

Cell Reports Methods, Volume 2

Supplemental information

**Rapid 3D-STORM imaging
of diverse molecular targets in tissue**

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Figure S1. Optical Diagram of Vutara, Related to Figure 1.

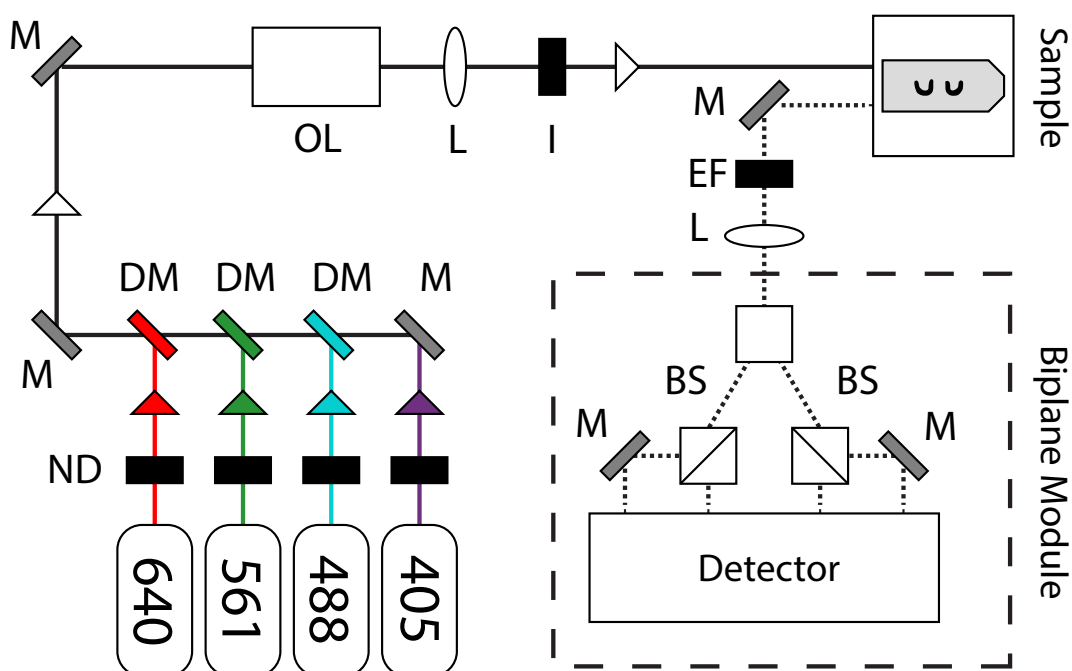


Figure S1. Optical Diagram of Vutara, related to Figure 1.

Layout and optical design of the Bruker Vutara SRX352, which allows 3D STORM imaging and PSF localization via a biplane module in place of a cylindrical lens. M: Mirror, DM: Dichroic mirror, ND: Neutral density filter, L: lens, BS: Beam splitter, I: iris/aperture, OL: Objective lens, EF: Emission filter.

Figure S2. RAIN-STORM resolution and localizations as a function of secondary labeling density, Related to Figure 1.

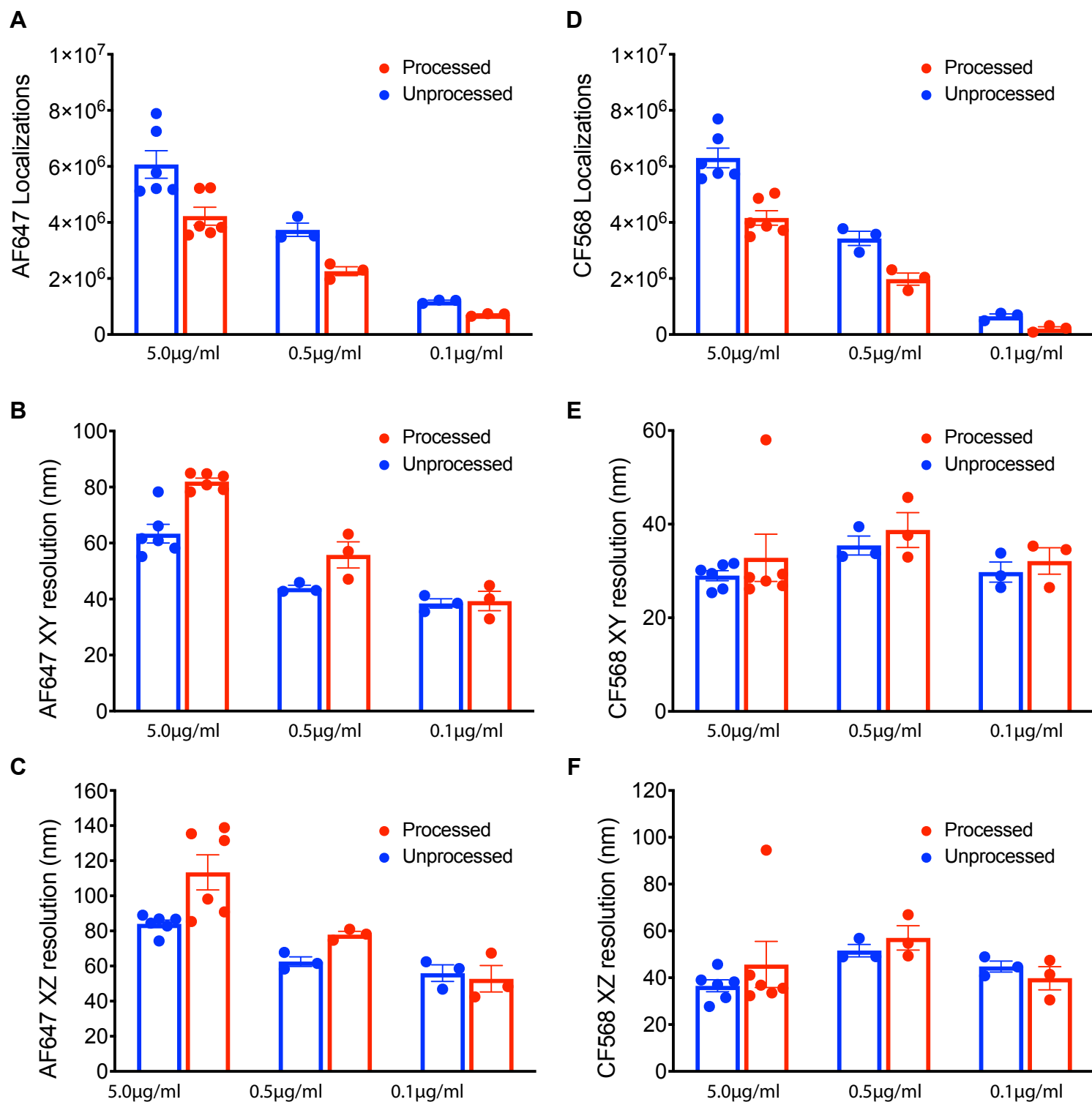
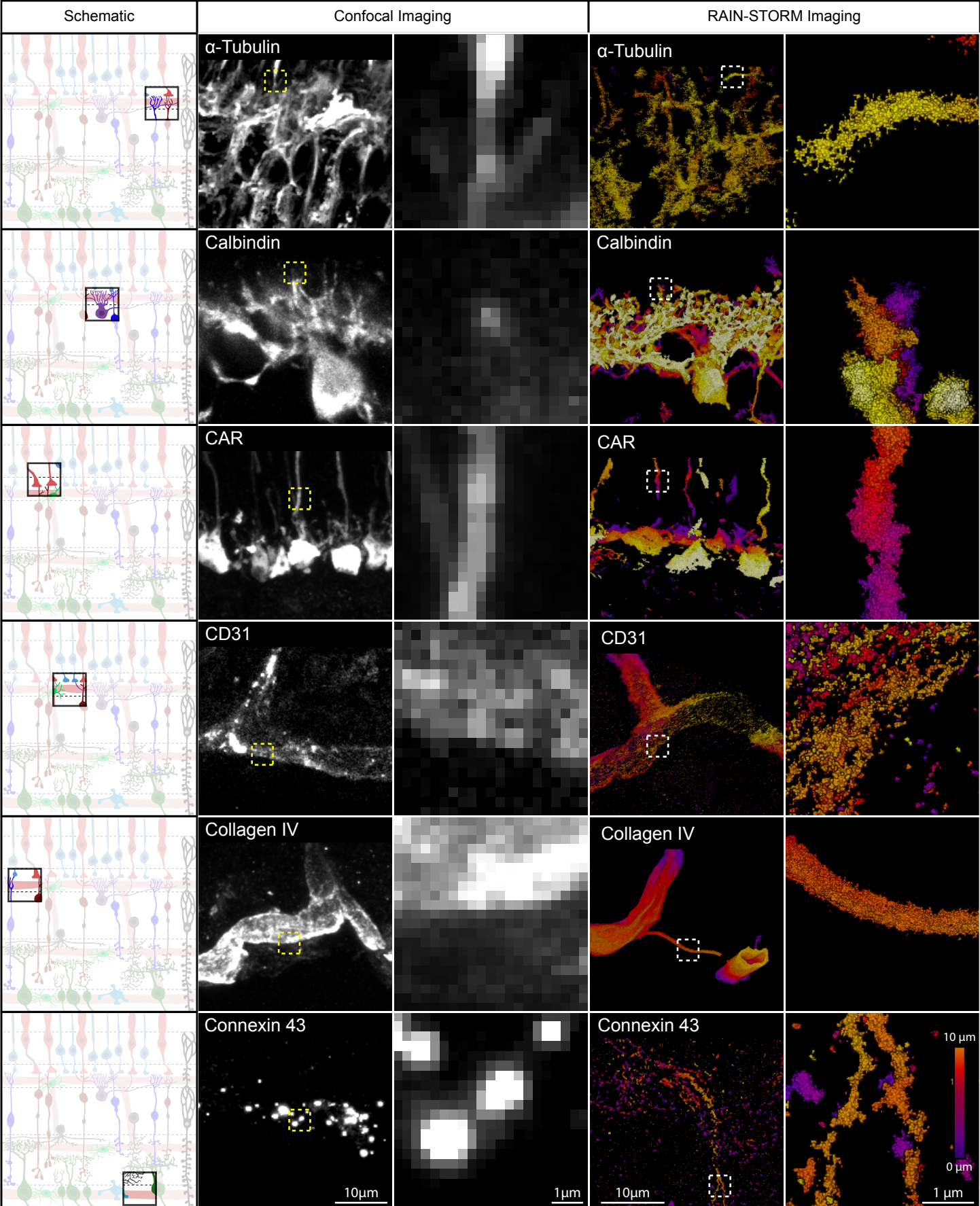
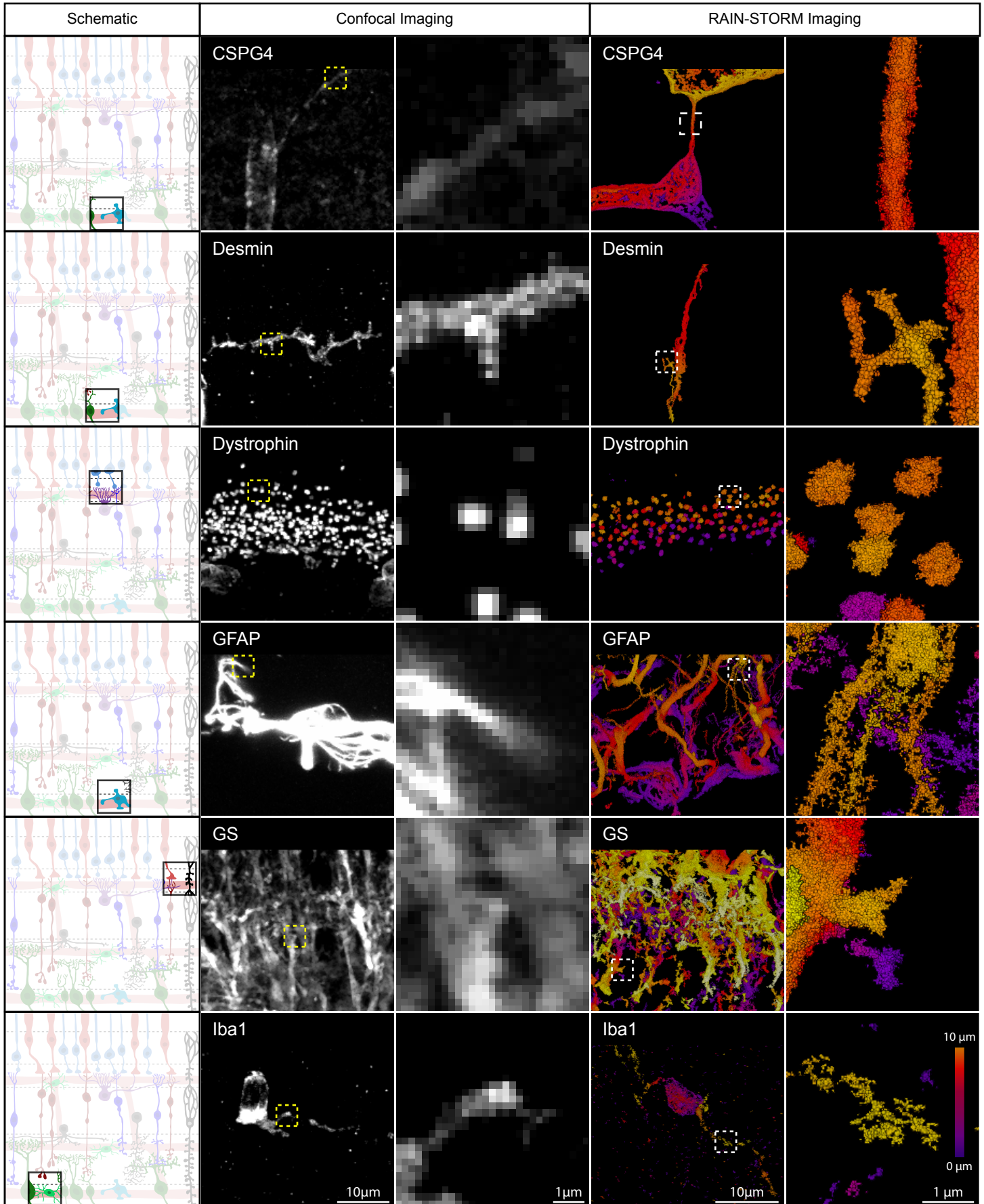


