Electronic Supporting Information

In-situ silver nanoparticle development for molecular-specific biological imaging via highly accessible microscopies.

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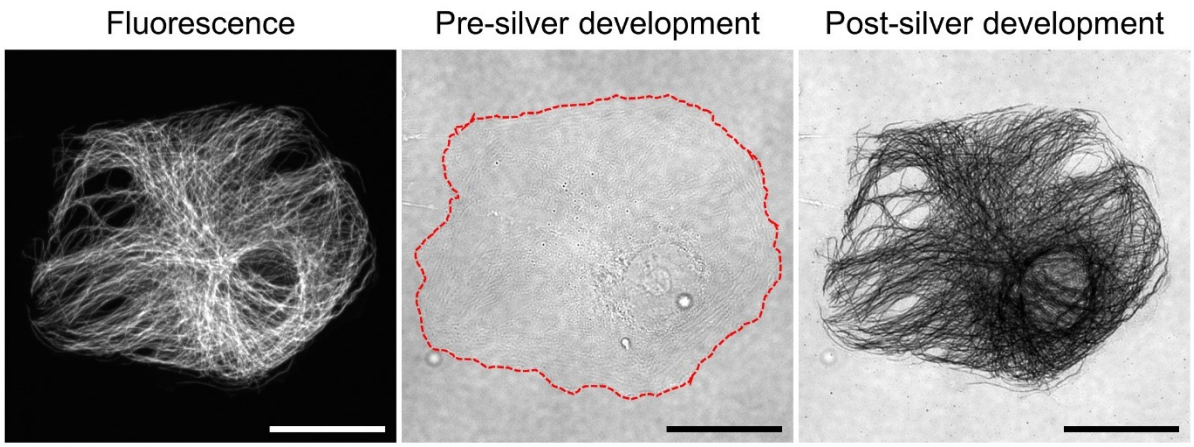
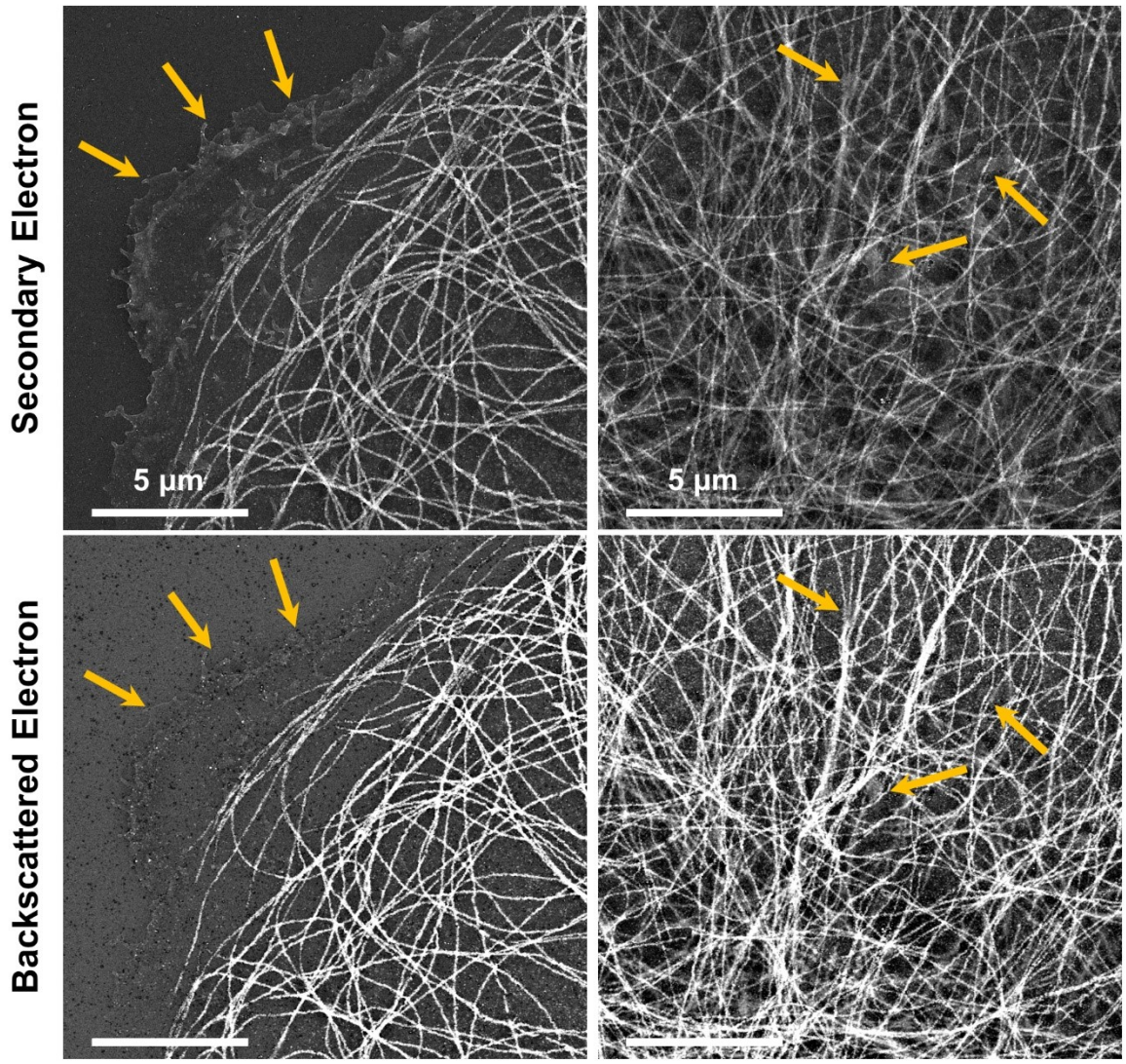
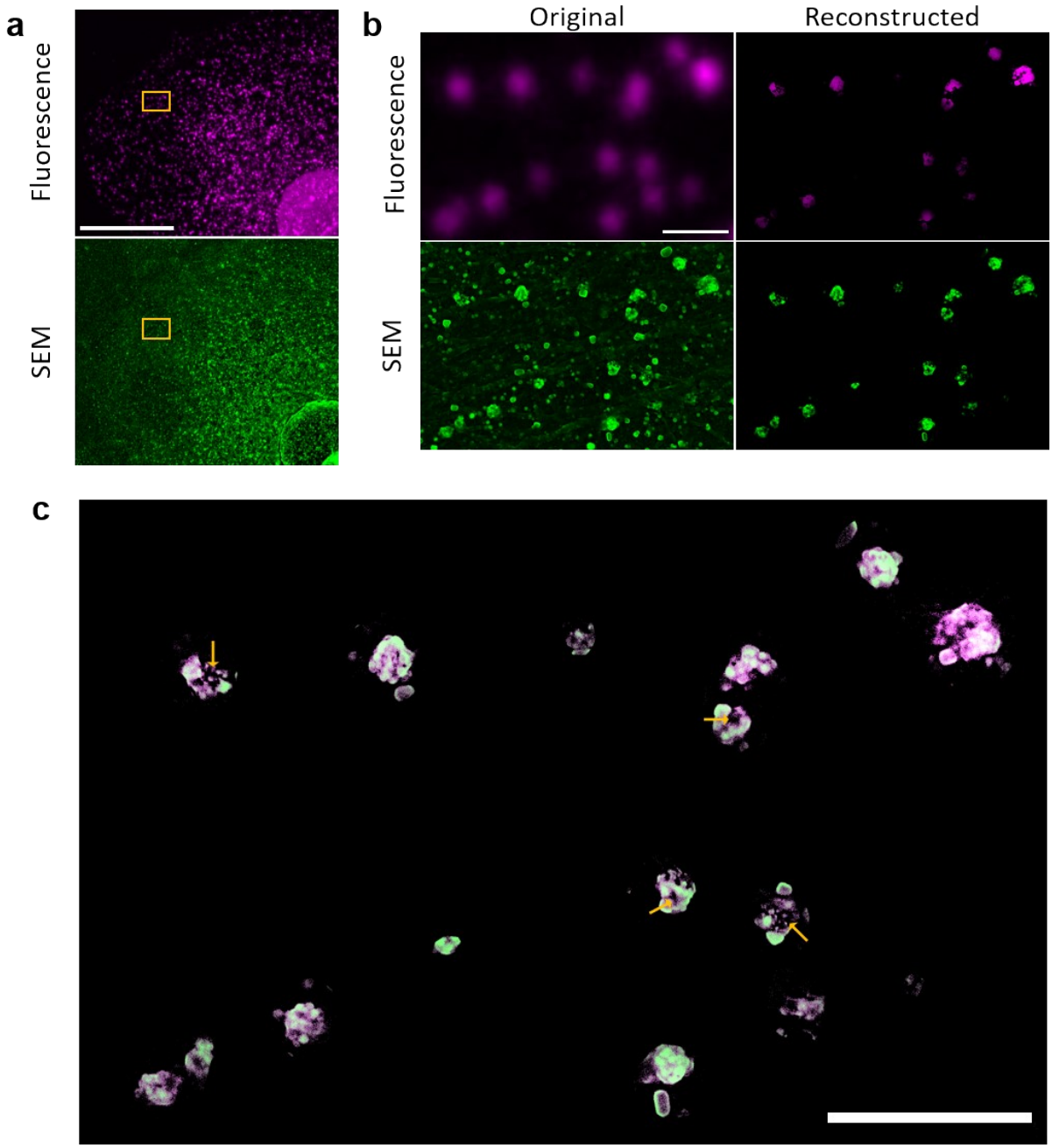


