert pair of $\psi_h(x)$ to satisfy the perfect reconstruction condition. For computational feasibility, $\psi_h(x)$ is obtained as the approximation of the Hilbert pair of $\psi_h(x)$.

The benefit of formulating such a dual-tree wavelet pair is that when transforming to a higher dimensional, the positive and negative frequency component is separated. This gives it the ability for better orientation detection. The higher dimensional complex dual-tree wavelet transform can be obtained by the tensor product of its 1D form. To maintain computational simplicity, the real part of each higher dimensional is subtracted. Reflecting in the outcome in fluorescence image, the 3D DTCW soft-thresholding subtracts the along-edge continuity information just as curvelet does in the 2D domain, which is used for denoising:

$$f = W_{DTCW-3D}^{-1} \mathcal{T}_\lambda (W_{DTCW-3D} g) \quad (5-5)$$

In order to simplify parameter tuning and consider that not all 3D images have good continuity, we hold the thresholding value for 3D DTCW coefficients as 1/3 of that for 3D framelet coefficients.

Supplementary Note 6: Simulation results

For lack of ground-truth images in practical fluorescence imaging, it is necessary to assess the performance of MRA through simulation. Here we summarize our simulation results to verify the effectiveness of MRA and contrast MRA with other algorithms. Our methods general include the following steps: (1) Establish ground-truth images; (2) Add noise and/or blurring to GT images; (3) Process images using established algorithms; (4) Evaluating the processed image quality using GT as reference.

Supplementary Note 6.1: Simulation to verify the effectiveness of MRA noise-control

To validate the effectiveness of the MRA deconvolution algorithm, we synthesized some simple structures to test their effectiveness in different noise levels (**Fig. S4**).

To evaluate the resolution enhancement ability of MRA deconvolution under different noise levels, we synthesized parallel lines distancing from 10 to 100 nm in a 1024×1024 image with pixel size statted as 2 nm (**Fig. S4a-c**). The line thickness is chosen as 2 pixels (i.e., 4 nm). Then we blurred the image using a PSF generated by Bessel approximation of a 488 nm excitation wavelength, 1.4 NA, and $2\times$ scaling factor, which simulates a super resolution imaging that ideally improves the image resolution by 2-fold. We use *imnoise* function in MATLAB to add Gaussian noise to the blurred Ground-truth, which generates noisy image with PSNR of 24, 14.8, 7.64 dB, which degenerates the original 60 nm resolution to 70, 70 and 100 nm. MRA deconvolution can faithfully recover the lost resolution and even provide a 10 nm resolution enhancement with respect to the original image when the PSNR is 24 dB. We also fabricate some circles with radius ranging from 10 to 100 nm and repeat a similar simulation (**Fig. S4d-f**). The results also support that MRA deconvolution can enhance the image resolution with high fidelity. However, such resolution enhancement is affected by noise level.

Supplementary Note 6.2: Simulation to compare MRA with other deconvolution techniques

We first compare the noise-control performance of MRA with variance-based regularization using two time-lapse images captured by widefield microscope and SIM (**Fig. 1e, h**). We use the captured time-lapse images as GT, blur them, add noise, and use Hessian and MRA algorithms to reduce noise. Then, we evaluate their SNR using GT images. Since we only want to compare the noise reduction performance of different regularizations and exclude the resolution scaling, the blur kernel used is the PSF at the time of capturing the image. Our blurring operation is equivalent to multiplying the frequency spectrum of an image by OTF again in the frequency domain. Due to the fact that the spectrum of the image obtained during microscopic imaging

is already within the OTF, this operation has almost no impact on the information in the image (since our software needs a PSF input to process, this also ensures the repeatability of the results). The type of the added noise is chosen as 50% Gaussian+ 50% Poisson. To do so, we use the MATLAB function *normrnd* and *poissrnd* to generate Gaussian and Poisson random numbers, then based on the power ratio of Gaussian and Poisson noise we adjust the amplitude of Poisson noise to make sure the power of the two noise types is equivalent.

We choose proper parameters to ensure the images obtained from the two algorithms are in equivalent resolution level (characterized by the FWHM value), and then calculate the SNR. Considering the good continuity of the data, we set the continuity parameter in Hessian as $\sigma=1$. The weight of Hessian penalty parameter is used as $\mu=150, 80, 60, 30, 20, 10$ for the lysosome image stack group 1-6; $\mu=150, 100, 80, 50, 80, 80$ for the microtubule image stack group 1-6. The chosen μ decrease with the decreasing image SNR to better control the noise. For the microtubule image stacks we did not decrease the μ value for group 5 and 6 because the signal of many whole microtubules would be wrongly recognized as noise and removed (**Fig. S6**), conversely resulting in a lower SNR value after deconvolution.

We also examine the performance of different regularizations applying to the iterative inverse filtering using simulation pattern²⁸ (**Fig. S9**). Considering the variety of parameter choice, we adjust the parameter value in TV-FISTA, Hessian-FISTA and MRA-FISTA to control the background noise to ~ 0.7 . From the result, it can be seen that even under relatively weak noise condition, Hessian and TV cannot support physical constrained inverse filtering to infer resolution. The high-frequency information inferred in physical constrained inverse filtering (with high variability features) is erased along with noise. But our MRA regularization, on the contrary, supports it well.

Besides the comparison with variance-based regularization, we also use the simulation pattern to validate some conclusions we made about the difference between MRA and MLE methods on resolution improvement. We first test the underlying mechanism: iterative inverse filtering and RL iteration on a noiseless simulation pattern contains rich structures (**Fig. S8**). We blurred the pattern with a Gaussian low-pass filter ($\sigma=3$ pixels) and then deconvolved it using iterative inverse filtering and standard RL iteration. The results showed that iterative inverse filtering successfully restored most of the high-frequency information of the image after sufficient iterations, but RL iteration showed artifacts in a few iterations. In fact, this conclusion is also relatively obvious in mathematics. In the absence of noise, the optimization problem $\min \|b-Ax\|_2^2$ is convex, meaning that forcing a gradient descent (i.e., the iterative inverse filtering) can always search its minimum.

Supplementary Note 7: Effect of parameters

In this note, we discuss the effect of each parameter in our algorithms.

(1) Noise related parameters: 3D-Framelet, 3D-DTCW, 2D-Framelet and 2D-Curvelet regularization parameter.

These parameters play a role in noise control. The effect of tuning 3D parameters is similar with the 2D cases. Here we mainly discuss the effect of 2D parameters. There are two reasons for the degradation of fluorescent images: blur and noise, which are compensated by the reduction of fidelity penalty $\|b-Ax\|_2^2$ and MRA sparsity penalty $\lambda_1\|Wx\|_1+\lambda_2\|Cx\|_1$ in our algorithm. The full cost function is $\|b-Ax\|_2^2+\lambda_1\|Wx\|_1+\lambda_2\|Cx\|_1$.

In our deconvolution model, the influence of λ_1 and λ_2 is notably conspicuous: a high parameter value corresponds to a higher MRA sparsity weight, thereby magnifying noise reduction outcome. On the contrary, a low parameter value corresponds to a higher fidelity term weight, thereby increasing the degree of deblurring. The parameter values determine the thresholding values for framelet and curvelet coefficients in the FISTA iteration, which is the direct reason why they dictating noise-control level. The effect of the fidelity term and MRA sparsity term is also to some extent contradictory. When MRA sparsity term dominates the total cost function (i.e., a higher regularization parameter applied), the noise would be finely controlled. But it is not conducive to improving resolution through inverse filtering due to the possibility of losing high-frequency details. On the contrary, if the fidelity term dominates the total function (i.e., a lower regularization parameter applied), the iterative inverse filtering would greatly expand the range of informative spectrum (i.e., deblurring). However, for lack of regularization in MRA sparsity, some residual noise in the image would be amplified.

Therefore, based on the noise condition of a specific image, we need to weigh the two aspects: resolution improvement and noise reduction. When the noise in the image is low, blurring is the major factor for image degeneration. At this point, λ_1 and λ_2 should be lower to increase the weight of the fidelity term to improve resolution. When the noise in the image is high, noise is the major factor for image degeneration, meaning that λ_1 and λ_2 value should be adjust higher to increase the level of noise reduction. Another issue should be reminded is that the attainable SNR through MRA deconvolution would decrease with the increase of noise level. And deblurring (i.e., resolution improvement) cannot be achieved in noisy conditions. Because the presence of large noise in image b , it becomes unreliable to deblur by reducing the fidelity term $\|b-Ax\|_2^2$. Given a specific image, the process of tuning λ_1 and λ_2 is seeking the optimal solution under its SNR level.

Taking simulated image as an example (**Fig. S27**), we adjust the parameters to achieve the highest SNR under different noise levels. Our simulation results also validate the above analysis: as the SNR decreases, the optimal filtering threshold should be higher. We also demonstrate this principle in real fluorescence images (**Figs. S28-30**), which shows adjusting the thresholding value mainly regulates the noise level and resolution enhancement in the deconvolution results. This effect is also demonstrated in the 3D scenario (**Fig. S31**).

Another issue is the ratio of λ_1 and λ_2 value. Tuning λ_1 and λ_2 is the process of choosing the weight of

denoising in framelet and curvelet domain, which should consider both the sample and noise features. Actually, we can see from **Fig. S5** that any single one of them still works to some extent, but a combination of the two multiscale bases yield better outcome. Each multiscale base has its only advantages and limitations. The logics of our combinations of framelet and curvelet lie in that to control a certain level noise, if only one of them is used, a higher cost in high-frequency information loss is needed (a higher thresholding value needed). As we stated before, the value of doing multiscale-base transform essentially is seeking a sparse representation of signal, meaning that most information is conserved in some high-value coefficients, and contrasting the signal in the low-value one.

For instance, an image decomposed by framelet, generating coefficients $\{f_1, f_2, \dots, f_n\}$, with $f_1 < f_2 < \dots < f_n$; for curvelet, generating coefficients $\{c_1, c_2, \dots, c_n\}$, with $c_1 < c_2 < \dots < c_n$. For convenience of discussion, we consider framelet coefficients $f > f_{k1}$ and curvelet coefficients $c > c_{k2}$ contain unneglectable information about the image (i.e., $f_{k1}-f_n, c_{k2}-c_n$ contain important information about the image). The low-value coefficients ($f_1-f_{k1-1}; c_1-c_{k2-1}$) are considered as noise. Due to the design to extract different features, we can assert that the perceived noise represented by f_1-f_{k1-1} and c_1-c_{k2-1} is different. To control the noise, if only one of them are used, a larger thresholding value is needed, which increases the possibility of removing coefficients representing important information. For example, if framelet is solely used and a thresholding value of f_{k1-1} is insufficient to control the noise to a satisfactory level, we would increase the framelet thresholding value, causing a loss in important information. In this case, adding an additional curvelet thresholding with thresholding value lower than c_{k2} is a better choice to further improve SNR. The mentioned criterion of important information threshold f_{k1} and c_{k2} are related with sample features.

We then further test different ratios of framelet and curvelet thresholding at different noise levels to the simulation pattern we used above. The results show that in most cases a combination of framelet and curvelet thresholding can improve the SNR more. Adjusting the ratio of λ_1 and λ_2 has a significantly lower impact on SNR than adjusting the thresholding value. It may be difficult to quantitatively describe the proportion of parameters that should be selected for a given fluorescence image without GT reference. On the one hand, this is because the framelet and curvelet thresholds not only have an impact on one's own sparsity, but also on the sparsity of the other part. On the other hand, given a noisy image, it is difficult to determine whether its low sparsity in a certain domain is due to noise or the characteristics of the sample itself (an example can be found in **Fig. S2**. The tubulin image shows higher curvelet sparsity than the mitochondrion image at high-SNR, which can be concluded as sample difference. While the addition of noise makes the curvelet sparsity in their noisy image look the same). Our parameter tuning results in show that in high-SNR cases (typically thresholding value $< 5e-3$) adjusting the ratio of framelet and curvelet thresholding has little effect on the final results (**Fig. S28**). While in noisy conditions, if the image contains rich structures with high along-edge continuity, adding more weight to curvelet thresholding can greatly boost the denoising performance while preserving high-frequency information. For example, a larger curvelet thresholding weight is very effective for the STED mitochondria image with rich cristae structures shown in **Fig. S30**.

- **Background related parameters: Bias parameter and background thresholding**

In SecMRA we add a bias thresholding mechanism to mitigate strong fluorescence background. Two parameters in the “Pre-debackground” and “2D-SecMRA deconvolution” step affect the results: bias parameter and background thresholding value. The background thresholding value is the uniform attenuation value (for a normalized image) applied to the image. It functions like conventional ways to increase the image’s sparsity and contrast. While a drawback for this attenuation is that low-intensity signals would also be removed during thresholding. The bias parameter is designed to detect background, bestowing it a larger weight during the background thresholding approach. In practical use, under a fixed bias parameter, increasing background thresholding would reduce the overall intensity of the image, which helps to attenuate the uniform low-intensity background. Under a given background thresholding, if the bias parameter is added, the intensity of the background concentration in the image would be attenuated. An example is shown in **Fig. S32**. Besides background attenuation, we also develop a “intensity correction” mode in SecMRA to process the case when the intensity of desired signal is low while the intensity in other part is high. In this mode, a higher bias parameter would attenuate the bright pixels in the image more.

Supplementary Note 8: Possible limitations of the algorithms

Although MRA and SecMRA have demonstrated commendable efficacy in facilitating fidelity-ensured computational super-resolution and denoising for fluorescence imaging, comprehending the occasion when they might not work well and might produce artifacts, remains imperative. The instances in which MRA and SecMRA might encounter challenges or engender artifacts are twofold: (1) Extreme noisy conditions: the noise may be too strong for the MRA to effectively extract signal information. (2) Normal noisy conditions: an improper selection of the regularization parameter may cause noise residue or thresholding of high-frequency details. We explain these two scenarios in more detail below:

(1) Extreme noisy conditions: the noise may be too strong for MRA to effectively extract signal information.

The term "extreme noisy conditions" as referred to in this discussion denotes a scenario where the image SNR falls below 1dB. A practical indicator for recognizing this condition is rooted in the chosen parameters: specifically, when the noise in the image is effectively eliminated, and if a 3D thresholding value >0.3 or a 2D thresholding value >0.1 is utilized, it indicates that the image undergoing processing might be situated in an extreme noisy condition. In such instances, careful attention should be taken toward the outcomes of the deconvolution process.

We illustrated this circumstance through the simulation presented in **Fig. 1** and **Fig. S7**. The simulation demonstrates that for data exhibiting good temporal continuity, as long as the SNR remains above 1 dB, MRA exhibits robust data recovery in the presence of noise, yielding a Pearson correlation coefficient > 0.8 when compared to GT data. Nevertheless, as the SNR descends further, noise could manifest structural attributes akin to "sharp edges" and "high continuity along edges," characteristics resembling signals. Consequently, the capacity of MRA to eliminate such noise becomes constrained. An illustrative example of this phenomenon is presented in the sixth column of **Fig. S7a**. Even when confronted with a negative SNR, MRA can still discern some useful signal-related information. However, at this level of noise, occasional convergence between noise structures and signal features can pose challenges for MRA in eliminating them. If users lack comprehensive familiarity with the intrinsic structure of the specimen, correct identification of residual noise could prove hard.

In summation, we advocate a noise level of 1 dB as a safety lower limit. Notably, this article also features an instance wherein MRA performs effectively, involving a critical scenario with an SNR of ~ 1 dB (resolving the ER signals with fast imaging speed shown in **Fig. S8**), showcasing its efficacy.

Furthermore, the variety of samples and the continuity of time-lapse data must be acknowledged. Our simulation provides a foundational reference, though under different conditions it may not apply. To facilitate advanced users in strictly ensuring the capability of MRA with specific biological experiments, we offer a solution to ascertain algorithmic capabilities via simulation. In addition, we make available an open-source program capable of introducing Gaussian-Poisson mixed noise. To enable users seeking to observe long-term,

or rapid biological dynamic processes where a low-SNR recording is expected, we propose the following course of action to verify the algorithm's capability:

- 1) First, capture images under good shooting conditions;
 - 2) Afterwards, use our simulation software to add noise to simulate the SNR level expected by users under low toxicity or fast conditions;
 - 3) Process simulated low SNR data using the MRA algorithm;
 - 4) Finally, compare and evaluate the effectiveness of the algorithm with high SNR images.
- (2) Normal noisy conditions: an improper selection of the regularization parameter may cause noise residual or thresholding of high-frequency details.**

We denote conditions that deviate from the realm of “extreme noisy conditions” as “normal noisy conditions”. In this case, an improper choice of parameters may cause noise residual or thresholding of high-frequency details.

The choice of regularization parameters should be based on the noise level of the image. We have to some extent made it an objective process by estimating the noise level using curvelet sparsity and automated the parameter choice. Nonetheless, precise estimation of image SNR remains challenging, engendering a manual tuning process during MRA/SecMRA application, potentially influencing outcomes. Lower regularization parameter values may not effectively control the noise during the iterative inverse filtering, resulting in amplified residual noise in the final image. Higher regularization parameter values may result in over-denoising and loss of high-frequency details. Additionally, besides the noise, if the sample contains less features that the two multiscale bases can sparsely represent, a higher regularization parameter value could also lead to loss of high-frequency details. The outcome of MRA is just like doing a low-pass filtering. The two phenomena can be found in **Figs. S28-31**.

A direct way to identify the possible residual noise or wrong thresholding to the high-frequency details is using the NanoJ-SQUIRREL software, which will indicate the location of the error and provide the overall resolution-scaled error. Based on the results in our article, for an 8-bit image, an overall error rate of less than 3 grayscale values is appropriate. While a comprehensive inspection of all deconvoluted images using NanoJ-SQUIRREL might be time-consuming. Furthermore, it is crucial to note that NanoJ-SQUIRREL computes the resolution-scaled error based on the original image, its fidelity contingent on the SNR of the original image.

To mitigate this, besides the NanoJ-SQUIRREL, we analyze change of the fidelity penalty before and after deconvolution, and also the parameter selection regarding the curvelet sparsity of the given noisy image, to give feedback about the parameter choice in the software. If the curvelet sparsity of the original image is high (typically indicating a high SNR) and the fidelity penalty of the image is not well reduced, we will remind the user that the chosen thresholding value is too large, which may suppress high-frequency details. If the original image's curvelet sparsity is not high (typically indicating a low SNR), but the processed image's fidelity penalty is significantly reduced, we will remind the user that the chosen thresholding is too small, which may result in residual noise artifacts.

Another issue should be taken care is the thresholding value in the spatiotemporal continuity denoising step. This operation is only effective for noisy time-lapse or 3D data with good continuity. If the continuity in the original image is not good, a large 3D thresholding value may also lead to artifacts. The method to identify this situation is to observe the images before and after processing. The emergence of structural information in the processed image absent in the pre-processing counterpart indicates an excessive 3D thresholding application.

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Supplementary Figures

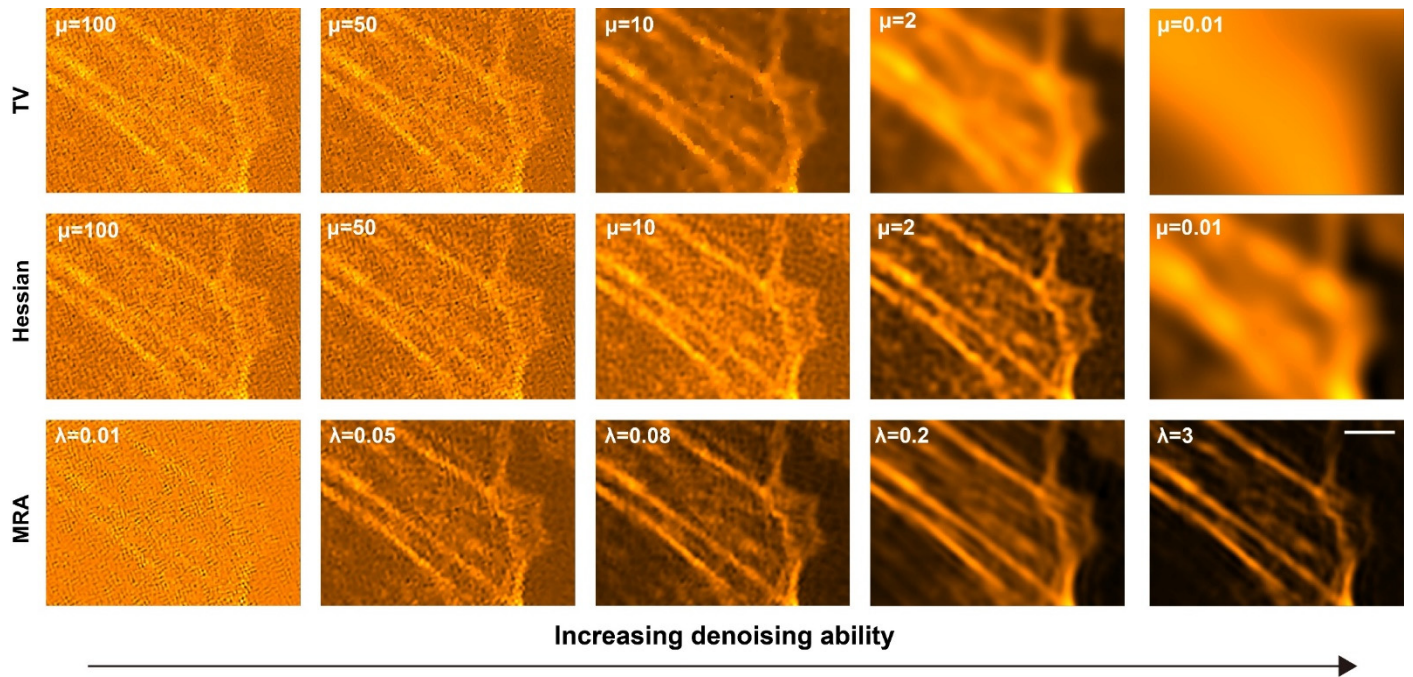


Fig. S1. Limitation of traditional variance-based regularization and the improvement by MRA regularization. TV, Hessian and MRA deconvolution are employed to deconvolute the noisy actin image with different denoising ability by tuning the regularization parameter. Scale bars: $2\ \mu\text{m}$.