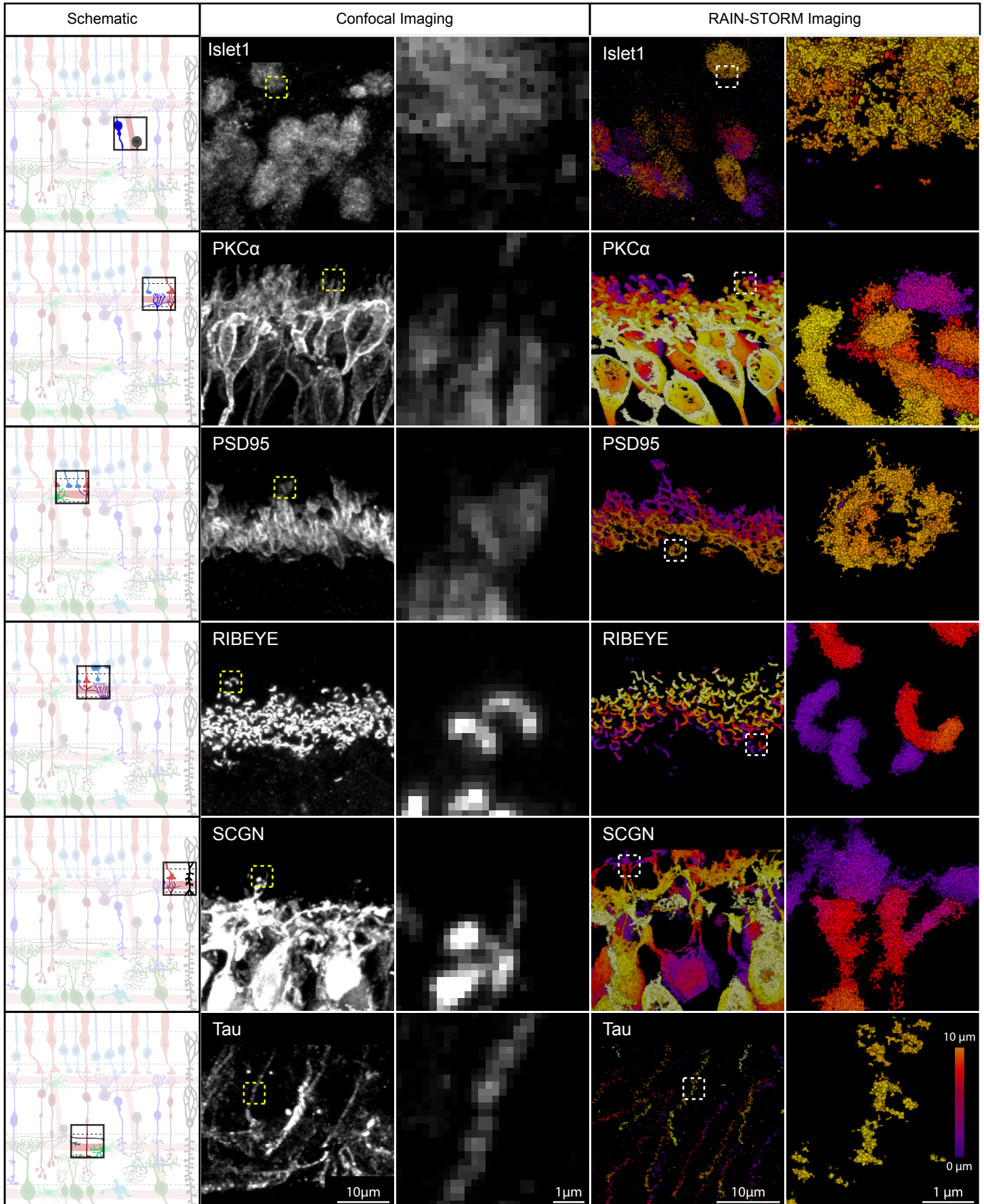
Figure S2. RAIN-STORM resolution and localization as a function of secondary labeling density, related to Figure 1.

A. Total localizations acquired when the concentration of the secondary antibody (AF647) is changed while the primary antibody concentration (Calbindin) is kept constant. Total acquired localizations decrease as secondary antibody concentration is reduced, showing $6.07 \times 10^6 \pm 0.490 \times 10^6$ localizations at $5.0 \mu\text{g/ml}$, $3.73 \times 10^6 \pm 0.239 \times 10^6$ at $0.5 \mu\text{g/ml}$, and $1.19 \times 10^6 \pm 0.038 \times 10^6$ at $0.1 \mu\text{g/ml}$. **B-C.** The associated XY (**B**) and XZ (**C**) resolutions as secondary antibody concentration is changed. At $5.0 \mu\text{g/ml}$, $R_{xy} = 63.4 \pm 3.3 \text{nm}$ and $R_{xz} = 84.0 \pm 2.1 \text{nm}$, at $0.5 \mu\text{g/ml}$, $R_{xy} = 44.0 \pm 1.0 \text{nm}$ and $R_{xz} = 62.6 \pm 2.8 \text{nm}$, while at $0.1 \mu\text{g/ml}$, $R_{xy} = 38.4 \pm 1.7 \text{nm}$ and $R_{xz} = 56.0 \pm 4.7 \text{nm}$. **D.** Total localizations acquired when the concentration of the secondary antibody (CF568) is changed while the primary antibody concentration (PSD95) is kept constant. As for AF647, total acquired localizations decrease as secondary antibody concentration is reduced showing $6.30 \times 10^6 \pm 0.347 \times 10^6$ localizations at $5.0 \mu\text{g/ml}$, $3.43 \times 10^6 \pm 0.254 \times 10^6$ at $0.5 \mu\text{g/ml}$, and $0.656 \times 10^6 \pm 0.079 \times 10^6$ at $0.1 \mu\text{g/ml}$. **E-F.** The associated XY (**E**) and XZ (**F**) resolutions as secondary antibody concentration is changed. At $5.0 \mu\text{g/ml}$, $R_{xy} = 29.0 \pm 1.1 \text{nm}$ and $R_{xz} = 36.6 \pm 2.6 \text{nm}$, at $0.5 \mu\text{g/ml}$, $R_{xy} = 35.5 \pm 2.0 \text{nm}$ and $R_{xz} = 51.6 \pm 2.6 \text{nm}$, while at $0.1 \mu\text{g/ml}$, $R_{xy} = 29.8 \pm 2.2 \text{nm}$ and $R_{xz} = 44.8 \pm 2.3 \text{nm}$. $N = 3$. Data are represented as the mean \pm the s.e.m.

Figure S3. Protein target validation and RAIN-STORM imaging of diverse molecular targets, Related to Figure 4.







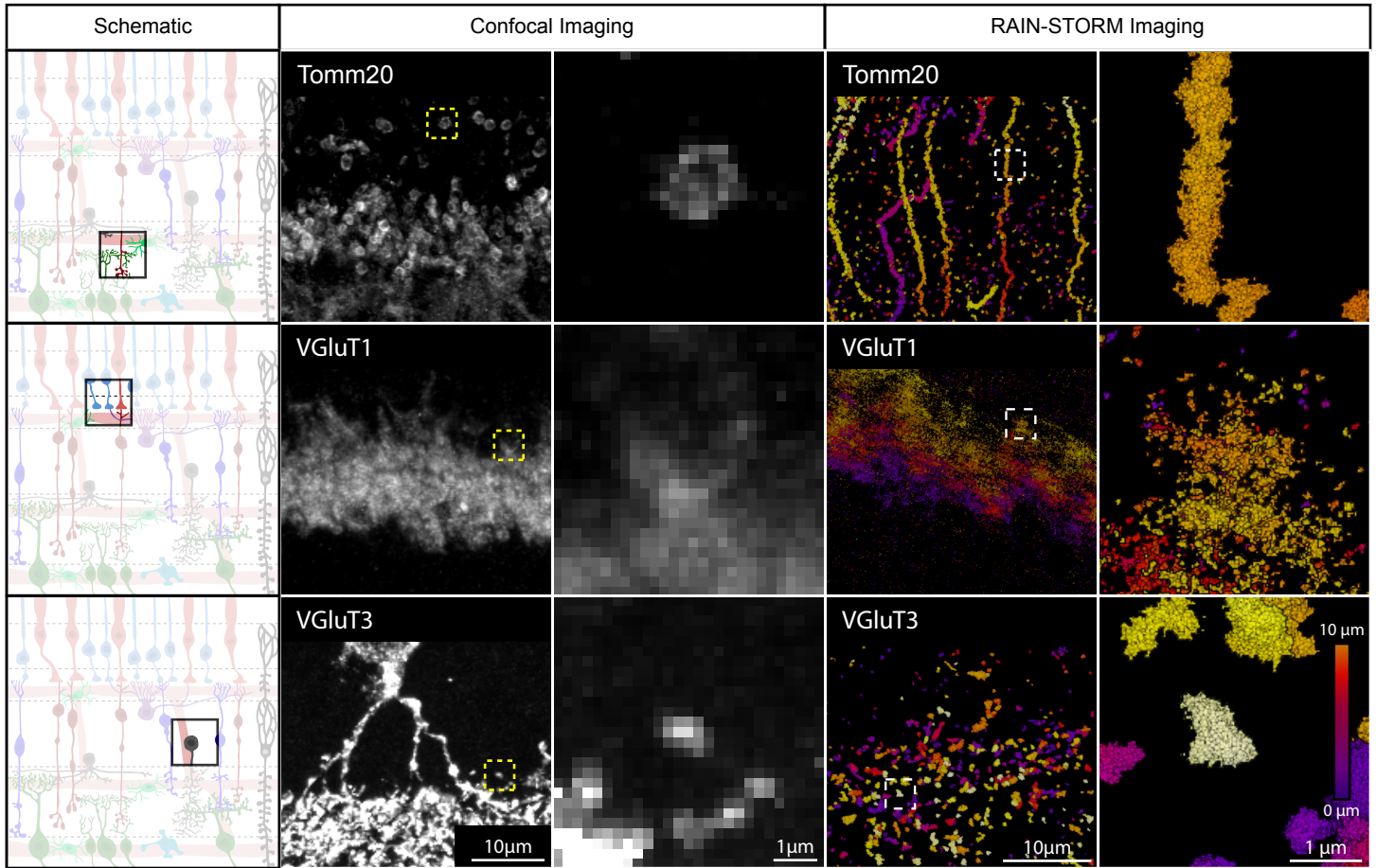


Figure S3. Protein target validation and RAIN-STORM imaging of diverse molecular targets, related to Figure 4.

A representative retina schematic for each target is shown in which the region of interest is boxed (left column). Cellular structure and labeling patterns from the fluorescence confocal based images were obtained (middle columns) and used as a baseline with which to compare the reliability and robustness of RAIN-STORM imaging for various targets. Boxed regions in confocal images are magnified in images to the right. For each target, tissue was then prepared using our optimized RAIN-STORM protocol (right most columns). Representative images for each antibody are shown, followed by a magnified view of the same general structure (boxed region) shown in the fluorescence image. RAIN-STORM optimized imaging was robust across these diverse targets and antibodies, demonstrating the combability of this protocol with a wide variety of proteins. Images are representative from N = 3 animals. All confocal images are intensity-based representations of fluorescence while STORM-based images show reconstructions formed from individual localizations. STORM images are color-coded by depth (purple, to yellow, 10 μ m). Scale bars = 10 and 1 μ m.

Figure S4. RAIN-STORM delivers robust imaging in the Nikon N-STORM system, Related to Figure 1.