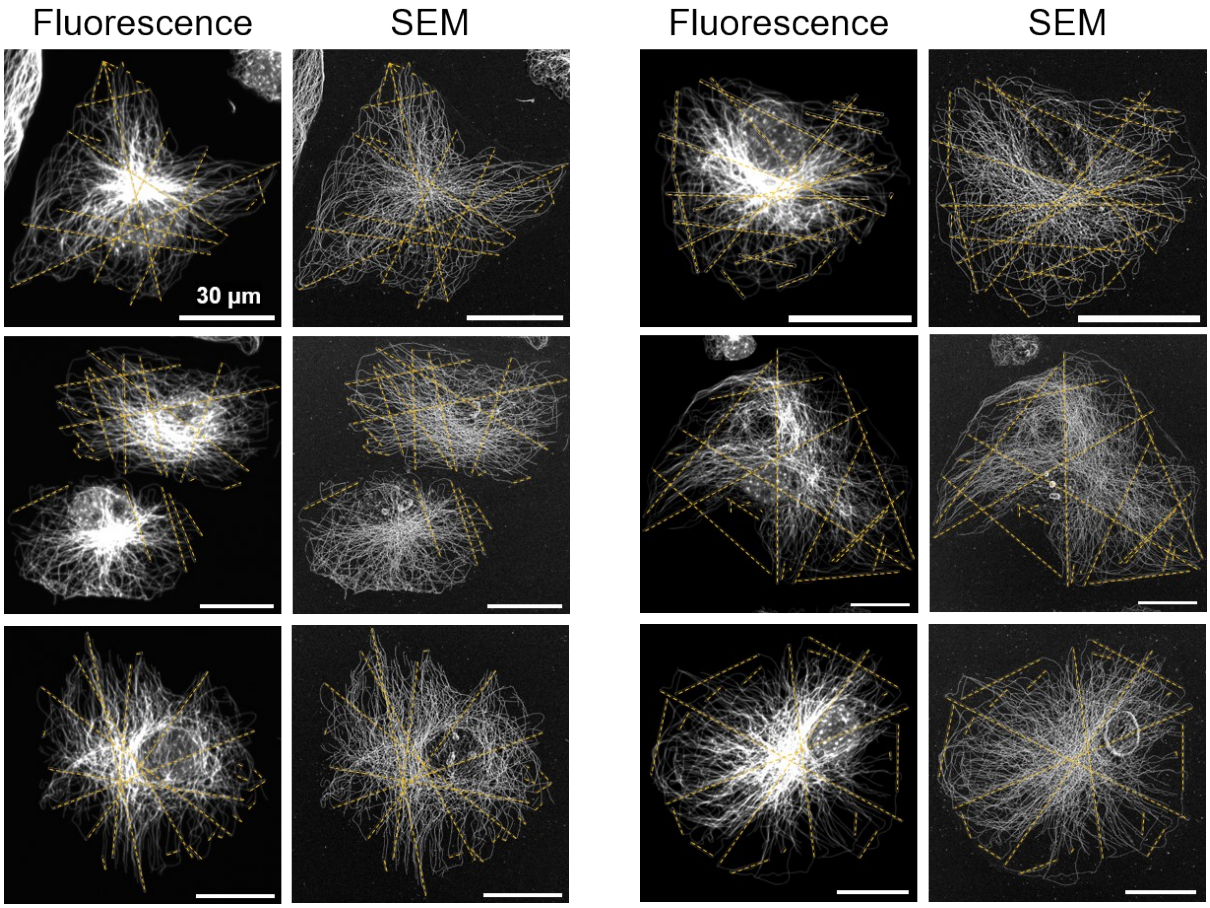
Supplementary Figure 1. Fluorescence and brightfield images of β-tubulin labeled BS-C-1 cell (pre- and post-silver development) from the same cell. Scale bar: 3 μm



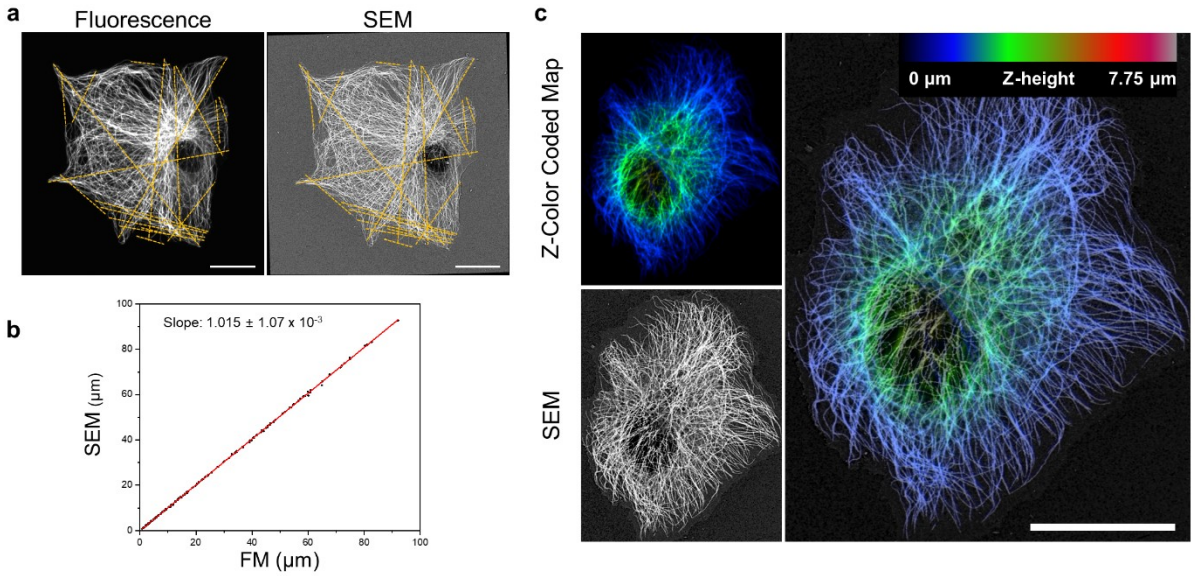
Supplementary Figure 2. SEM images of β-tubulin labeled as BS-C-1 cell using an SE-detector and a BSE detector. Yellow arrows indicate organic layers, including a cell membrane.



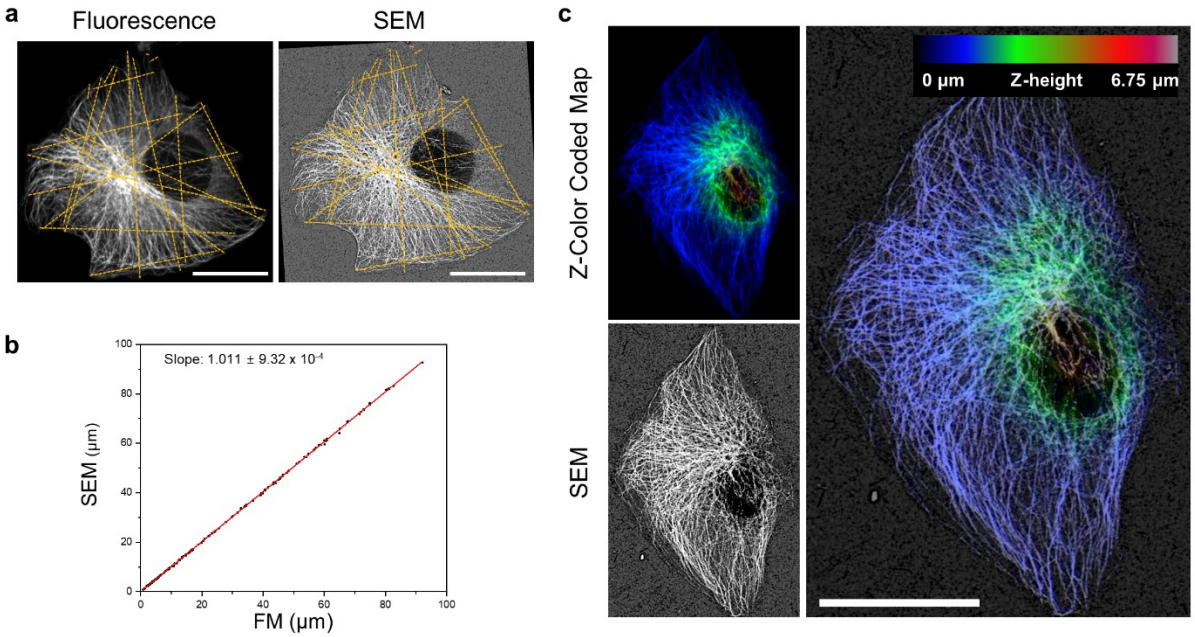
Supplementary Figure 3. Clathrin-coated pit of labeled BS-C-1 cell images. (**a**) Low-magnification images of labeled BS-C-1 (magenta: fluorescence, green: SEM). (**b**) Magnified original and reconstructed FM and SEM images from the yellow-boxed region in a. (**c**) Overlay image of reconstructed fluorescence and SEM images in b (yellow arrows: the internal hollow space of clathrin-coated pits). Scale bar: a) 20 μm, b, c) 1 μm.



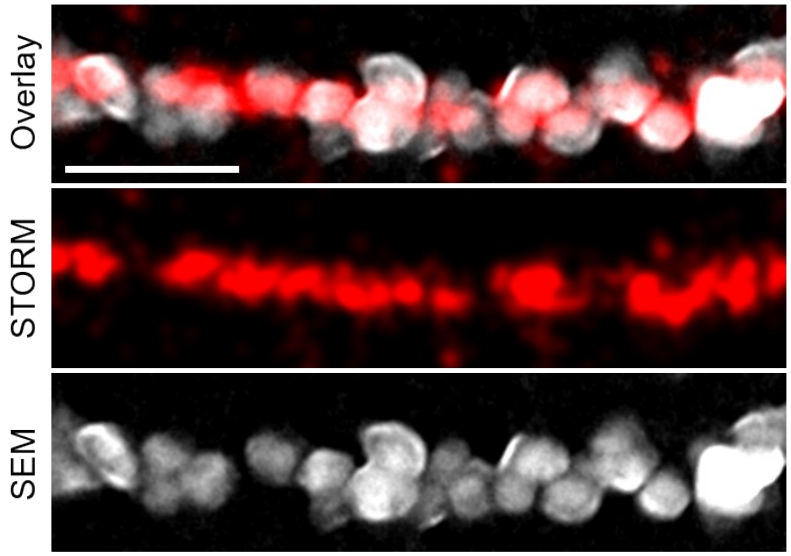
Supplementary Figure 4. Additional β-tubulin labeled BS-C-1 cell fluorescence microscopy and SEM images with lines for the confirmation of macroscopic deformation by cell size comparison.