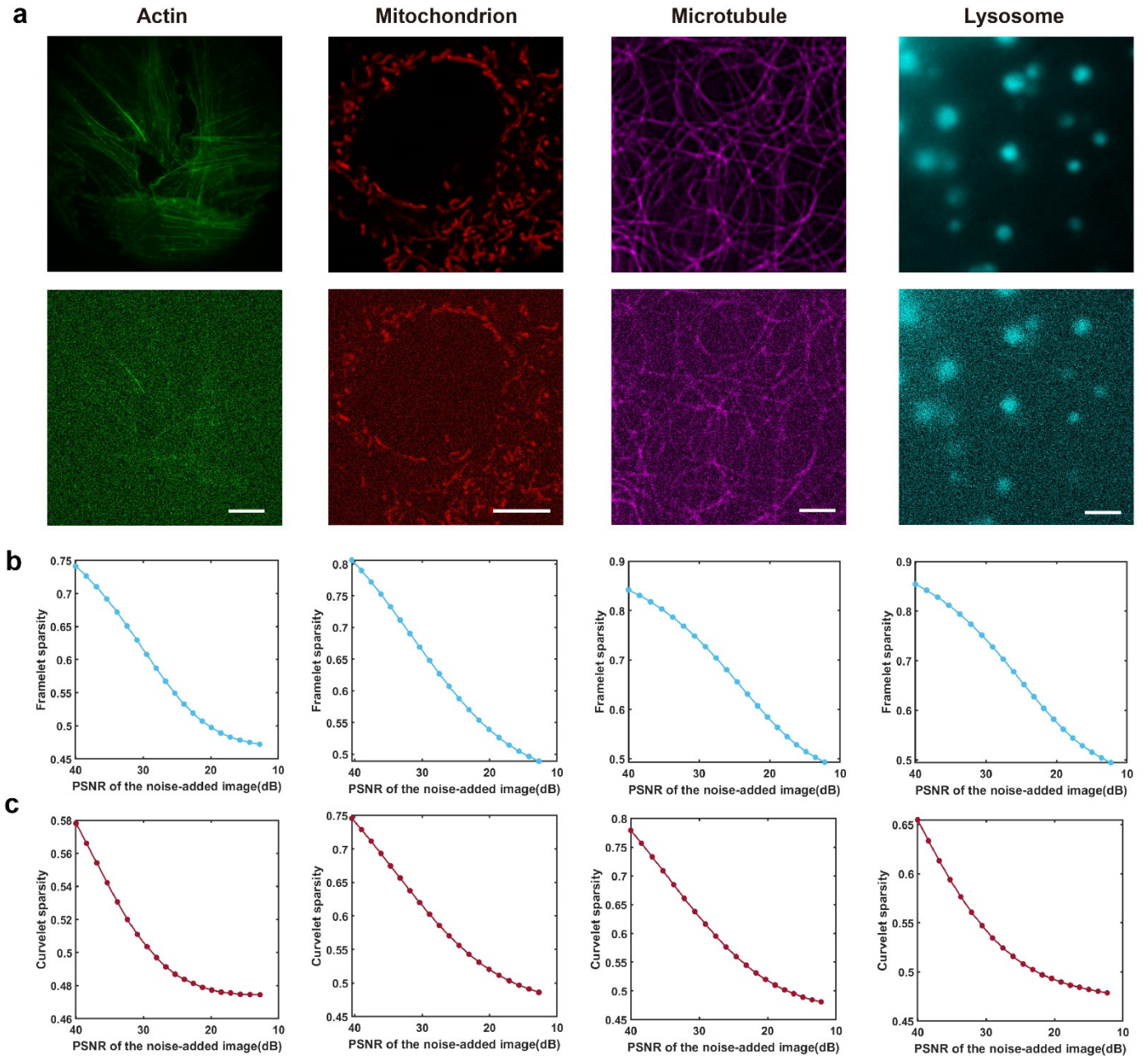


Fig. S2. Verification of the fluorescence image sparsity in framelet and curvelet domain. **a.** The clean image of the cell structures (first row) and the one with 50% Gaussian + 50% Poisson noise added (second row). **b.** The sparsity of the image under framelet representation as the function of the input noise power. **c.** The sparsity of the image under curvelet representation as the function of the input noise power. Scale bars: 10 μm (Mitochondrion), 5 μm (Actin), 2 μm (Microtubule, Lysosome).

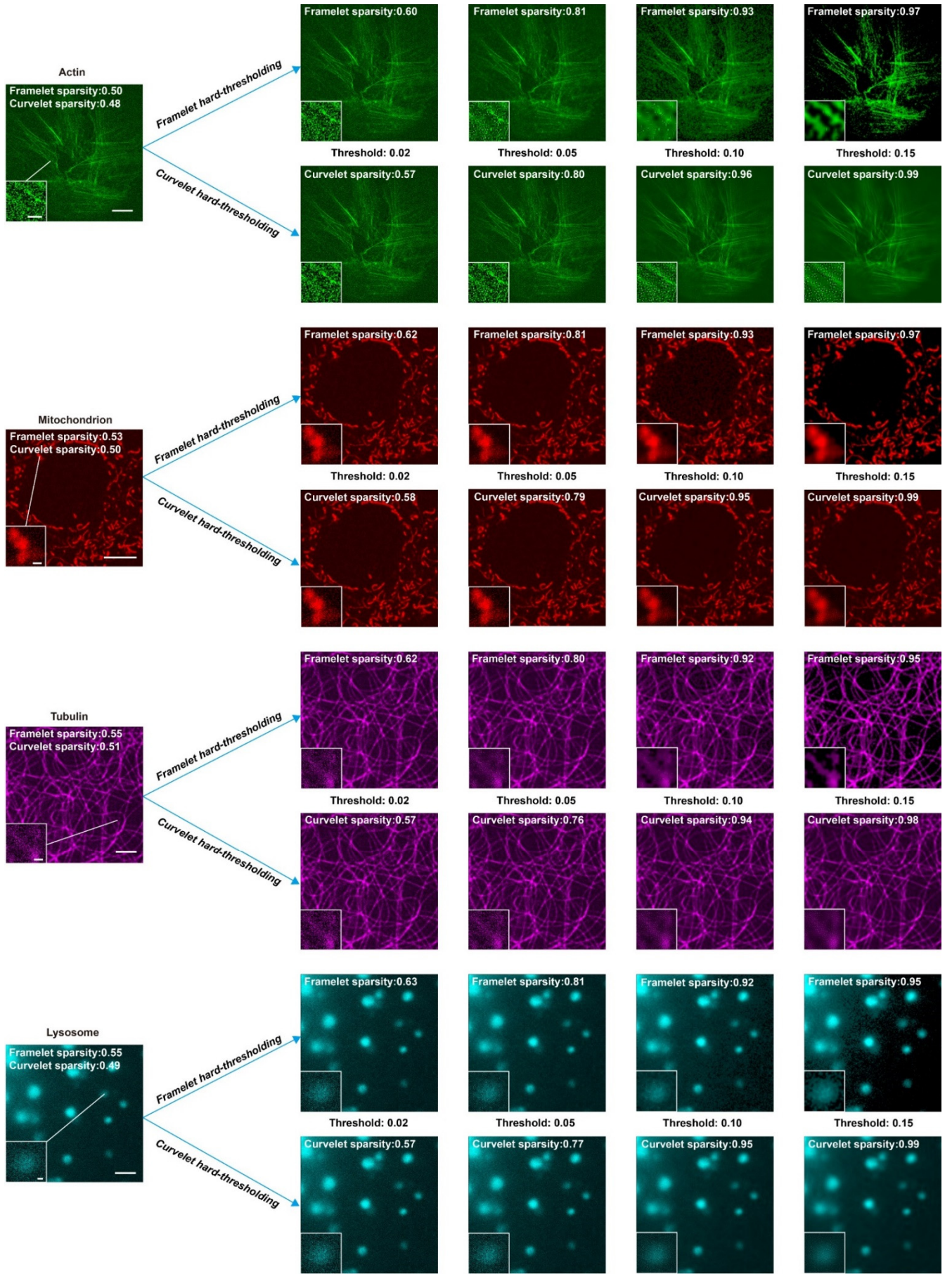


Fig. S3. The effect of framelet-domain and curvelet-domain hard thresholding on the noisy fluorescence image. Scale bars: 10 μm (Mitochondrion), 5 μm (Actin), 2 μm (Tubulin, Lysosome), 1 μm (Mitochondrion subgraph, Lysosome subgraph), 0.5 μm (Actin subgraph, Tubulin subgraph).

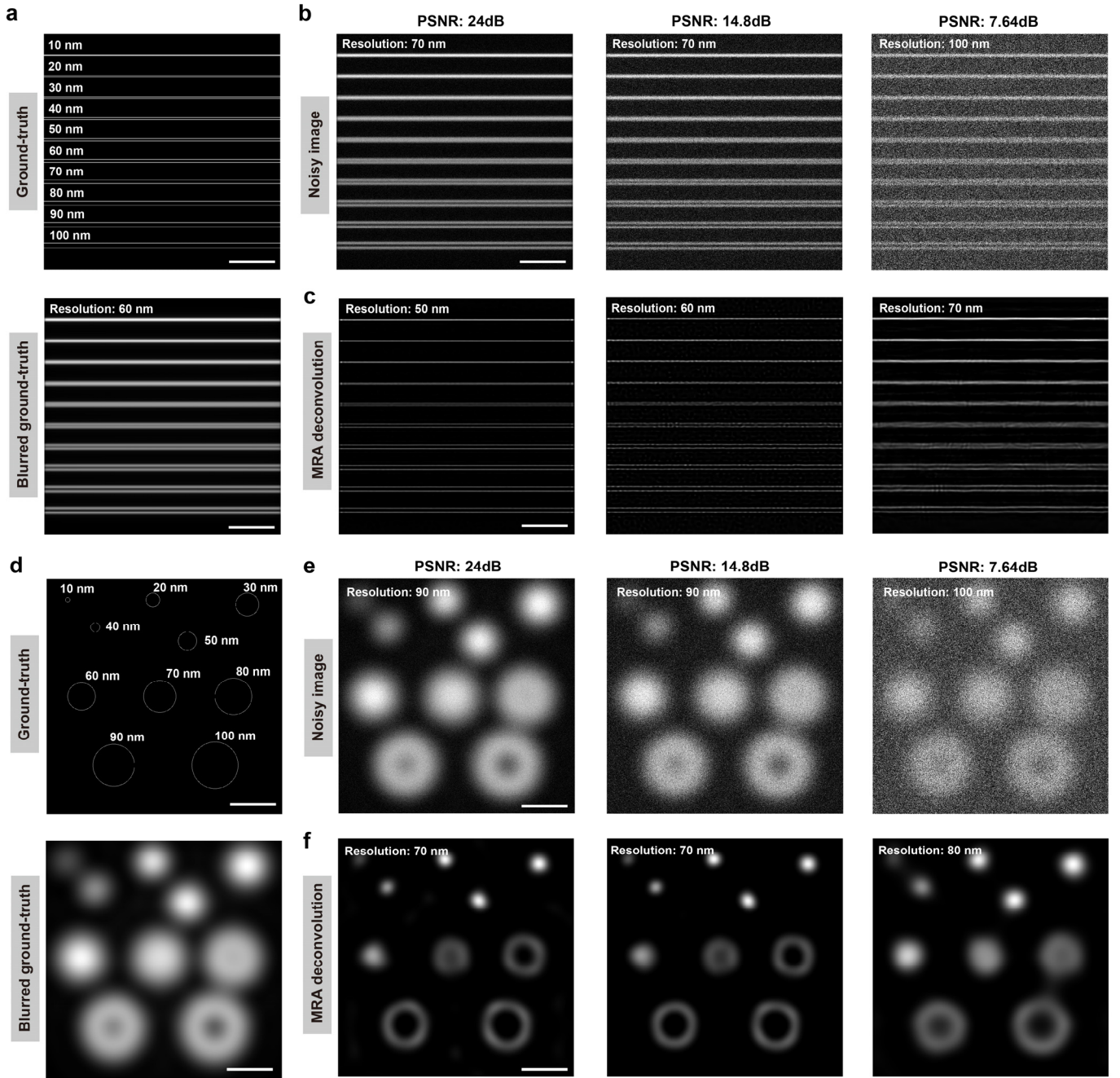
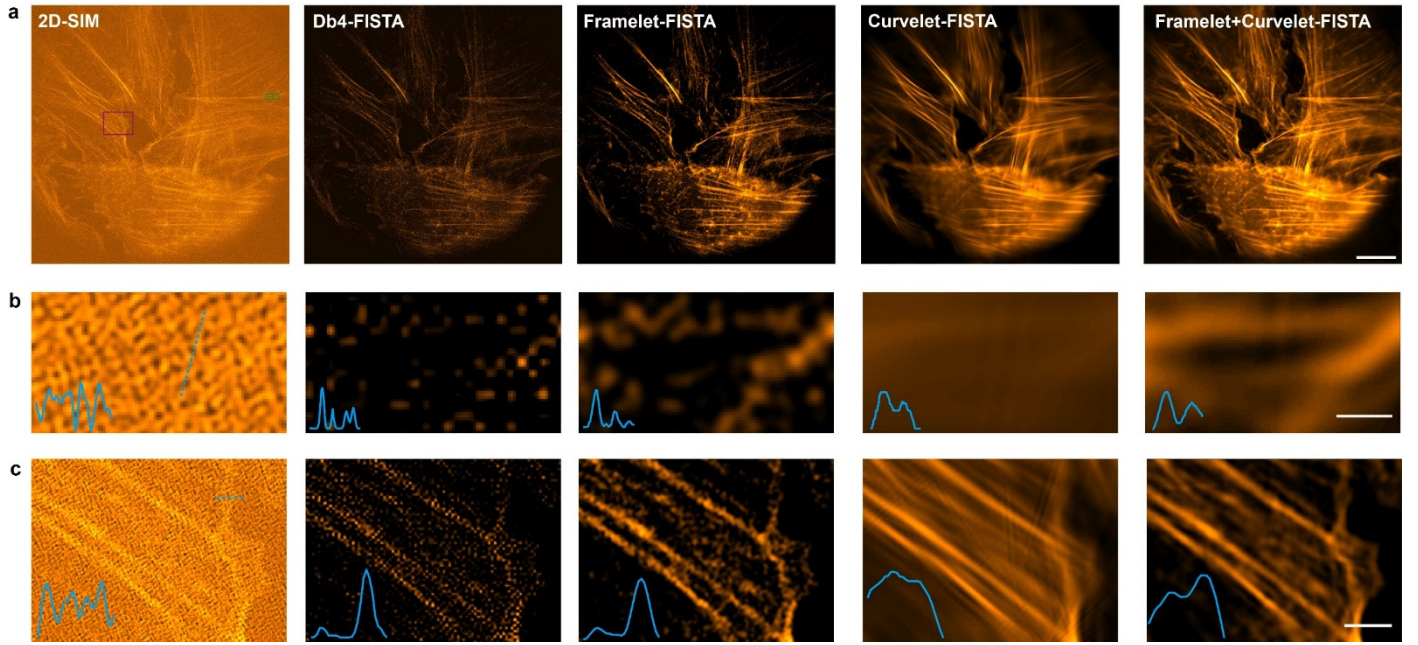


Fig. S4. MRA deconvolution algorithm recovers straight lines and circles from blurring and noise. **a.** The ground-truth straight line pairs, with spacing from 10 nm to 100 nm. The bottom is the blurred ground-truth. **b.** The blurred ground-truth added with different levels of Gaussian noise. **c.** The outcome of the MRA deconvolution. **d.** The ground-truth circles, with spacing from 10 nm to 100 nm. The bottom is the blurred ground-truth with a PSF size of. **e.** The blurred ground-truth added with different levels of Gaussian noise. **f.** The outcome of the MRA deconvolution. Scale bar: 0.1 μm .



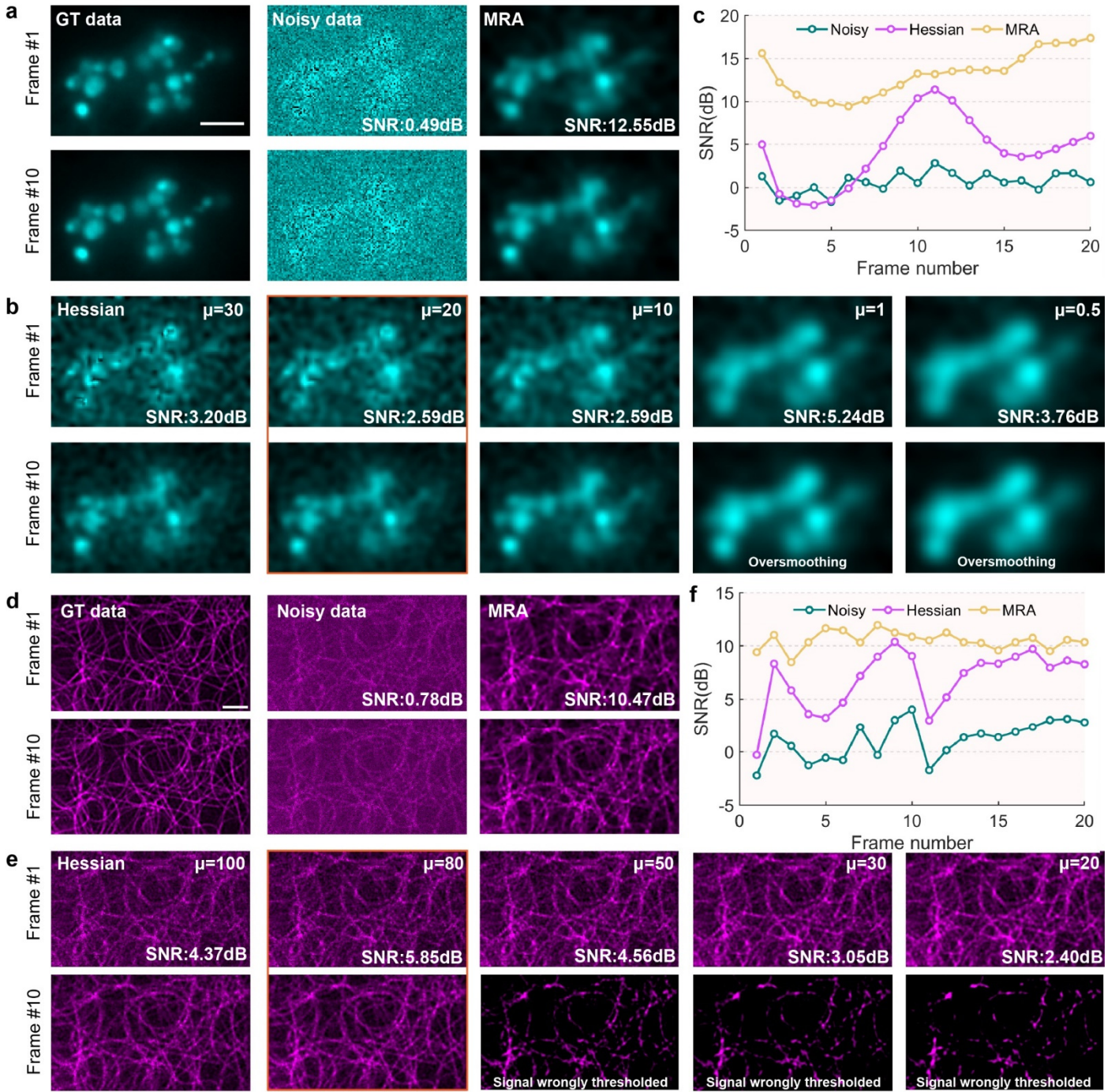


Fig. S6. Detailed comparison of MRA and Hessian regularization on the image stacks shown in Fig. 1d.
a. d. The GT, noisy and MRA deconvolved lysosome and microtubule images. **b. e.** The Hessian deconvolution results at different parameter values. The continuity parameter σ is fixed as 1. In the lysosome image stack, with the increase of noise-control level (decrease of μ), Hessian deconvolved image shows over-smoothing problem because global continuity was overly strengthened. In the microtubule image stack, with the increase of noise-control level (decrease of μ), Hessian was unable to distinguish between signals and noise correctly and removed many signal components. This phenomenon did not occur in the lysosome image stack because the signal intensity was mostly strong. **c. f.** SNR of single frame in noisy, Hessian deconvolved (employ the parameter used in the panel marked by the orange box), and MRA deconvolved lysosome and microtubule data. Scale bars: $2 \mu\text{m}$ (a, d).