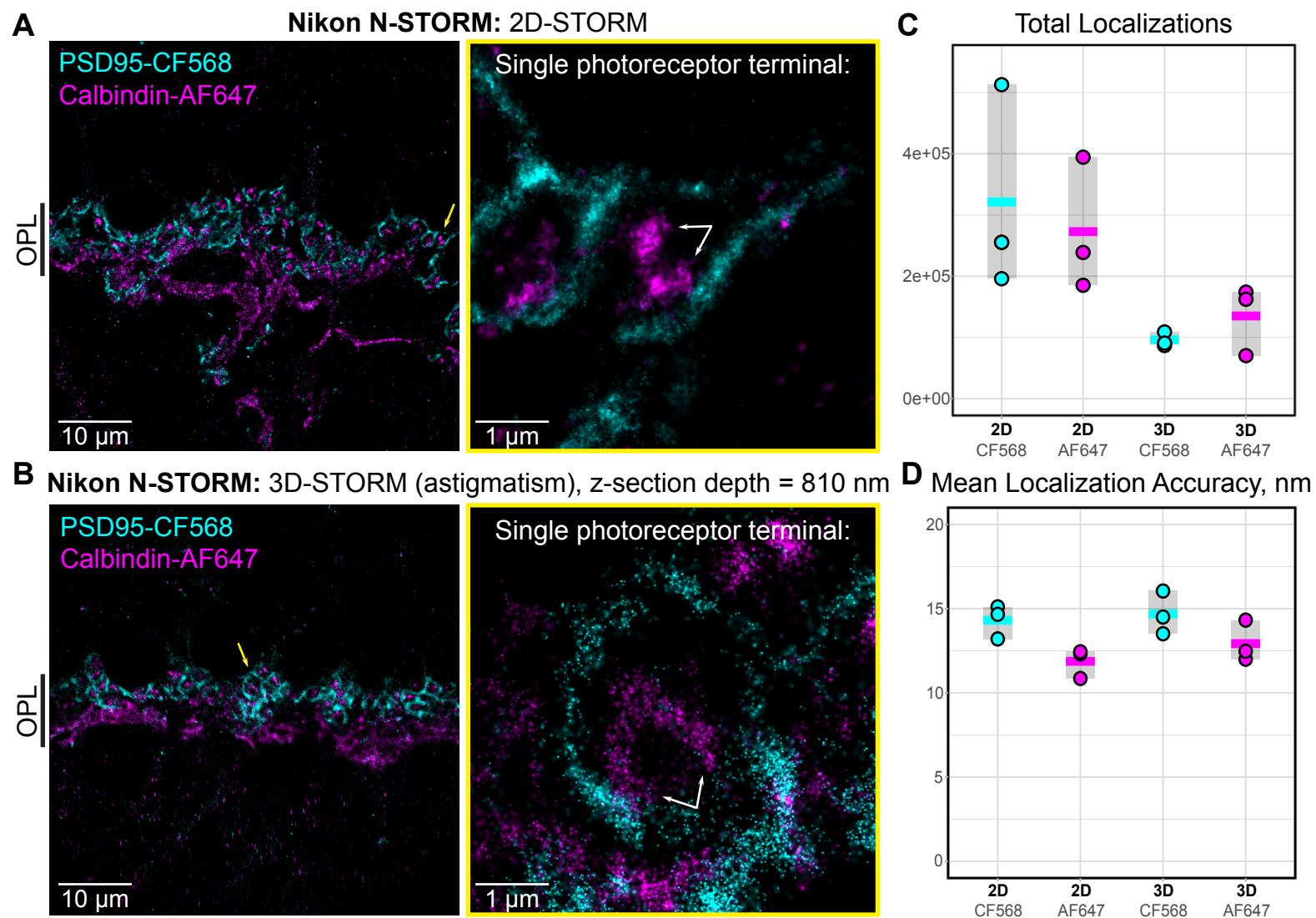


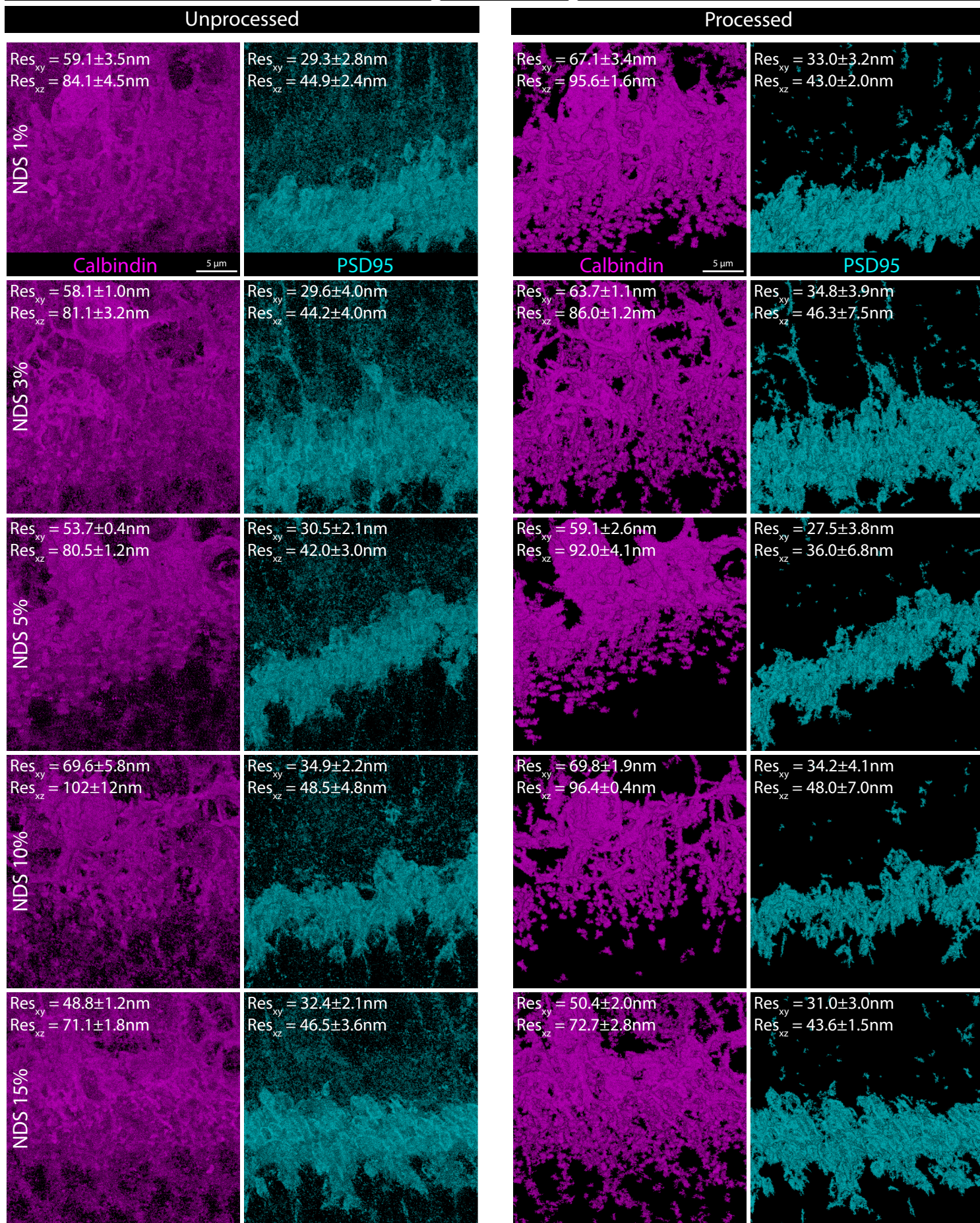
Figure S4: RAIN-STORM delivers robust imaging in the Nikon N-STORM system.

Mouse retina cryosections were prepared using our optimized RAIN-STORM protocol. Horizontal cells in the outer plexiform layer (OPL) were immunolabeled with Calbindin + AF647 secondary labeling (magenta), and pre-synaptic photoreceptor terminals (spherules and pedicles) were labeled with PSD-95 + CF568 secondary labeling (cyan). STORM acquisition and analyses were performed on a Nikon N-STORM system. **A.** A 2D-STORM example reconstruction is shown with an adjacent magnified example of an individual photoreceptor terminal (yellow arrow) containing distinct Calbindin+ horizontal cell processes (white arrows). Analysis settings for 2D-STORM data were based on (Robichaux et al., 2019) and were as follows: Minimum PSF height: 1,000, Maximum PSF height: 65,636, Minimum PSF Width: 200 nm, Maximum PSF Width: 400 nm, Initial Fit Width: 300 nm, Max Axial Ratio: 1.15, Max Displacement: 1 pixel. **B.** A 3D-STORM example reconstruction after Z-position astigmatism fitting is shown with another example magnified photoreceptor terminal (yellow arrow) encircling horizontal cell processes (white arrows). More permissive 3D-STORM analysis settings were used to enable astigmatism fitting: Minimum PSF height: 1,000, Maximum PSF height: 65,000, Minimum PSF Width: 200 nm, Maximum PSF Width: 700 nm, Initial Fit Width: 300 nm, Max Axial Ratio: 2.5, Max Displacement: 1 pixel. The z-sectioning depth of 3D-STORM reconstructions was 810 nm. All data points in the STORM reconstructions were visualized as “Gaussians” based on individual brightness and localization accuracy values. **C.** Total localizations and **D.** mean localization accuracy values are plotted as circles for both channels from 3 replicate 2D- and 3D-STORM acquisitions demonstrating reproducibility and high-quality fitting. Gray bars indicate the range, and

horizontal lines indicate the mean values. Graphs were generated using PlotsOfData (Postma and Goedhart, 2019).

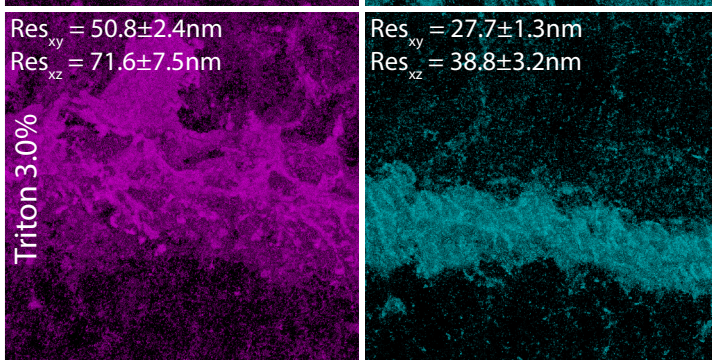
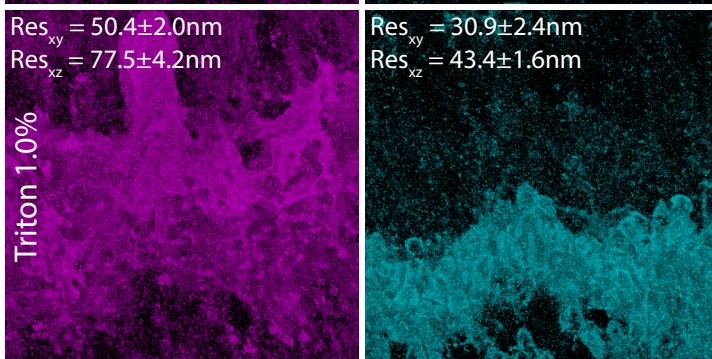
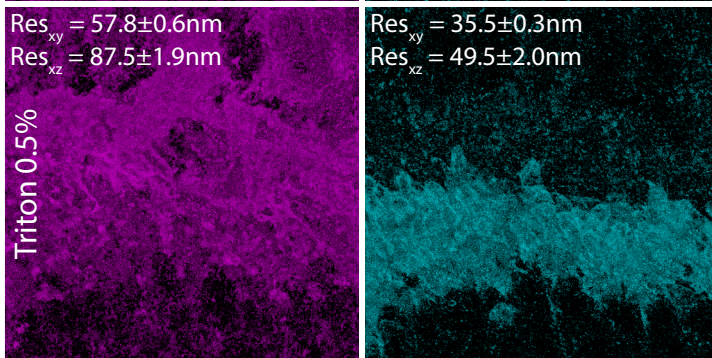
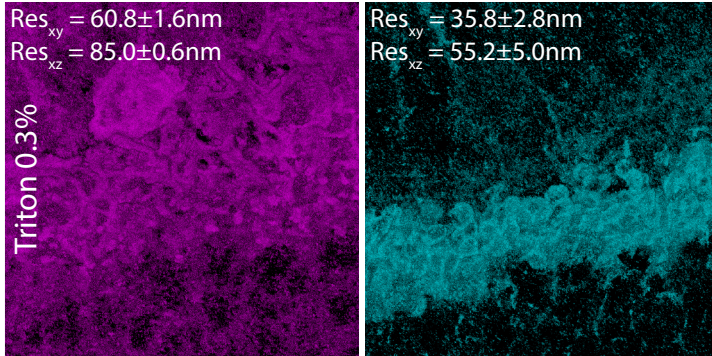
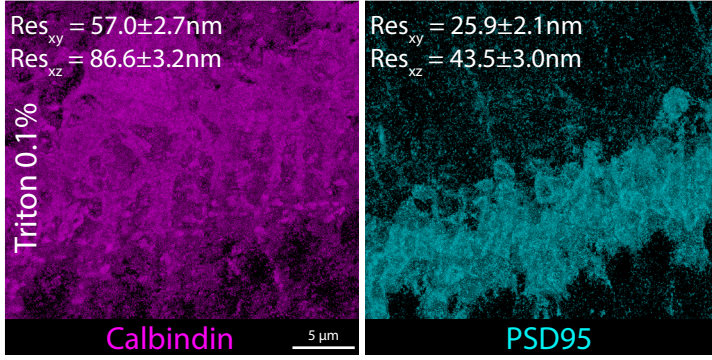
Data S1. Sample and imaging conditions affect image quality and metrics, Related to Figure 4.