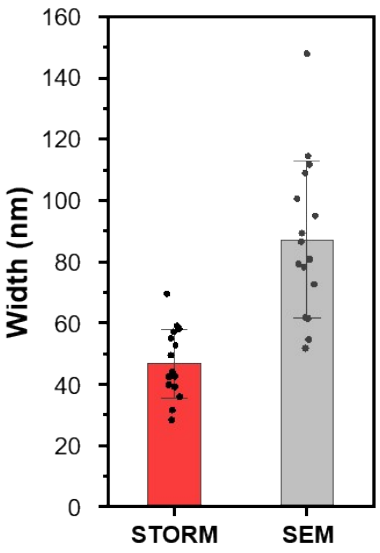


Supplementary Figure 5. Characterization of deformation during HMDS dehydration. (**a**) β-tubulin labeled BS-C-1 cell images with lines for confirmation of macroscopic deformation by cell size comparison. (**b**) Plot of lengths measured from the SEM image against those from the same position on the FM image. The points in b were obtained through 20 measurements, each from seven different cells, and a linear fit is shown as a red line (R-square: 0.99985). (**c**) Correlative analysis of the Z-color-coded map of confocal microscopy and SEM images. Step size in the color-coded map is 0.25 µm, and the overlay opacity is 50%. All SEM images were obtained using a BSE detector due to the organic layers. Scale bar: a) 20 µm, c) 20 µm.



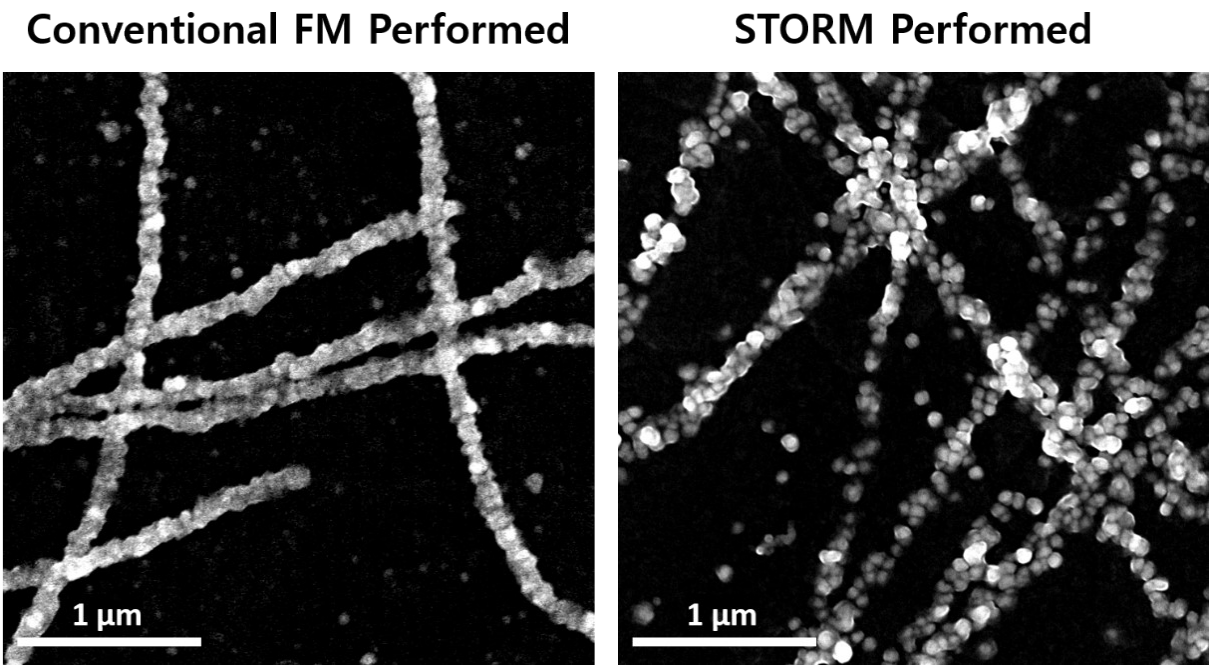
Supplementary Figure 6. Characterization of deformation during CPD. (**a**) β-tubulin labeled BS-C-1 cell images with lines for confirmation of macroscopic deformation by cell size comparison. (**b**) Plot of lengths measured from the SEM image against those from the same position on FM image. The points in b were obtained through 20 measurements, each from seven different cells, and a linear fit is shown as a red line (R-square: 0.99988). (**c**) Correlative analysis of the Z-color-coded map of confocal microscopy and SEM images. Step size in the color-coded map is 0.25 µm, and the overlay opacity is 50%. All SEM images were obtained using a BSE detector due to the organic layers. Scale bar: a) 20 µm, c) 20 µm.



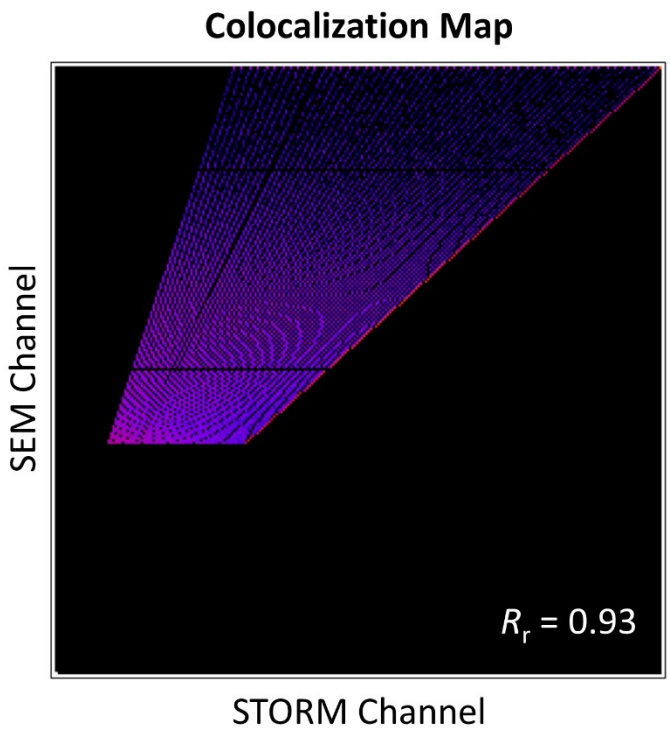


	Width Avg. ± STD (nm)
STORM	46.703 ± 10.801
SEM	87.181 ± 24.739

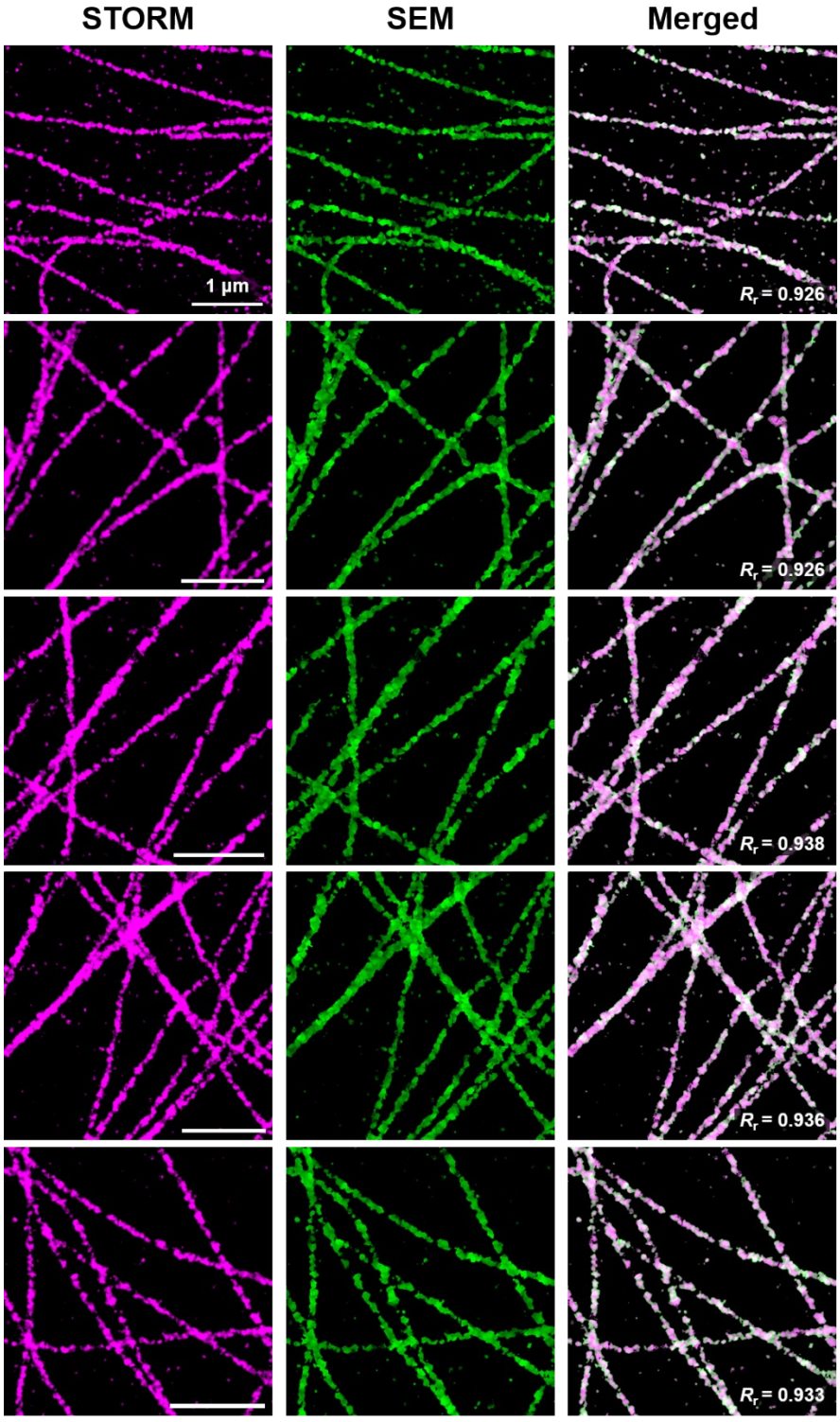
Supplementary Figure 7. Characterization of deformation during drying under ambient conditions. Width comparison of the regions between the stochastic optical reconstruction microscopy (STORM) and SEM images where STORM imaging was performed. When Gaussian fitting is possible, the full width at half-maximum (FWHM) value is used as the width, and when the saturation value appears, the length between the two peaks is used as the width value (STORM: 46.703 ± 10.801 nm, SEM: 87.181 ± 24.739 nm). Scale bar: 300 nm