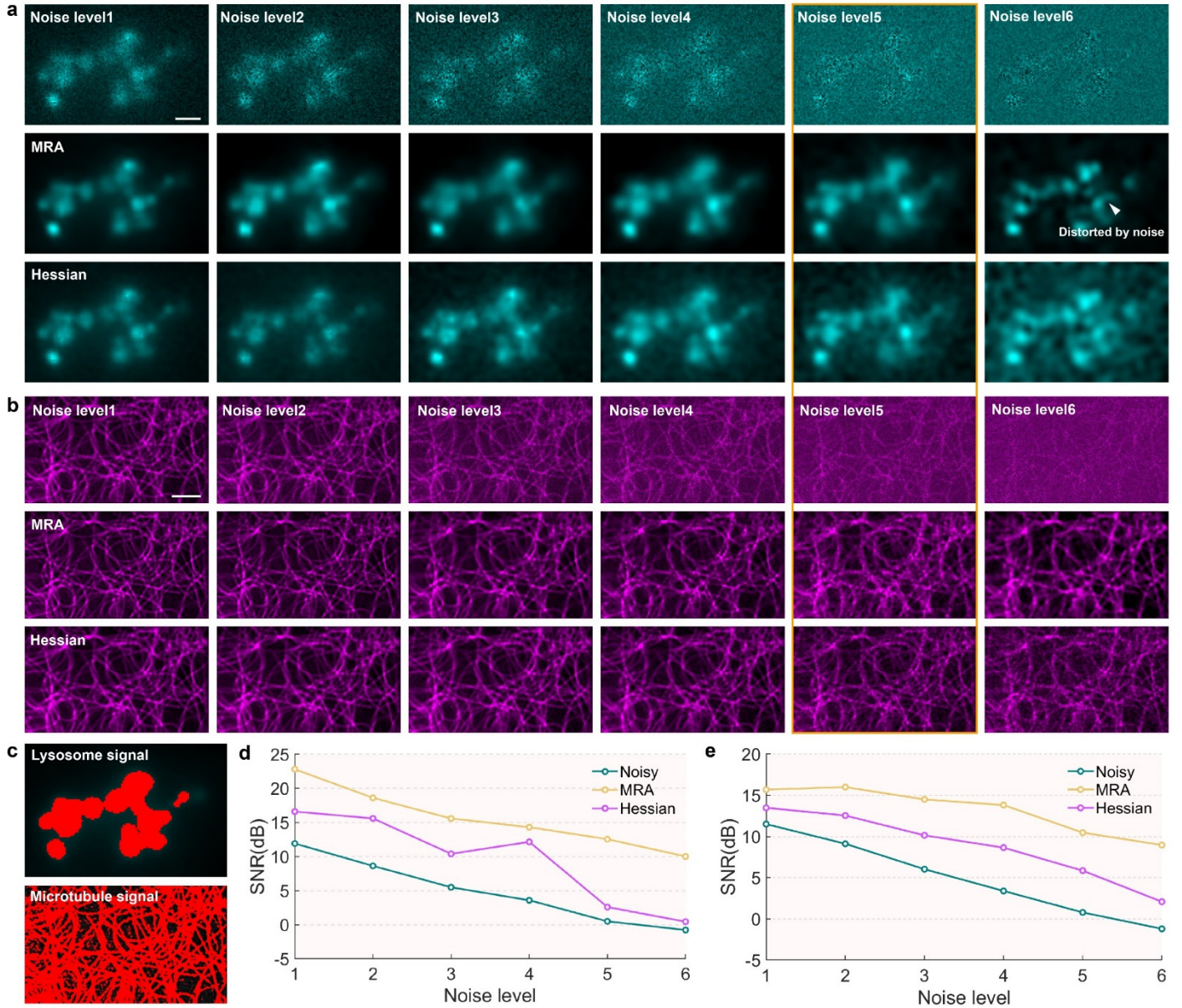


Fig. S7. Comparison of MRA regularization and variance-based regularization at different noise levels. Lysosome **a.** and microtubule **b.** time-lapse images with varying noise levels, and the corresponding MRA and Hessian deconvolution results. The noise type is set as 50% Gaussian mixed 50% Poisson noise. The middle frame of the whole image stack ($n=20$) is displayed. The red boxed group denoted the noise level shown in Fig. 1d and g, which show the first frame of the image stack. **c.** The region that utilized to measure the average signal intensity in the calculation of SNR. The thresholding values for the lysosome and microtubule images are 19.6% and 11.8% of the maximum respectively. **d., e.** The SNR of the noisy, MRA deconvolved and Hessian deconvolved lysosome and microtubule data at different noise level. Scale bars: 1 μm (a), 2 μm (b).

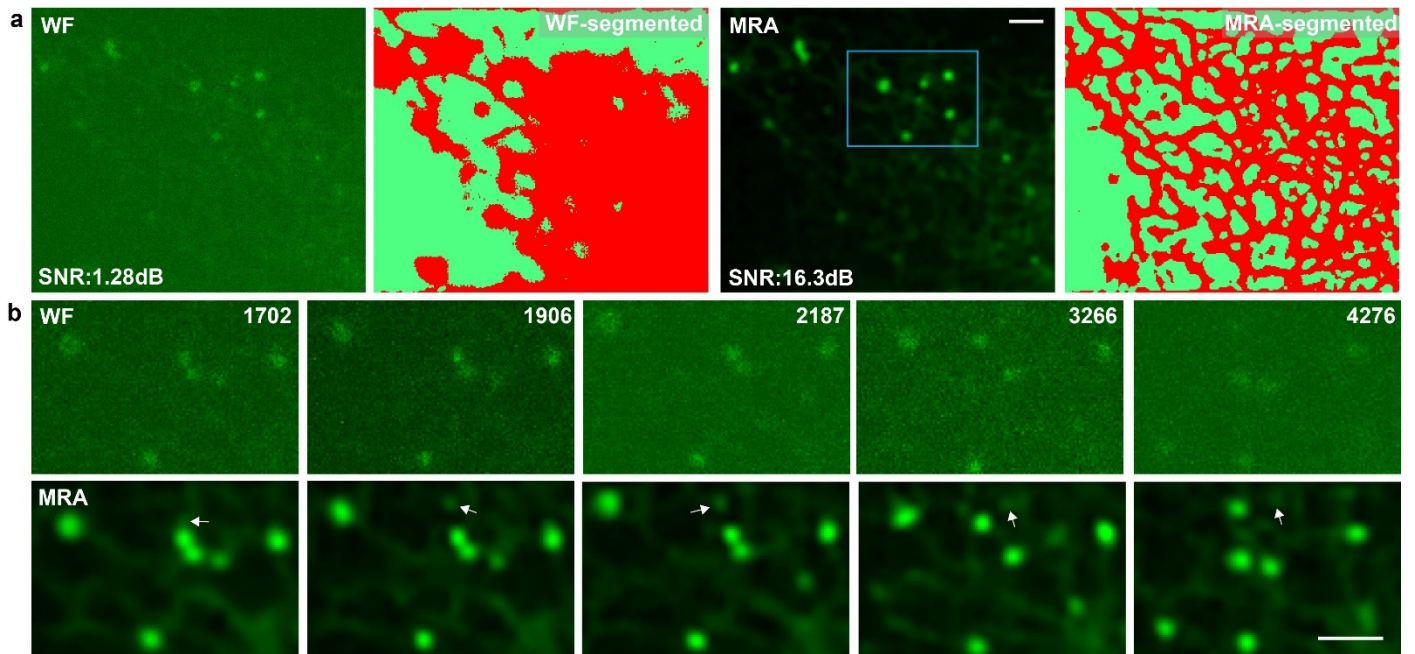


Fig. S8. MRA supports fast imaging of ER tubules. **a.** Widefield time-lapse images of ER tubules in COS-7 cells labeled by KEDL-GFP plasmid, and the MRA deconvolution result. The recording contains a total frame of 4,500 with a frame rate of ~ 417 fps. The ER network is segmented using TWS⁴⁸, a machine-learning toolbox for organelle segmentation. **b.** The magnified blue boxed region in **a**. The number on the right top denotes the frame number. Scale bars: $2\ \mu\text{m}$ (a, b).

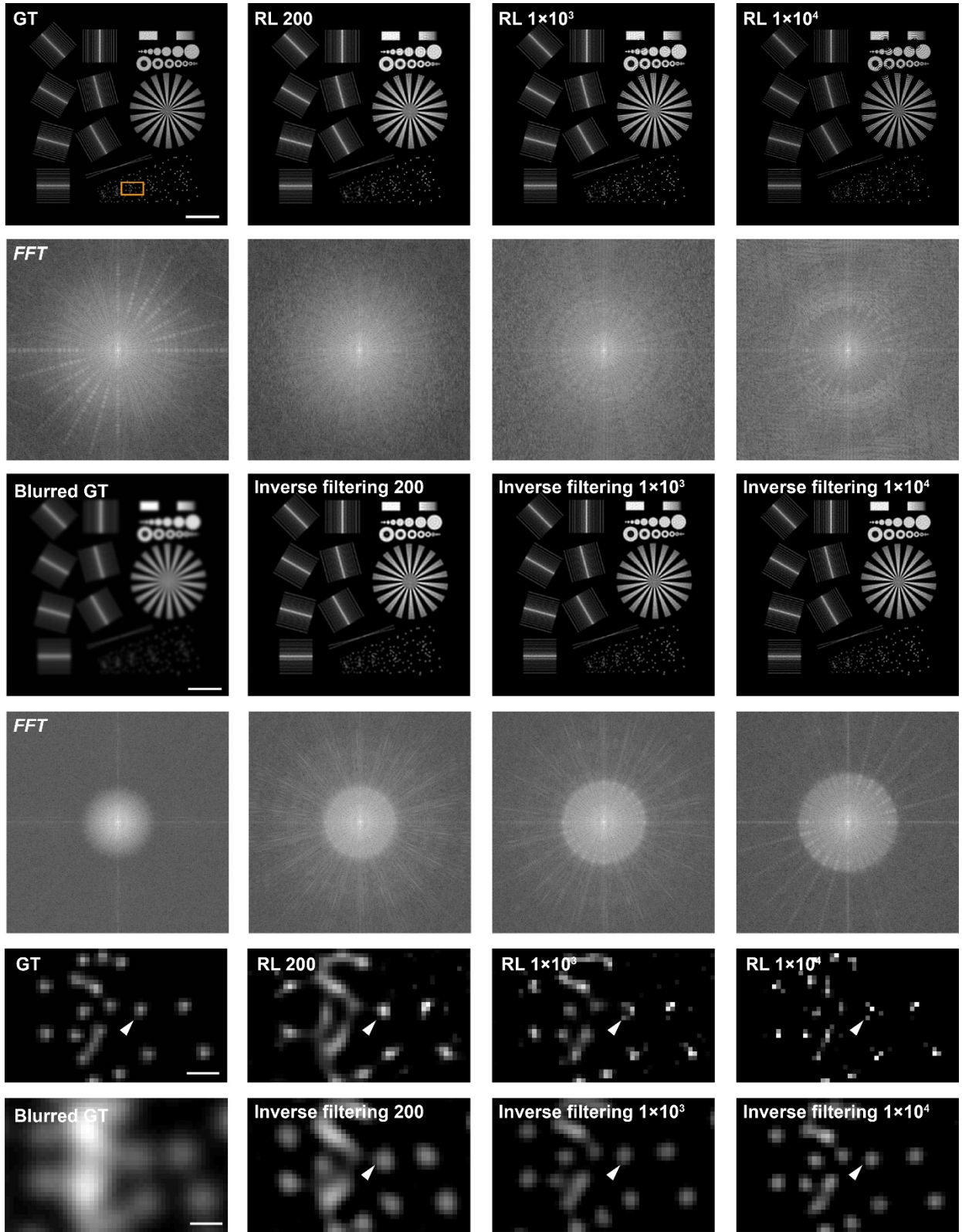


Fig. S9. Simulation of Richardson-Lucy and physics-rooted iterative inverse filtering on a noiseless blurred image. The raw image²⁸ is blurred by a Gaussian kernel ($\sigma=3$) with no noise addition, and is then deconvolved by standard RL and iterative inverse filtering with no regularization. Since the noise-free inverse problem is convex, a sufficient number of iterative inverse filtering (i.e. gradient descent) can perfectly transform the blurred image to ground-truth as a numerical solution. The analytical solution of the noise-free inverse problem is to divide the spectrum of the blurred image by the system OTF, and then transform it back into the spatial domain, also termed as direct inverse filtering. Scale bars: $5\ \mu\text{m}$ (overview), $0.5\ \mu\text{m}$ (subgraph).

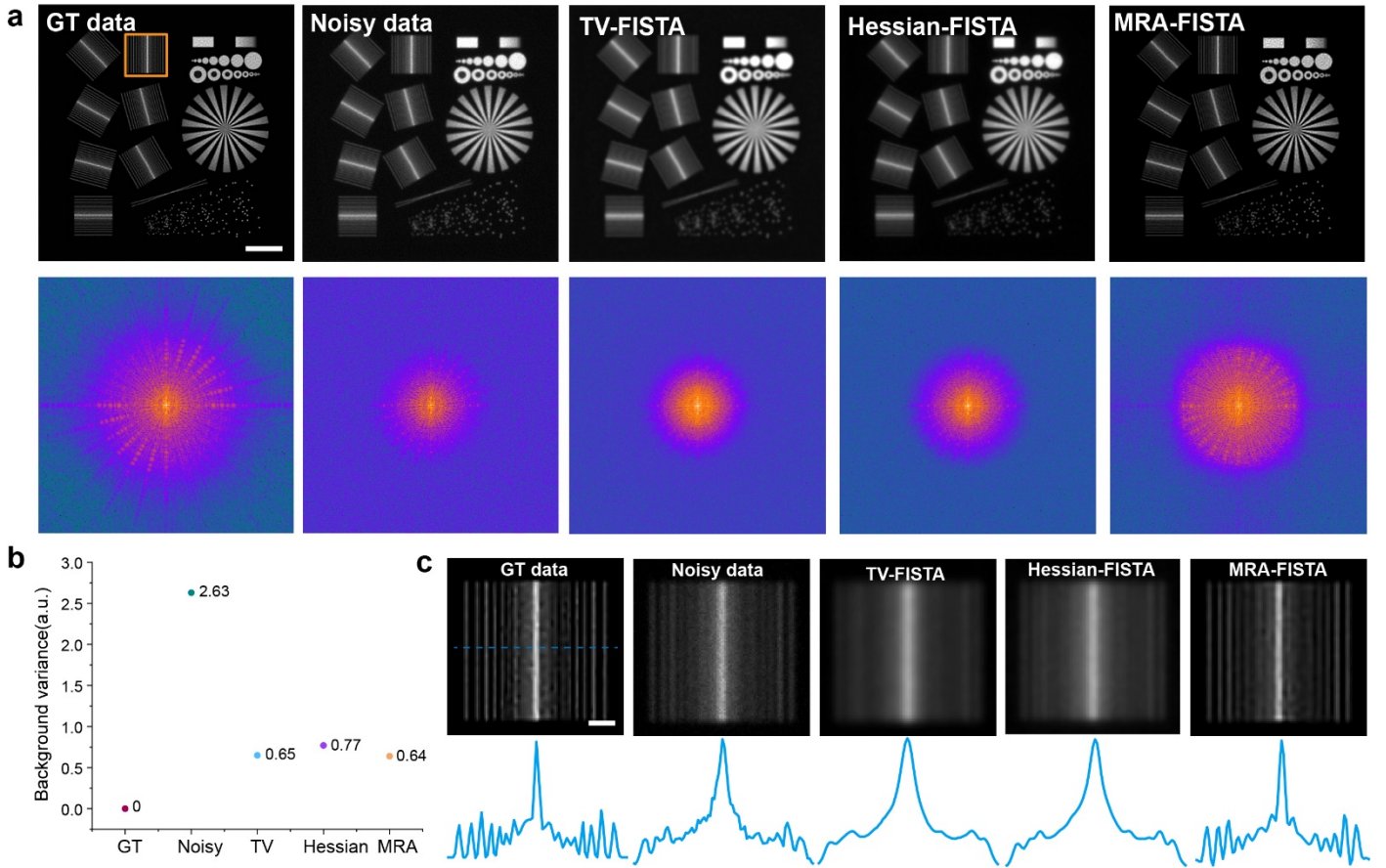


Fig. S10. Comparison of different regularization supporting the iterative inverse filtering. **a.** The ground-truth data²⁸ was blurred using a simulated widefield microscopy PSF (wavelength=685 nm, NA=1.49, pixel size=65 nm) and added with moderate noise (50% Gaussian+50% Poisson, PSNR: 23.98 dB). Three regularization schemes: TV, Hessian and MRA are utilized to control the noise during iterative inverse filtering for recovering the noisy data. The Fourier spectrum of each graph is shown below, which shows that though TV and Hessian control the noise in the image, they hinder the inference of high-frequency information through iterative inverse filtering. **b.** The noise is characterized by the background variance in each image. The noise is controlled at an equivalent level by adjusting the regularization parameter. **c.** Magnified orange boxed region in a. By reducing the global variance, TV and Hessian remove some random noise. However, the inverse filtering inferred high-frequency information (also in a high variance) is simultaneously removed. While MRA regularization can preserve the high-frequency information inferred by the inverse filtering while eliminating random noise. Scale bars: 10 μm (a), 1 μm (c).

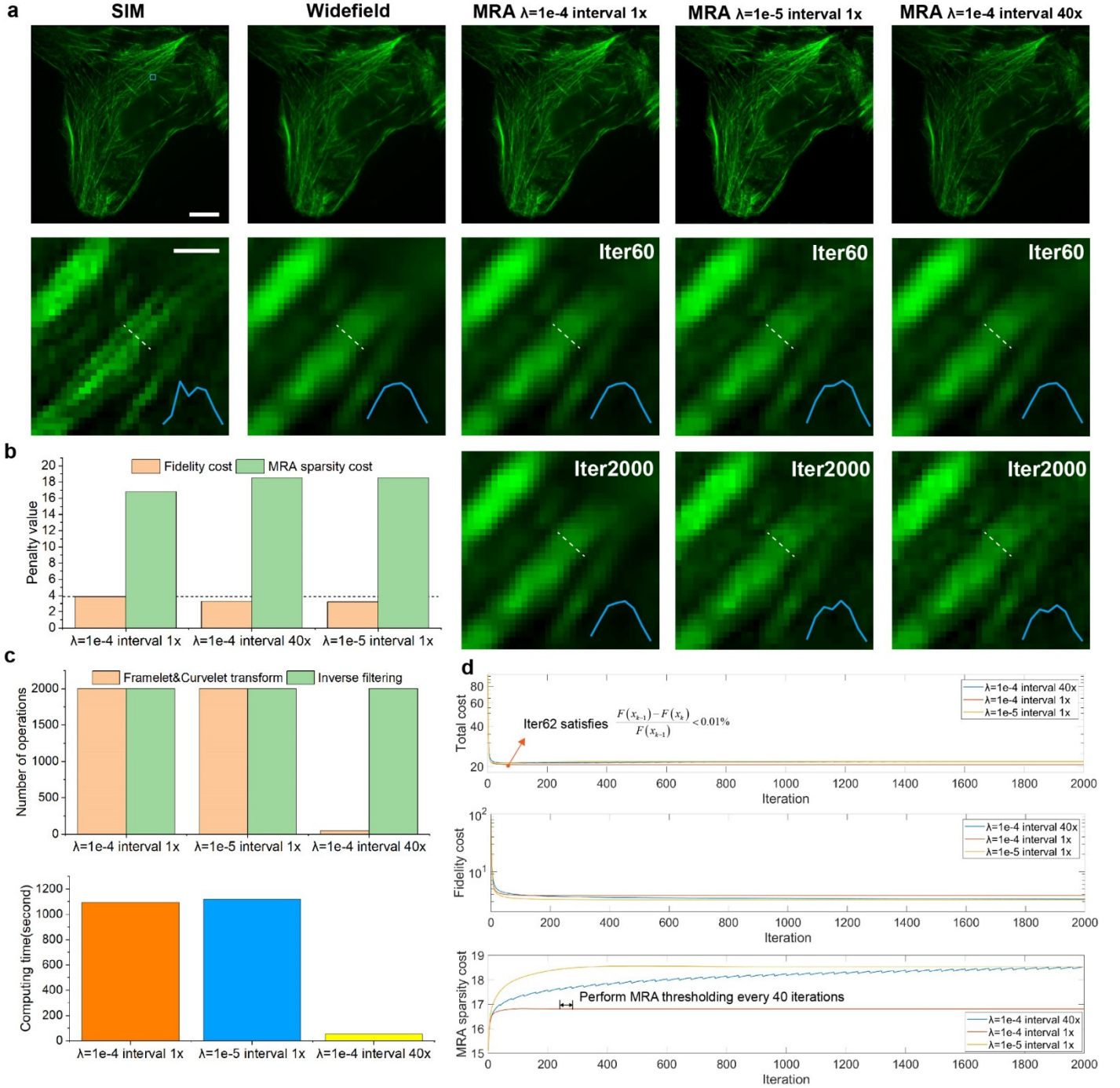


Fig. S11. Acceleration of FISTA iteration in a high-SNR actin image. **a.** Two regularization parameters ($1e-4$ and $1e-5$) were chosen to deconvolved the widefield data. The $1e-4$ value was also tried by the accelerated scheme which performs once MRA thresholding in every 40 iterations. **b.** The fidelity cost and MRA sparsity cost of each group. **c.** The operation number in each group and the computing time. **d.** The total cost function value, fidelity cost value and MRA sparsity cost value as the change of iteration time. The iteration number needed for the convergence of the total cost function is marked. Under the typical iteration numbers (Iter 60) when the global cost function converges, none of the three schemes effectively restored high-frequency information. After more iterations (Iter 2000) the $1e-5$ interval 1x and $1e-4$ interval 40x recovered the selected high-frequency information. The fidelity cost in $1e-5$ interval 1x and $1e-4$ interval 40x group are better reduced compared with the $1e-4$ interval 1x group. Meanwhile, the computation $1e-4$ interval 40x group only takes $\sim 1/20$ of the time that the other two groups need. Scale bars: $10 \mu\text{m}$ (a), $0.5 \mu\text{m}$ (a subgraph).