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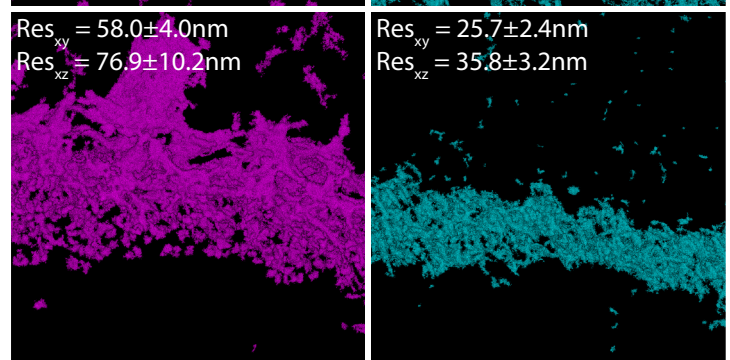
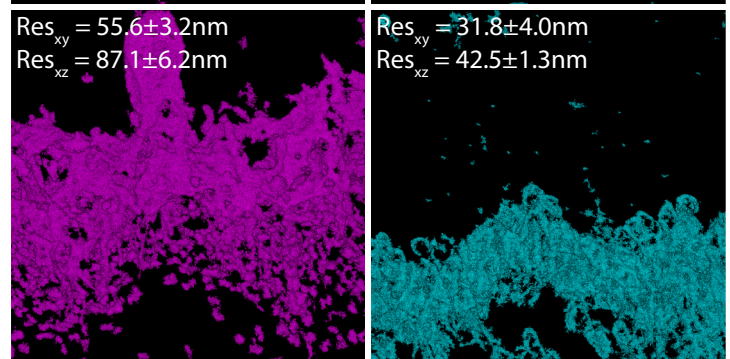
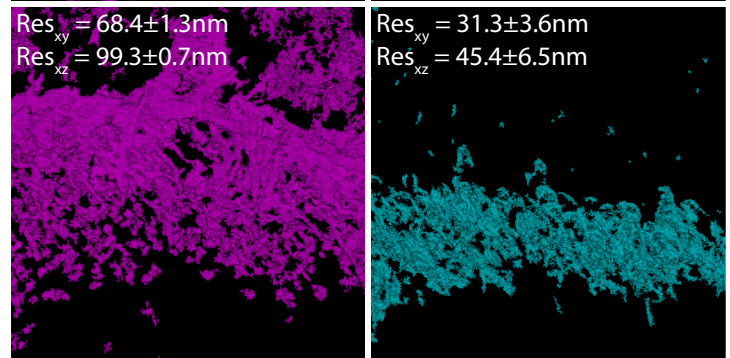
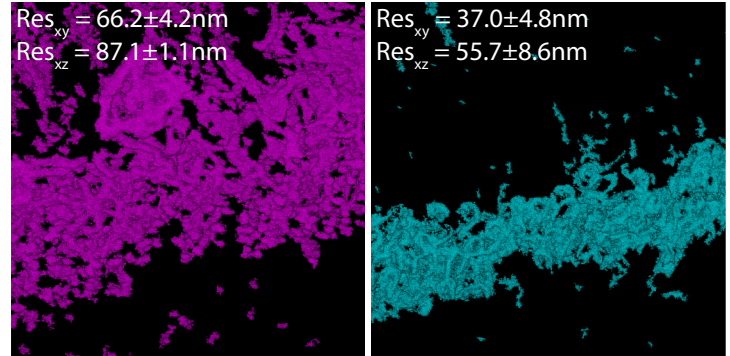
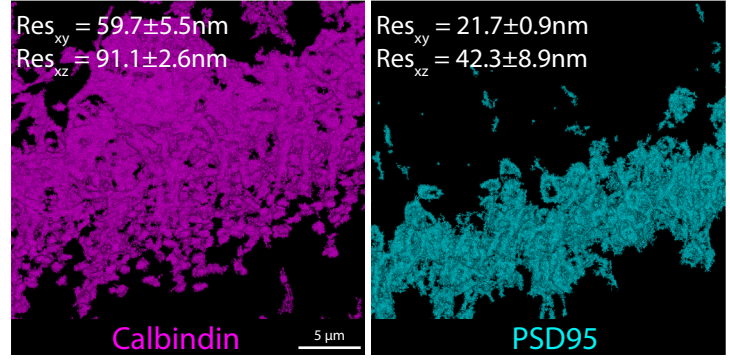


Staining - Blocking, Triton-X100

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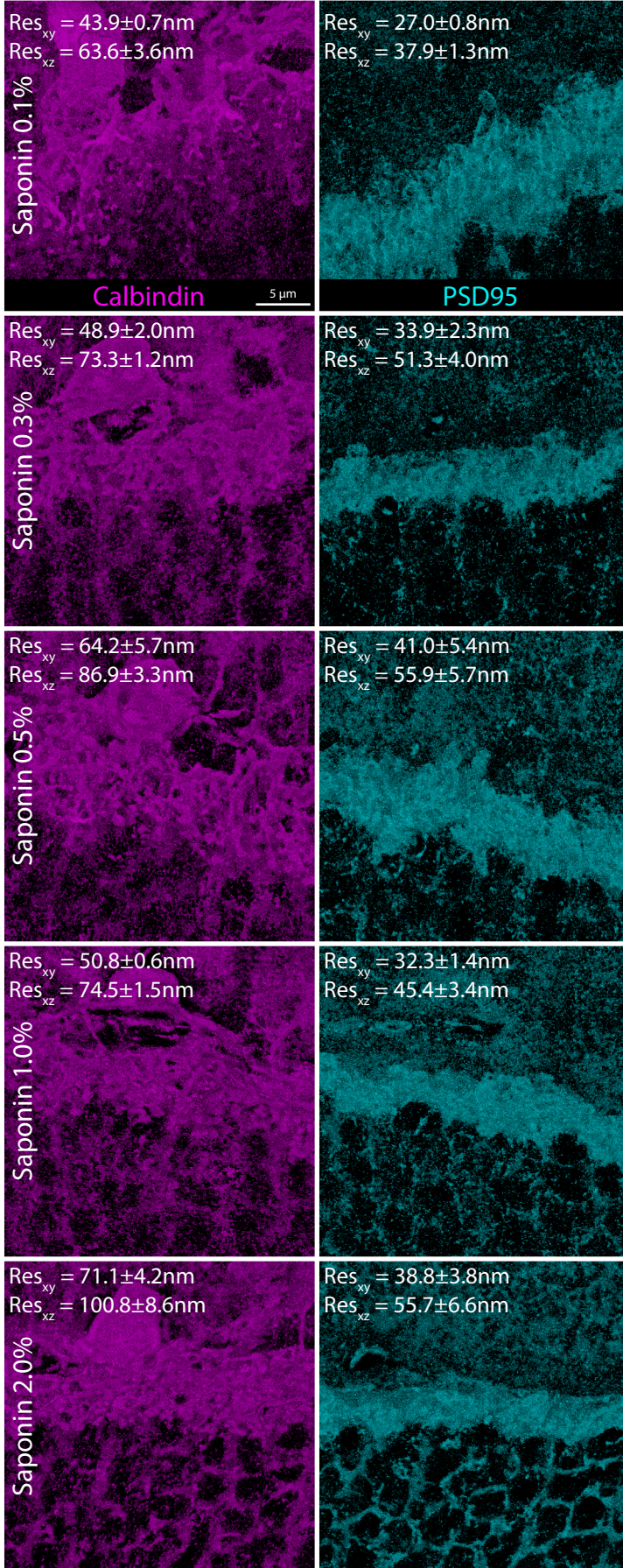


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Staining - Blocking, Saponin

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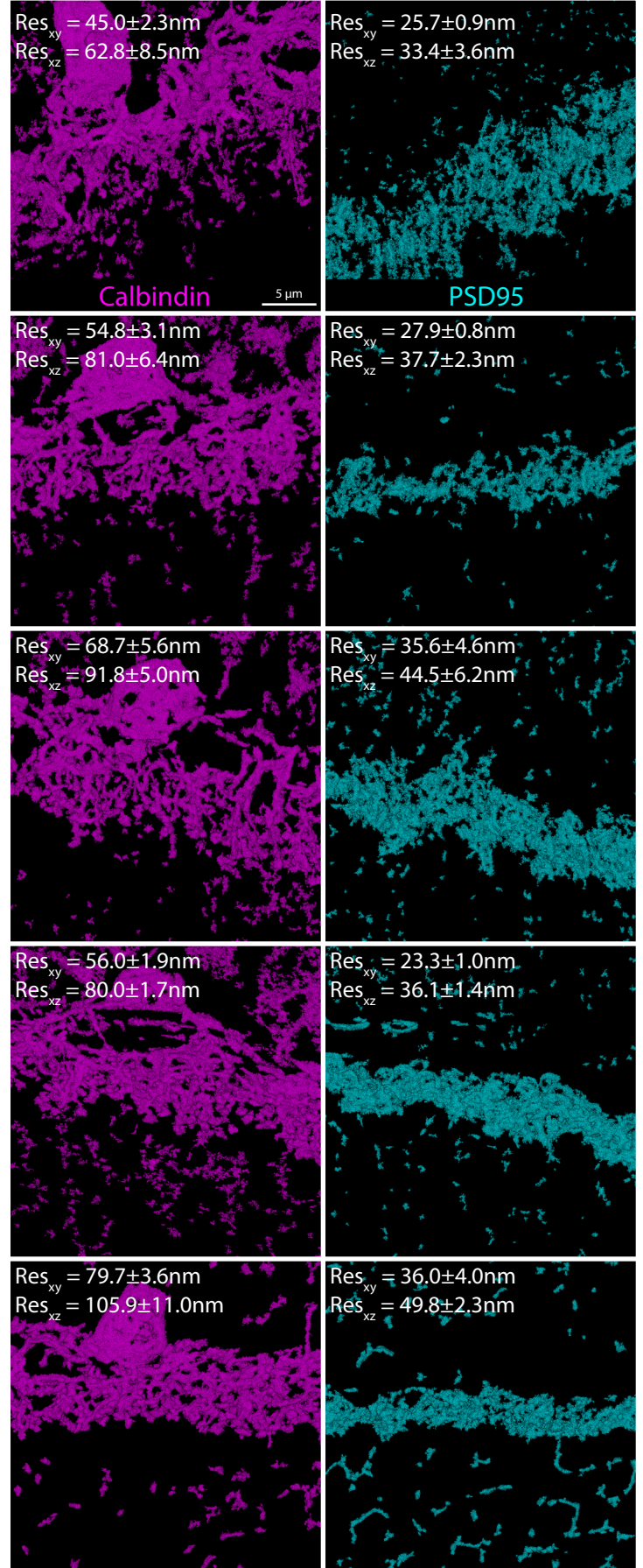
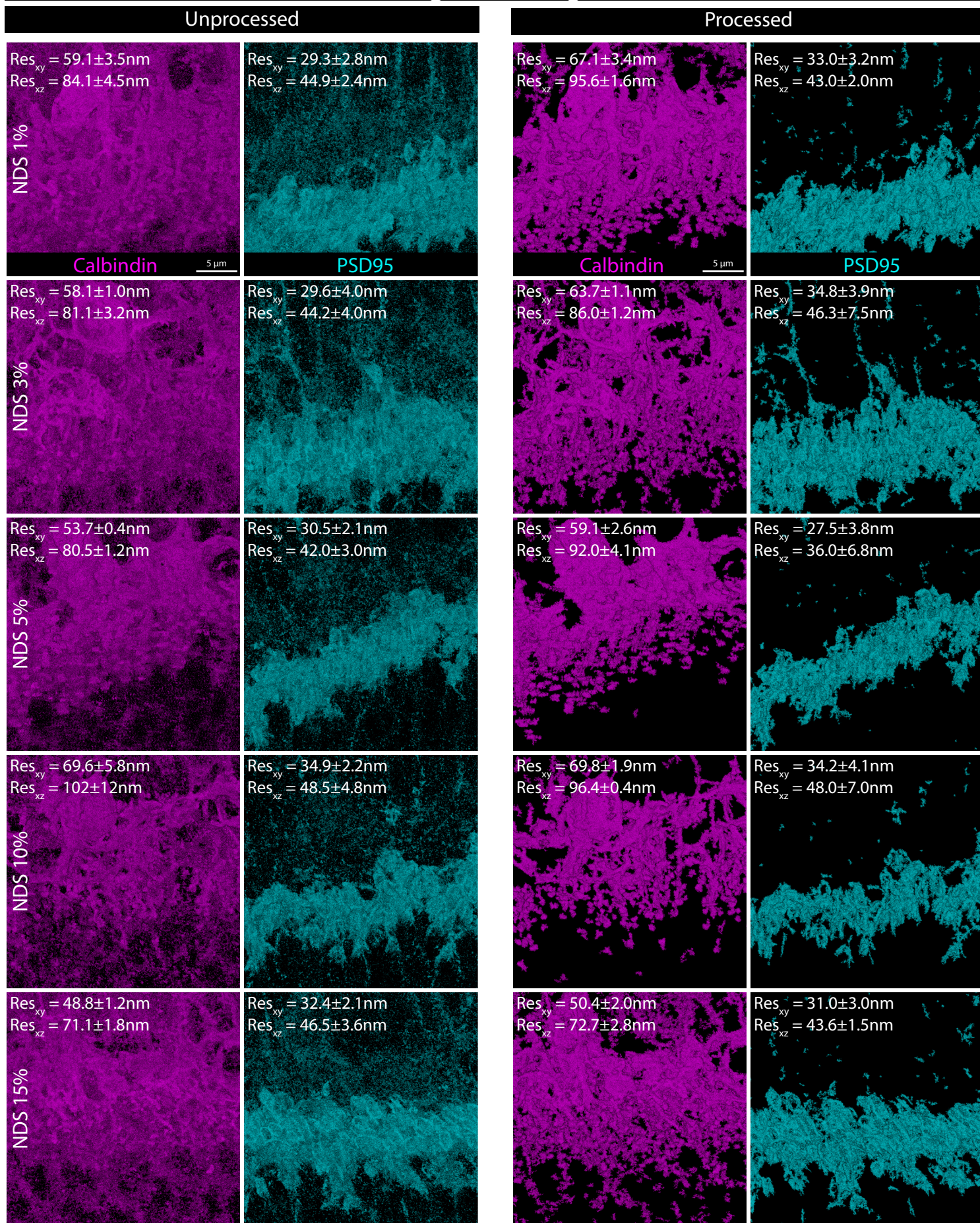