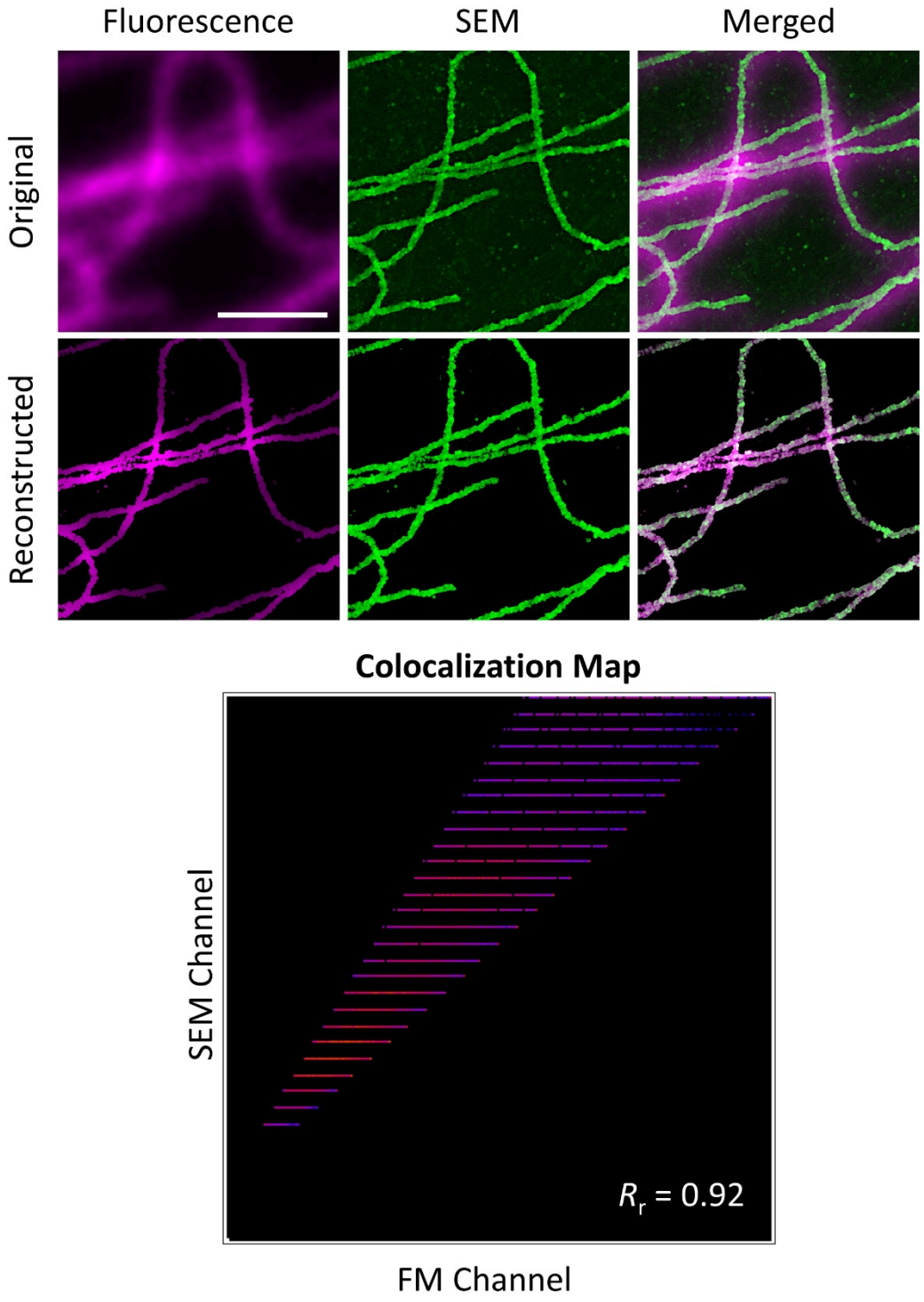
Supplementary Figure 8. Comparison of SEM images after the silver-development of samples subjected to conventional FM imaging and STORM imaging.



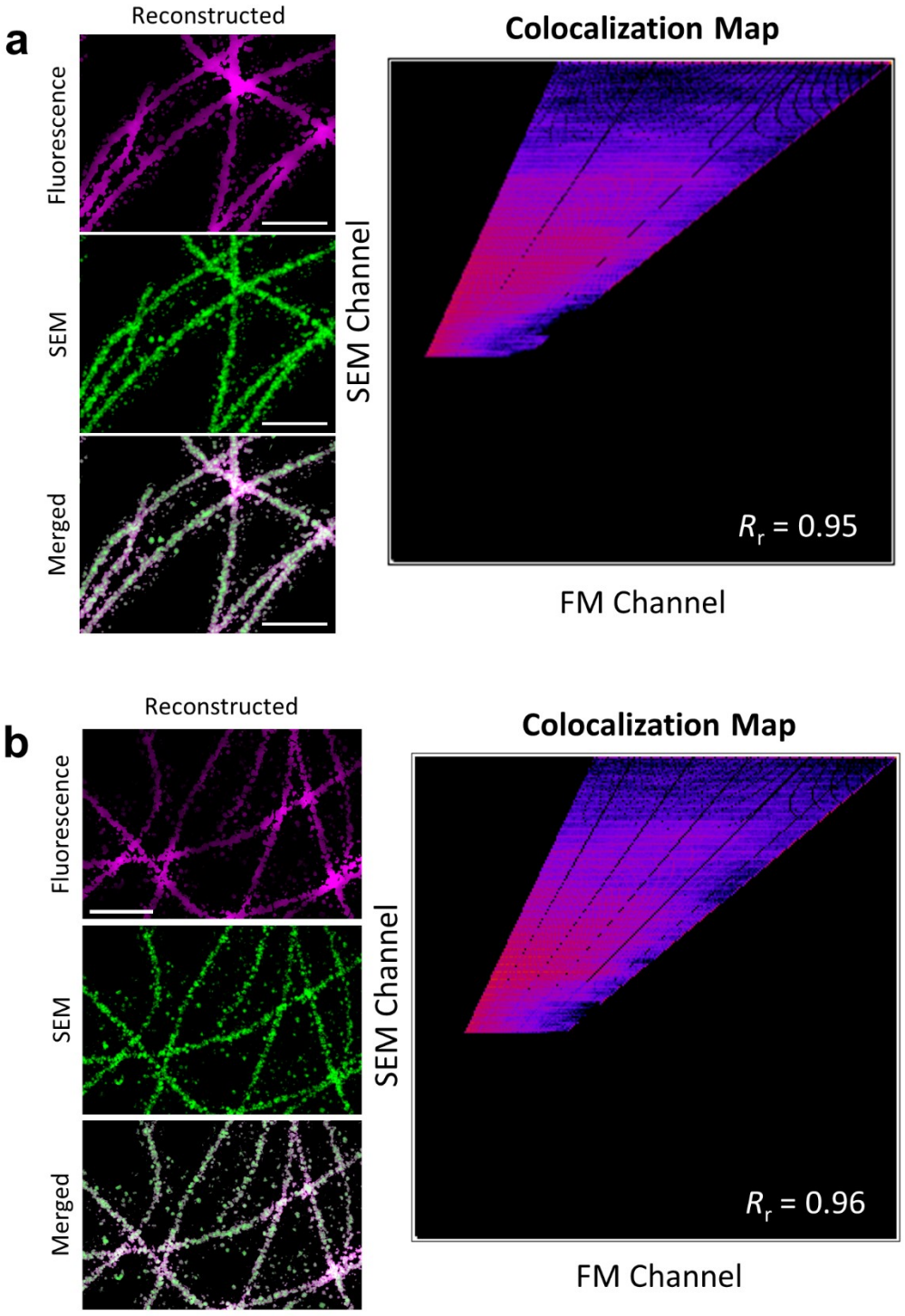
Supplementary Figure 9. Colocalization map between signal-reconstructed STORM and SEM image with high Pearson's coefficient (R_r = 0.93).



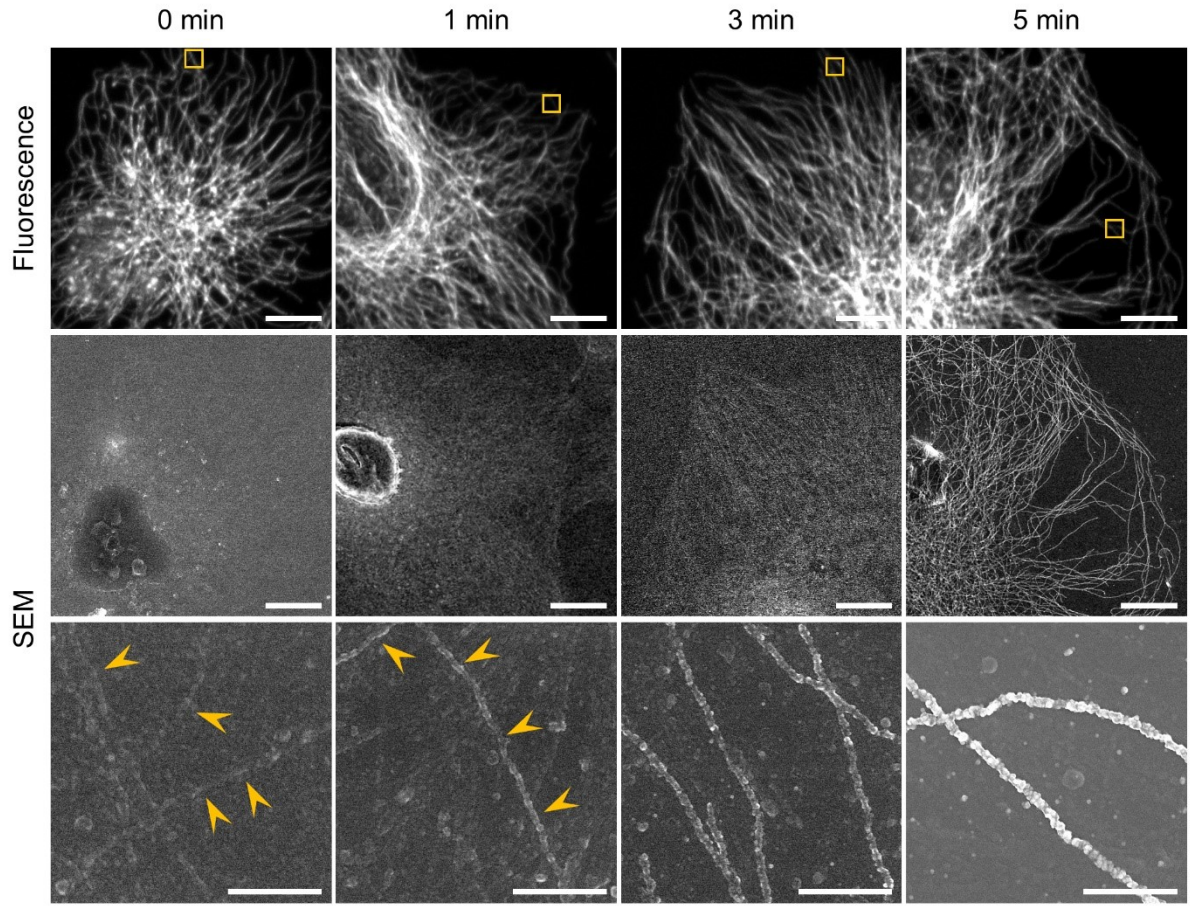
Supplementary Figure 10. Additional signal-reconstructed images of STORM, SEM, and merged images at the different cells showed a high correlation with Pearson's coefficient (magenta: STORM, green: SEM).