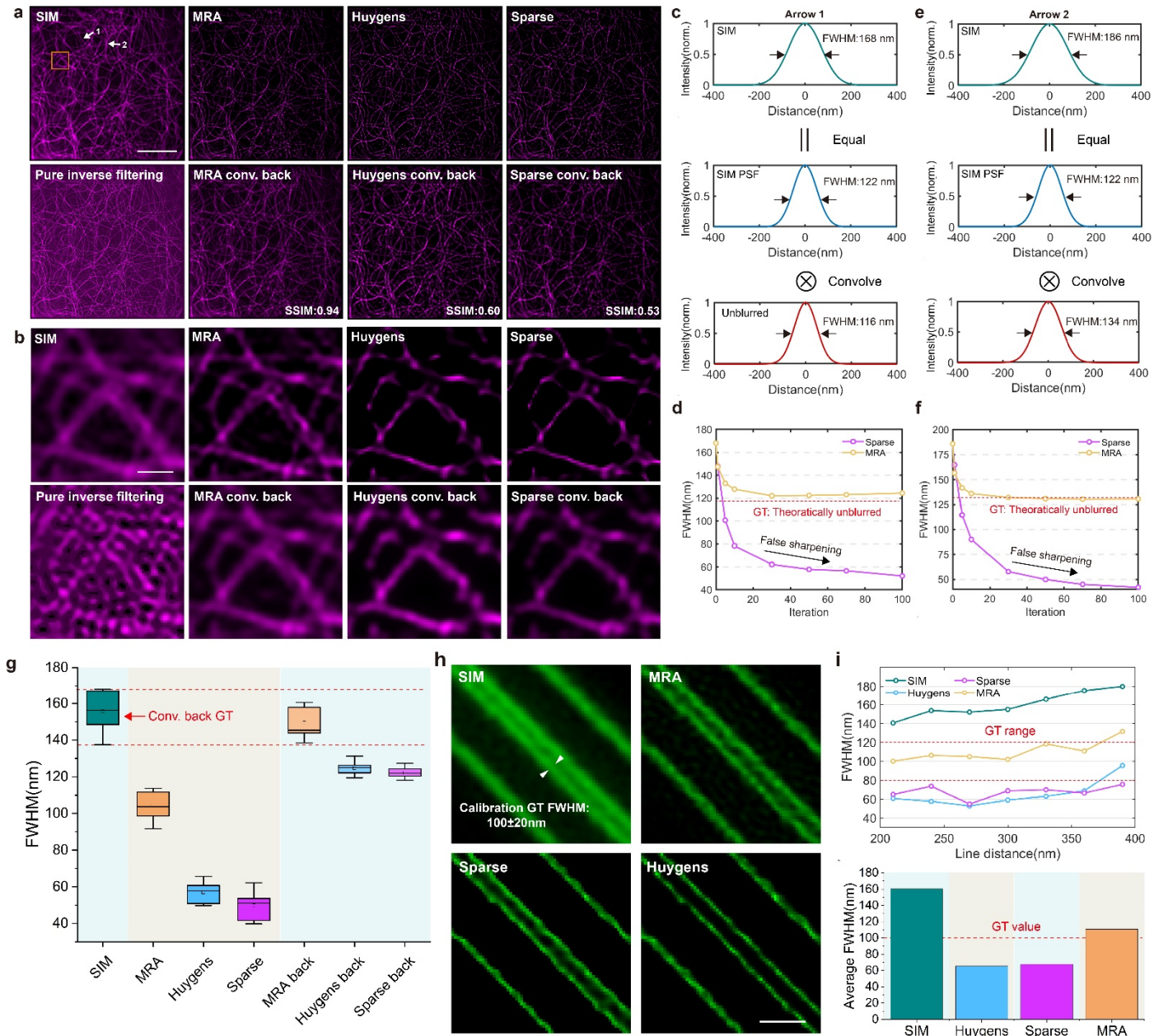


Fig. S12. Quantitative assessment of the fidelity of physical and statistical deconvolution. **a.** SIM microtubule image, and the pure inverse filtering, MRA, Huygens, and Sparse deconvolution results, and the corresponding convolved back results. The SSIM value is calculated using the raw SIM image as a reference. **b.** Magnified orange boxed region in a. **c. e.** Determination of theoretically unblurred FWHM values of the structures denoted by the white arrow 1 and 2 in a. **f. g.** Variation of the FWHM values in the structure denoted by arrow 1 and arrow 2 as the change of iteration times in MRA and the RL iteration times in Sparse. **g.** Box chart of the FWHM of microtubule filaments in each graph ($n=10$). Centerline: median. Square box: average value. Box range: data ranking 25%-75%. Whiskers: the minimal and maximum value. **h. i.** Quantitative assessment of different algorithms through the calibrated fluorescent lines in Argo-SIM panel. The FWHM sampling uses a 240-nm-spaced fluorescence line as an example, is shown in f. The FWHM calibrated by the manufacturer is 100 ± 20 nm. For SIM, Huygens, Sparse, and MRA deconvolved images, we measured the FWHM of fluorescent lines distanced from 210 to 390 nm. The results are shown in g. MLE methods tend to over infer the FWHM value. Due to the presence of noise, MRA may not be able to deconvolve the image to an ideal diffraction-free state, resulting in a FWHM value slightly higher than the calibration value. While different from MLE methods, MRA would not do excessive inference. Scale bars: $5 \mu\text{m}$ (a), $0.5 \mu\text{m}$ (b, h).

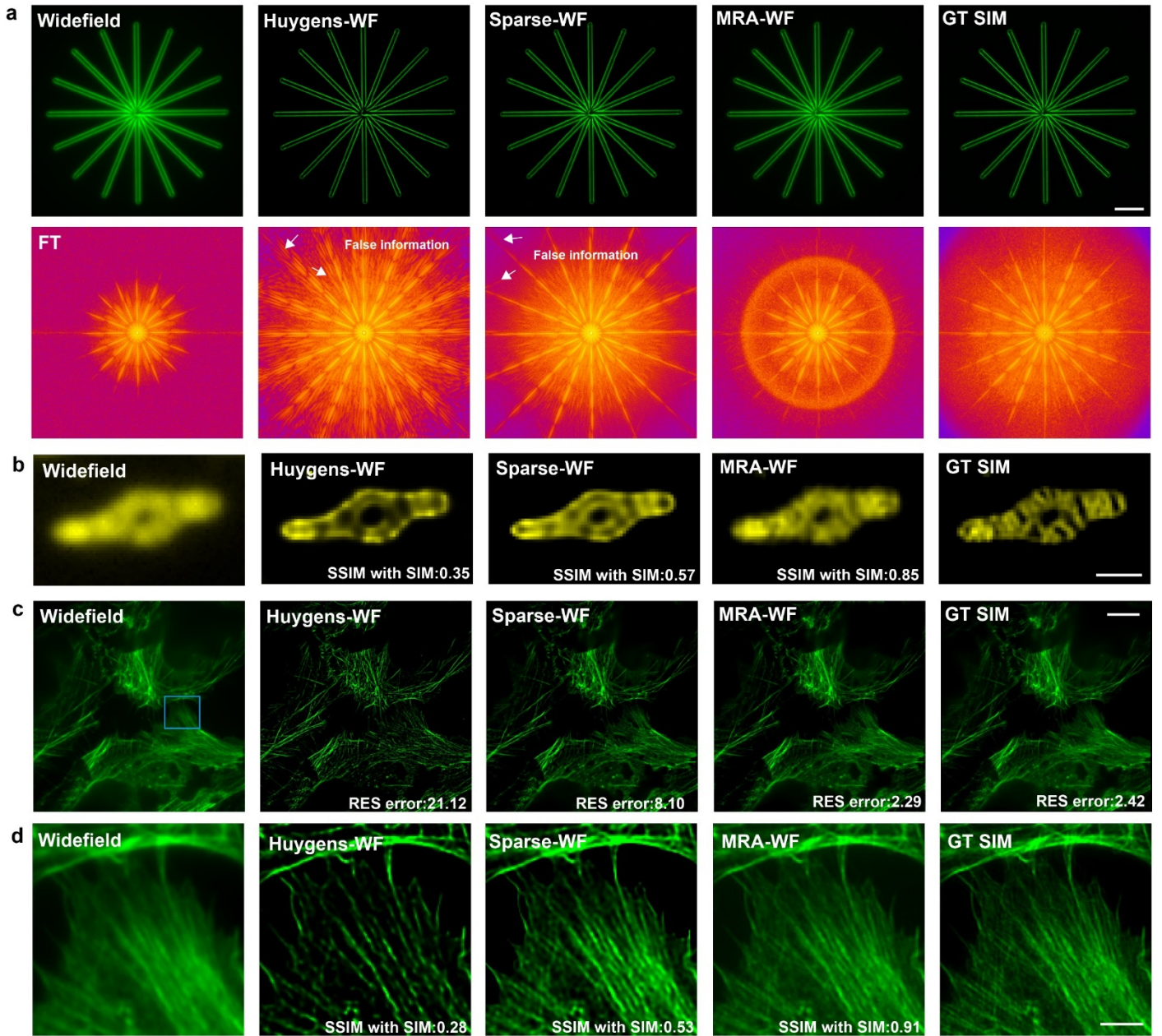


Fig. S13. Evaluation of the fidelity of MRA deconvolution and MLE deconvolution methods through widefield-SIM image pairs. a. The widefield image of a pattern in Argo-SIM panel, and the Huygens, Sparse, MRA deconvoluted results, and the SR-SIM result. Below is the Fourier spectrum of the corresponding image. **b.** The widefield image of mitochondria in U2OS cell, and the Huygens, Sparse, MRA deconvoluted results, and the SR-SIM result. **c.** The widefield image of actin in U2OS cell, and the Huygens, Sparse, MRA deconvoluted results, and the SR-SIM result. **d.** Magnified blue boxed region in c. Scale bars: 5 μm (a), 1 μm (b), 10 μm (c), 2 μm (d).

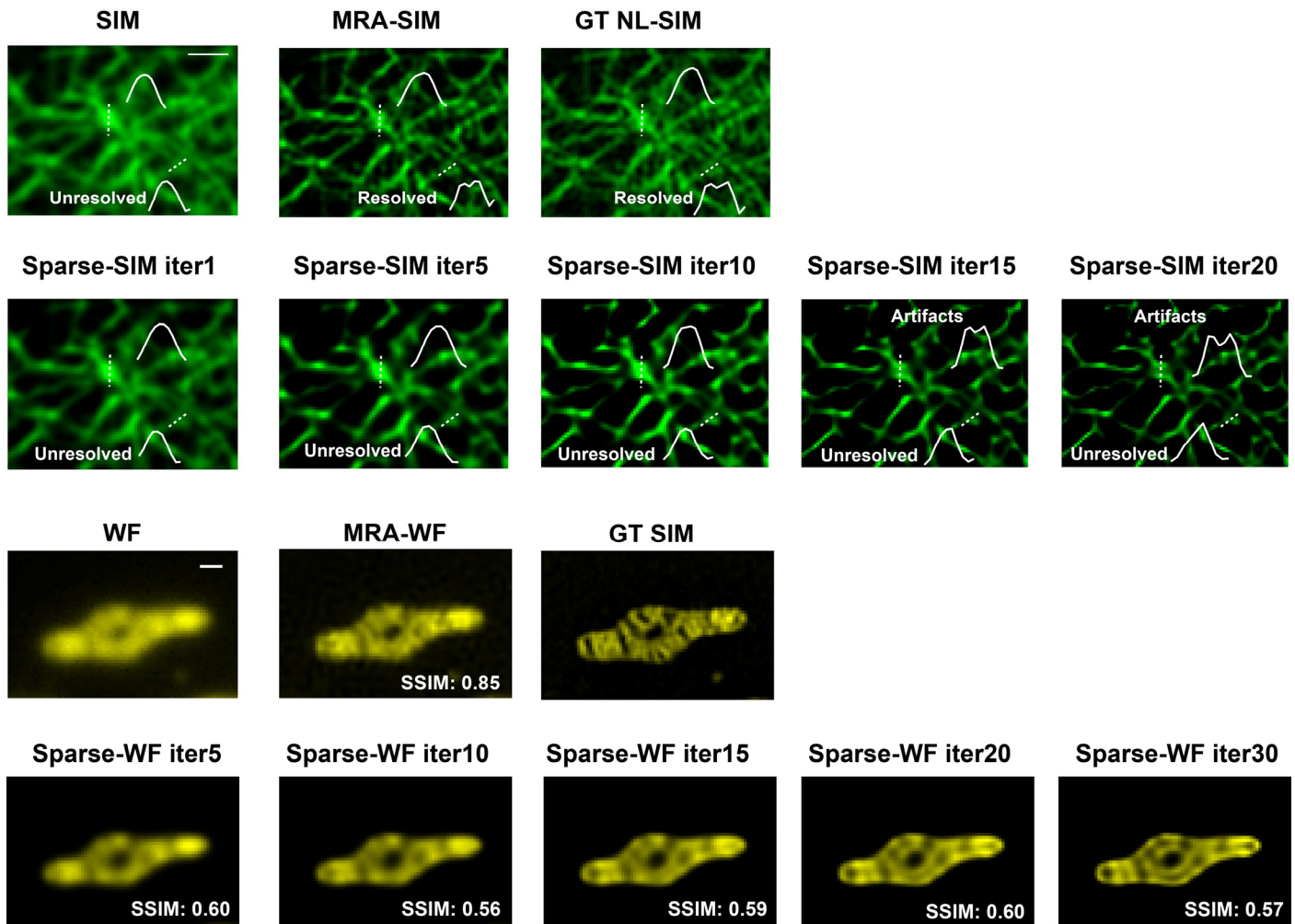


Fig. S14. Examination of the effect of RL iteration times in Sparse deconvolution. The data from Fig. 2 and Fig. S13 are used as example images. The results reveal that reducing the iteration number of MLE deconvolution can reduce the over-sharpening problem. But it still fails to infer similar high-frequency information as MRA does that can be verified by physical-SR microscope. Scale bars: $0.5 \mu\text{m}$.

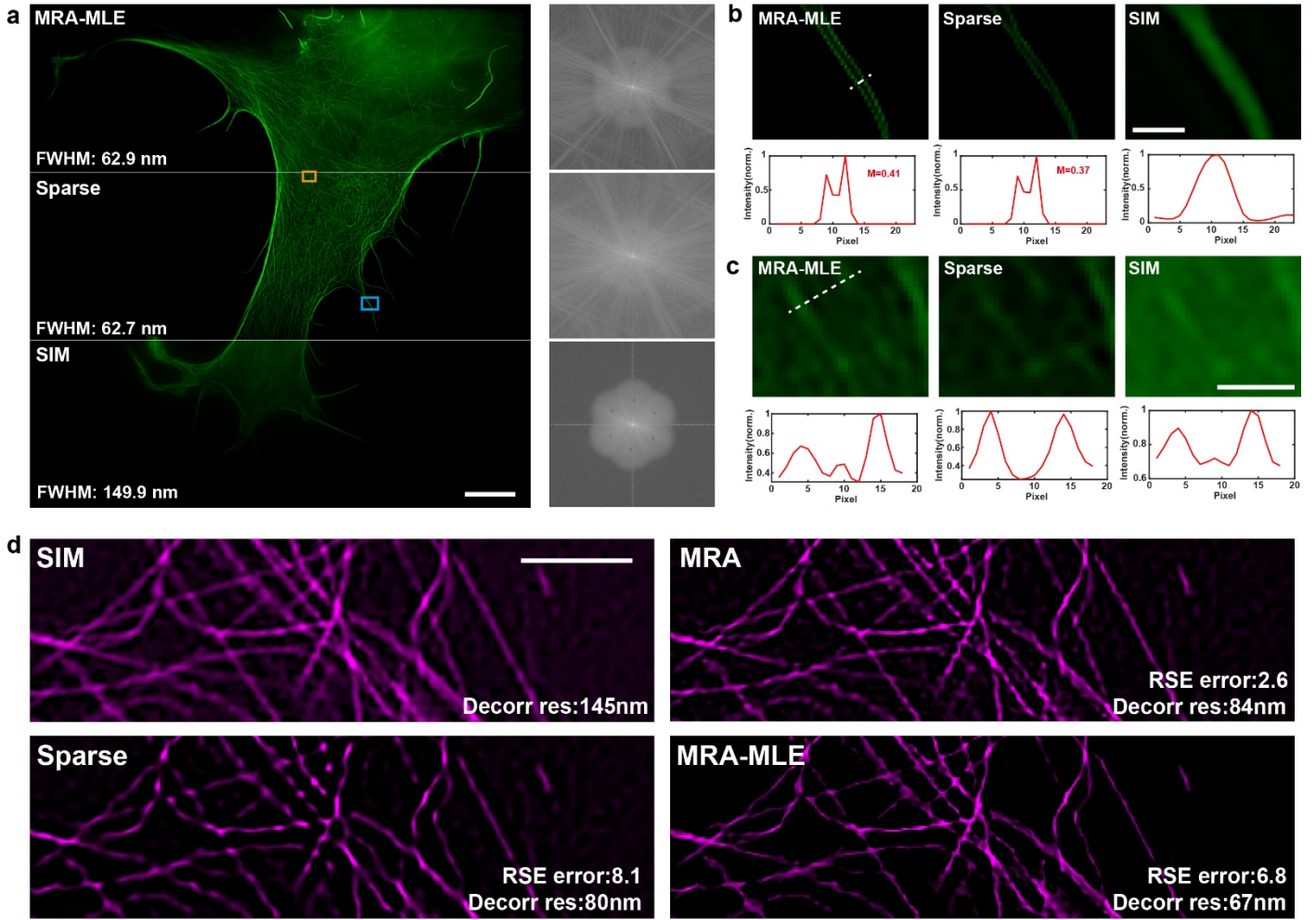


Fig. S15. Resolution improvement by MRA-MLE scheme. **a.** Raw SIM actin image¹⁴, and the corresponding Sparse and MRA-MLE deconvolution result. **b.** Magnified blue boxed region in **a**. **c.** Magnified orange boxed region in **a**. **d.** SIM image of microtubules in COS-7 cell and its deconvolution results by MRA, Sparse, and MRA-MLE. These results show that using MRA regularization can also improve the performance of MLE inference, in which MRA regularization helps maintain the integrity of the structure in the original image. However, the drawback of MLE inference being prone to artifacts still exists in MRA-MLE, which is not a good solution for the inverse problem. Scale bars: 5 μm (**a**), 0.5 μm (**b**, **c**), 2 μm (**d**).

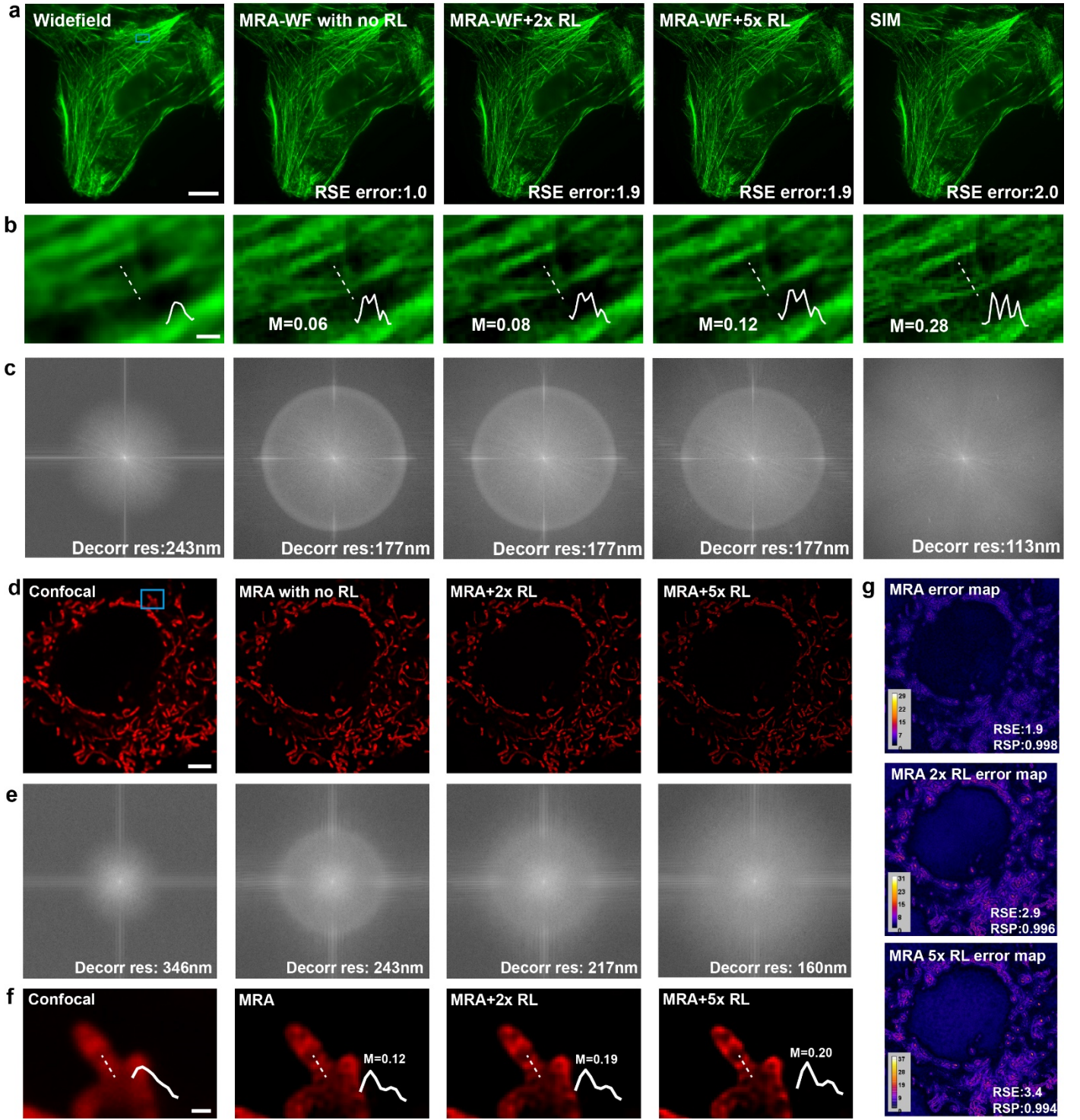


Fig. S16. Effect of RL iteration. **a.** Images of widefield, MRA+0x RL, MRA+2x RL, MRA+5x RL and SIM. **b.** Magnified blue boxed region in **a**. M represents modulation degree. **c.** The Fourier spectrum of the images displayed in **a**. The resolution estimated decorrelation analysis also shows no improvement after RL iteration. **d.** Images of confocal, MRA+0x RL, MRA+2x RL, and MRA+5x RL. **e.** The corresponding Fourier spectrum of the images in **a**. **f.** Magnified blue boxed region in **a**. **g.** The error map of the three deconvolved images. In this case, executing RL a few times can significantly improve the contrast and numerical resolution of the image. However, the increase in RSE is also very significant, meaning the damage of fidelity. A significant increase in edge error can be seen in the error map. Therefore, the use of RL should also be cautious. Scale bars: $10\ \mu\text{m}$ (**a**), $5\ \mu\text{m}$ (**d**), $0.5\ \mu\text{m}$ (**b**, **f**).