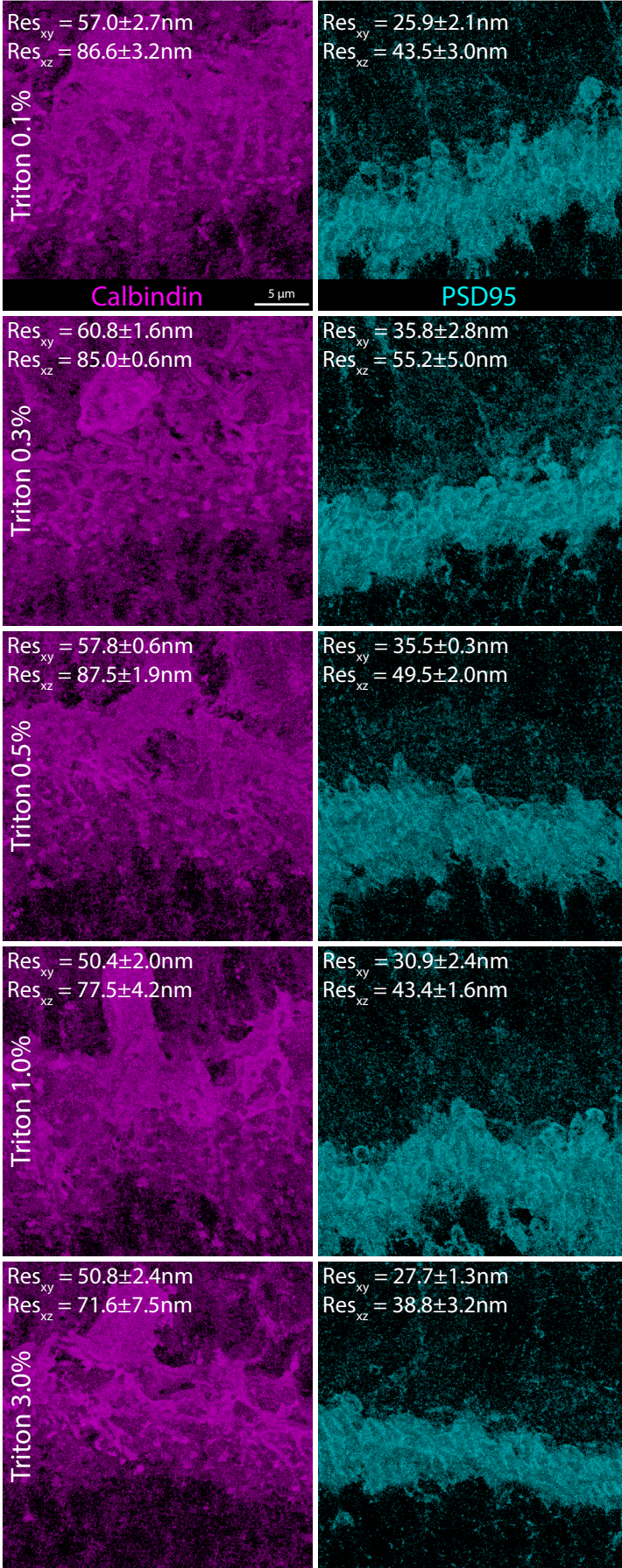
Figure S4. Sample and imaging conditions affect image quality and metrics, Related to Figure 4.

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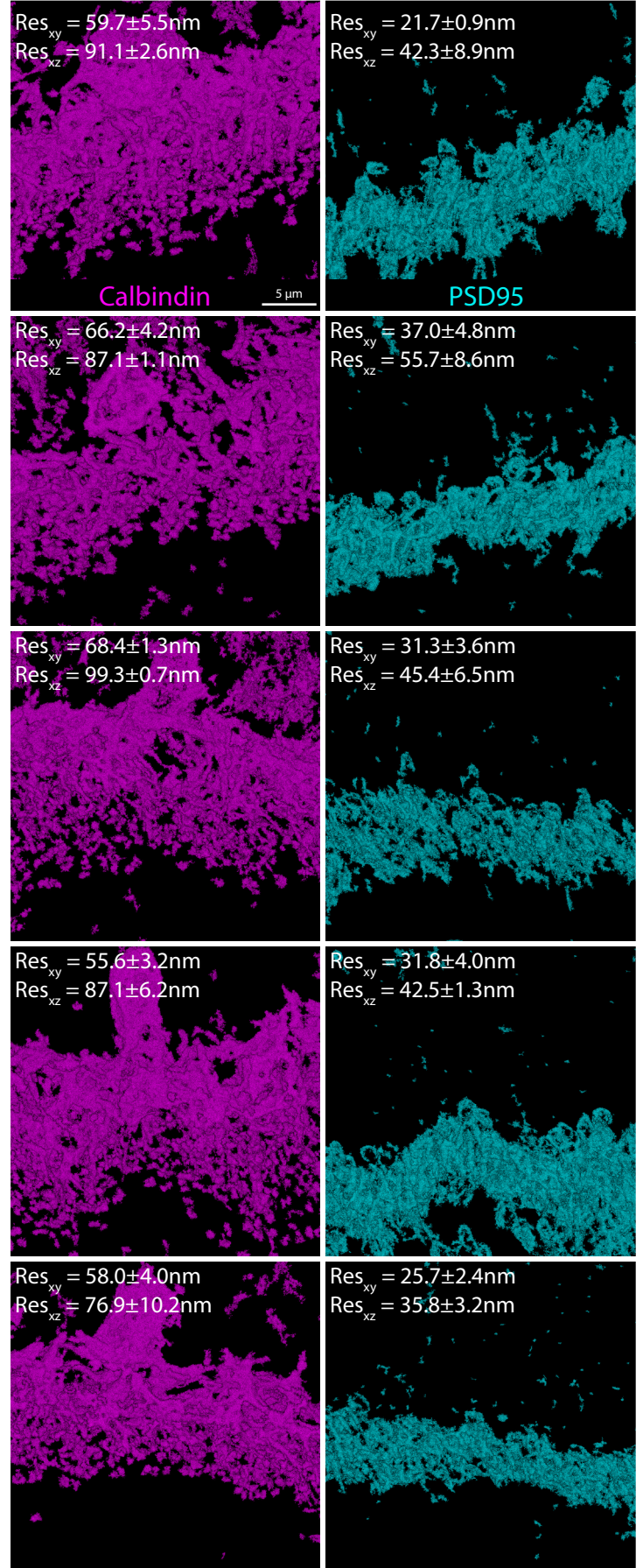