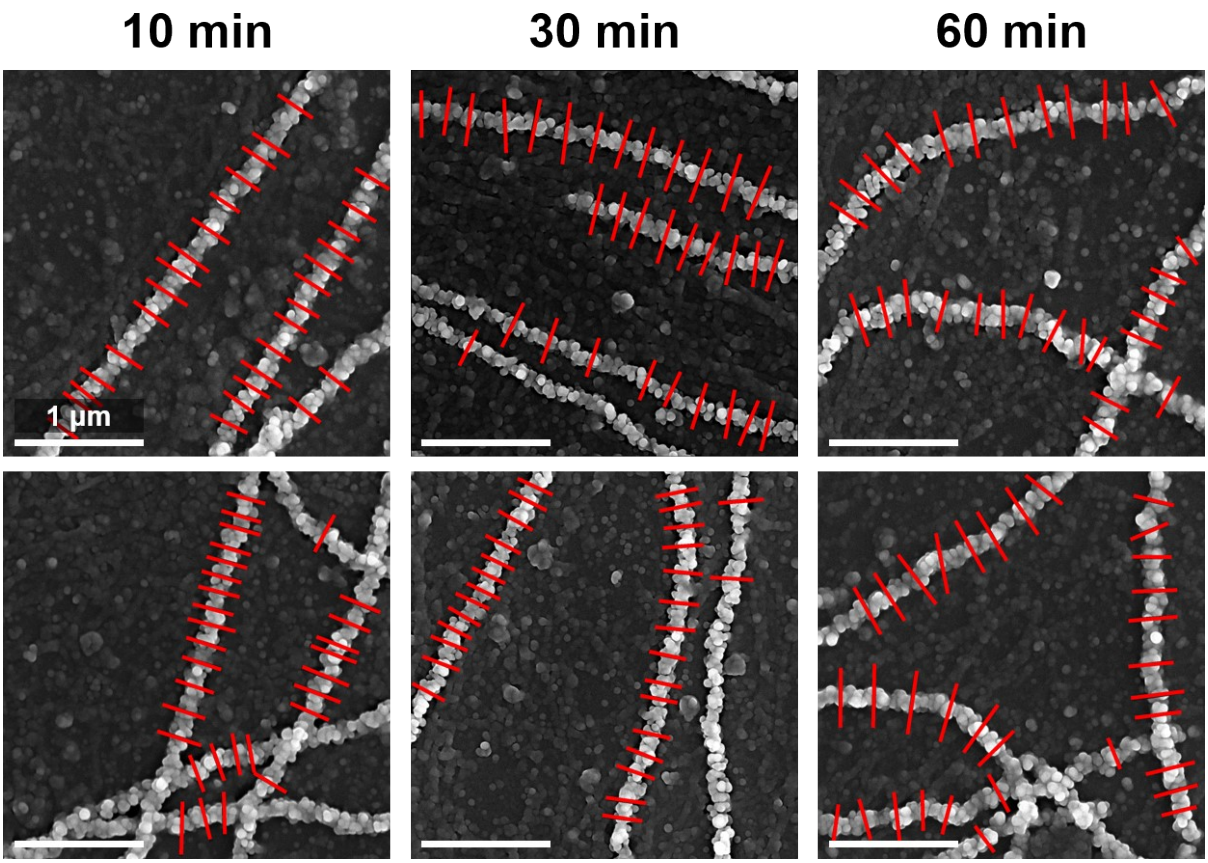
Supplementary Figure 11. Original (Figure 1c) and reconstructed FM, SEM, and merged images with colocalization map between signal-reconstructed FM and SEM image with high Pearson's coefficient (R_r = 0.92). Scale bar: 2μm



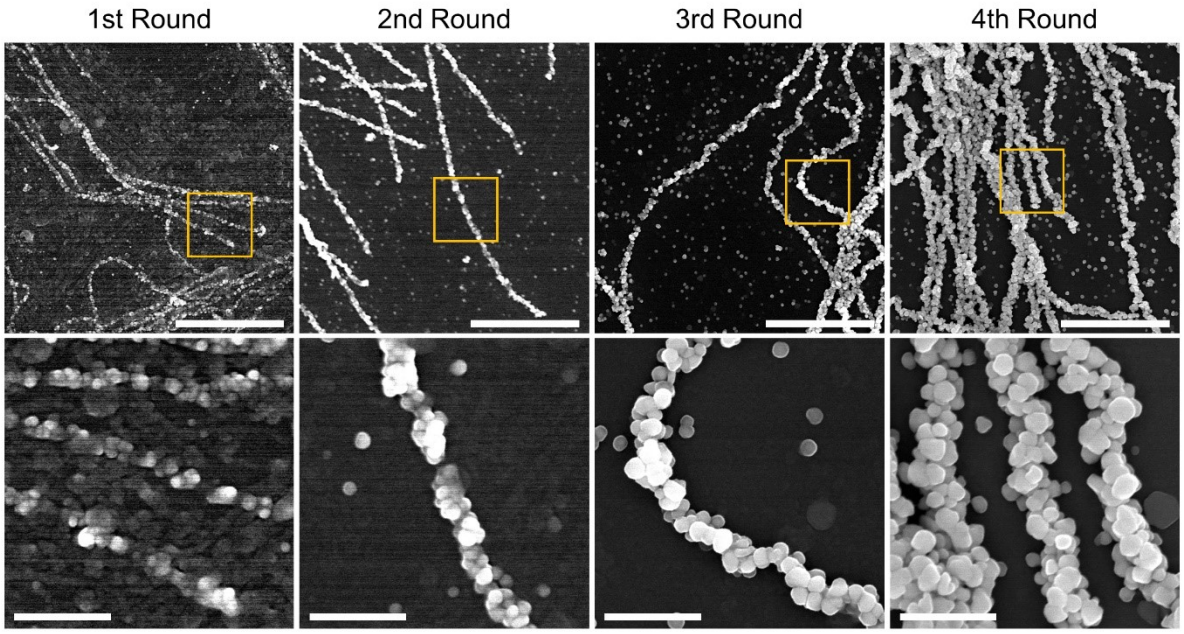
Supplementary Figure 12. Reconstructed FM, SEM, and merged images with a colocalization map between signal-reconstructed FM and SEM images through (**a**) HMDS (R_r = 0.95), and (**b**) CPD (R_r = 0.96) dehydration process. Scale bar: 1 μm.



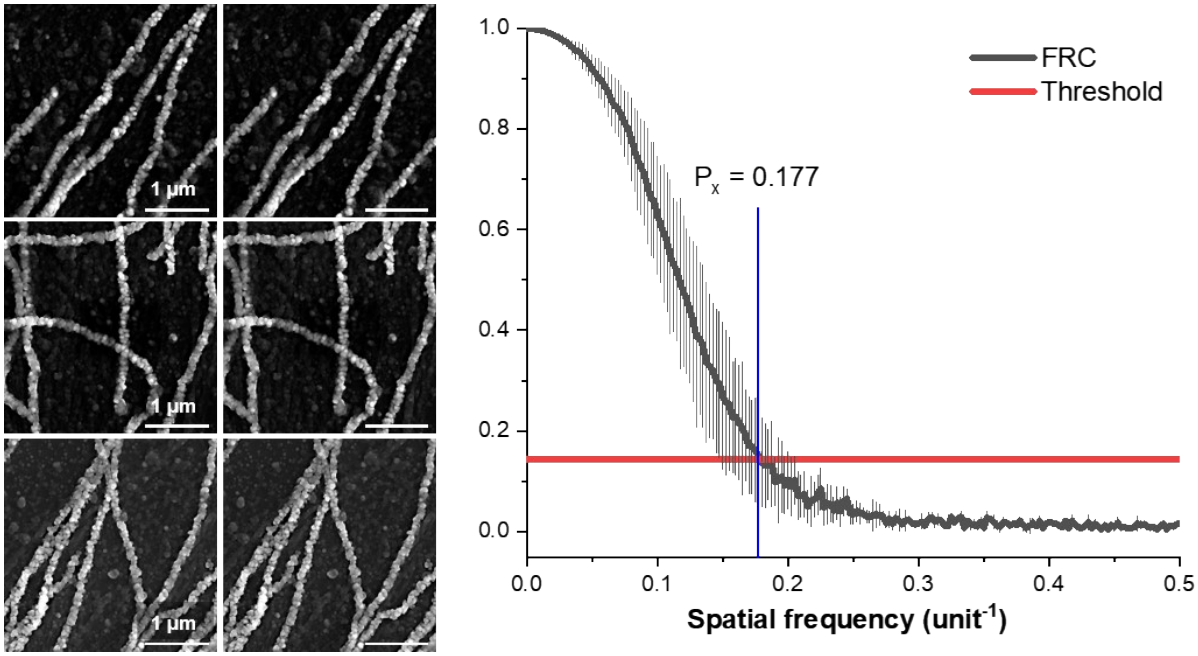
Supplementary Figure 13. Change in silver-tubulin structures according to silver development time. All images were obtained from cytoskeleton extracted BS-C-1 cells with silver particle labeled β-tubulin. Images according to silver development time. Bottom images show magnified SEM images of the yellow boxed region in FM images each. Arrowheads in the magnified images indicate the tubulin structures. Scale bar: 10 μm, 1 μm



Supplementary Figure 14. Additional SEM images of β-tubulin labeled BS-C-1 cells according to silver development time with red lines for the FWHM value calculation.