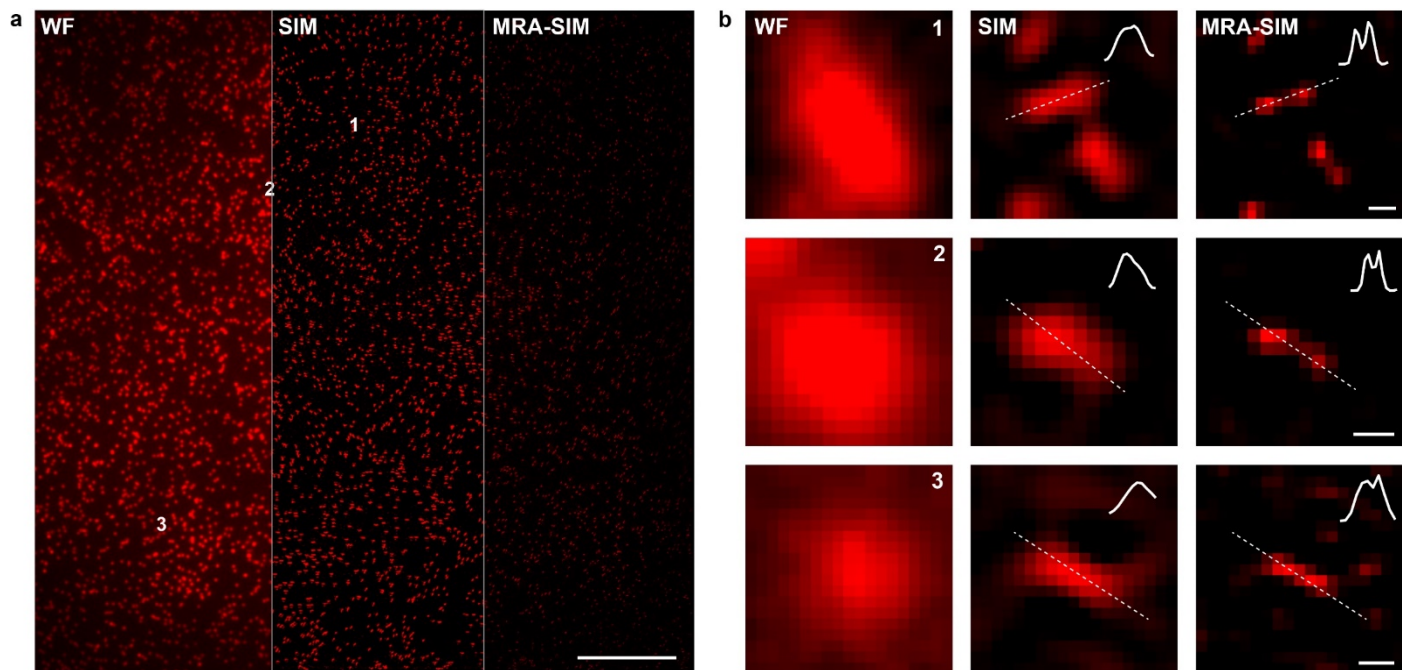


Fig. S17. Resolving of the Nanoruler 70R sample through MRA. **a.** The overview of the widefield, SIM and MRA-SIM Nanoruler image. It contains marked fluorescent spots with 70 nm distance. **b.** Some representative examples of resolving the 70-nm distanced spots. Scale bars: 10 μm (a), 0.1 μm (b).

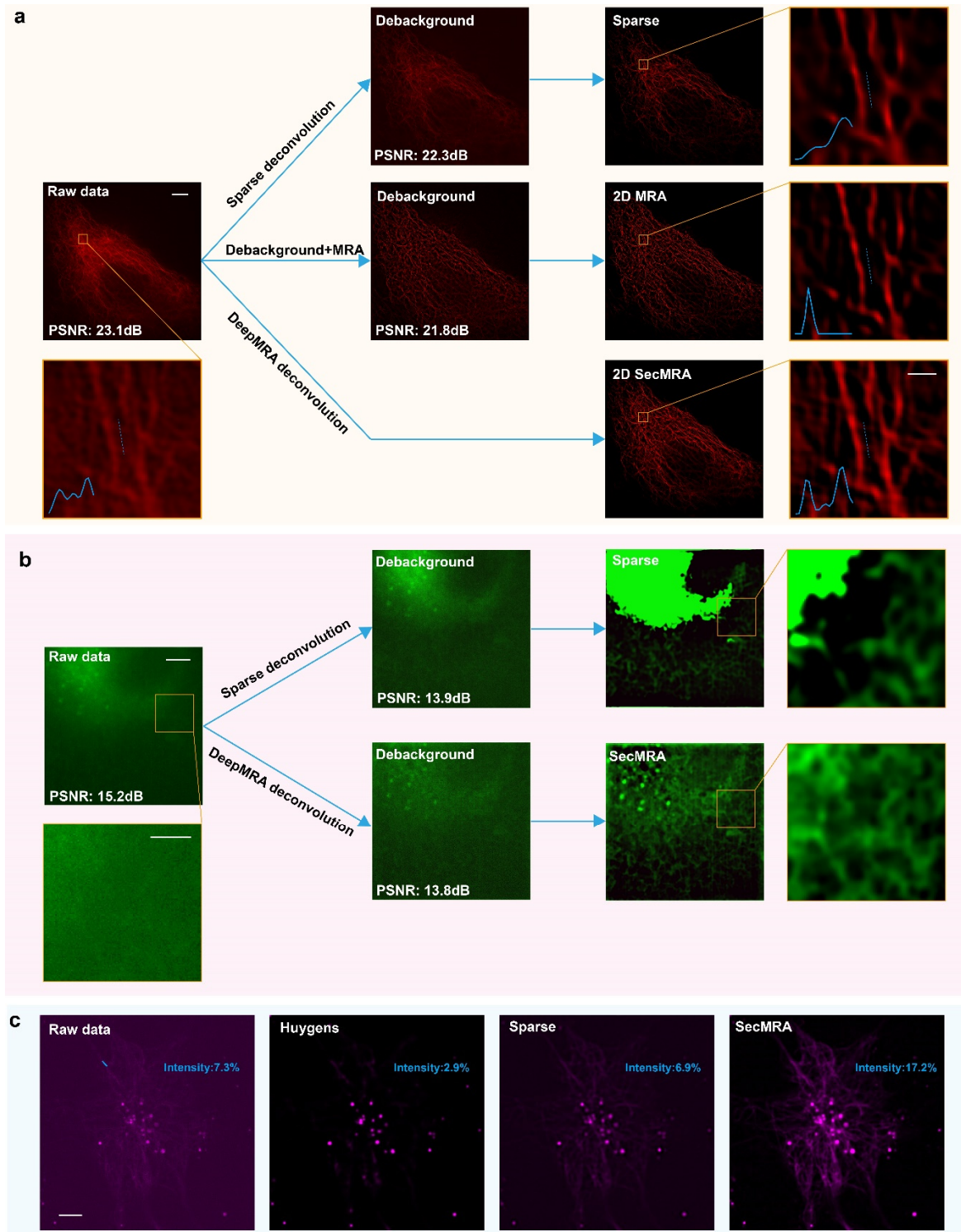


Fig. S18. Comparison of different deconvolution schemes for fluorescence images suffered from strong background or unbalanced intensity distribution. **a.** Deconvolution of microtubule image using three schemes: Sparse, debackground-MRA, and SecMRA. **b.** Deconvolution of ER image using two schemes: Sparse, and SecMRA. In graph a and b, SecMRA better preserves low-intensity signals while eliminating background. **c.** Deconvolution of microtubule image with ultra-bright point interference using Huygens, Sparse, and SecMRA. The intensity denotes the ratio of the average intensity along the blue line to the maximum grayscale value of the entire image. Both Huygens and Sparse show a tendency to attenuate the low-intensity signal and amplify the bright region in the deconvolution process. The intensity correction mode of SecMRA can selectively amplify the desired signal portion and enhance visual perception. Scale bars: 5 μm (a Left, b Left, c), 0.5 μm (a Right), 2 μm (b Right).

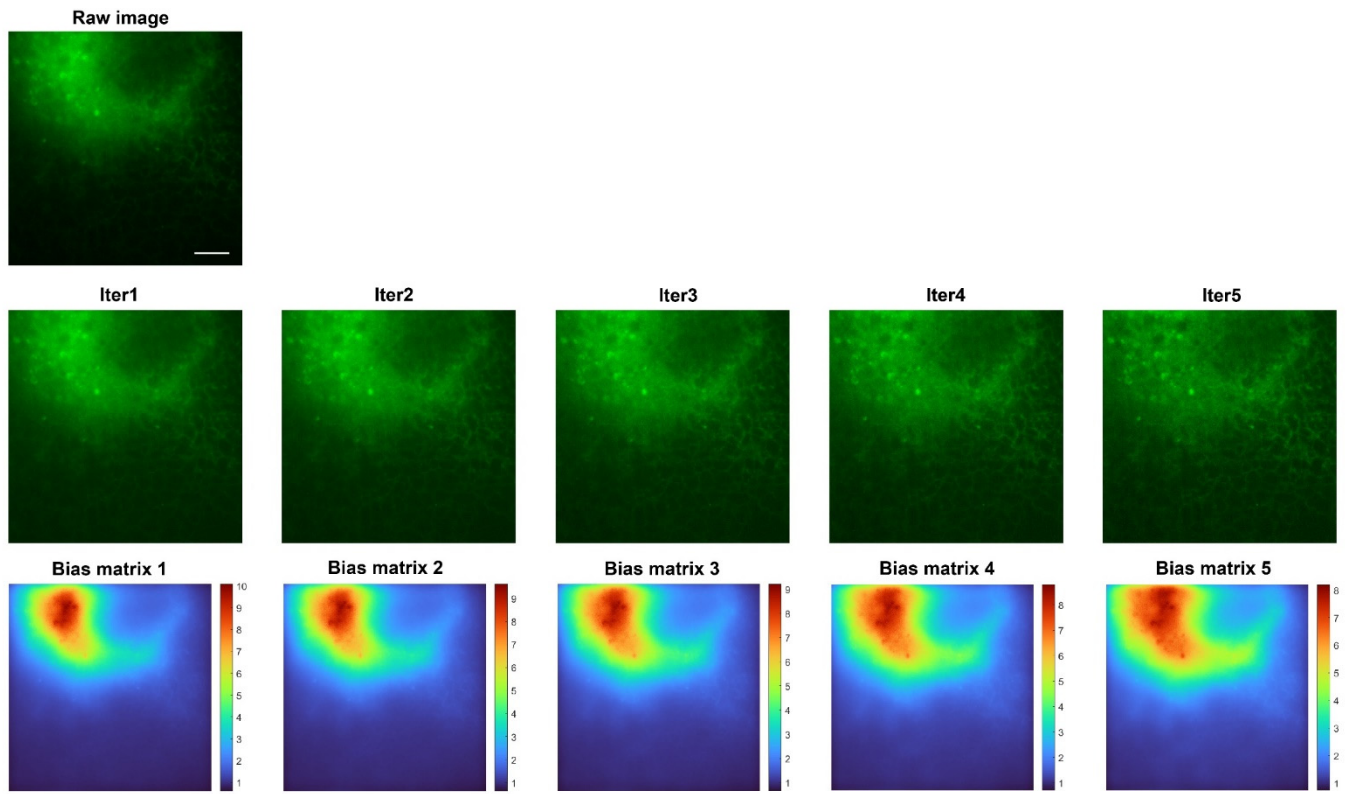


Fig. S19. Iteration process of the ER image preliminary background subtraction that displayed in Fig. 5. Scale bar: $5\ \mu\text{m}$

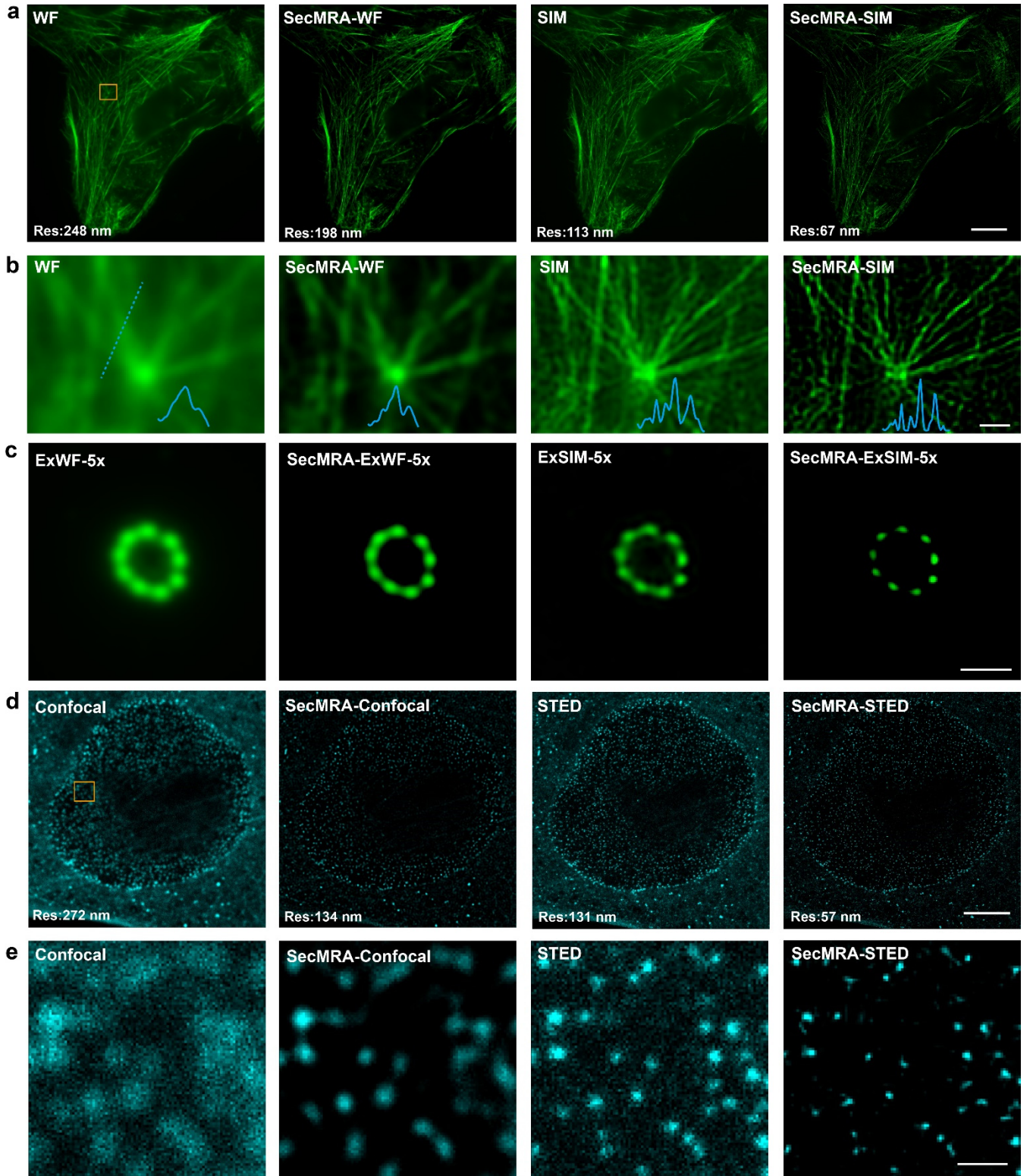


Fig. S20. SecMRA deconvolution improves fluorescence image resolution. **a.** U2OS cell actin image captured by wide-field microscopy and SIM, and the corresponding SecMRA deconvolution results. **b.** Magnified yellow boxed region in **a**. The intensity profile of the dotted line is shown at the left bottom of each graph. **c.** Expansion microscopy image of the centriole, and the corresponding SecMRA deconvolution results. **d.** An open-source mouse embryonic fibroblasts nuclear pore image captured by confocal and STED microscopy¹¹, and the corresponding SecMRA deconvolution results. **e.** Magnified orange boxed region in **d**. Scale bars: 10 μm (**a**), 1 μm (**b**), 0.2 μm (**c**), 5 μm (**d**), 0.5 μm (**e**).

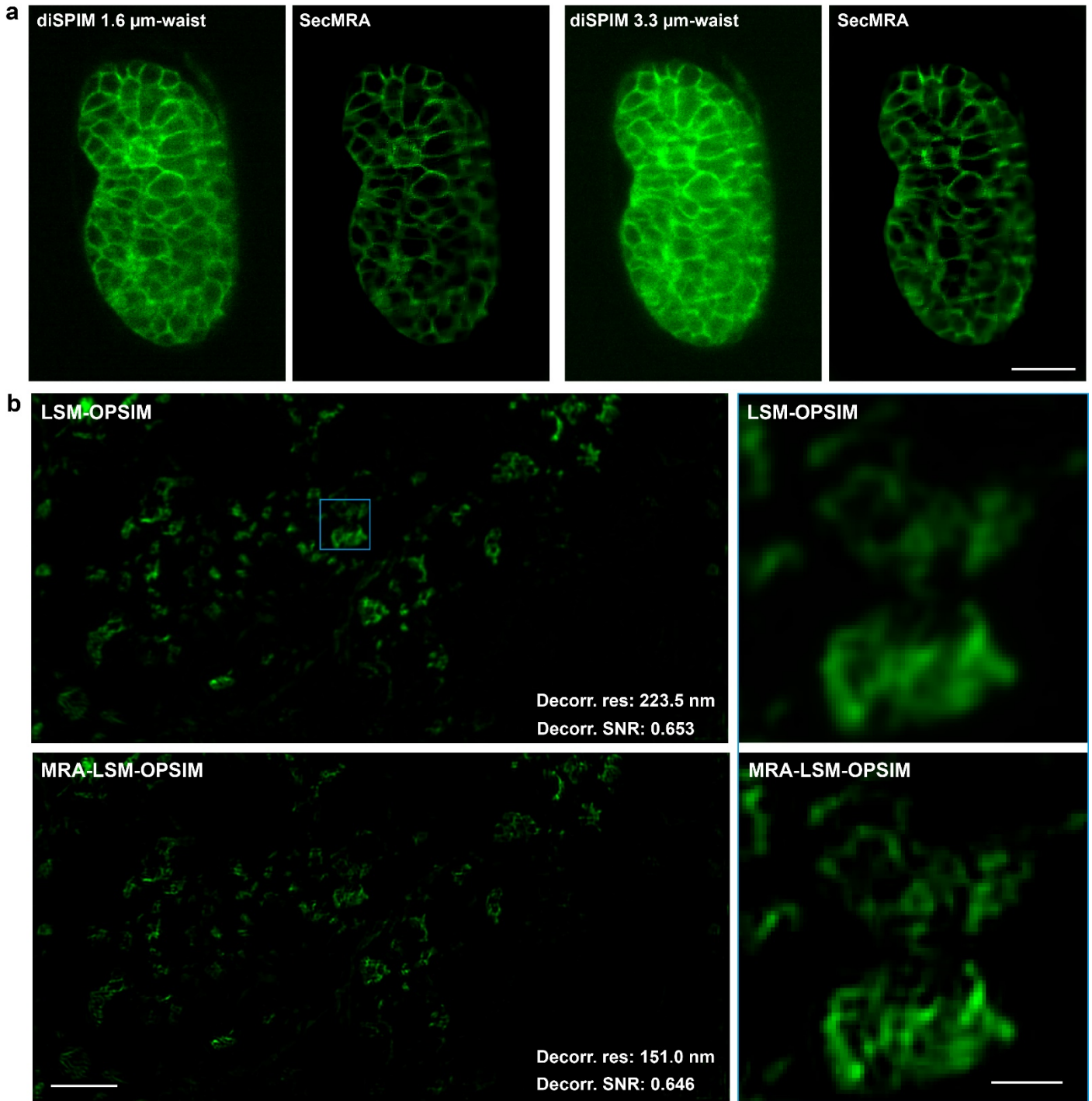


Fig. S21. SecMRA assists light-sheet microscopy (LSM) imaging. **a.** An open-source diSPIM image of *C. elegans* embryos with 1.6 and 3.3 μm Gaussian beam waist³², and the corresponding SecMRA deconvolution result. **b.** An open-source OPSIM image of spinal cord slice³¹ and the MRA deconvolution result. Scale bars: 10 μm (a), 5 μm (b Left), 1 μm (b Right).