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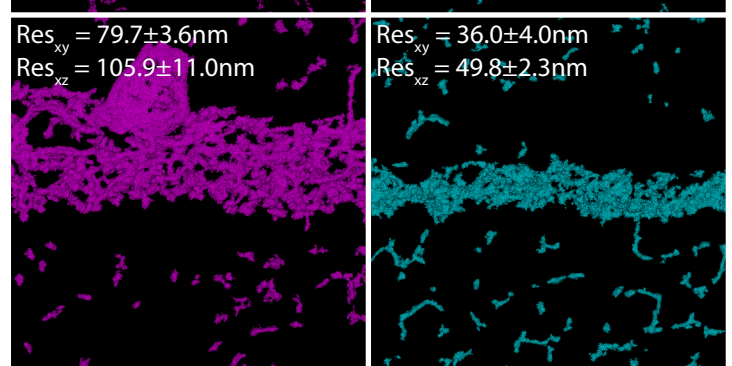
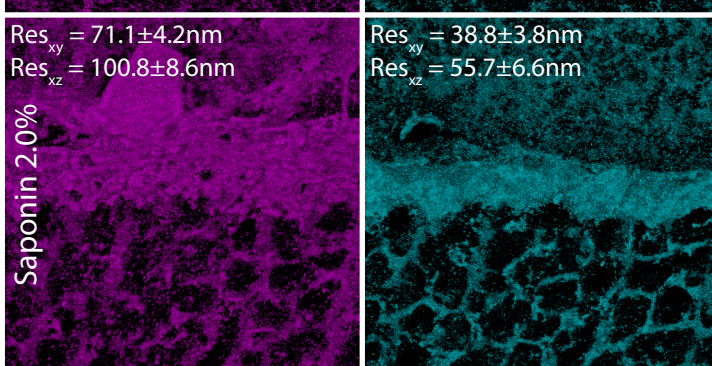
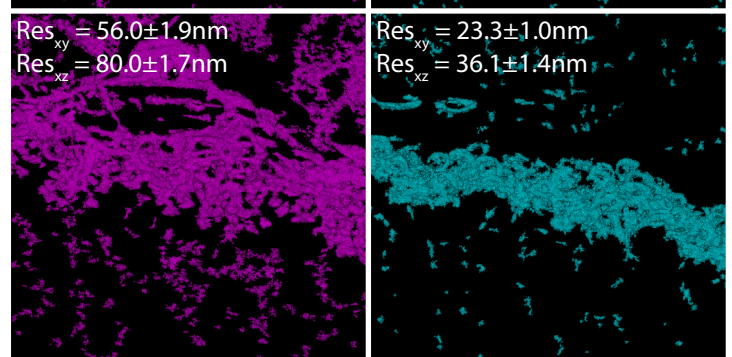
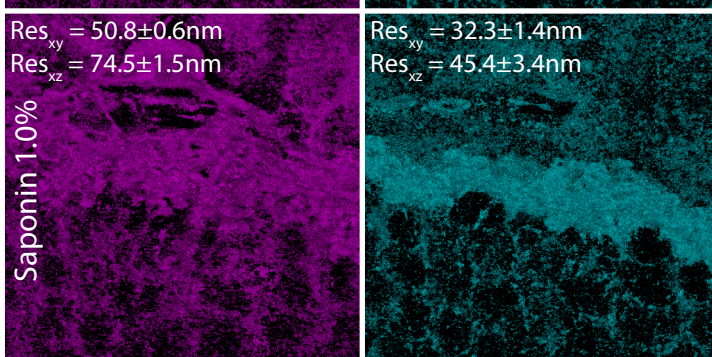
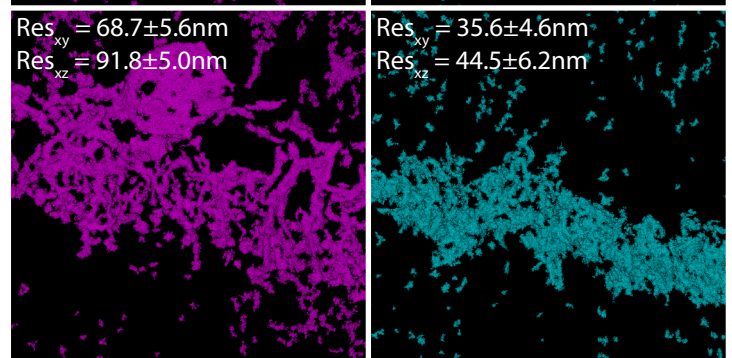
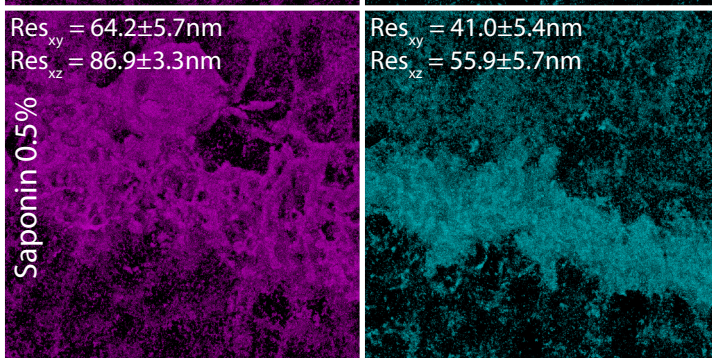
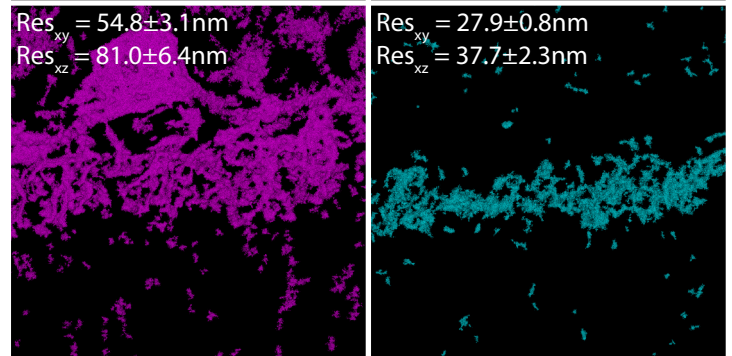
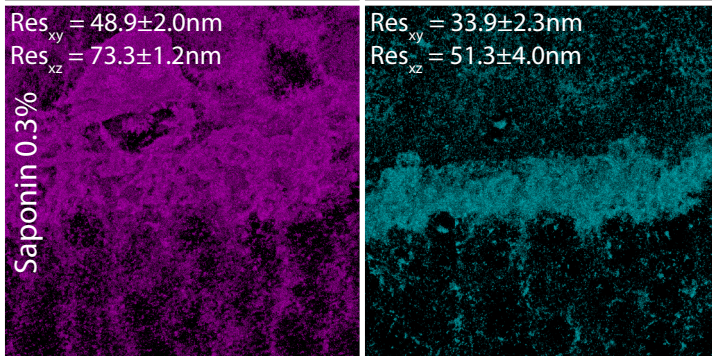
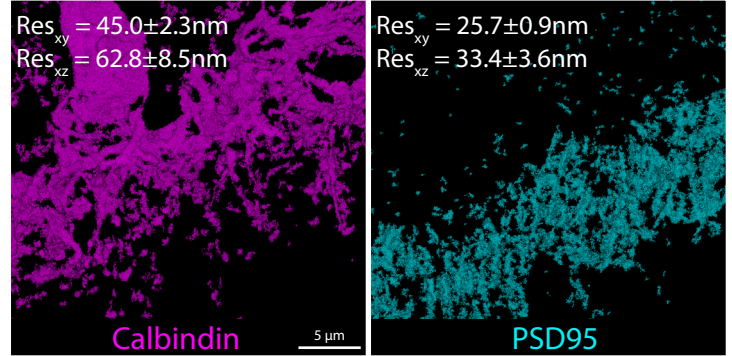
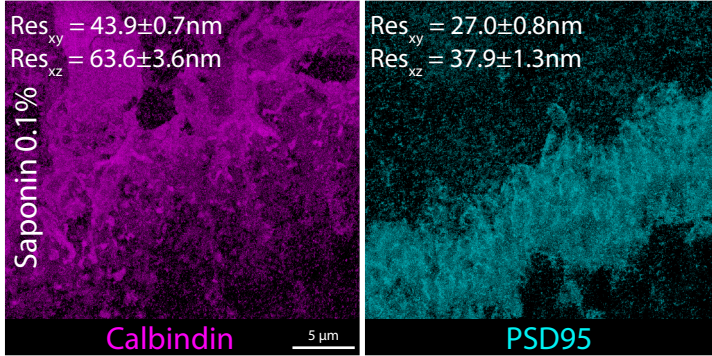
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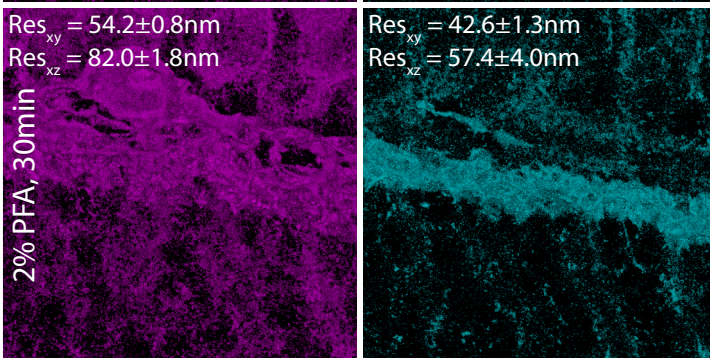
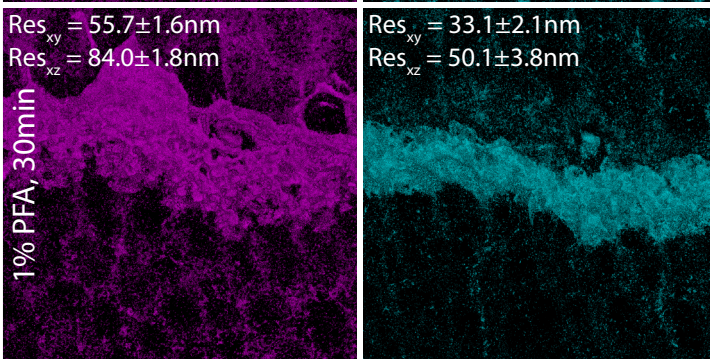
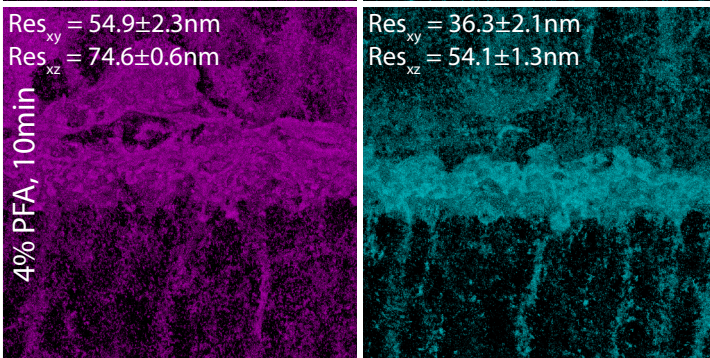
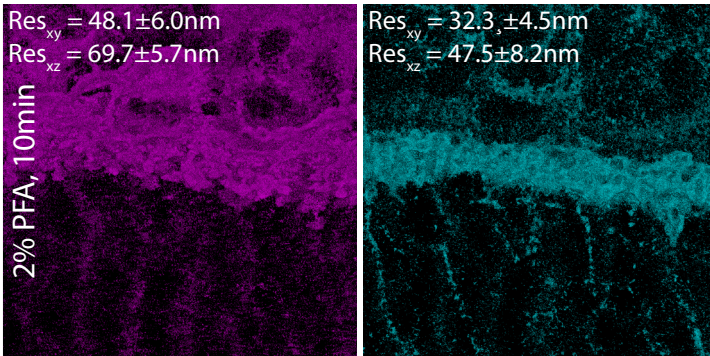
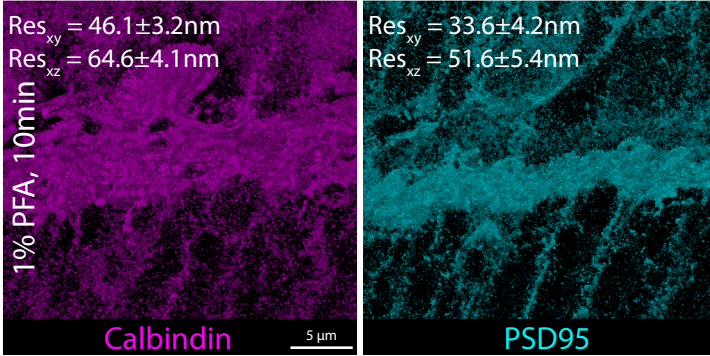
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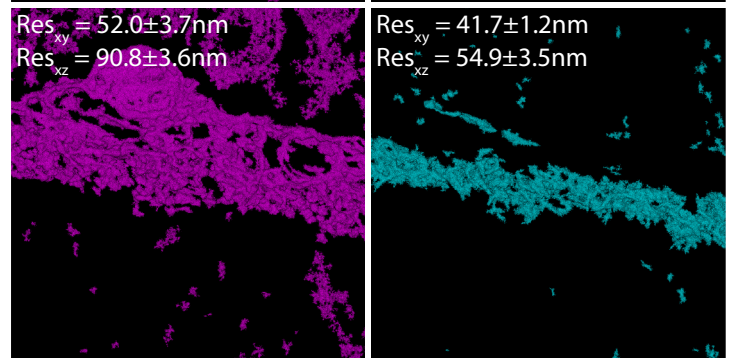
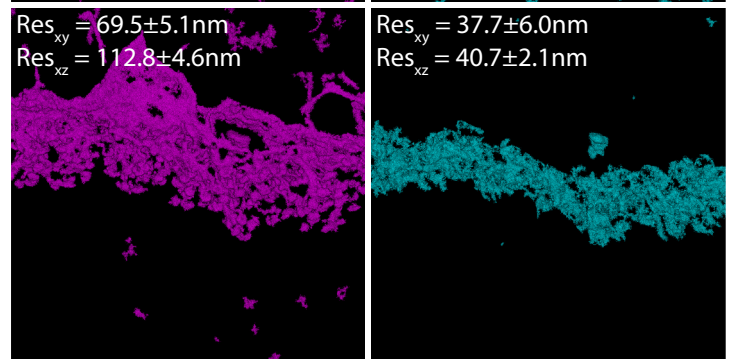
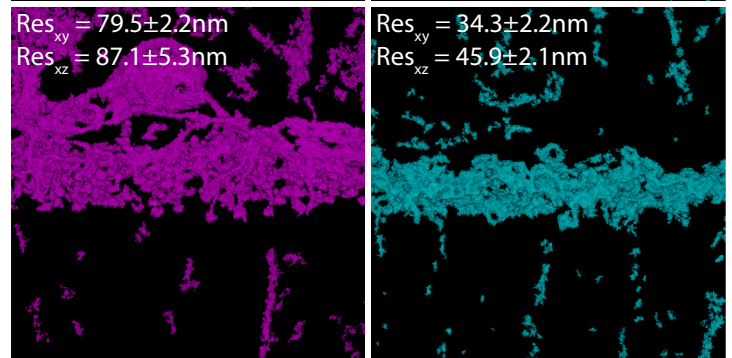
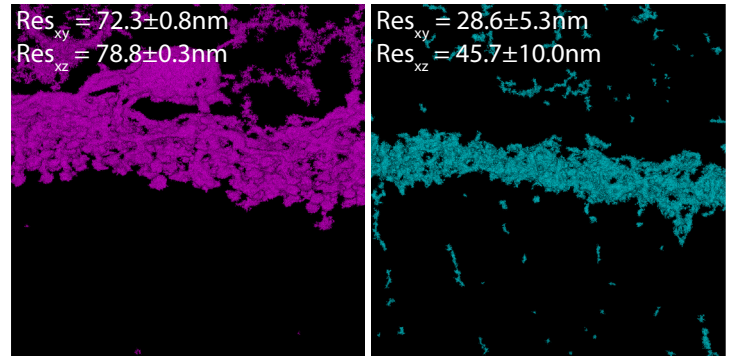
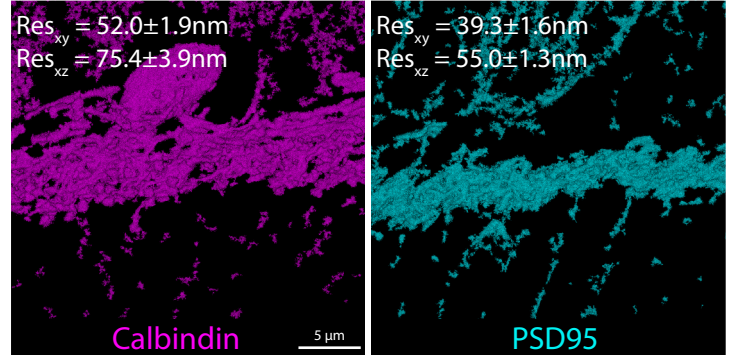


Staining - Post-Fixation, Time & Concentration

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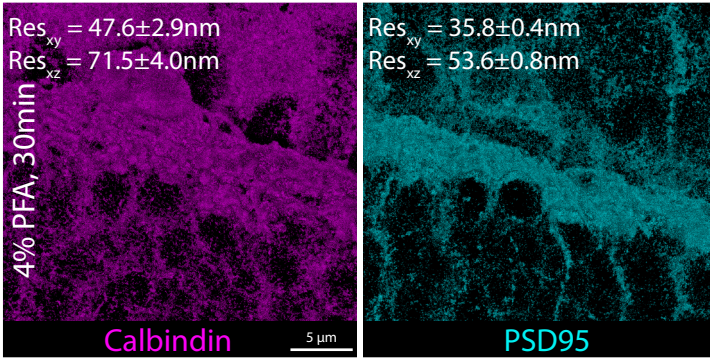


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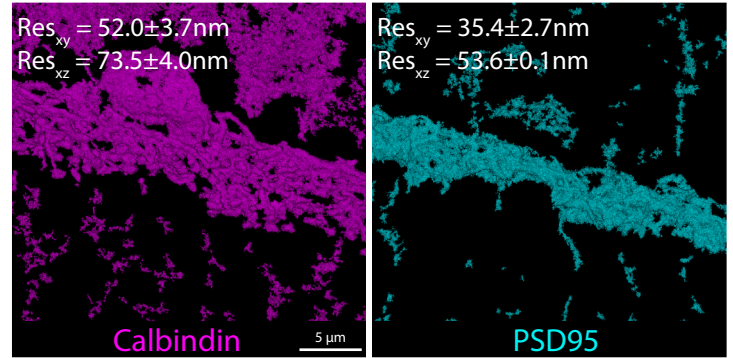