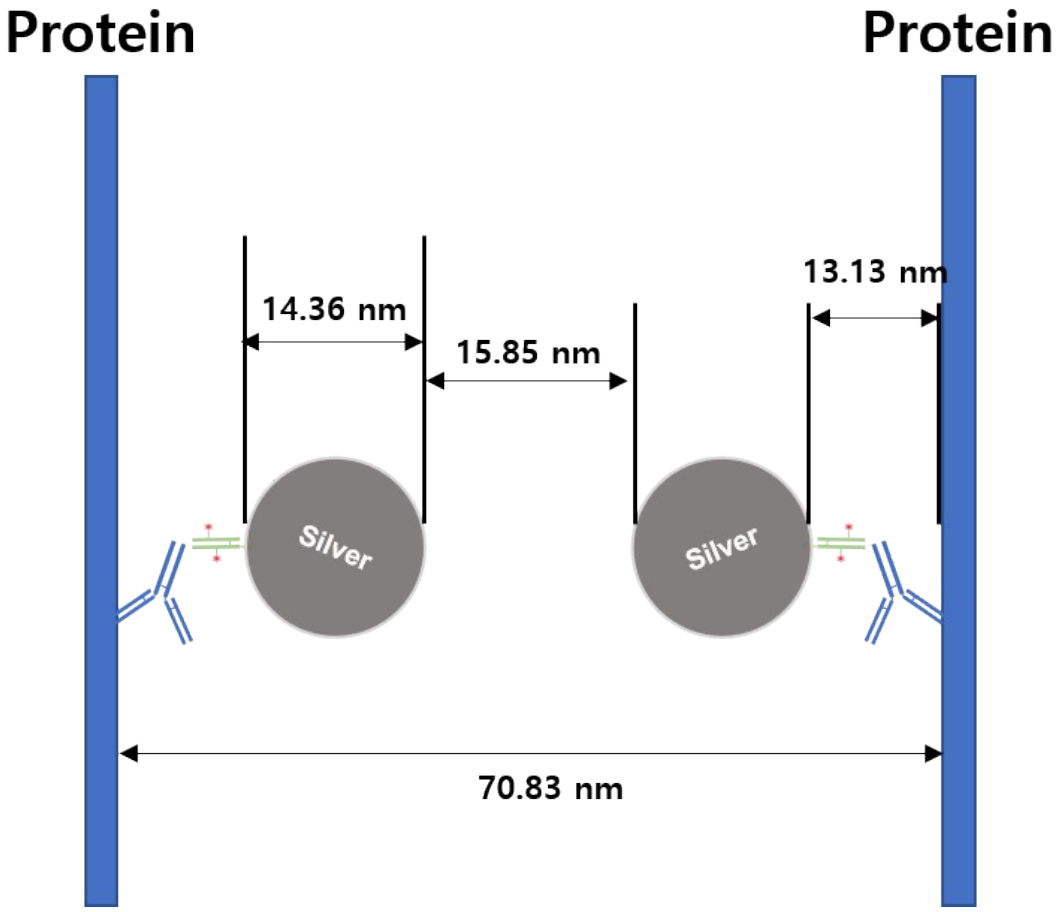


Supplementary Figure 15. Change in silver-tubulin size according to the round of 5 min silver development. All images were obtained from cytoskeleton extracted BS-C-1 cells with silver particle labeled β-tubulin. Bottom images show magnified SEM images of the yellow boxed region. Scale bar: 3 μm, 500 nm

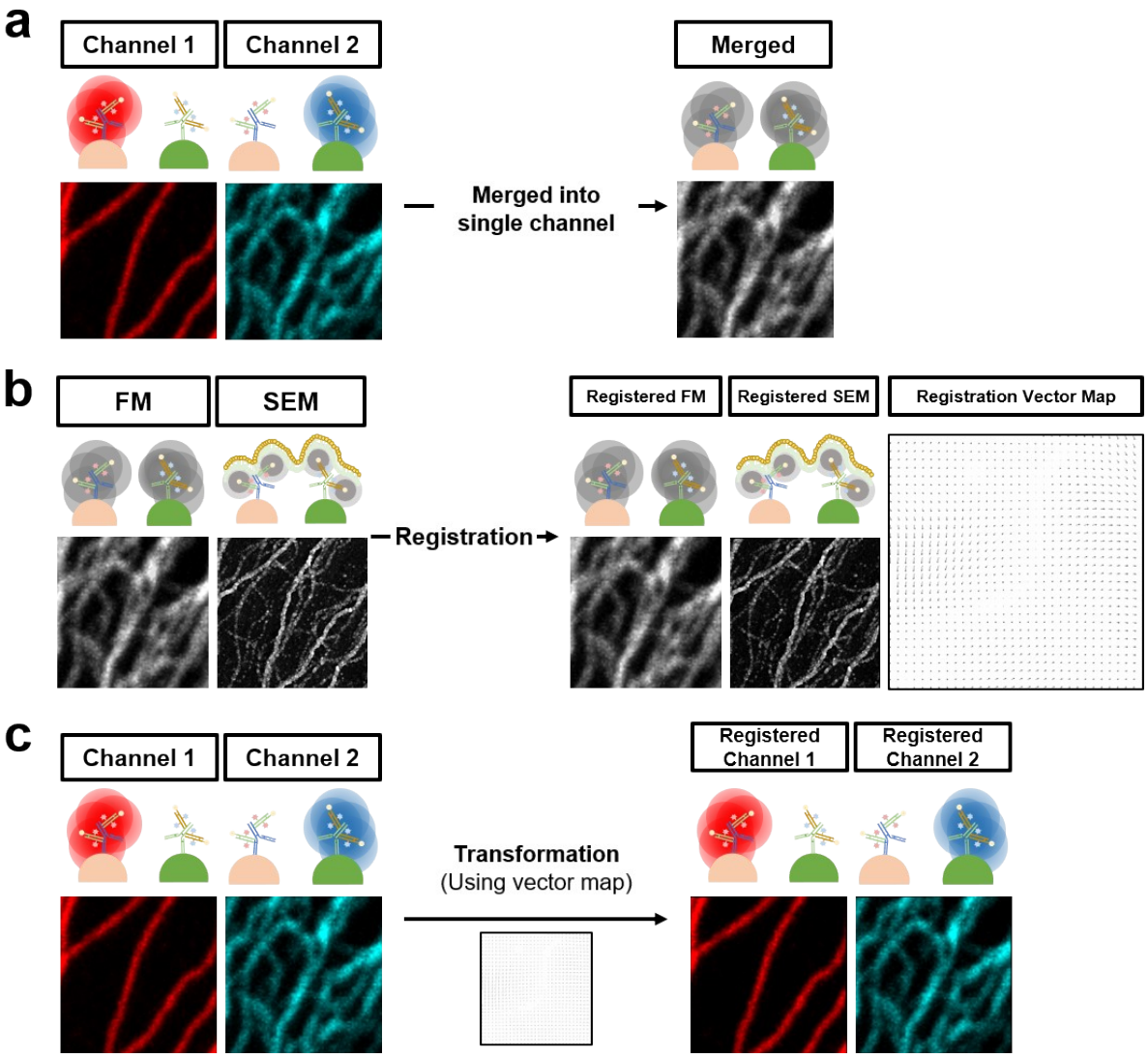


Supplementary Figure 16. Consecutive SEM image sets for the Fourier ring correlation (FRC) method and FRC curve with the threshold line (= 1/7)[1]. In FRC curve, the auxiliary lines represent the standard deviation of each point. The intersection of the FRC curve and the threshold is P_x, which is 0.177 unit^{-1}. Unit: 1.984 nm.

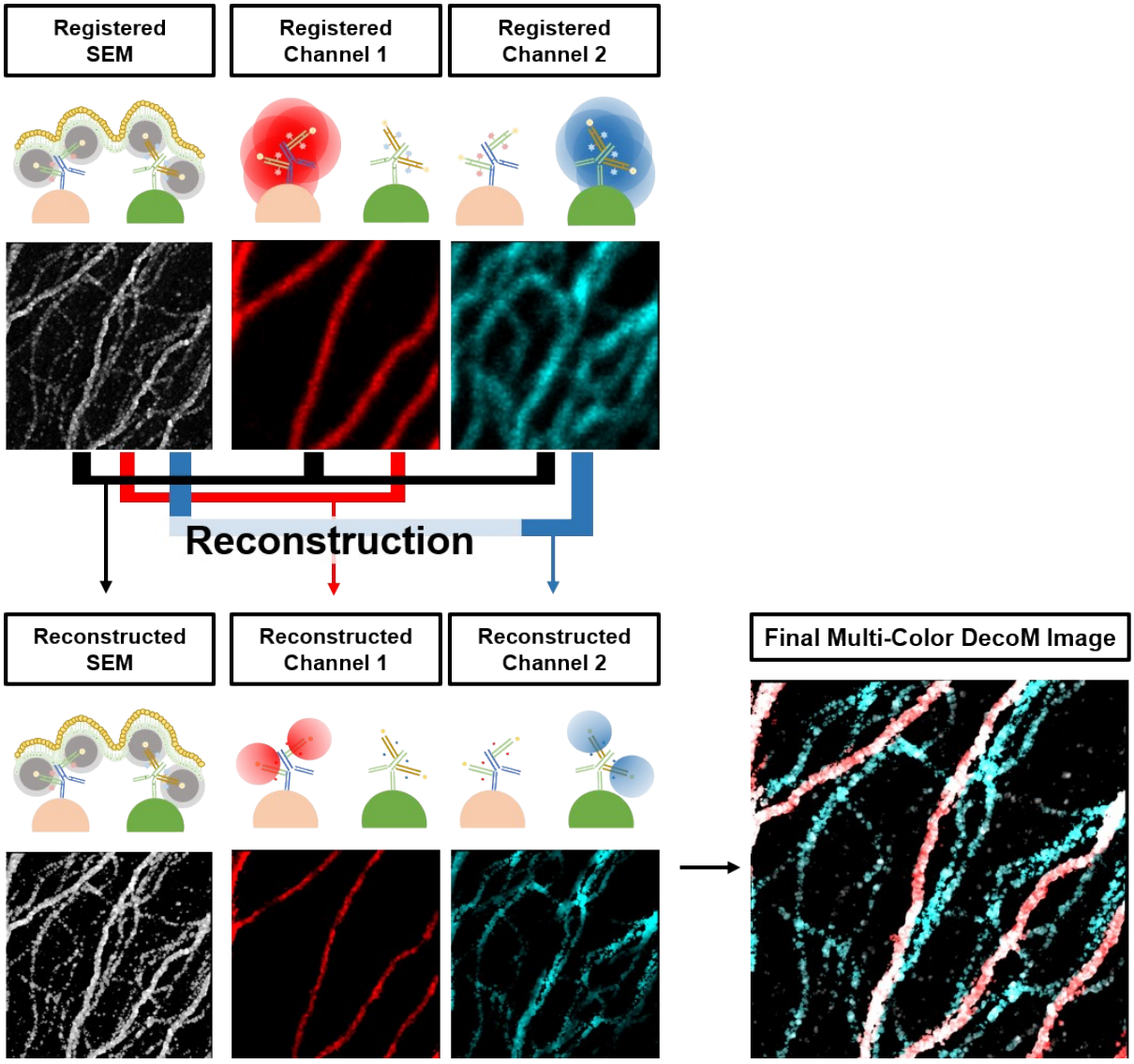
$$Final\ resolution\ from\ FRC\ code\ (nm) = \frac{\sqrt{2}}{P_x} = 15.85$$