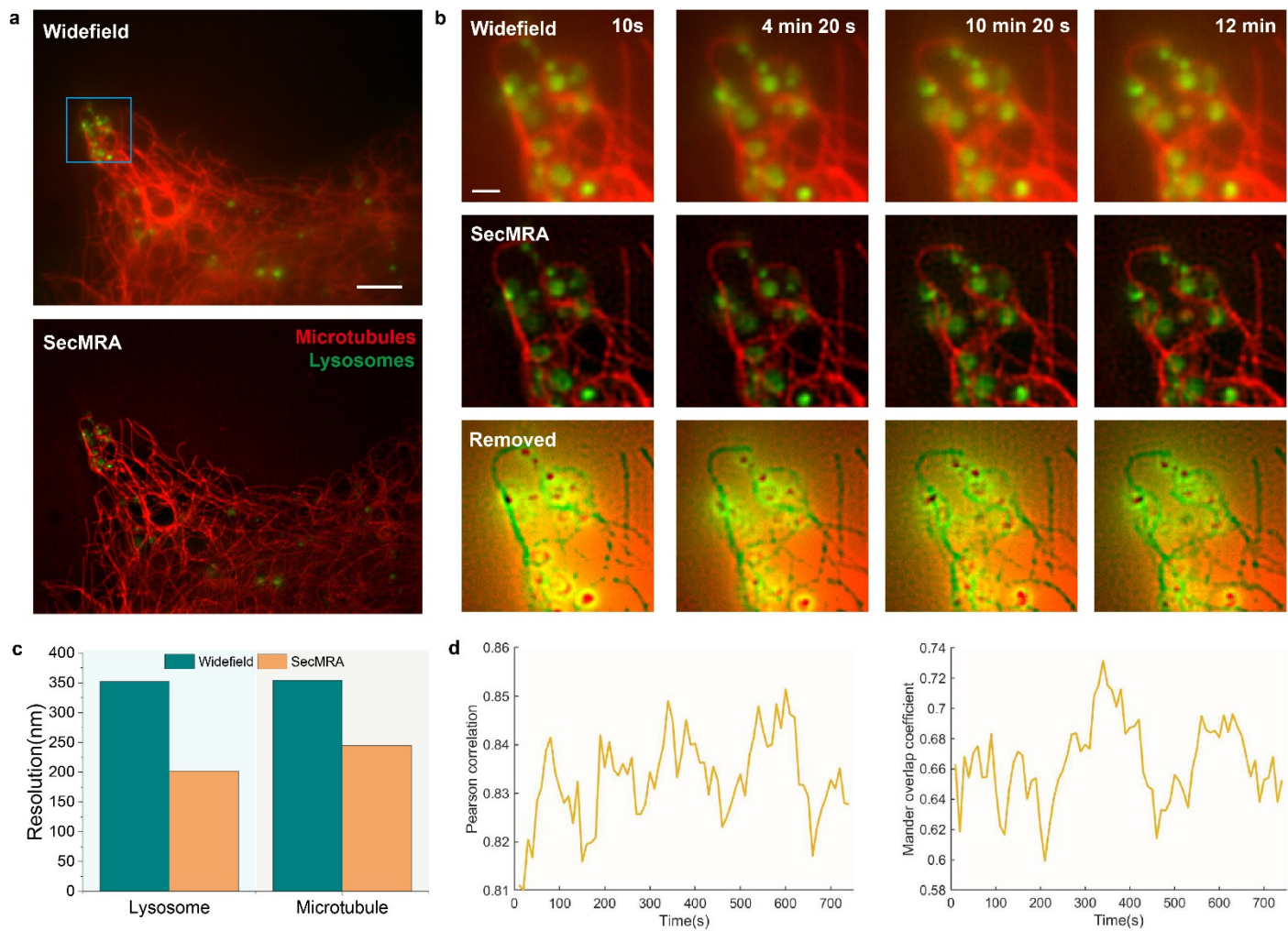


Fig. S22. SecMRA deconvolution assists imaging of microtubule interaction with lysosome in widefield imaging modality. **a.** The widefield time-lapse images of co-labeled lysosomes (green) and microtubules (red), and the SecMRA deconvolution results. The imaging lasts 12 minutes and 20 seconds with 74 frames. **b.** Magnified blue boxed region in a, in which we observe the close bond of the lysosomes and microtubules. **c.** The average resolution of the widefield and SecMRA deconvolved lysosome and microtubule image stack. **d.** The Pearson correlation and Mander's overlap coefficient value of microtubules and lysosomes during the whole recording time. Scale bars: 5 μm (a), 1 μm (b).

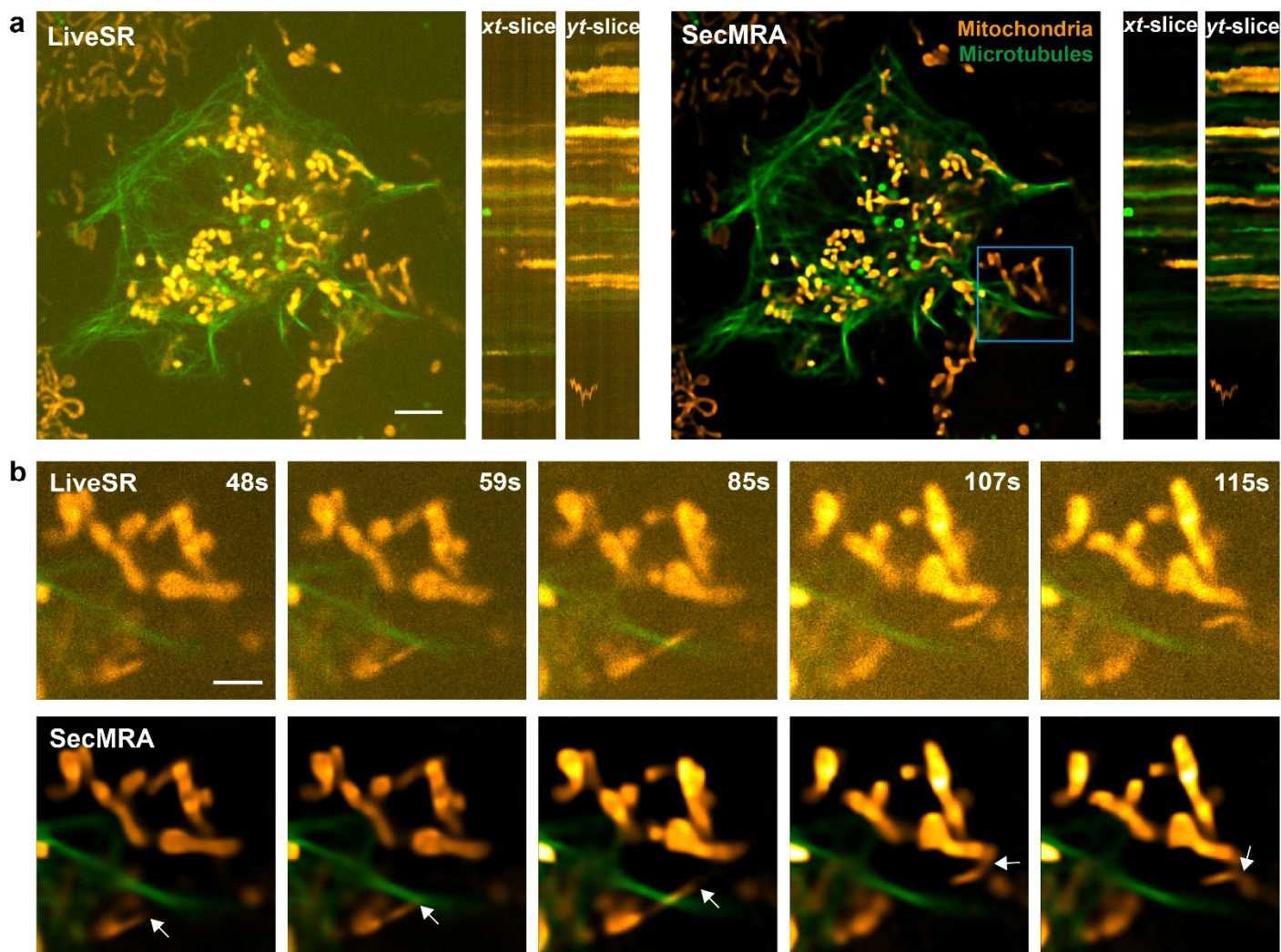


Fig. S23. SecMRA deconvolution assists imaging of mitochondria and microtubules interaction. **a.** The LiveSR time-lapse images of co-labeled mitochondria and microtubules in U2OS cell, and the SecMRA deconvolution result. It consists of 120 frames with a duration of 120 s. **b.** Magnified blue boxed region in **a**, in which we observe consecutive mitochondrial fission and fusion process. A mitochondrial division event occurs approximately between the 49th and 59th seconds of the recording period. Subsequently, a portion of the mitochondrial division approached another mitochondrion at the 107th second. After ~8 seconds, it separated again and fused with another neighboring mitochondrion. Scale bars: 5 μm (a), 2 μm (b).

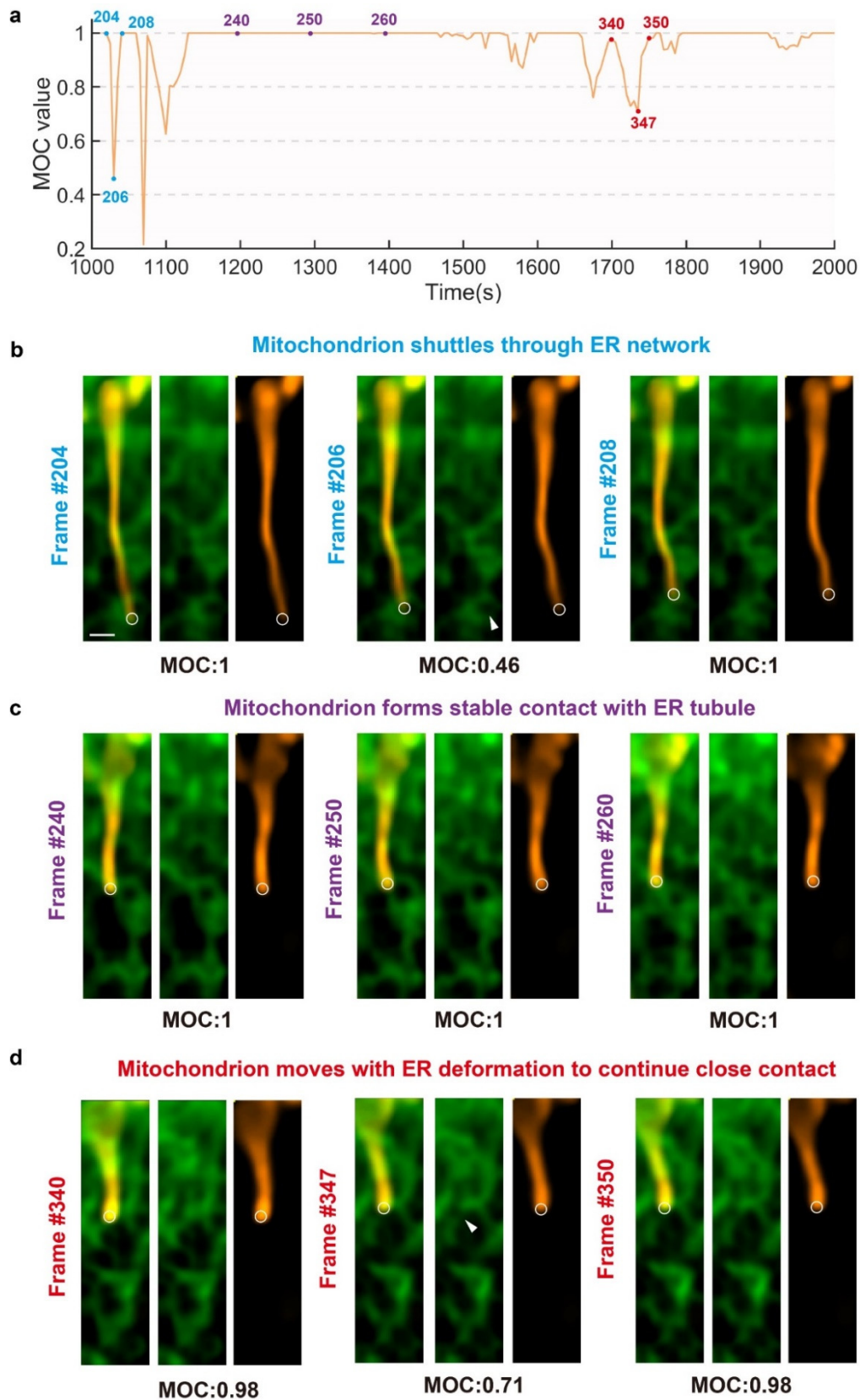


Fig. S24. Interpretation of the MOC curve in Fig. 6. a. The MOC curve of the mitochondrion distal end with the ER network. The marked point denotes the representative frame number in different stages. **b.** The frames depict the mitochondrion shuttles through the ER network. **c.** The frames depict the mitochondrion forms stable contact with ER tubule. **d.** The frames depict the mitochondrion moves with ER deformation to continue close contact. The left image in each panel shows the raw two-color fluorescence images. The middle image shows the ER channel. The right channel shows the mitochondria channel. Scale bar: 1 μm .

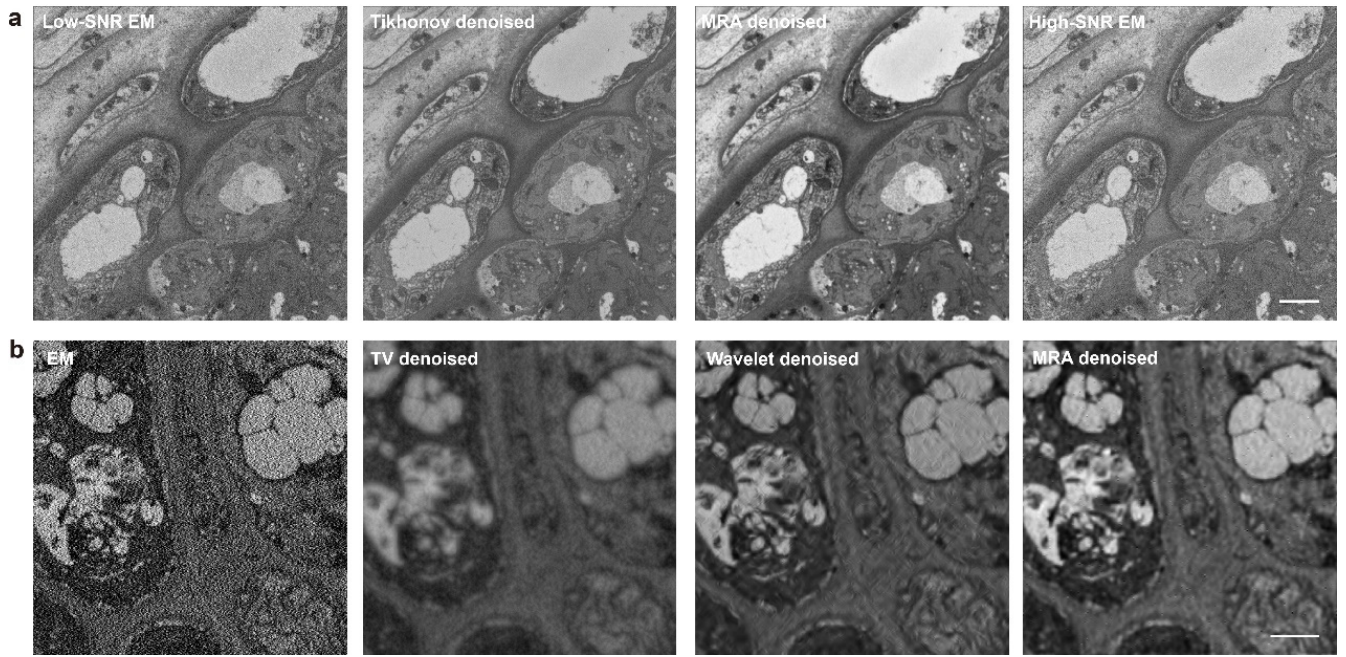


Fig. S25. Denoising of scanning electron microscopy image by MRA. **a.** An open-source low-SNR scanning electron microscope image of Arabidopsis root tip56³⁰, Tikhonov denoised image, MRA denoised image, and high-SNR image. **b.** An open-source scanning electron microscope image of Arabidopsis root56³⁰, TV-denoised image, wavelet denoised image, and MRA denoised image. Scale bars: 2 μm (a), 1 μm (b).

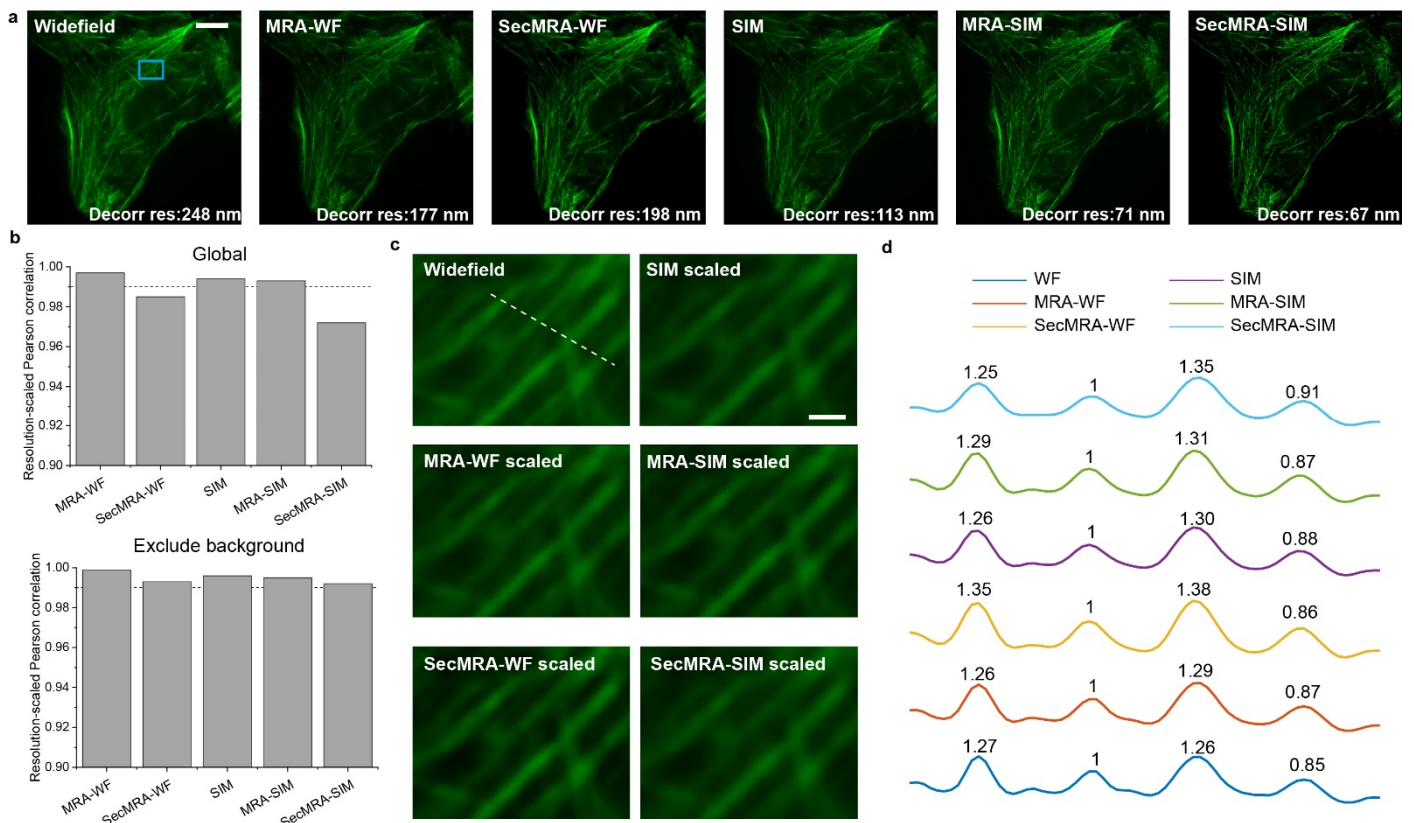


Fig. S26. Evaluation of the linearity of MRA and SecMRA deconvolved images. **a.** The actin image in both widefield and SIM modality is shown in Fig. 2. MRA and SecMRA deconvolution are used to deconvolve the two images respectively. **b.** The resolution-scaled Pearson correlation value was calculated using NanoJ-SQUIRELL software. The cases include and exclude background are calculated respectively. **c.** Magnified blue box region in **a**. The SR image is re-scaled according to the similar resolution level with the widefield image. **d.** The intensity profiles along the dotted line in **c**. Scale bar: 10 μm (**a**), 1 μm (**c**).