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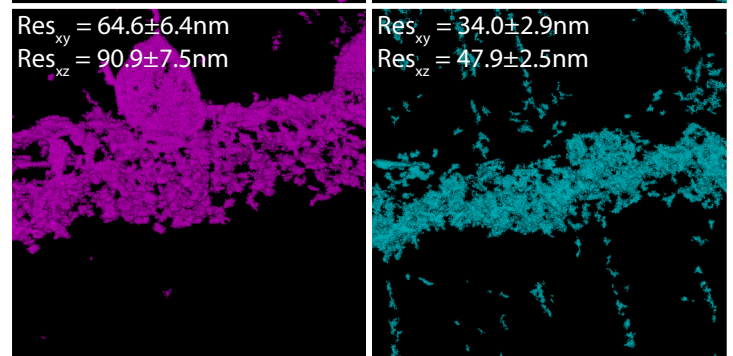
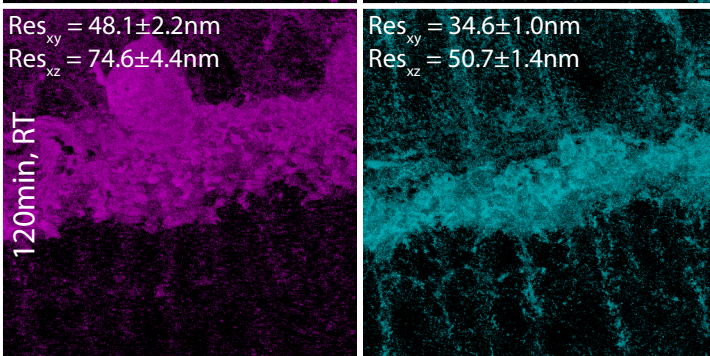
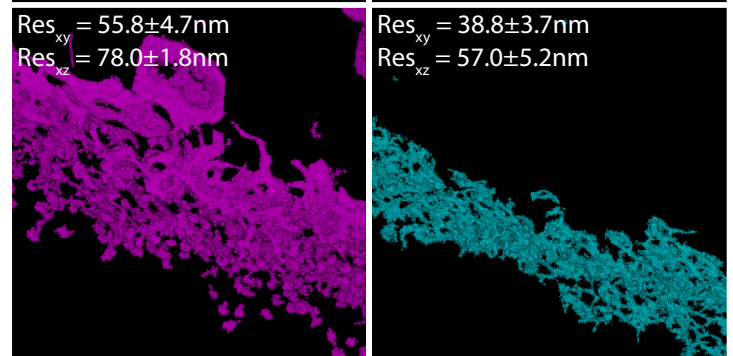
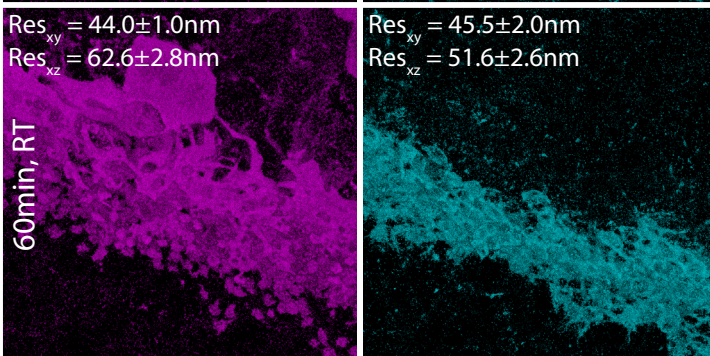
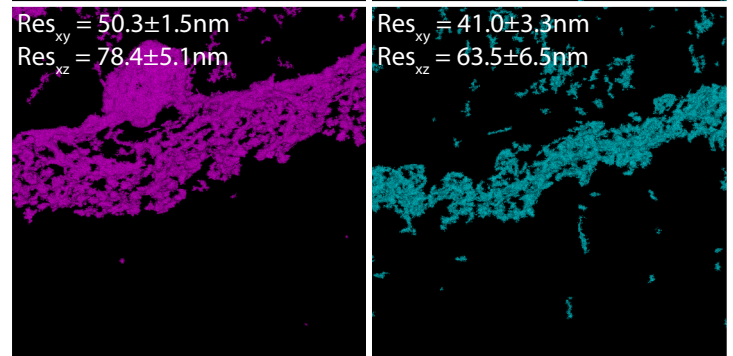
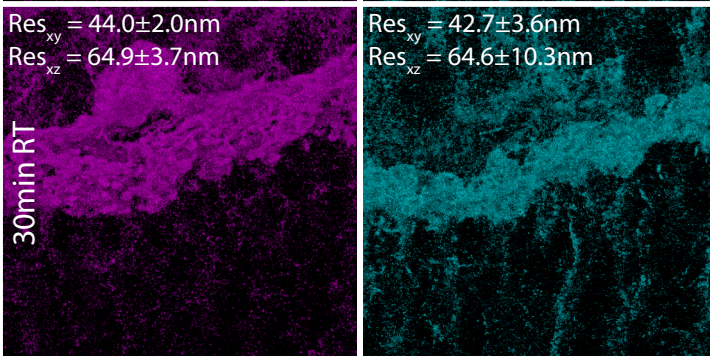
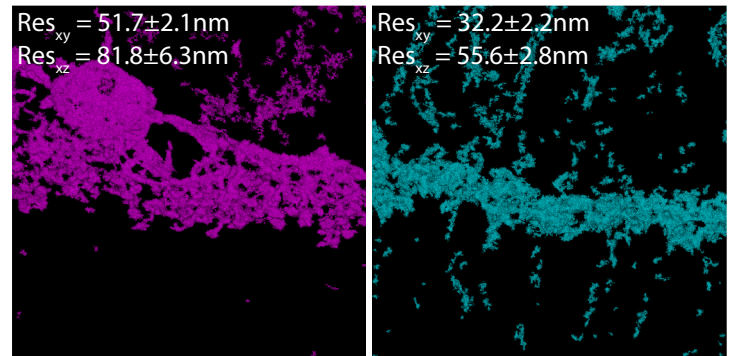
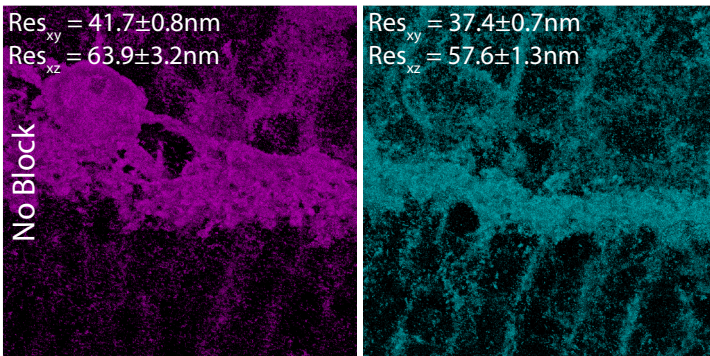
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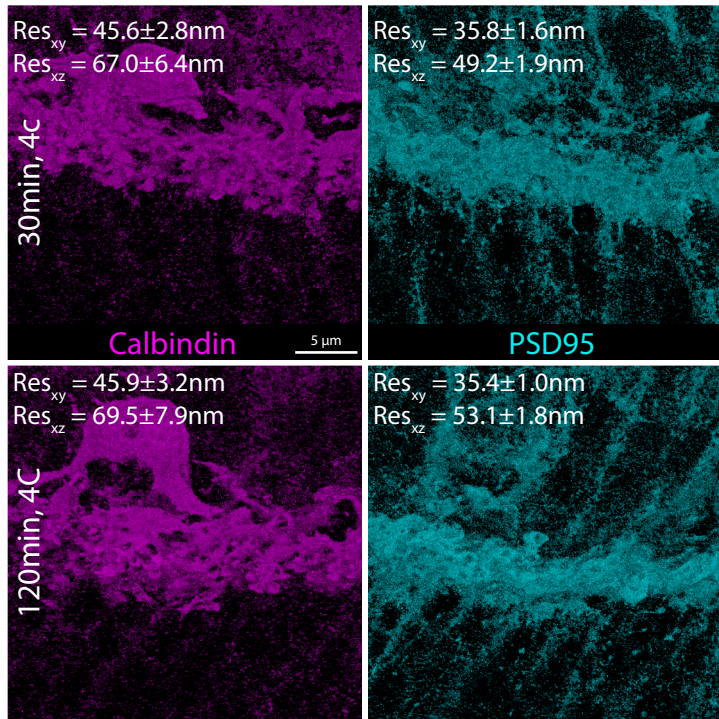


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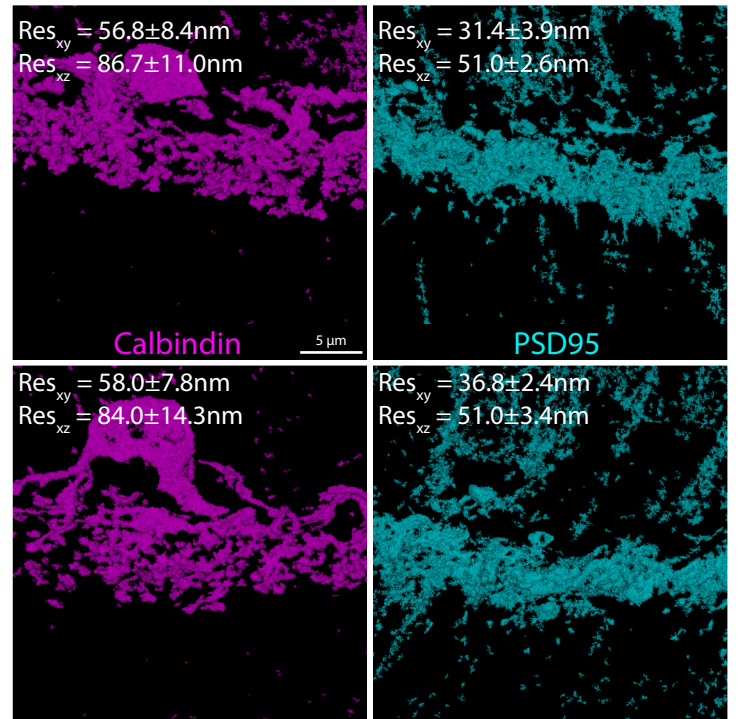


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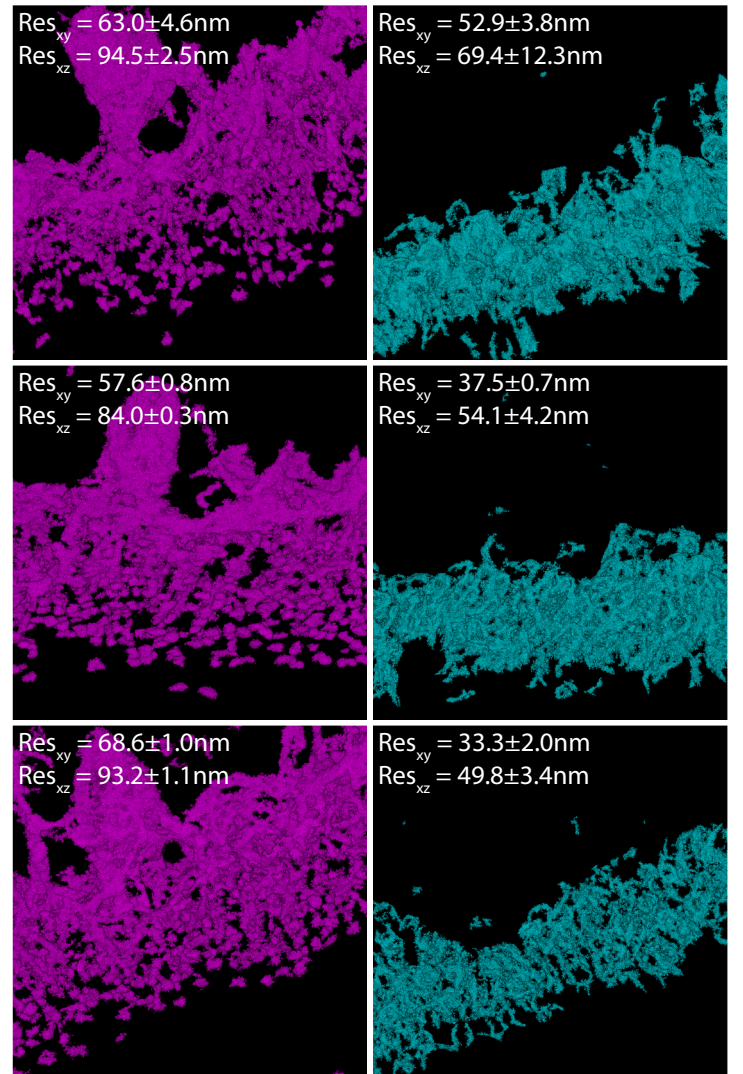
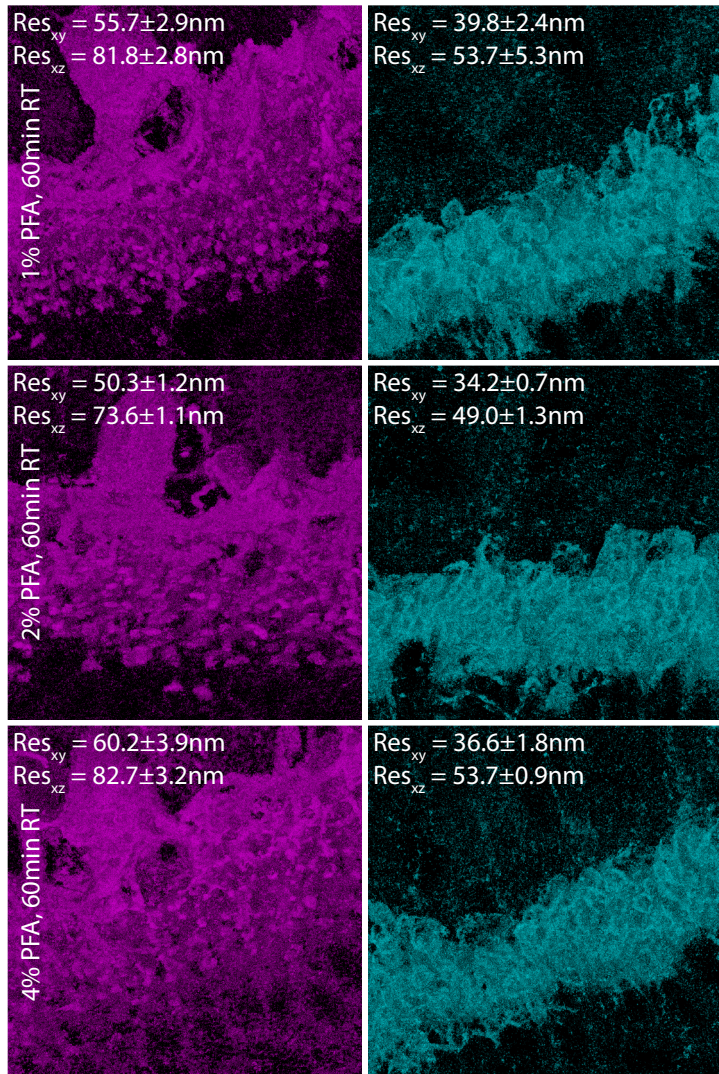
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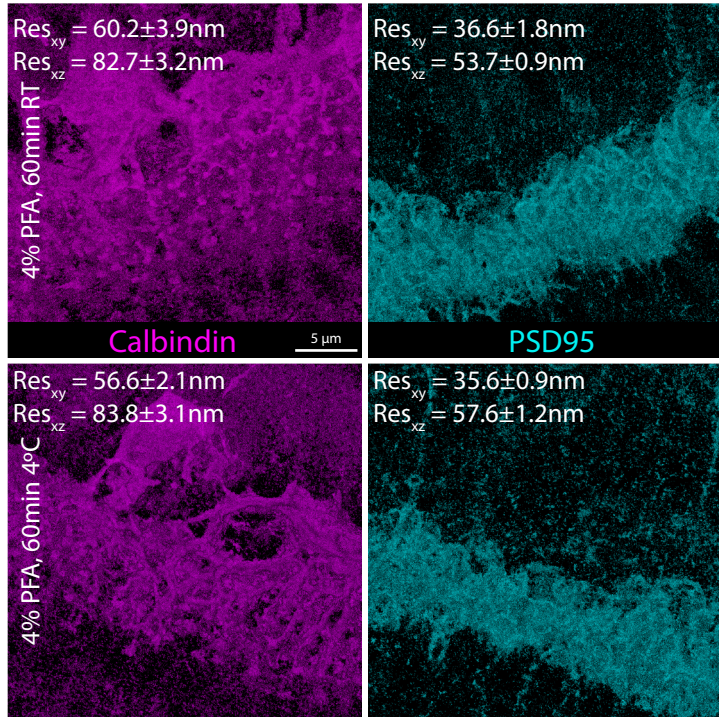