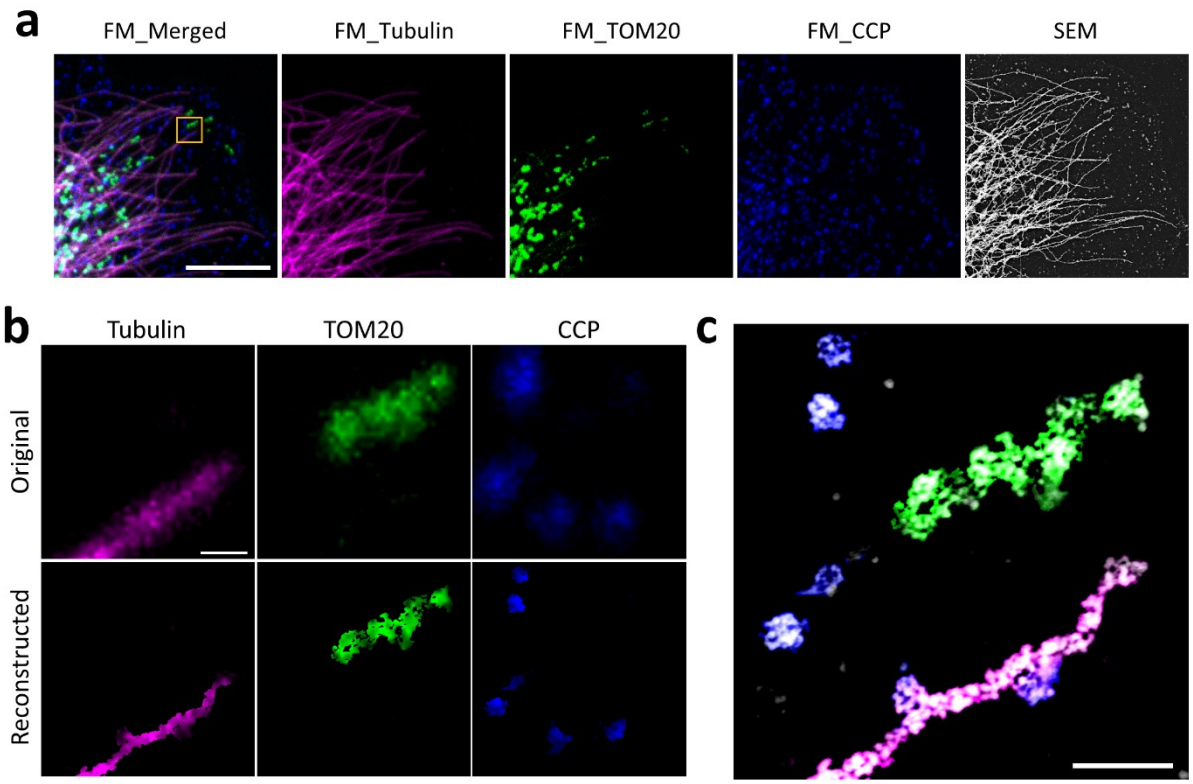
Supplementary Figure 17. Schematic of silver-labeled target protein complexes to describe the resolution of DecoM.



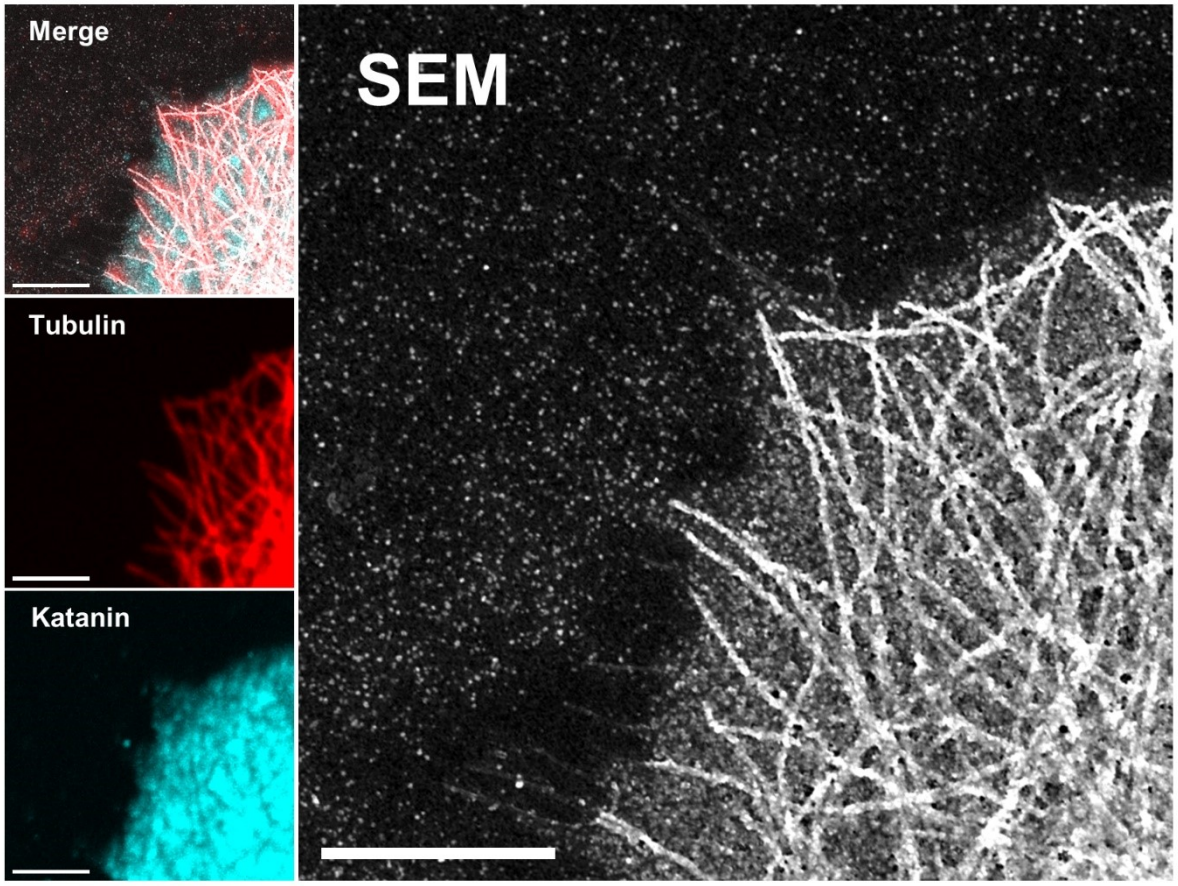
Supplementary Figure 18. Registration schematic of multiplexed FM and SEM image. (a) Merging process of each FM channel image with corresponding images. (b) Registration of the merged FM and SEM images with corresponding images and registration vector map. (c) Transformation of each FM channel with corresponding images.



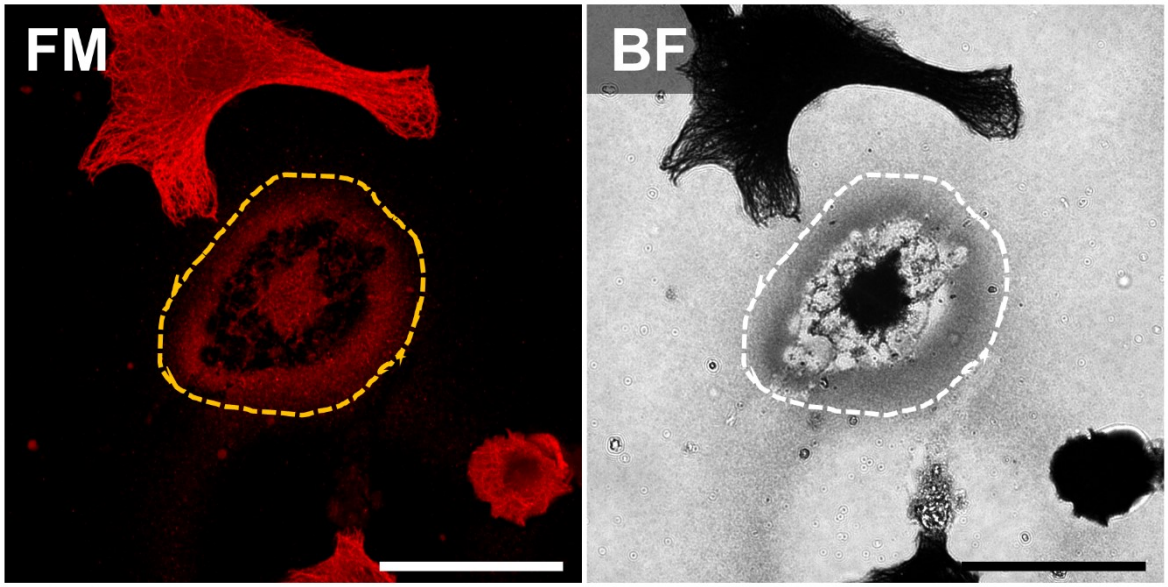
Supplementary Figure 19. Reconstruction schematic of the registered image. The reconstruction process involves background removal and pixel extraction.