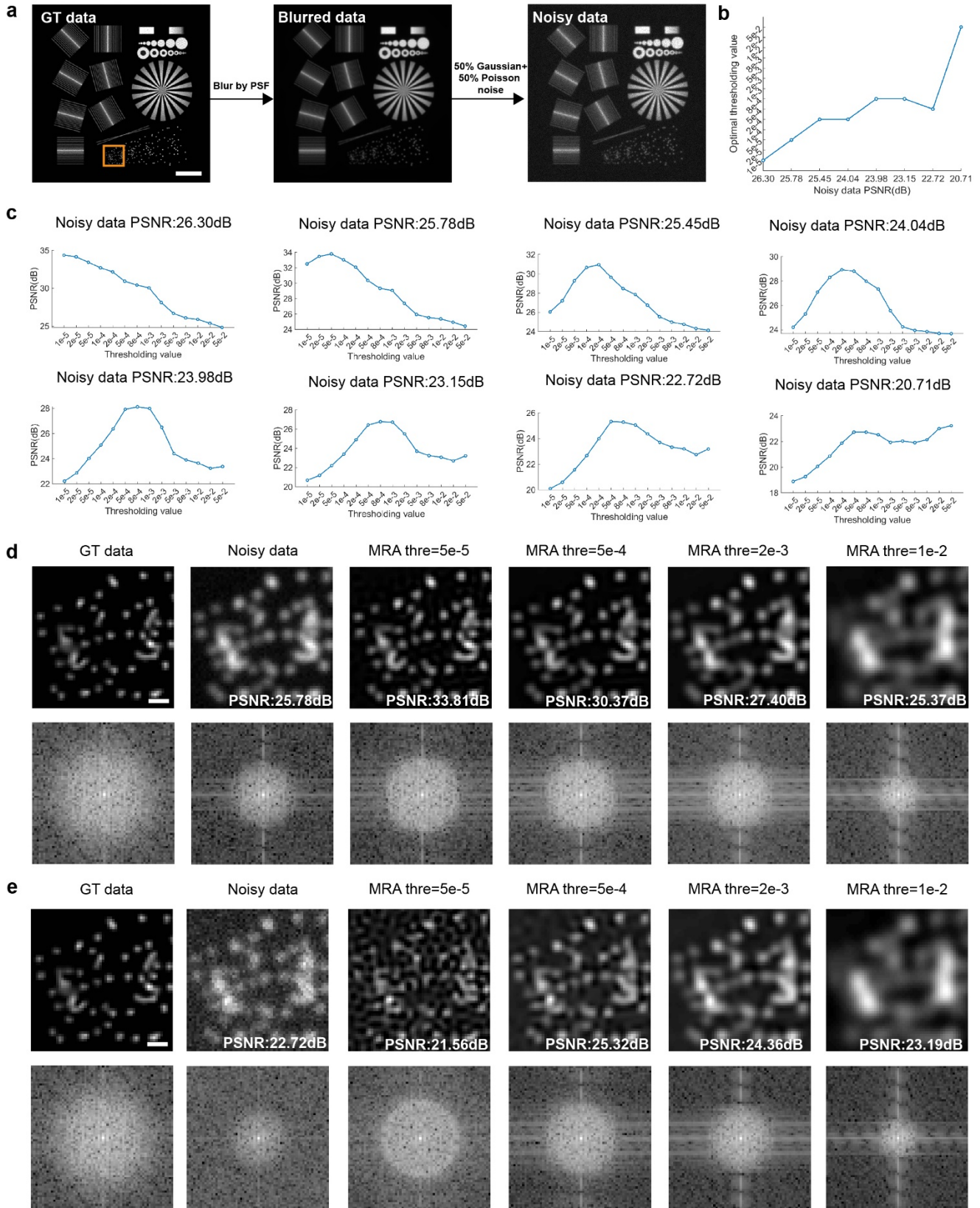


Fig. S27. Simulation to investigate the optimal thresholding values at different Gaussian noise levels. a. The procedure of degrading the ground-truth data²⁸. Simulated PSF parameter: wavelength=685 nm, NA=1.49, pixel size=65 nm. **b.** Optimal thresholding values in the selected 14 choices. The framelet and curvelet thresholding values are the same. **c.** PSNR obtained through different thresholding values at different noise level. **d. e.** Magnified orange boxed regions in a at two representative noise levels, with the Fourier spectrum shown in the second row. Scale bars: 5 μm (a), 0.5 μm (d, e).

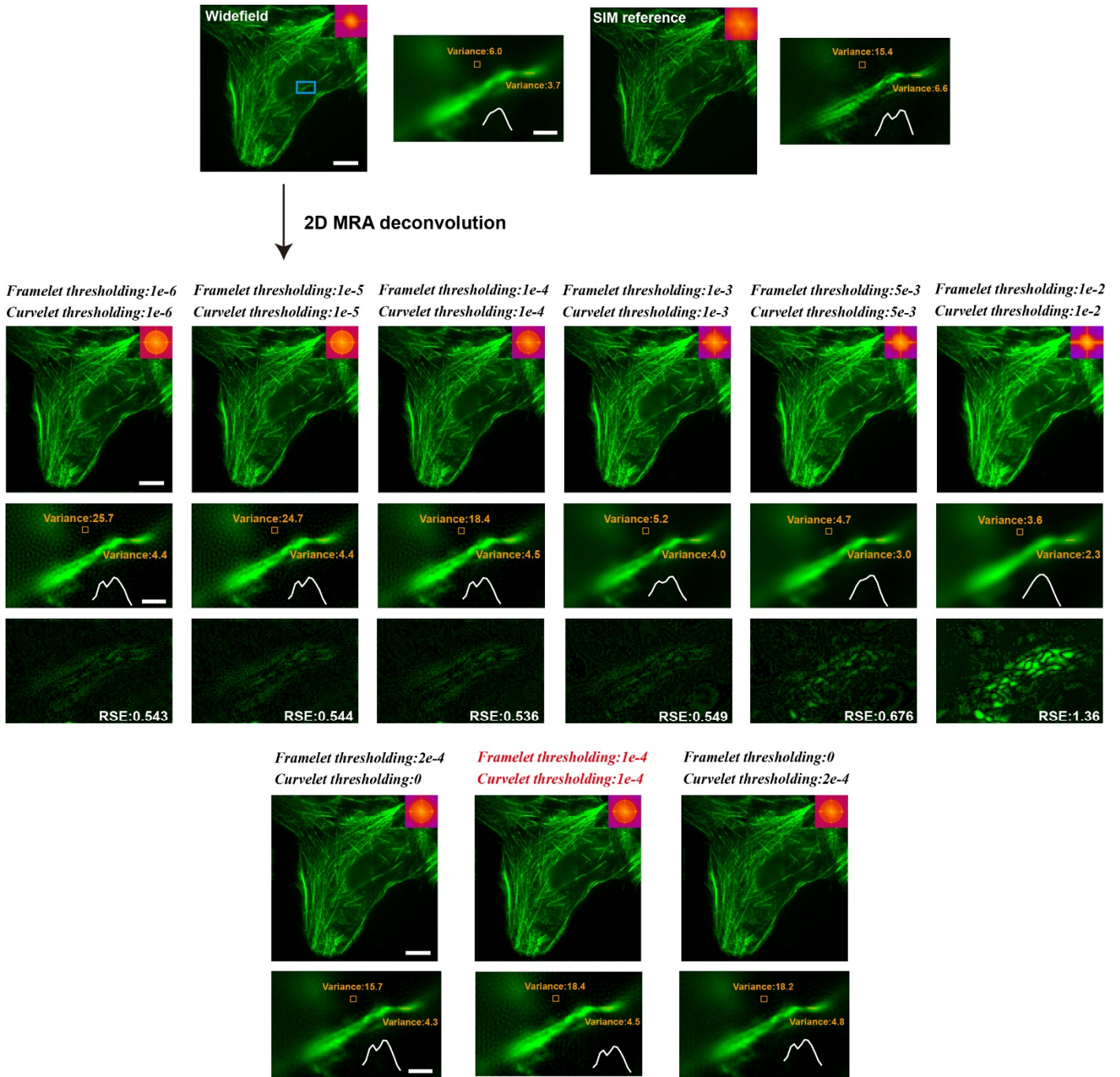


Fig. S28. Effect of adjusting MRA parameter in the widefield actin image shown in Fig. 2. The results show that a high thresholding value tends to smooth the edge. While a low thresholding value tends to generate sharper image. The SIM image as a physical-SR reference indicates that a $1e-4$ thresholding value seems more rational. In visual perception, $1e-4$ thresholding generates image with moderate noise and sharp edge. The ratio of framelet and curvelet thresholding influences little on the final results since this is scenario with weak regularization. Scale bars: $5 \mu\text{m}$ (overview), $1 \mu\text{m}$ (subgraph).

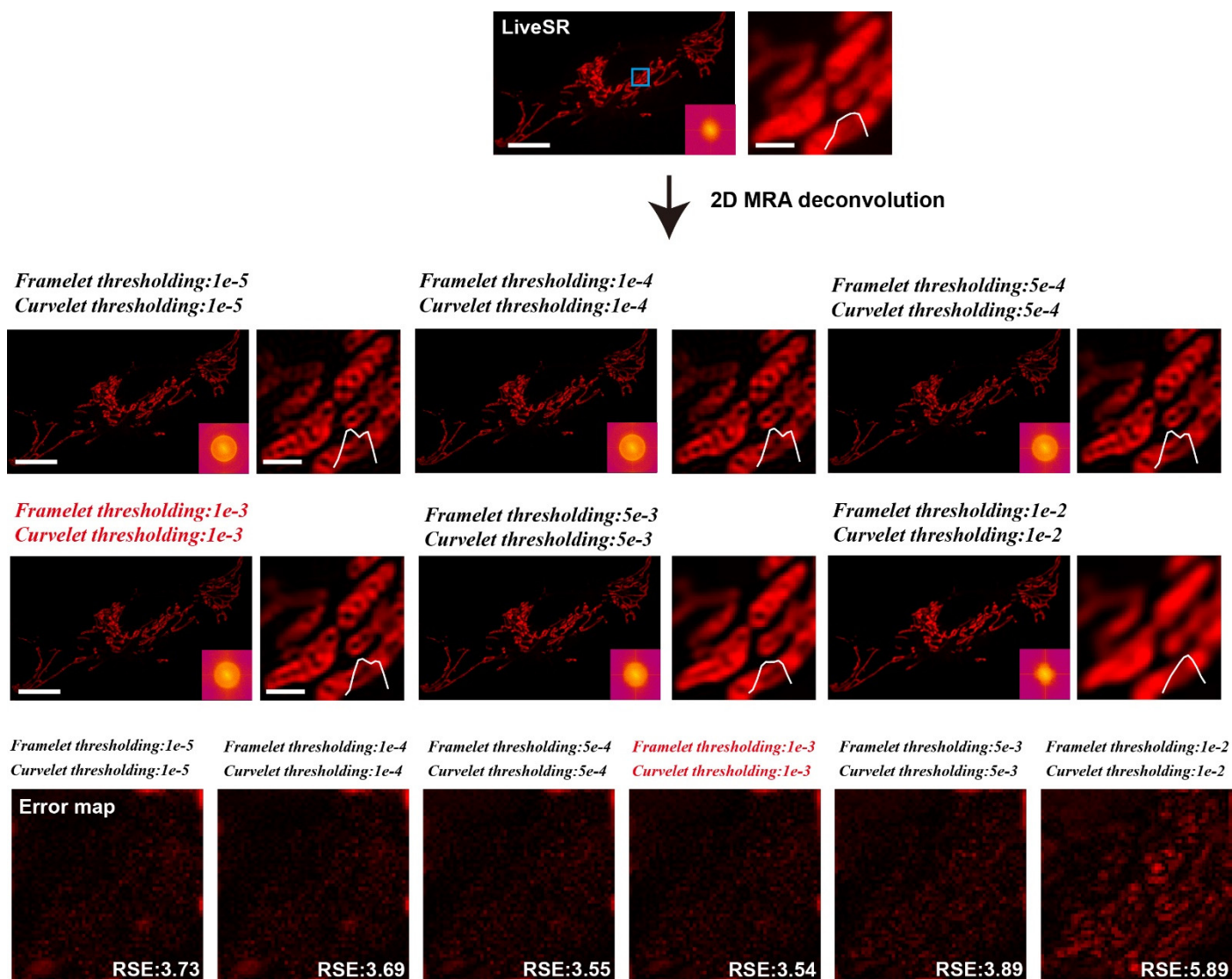


Fig. S29. Effect of adjusting MRA parameter in the LiveSR mitochondria image shown in Fig. 3. The results show that a high thresholding value tend to smooth the edge. While a low thresholding value tends to generate sharper image. The effect is similar as observed in Supplementary Fig. 32. Scale bars: $10\ \mu\text{m}$ (overview), $1\ \mu\text{m}$ (subgraph).

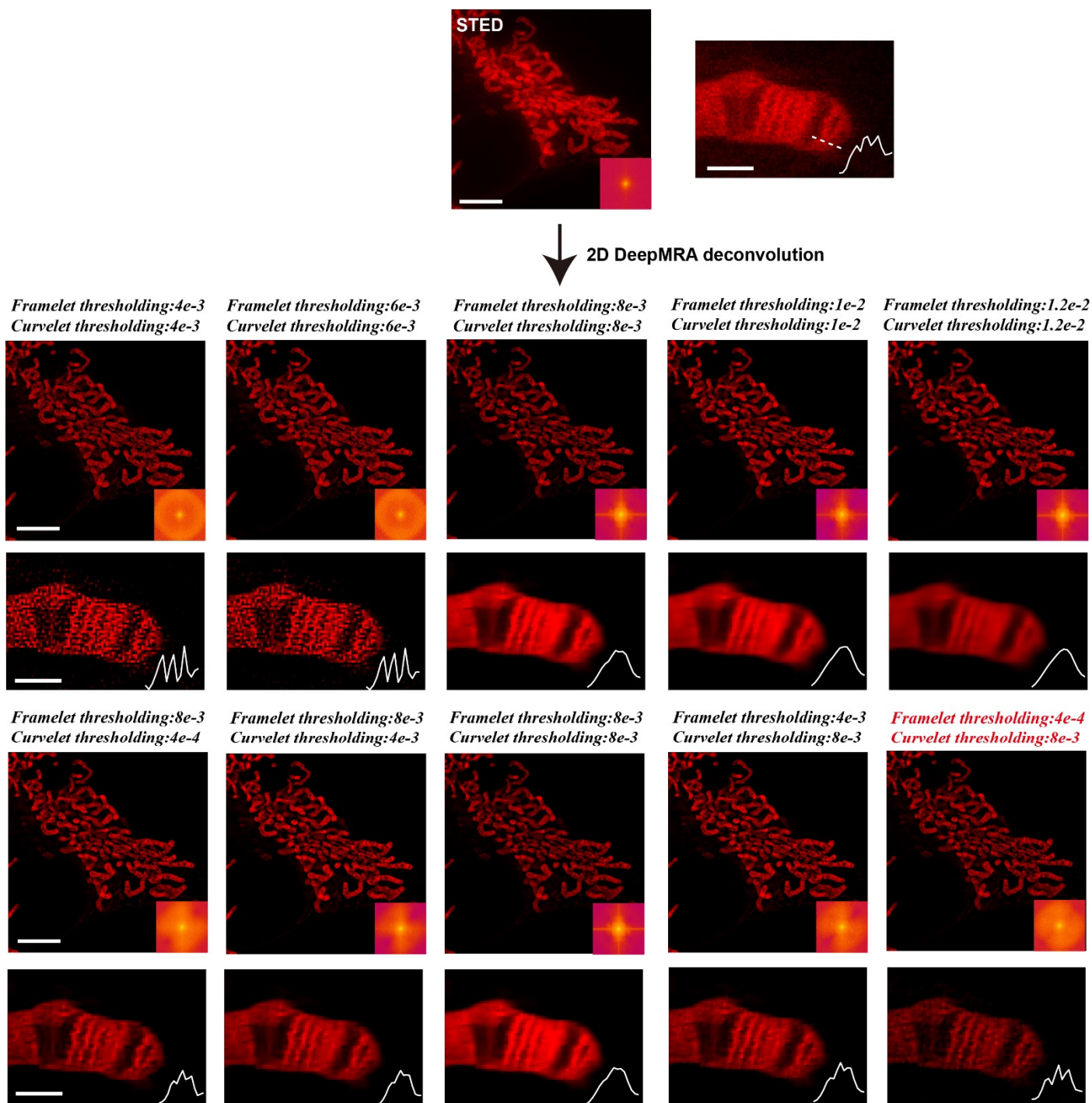


Fig. S30. Effect of adjusting MRA parameter in the STED mitochondria image shown in Fig. 4. Adjusting the thresholding value yields similar effect as described in Supplementary Fig. 32. A large portion of curvelet thresholding better fits this image. Scale bars: $5\ \mu\text{m}$ (overview), $0.5\ \mu\text{m}$ (subgraph).

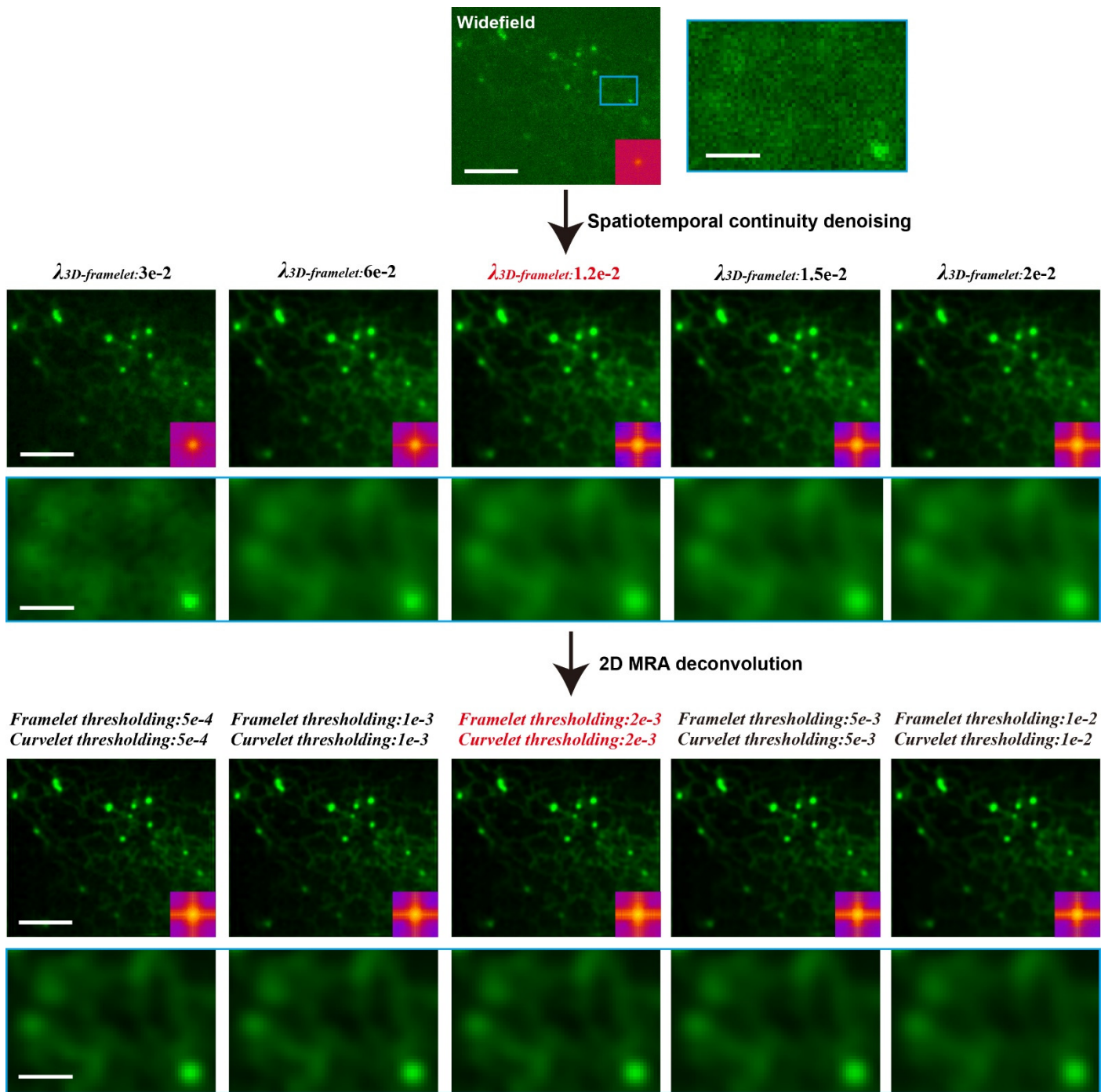


Fig. S31. Effect of adjusting MRA parameter in the widefield ER image shown in Fig. 1. The effect of adjusting the 3D-framelet thresholding yields similar results as we observe in 2D cases. Scale bars: 5 μm (overview), 0.5 μm (subgraph).

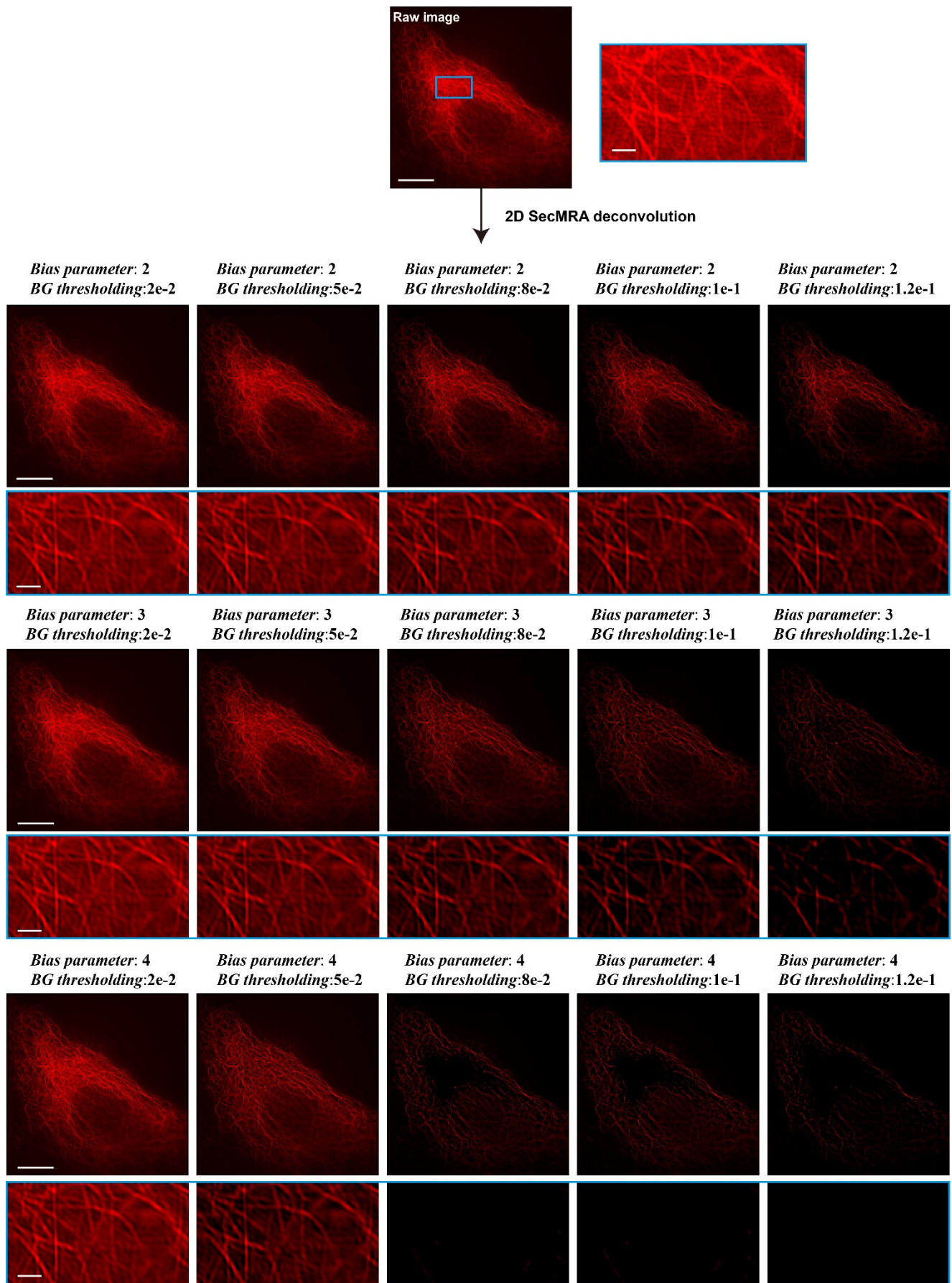


Fig. S32. Effect of the bias coefficient and bias thresholding parameter on the 2D-SecMRA deconvolution of the tubulin image displayed in Fig. 5. The red mark denotes the parameter adopted in the main text. Scale bars: 10 μm (overview), 2 μm (subgraph).

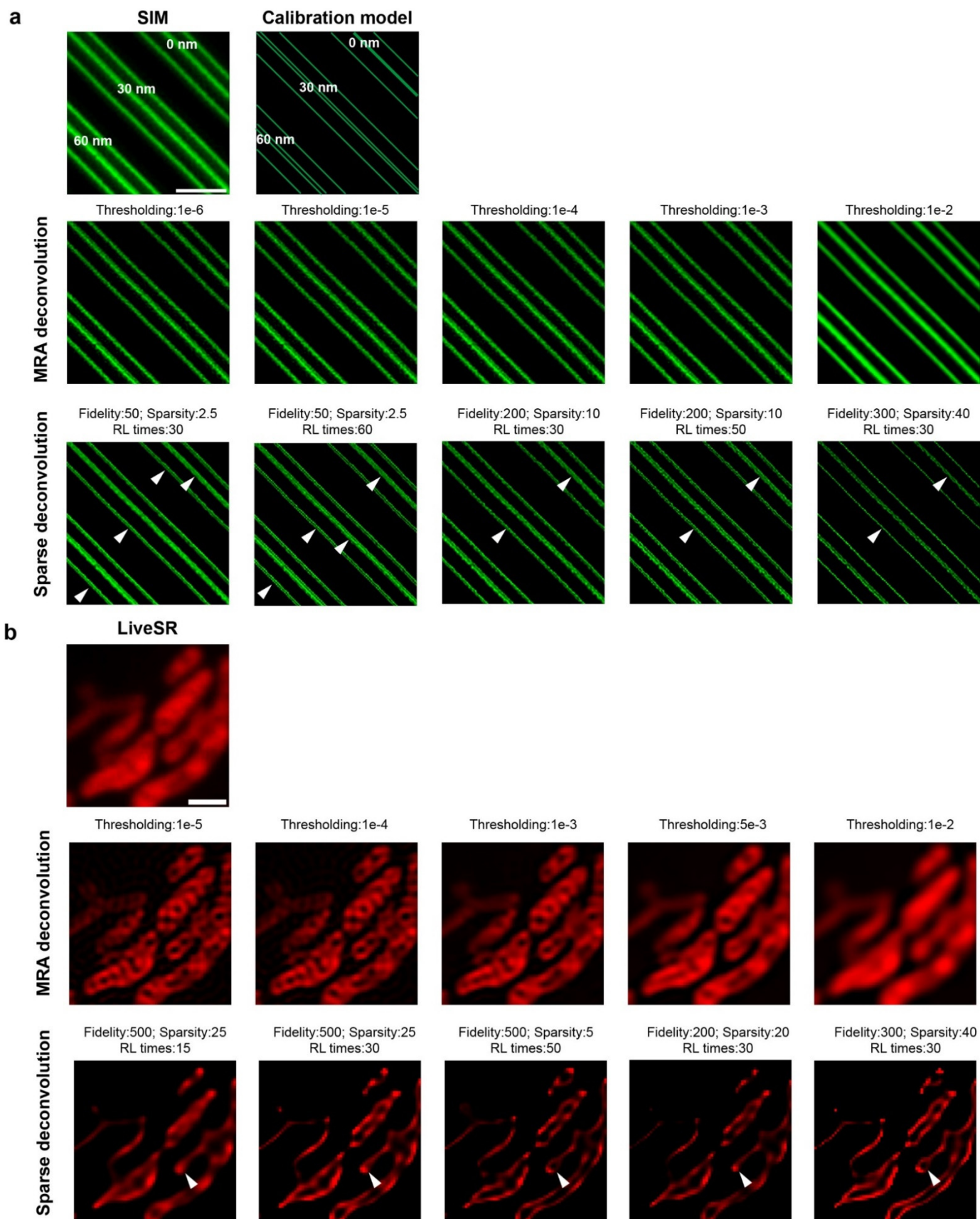


Fig. S33. Effect of parameter tuning in our method and denoise-MLE scheme. a. The Argo-SIM standard slide, which was previously shown in Fig. 3. **b.** The mitochondria image captured by LiveSR, which was previously shown in Fig. 3. The results show that denoise-MLE scheme shows rather different results as the change of denoising parameters and MLE iteration times. The high-frequency information obtainable through MRA remains consistent and is not substantially influenced by parameters. Scale bars: $2\ \mu\text{m}$ (a), $1\ \mu\text{m}$ (b).

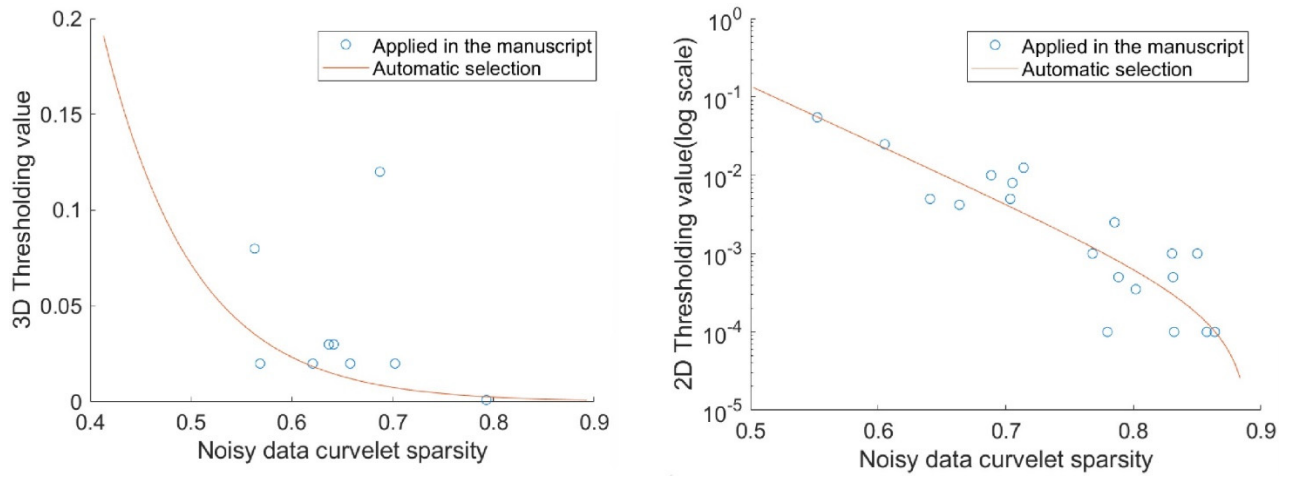


Fig. S34. Automated MRA parameters selection. The automatic selection curve is an exponential function fitter from the parameters used in the manuscript, with exclusion of some outliers. Automatic parameter selection in most cases closely approximates our final parameter selection in the manuscript. We have slightly reduced the automatic selection of 3D thresholding parameters, as not all time series images have good continuity.

Supplementary Tables

Table S1. Linearity preservation of MRA and SecMRA in representative data

Data	RSP			RSP excluding background		
Fig. 2e	MRA-WF	SecMRA-WF	SIM	MRA-WF	SecMRA-WF	SIM
	0.997	0.985	0.994	0.999	0.993	0.996
	MRA-SIM		SecMRA-SIM	MRA-SIM		SecMRA-SIM
	0.993		0.972	0.995		0.992
Fig. 4e	MRA-Confocal	SecMRA-Confocal	SoRa	MRA-Confocal	SecMRA-Confocal	SoRa
	0.993	0.903	0.885	0.993	0.947	0.886
	MRA-SoRa		SecMRA-SoRa	MRA-SoRa		SecMRA-SoRa
	0.885		0.763	0.986		0.886
Fig. S13a	MRA-WF	SecMRA-WF	SIM	MRA-WF	SecMRA-WF	SIM
	0.998	0.993	0.971	0.999	0.993	0.991
	MRA-SIM		SecMRA-SIM	MRA-SIM		SecMRA-SIM
	0.965		0.936	0.986		0.973

RSP denotes the resolution-scaled Pearson correlation. The RSP excluding background is calculated by segmenting the image using trainable weka segmentation, and only considering the difference in signal part in the calculation. In the widefield-SIM imaging pairs the basis for evaluation is the widefield image. In confocal-SoRa imaging pair the basis for evaluation is the confocal image.

Table S2. Imaging parameter used for capturing the data used in this paper

Data	Imaging technique	Objective lens	Excitation wavelength	Numerical aperture	Pixel size	SBR (dB)
Fig. 1b	SIM	100x	561 nm	1.49	32.5 nm	(in article)
Fig. 1e	Widefield	100x	488 nm	1.49	65 nm	8.03
Fig. 1h	SIM	100x	488 nm	1.49	32.5 nm	5.65
Fig. 2a	Confocal	100x	637 nm	1.4	70 nm	17.17
Fig. 2e	Widefield	100x	488 nm	1.49	65nm	7.64
Fig. 2e	SIM	100x	488 nm	1.49	32.5 nm	7.19
Fig. 3a	SIM	100x	488 nm	1.49	32.5 nm	8.31
Fig. 3b	SIM	100x	488 nm	1.49	32.5 nm	10.58
Fig. 3e	Widefield	100x	488 nm	1.49	65 nm	14.81
Fig. 3g	LiveSR	100x	637 nm	1.49	65 nm	15.62
Fig. 4b	Widefield	20x	405 nm	1.2	550 nm	2.74
Fig. 4b	SD-Confocal	20x	405 nm	1.2	550 nm	5.36
Fig. 4c	SoRa	100x	488 nm	1.45	39.28 nm	4.59
Fig. 4d	Widefield	100x	488 nm	1.49	65 nm	3.49
Fig. 4e	SD-Confocal	100x	561 nm	1.45	110 nm	3.77
Fig. 4e	SoRa	100x	561 nm	1.45	39.28 nm	4.10
Fig. 4f	LiveSR	100x	637 nm	1.49	65 nm	1.14
Fig. 4g	SIM	100x	488 nm	1.49	65 nm	0.33
Fig. 4h	STED	100x	637 nm	1.4	22.72 nm	5.36
Fig. 5a	Widefield	100x	488&637 nm	1.49	65 nm	8.55&5.09
Fig. 5e	SIM	100x	488&637 nm	1.49	65 nm	8.91&3.55
Fig. 6a	LiveSR	100x	488&637 nm	1.49	65 nm	1.85&5.95
Fig. 6c	Widefield	100x	488&637 nm	1.49	65 nm	1.01&4.99
Fig. S8	Widefield	100x	488 nm	1.49	65 nm	0.43
Fig. S12	SIM	100x	637 nm	1.49	65 nm	9.89
Fig. S13a	Widefield	100x	488 nm	1.49	65 nm	4.53
Fig. S13a	SIM	100x	488 nm	1.49	32.5 nm	14.39
Fig. S13b	Widefield	100x	561 nm	1.49	65 nm	4.53
Fig. S13b	SIM	100x	561 nm	1.49	32.5 nm	9.25
Fig. S13c	Widefield	100x	488 nm	1.49	65 nm	11.13
Fig. S13c	SIM	100x	488 nm	1.49	32.5 nm	10.58
Fig. 20c	Ex-WF	100x	488 nm	1.49	65 nm	6.70
Fig. 20c	Ex-SIM	100x	488 nm	1.49	32.5 nm	11.96

Table S3. Parameter of MRA deconvolution used in this paper

Data	Spatiotemporal continuity denoising	2D MRA deconvolution		
	$\lambda_{3D\text{-Framelet}}$	$\lambda_{\text{Framelet}}$	$\lambda_{\text{Curvelet}}$	RL times
Fig. 1b 3%	\	0.05	0.06	2
Fig. 1b 9%	\	0.05	0.05	2
Fig. 1b 20%	\	0.01	0.04	2
Fig. 1b 100%	\	0.004	0.01	2
Fig. 1e	0.12	0.01	0.01	0
Fig. 1h	0.01	0.006	0.006	0
Fig. 2a	\	0.0005	0.0005	0
Fig. 2e	\	0.0001	0.0001	0
Fig. 2i	\	0.0001	0.0001	2
Fig. 3a	\	0.00001	0.00001	0
Fig. 3b	\	0.0001	0.0001	0
Fig. 3e	\	0.0001	0.0001	2
Fig. 3g	\	0.001	0.001	2
Fig. S8	0.12	0.005	0.005	2
Fig. S12a	\	0.001	0.001	2
Fig. S13a	\	0.0001	0.0001	2
Fig. S13c	\	0.0005	0.0005	2
Fig. S13d	\	0.0001	0.0001	2
Fig. S21b	\	0.00001	0.00001	2

Note: The PSF estimation uses the imaging parameters, and considers resolution scaling factor for SR technique: LiveSR 1 $\times$, SIM 2 $\times$.

Table S4. Parameter of SecMRA deconvolution used in this paper

Data	Preliminary debackground		Spatiotemporal continuity denoising	2D SecMRA deconvolution			
	k	λ_{bias}		k	λ_{bias}	$\lambda_{Framelet}$	$\lambda_{Curvelet}$
Fig. 4b WF	\	\	\	2	0.45	0.001	0.001
Fig. 4b SD-Confocal	\	\	\	2	0.3	0.001	0.001
Fig. 4c	2	0.15	\	3	0.15	0.002	0.008
Fig. 4d	4	0.08	\	4	0.05	0.008	0.008
Fig. 4e Confocal	\	\	\	2	0.15	1e-6	1e-6
Fig. 4e SoRa	\	\	\	1	0.15	0.001	0.001
Fig. 4f	\	\	\	3	0.12	0.003	0.005
Fig. 4g	\	\	\	3	0.12	0.005	0.008
Fig. 4h	\	\	\	3	0.18	0.0004	0.008
Fig. 5a Tubulin	\	\	\	3	0.2	0.0002	0.0005
Fig. 5a Lysosome	\	\	\	1	0.18	1e-5	0.0005
Fig. 5e Tubulin	\	\	\	3	0.1	0.0005	0.02
Fig. 5e Lysosome	\	\	\	3	0.15	0.01	0.01
Fig. 6a Tubulin	\	\	0.02	8	0.03	0.0001	0.0001
Fig. 6a Mito	\	\	0.02	3	0.08	0.0001	0.0001
Fig. 6c ER	4	0.06	0.03	4	0.08	0.0005	0.0005
Fig. 6c Mito	4	0.08	0.001	4	0.0001	0.0001	0.0001
Fig. S20a WF	\	\	\	2	0.15	0.0001	0.0001
Fig. S20a SIM	\	\	\	1	0.1	0.0005	0.0005
Fig. S20c Ex-WF	\	\	\	1	0.65	1e-5	1e-5
Fig. S20c Ex-SIM	\	\	\	1	0.35	1e-5	1e-5
Fig. S20d Confocal	\	\	\	3	0.08	1e-5	1e-5
Fig. S20d STED	\	\	\	0.5	0.12	0.001	0.001
Fig. S21a 1.6 μm	\	\	\	2	0.25	0.01	0.01
Fig. S21a 3.3 μm	\	\	\	2.5	0.22	0.01	0.008

Note: The PSF estimation uses the imaging parameters, and considers resolution scaling factor for SR technique: LiveSR 1 $\times$, SoRa 1.5 $\times$, SIM 2 $\times$, STED 6 $\times$ for Fig. 4g and 3 $\times$ for Fig. S20d; Fig. 6a Tubulin, employed the ‘Intensity correction’ mode in the SecMRA deconvolution software, the other data were processed with the ‘Background removal’ mode.

Table S5. Parameter of other deconvolution algorithms used in this paper

Data	Algorithm	Parameter
Fig. 1e	Hessian	Mu: 20, Sigma=1
Fig. 1h	Hessian	Mu: 80, Sigma=1
Fig. 2a	Sparse	Fidelity:1000, Sparsity: 50, RLtimes:30, Iteration: 100
Fig. 2a	Huygens	Deconvolution wizard, SNR: 10
Fig. 2e	Sparse	Fidelity:800, Sparsity: 50, RLtimes:30, Iteration: 100
Fig. 2e	Huygens	Deconvolution wizard, SNR: 40
Fig. 2h, i	Sparse	Fidelity:500, Sparsity: 8, RLtimes:30, Iteration: 100
Fig. 2h, i	Huygens	Deconvolution wizard, SNR: 40
Fig. S12a	Sparse	Fidelity:500, Sparsity: 20, RLtimes:30, Iteration: 100
Fig. S12a	Huygens	Deconvolution wizard, SNR: 53
Fig. S12h	Sparse	Fidelity:500, Sparsity: 25, RLtimes:30, Iteration: 100
Fig. S12h	Huygens	Deconvolution wizard, SNR: 40
Fig. S13a	Sparse	Fidelity:400, Sparsity: 20, RLtimes:30, Iteration: 100
Fig. S13a	Huygens	Deconvolution wizard, SNR: 80
Fig. S13c	Sparse	Fidelity:800, Sparsity: 50, RLtimes:30, Iteration: 100
Fig. S13c	Huygens	Deconvolution wizard, SNR: 40
Fig. S13d	Sparse	Fidelity:800, Sparsity: 40, RLtimes:30, Iteration: 100
Fig. S13d	Huygens	Deconvolution wizard, SNR: 50
Fig. S15a	Sparse	Strong background (HI), Fidelity:15, Sparsity: 0.4, RLtimes:10, Iteration: 100
Fig. S15b	Sparse	Strong background (LI), Fidelity:5, Sparsity: 0.1, t(z) axial continuity: 1, RLtimes:10, Iteration: 100
Fig. S15c	Sparse	Fidelity:5, Sparsity: 0.5, t(z) axial continuity: 0.5, RLtimes:50, Iteration: 100
Fig. S15c	Huygens	Deconvolution wizard, SNR: 14

Table S6. Typical computing time of MRA/SecMRA

Data	Size	MRA/SecMRA computing time
Fig. 2a	600*600*1	~14 seconds
Fig. 2e	1024*1024*1	~45 seconds
Fig. 3b	2048*2048*1	~4 minutes 46 seconds
Fig. 5e	1524*1524*1	~40 seconds
Fig. 6a	706*706*10	~2 minutes 8 seconds
Fig. 6c	512*512*20	~3 minutes 25 seconds
Fig. S8	301*256*50	~21 seconds
Fig. S12a	600*600*1	~17 seconds

CPU: 12th Gen Intel(R) Core(TM) i7-12700 2.10 GHz