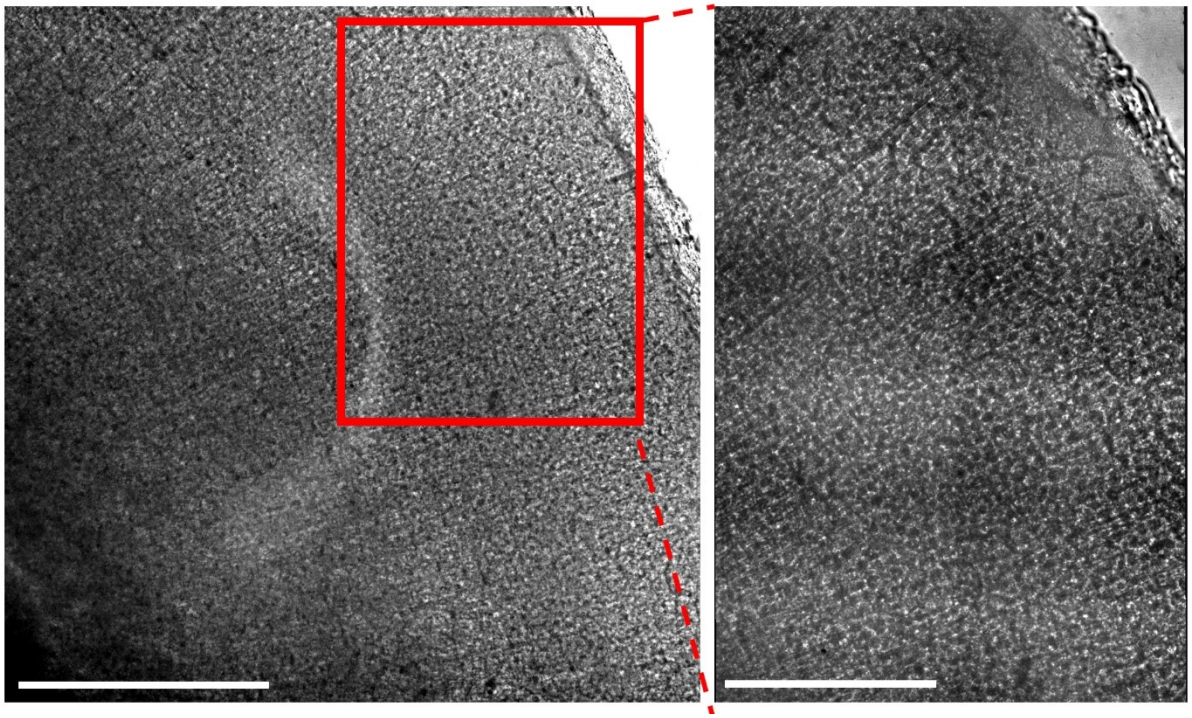
Supplementary Figure 20. Three-color DecoM images. (**a**) Low-magnification FM and SEM (gray) images labeled with anti-tubulin (magenta), TOM20 (green), and CCP (blue) in a BS-C-1 cell. (**b**) Magnified original and reconstructed FM images from the yellow-boxed region in a. (**c**) Overlay image of reconstructed three-color fluorescence images and SEM image. Scale bar: a) 10 μm, b, c) 500 nm.



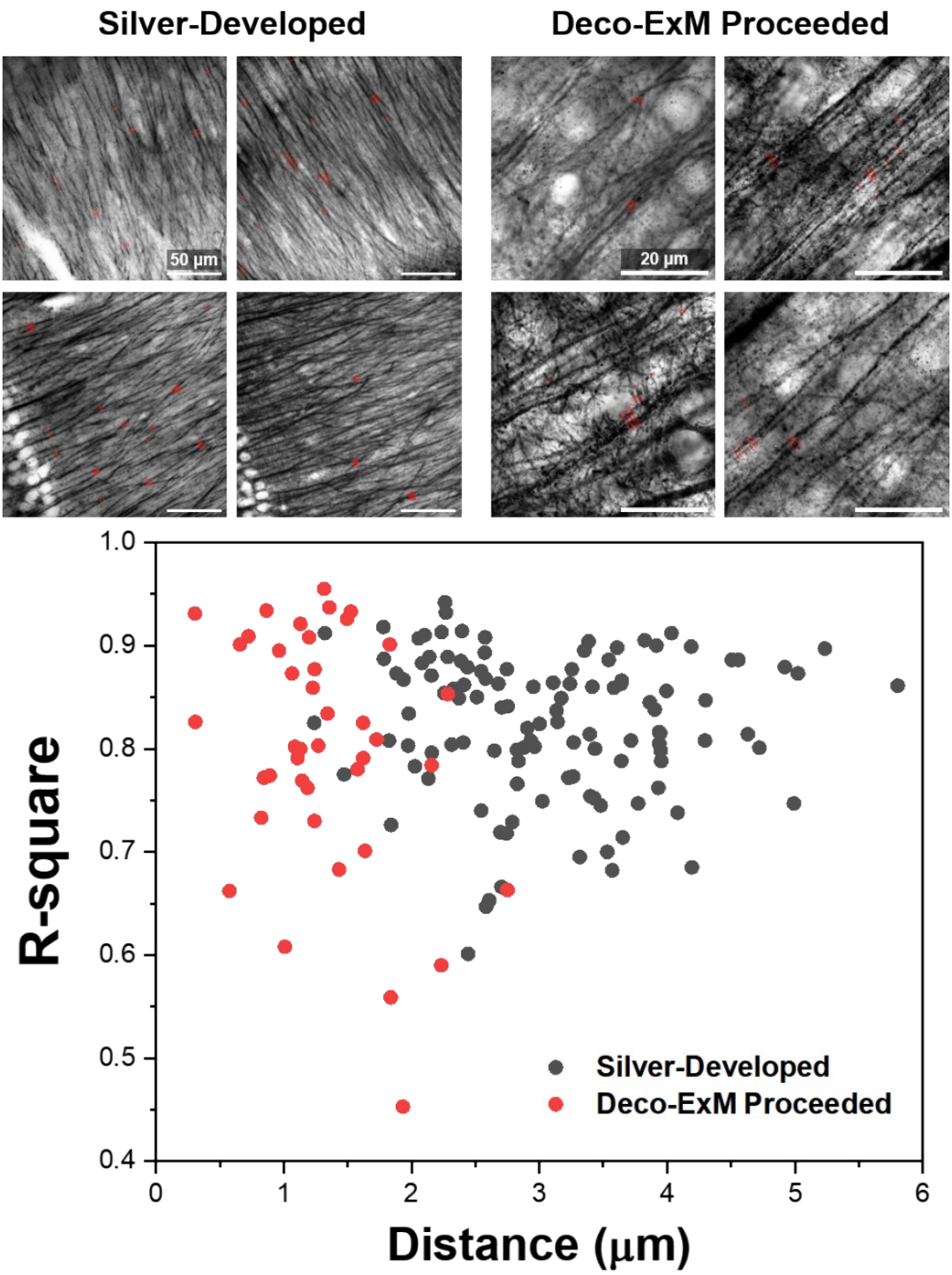
Supplementary Figure 21. Scattered tubulin monomers generated by overexpression of a catalytic subunit of katanin, Kp60 (red: β-tubulin, cyan: katanin). Scale bar: 5 μm



Supplementary Figure 22. FM and BF images of dead cells with the scattering of tubulin monomers around the cell. (Dotted lines: scattering region) Scale bar: 50 μm



Supplementary Figure 23. BF images of a fixed-only brain slice (thickness: 150 µm). Scale bar: 500 µm, 300 µm



Supplementary Figure 24. Additional brightfield images of silver-developed brain slice and Deco-ExM proceeded hydrogel-tissue composites with red lines for double Gaussian fitting and the distribution map of R-square (double Gaussian fits) vs. the distance between dendrites.

Plasmid	Type	Tag	Vendor	Cat #	ACCN
SPAST	Human Tagged ORF Clone	Myc-DDK	Origene	RC214100	NM_199436
KATNAL1	Human Tagged ORF Clone	Myc-DDK	Origene	RC200828	NM_032116

# Primary Antibody	Host	Target	Vender	Cat #
1	Rabbit	β-tubulin	Abcam	Ab6046
2	Rabbit	Vimentin	Abcam	Ab45939
3	Rabbit	Clathrin heavy chain	Abcam	Ab21679
4	Rabbit	MAP2	Abcam	Ab32454
5	Rabbit	Alexa 488	ThermoFisher	A11094
6	Mouse	α-tubulin	Sigma	T5168
7	Mouse	FLAG	Sigma	F1804
8	Mouse	TOM20	Santa cruz	sc-17764
9	Mouse	Myc	Santa cruz	sc-40
10	Rat	Tubulin	Abcam	ab6160

# Secondary Antibody	Host	Target	Isotype	Dye	Vendor	Cat #
1	Goat	Mouse	Fab’	Alexa 488	Nanoprobes	7202
2	Goat	Rabbit	Fab’	Alexa 546	Nanoprobes	7404
3	Goat	Rabbit	Fab’	Alexa 647	Nanoprobes	7504
4	Goat	Rabbit	IgG	Alexa 546	ThermoFisher	A27034
5	Goat	Rabbit	Fab’	None	Nanoprobes	2004
6	Goat	Rat	Fab’	Alexa 647	Nanoprobes	7508

Supplementary Table 1. List of plasmids and antibodies

References

1 R. P. J. Nieuwenhuizen, K. A. Lidke, M. Bates, D. L. Puig, D. Grünwald, S. Stallinga and B. Rieger, *Nat. Methods*, 2013, **10**, 557–562.