Tissue Preparation - Primary Fixation, PFA Concentration

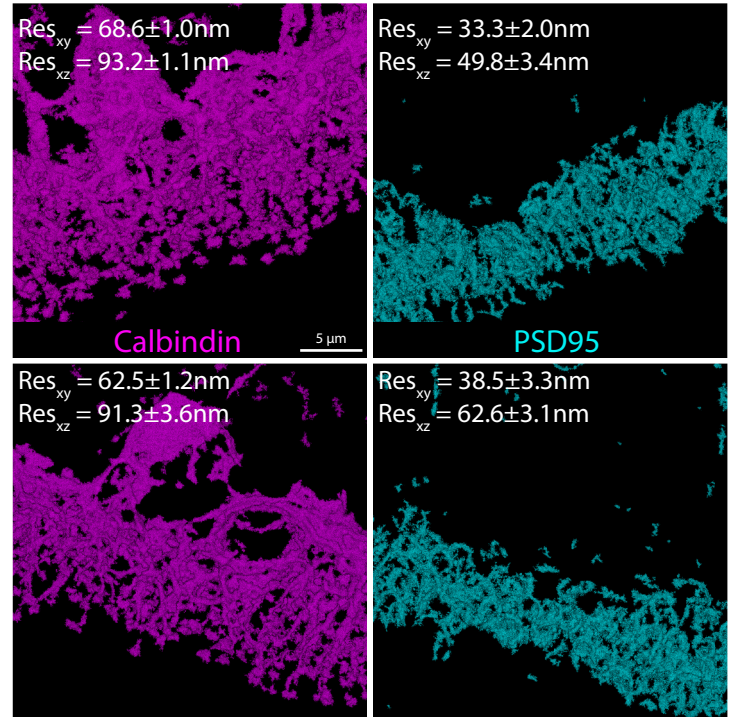


Tissue Preparation - Primary Fixation, Temperature

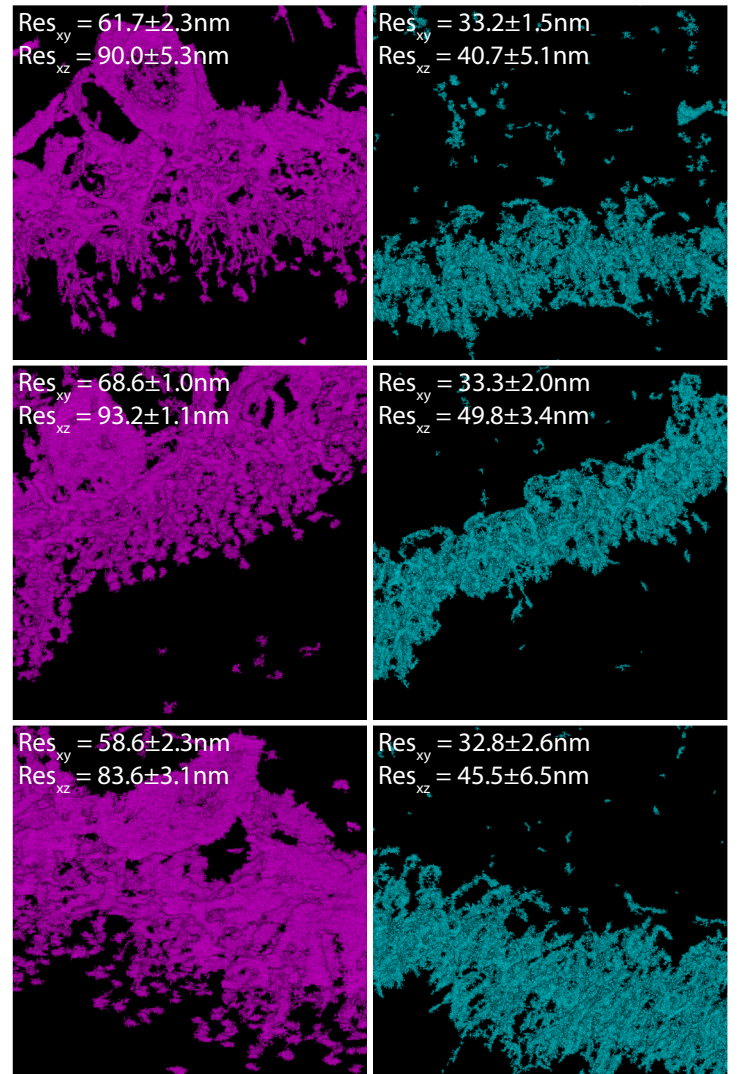
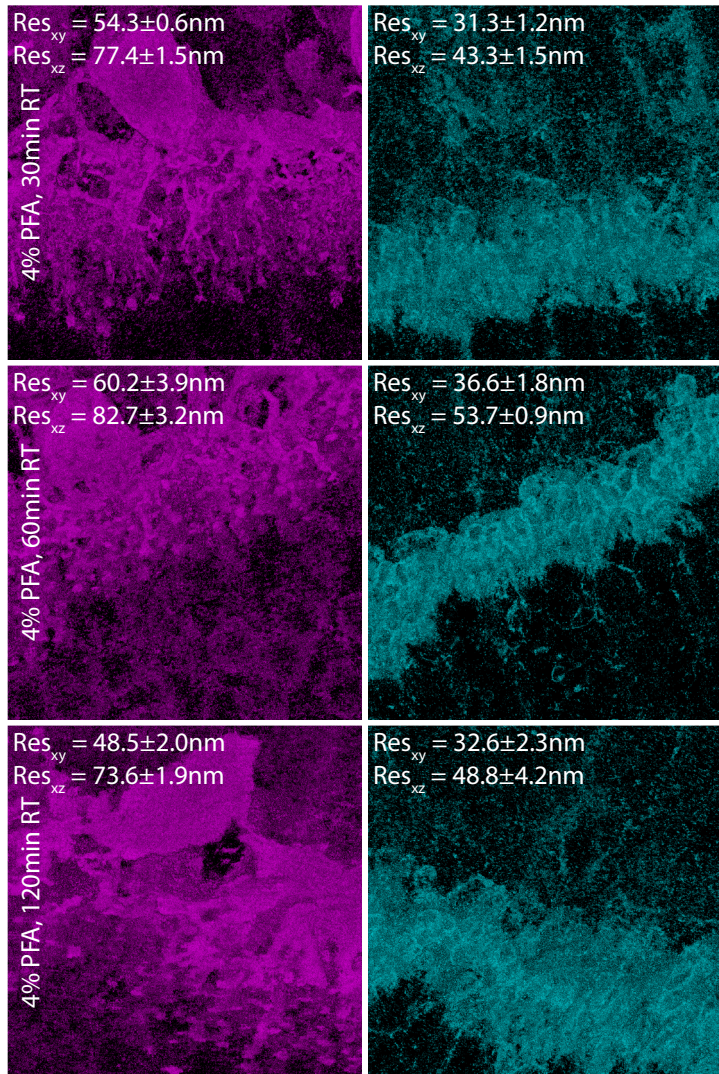
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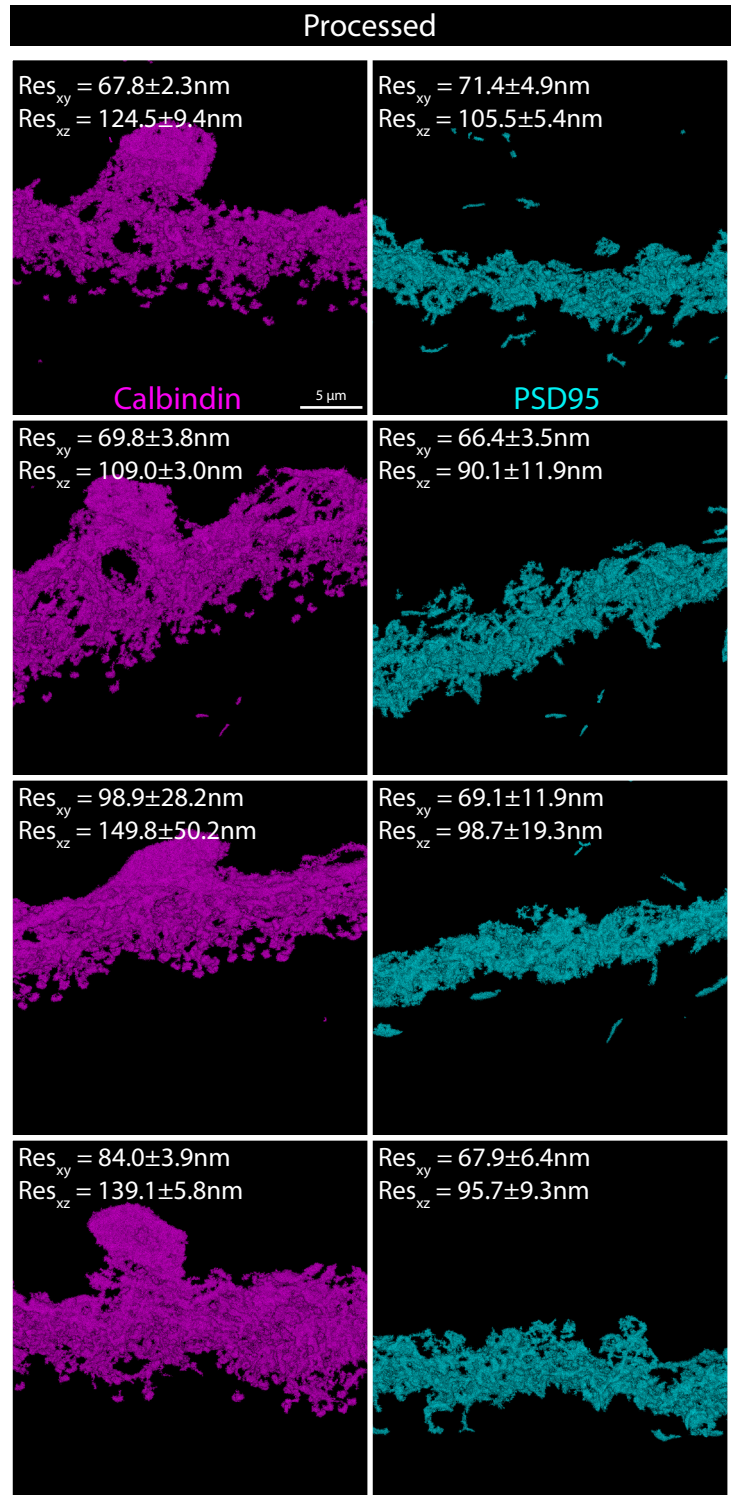
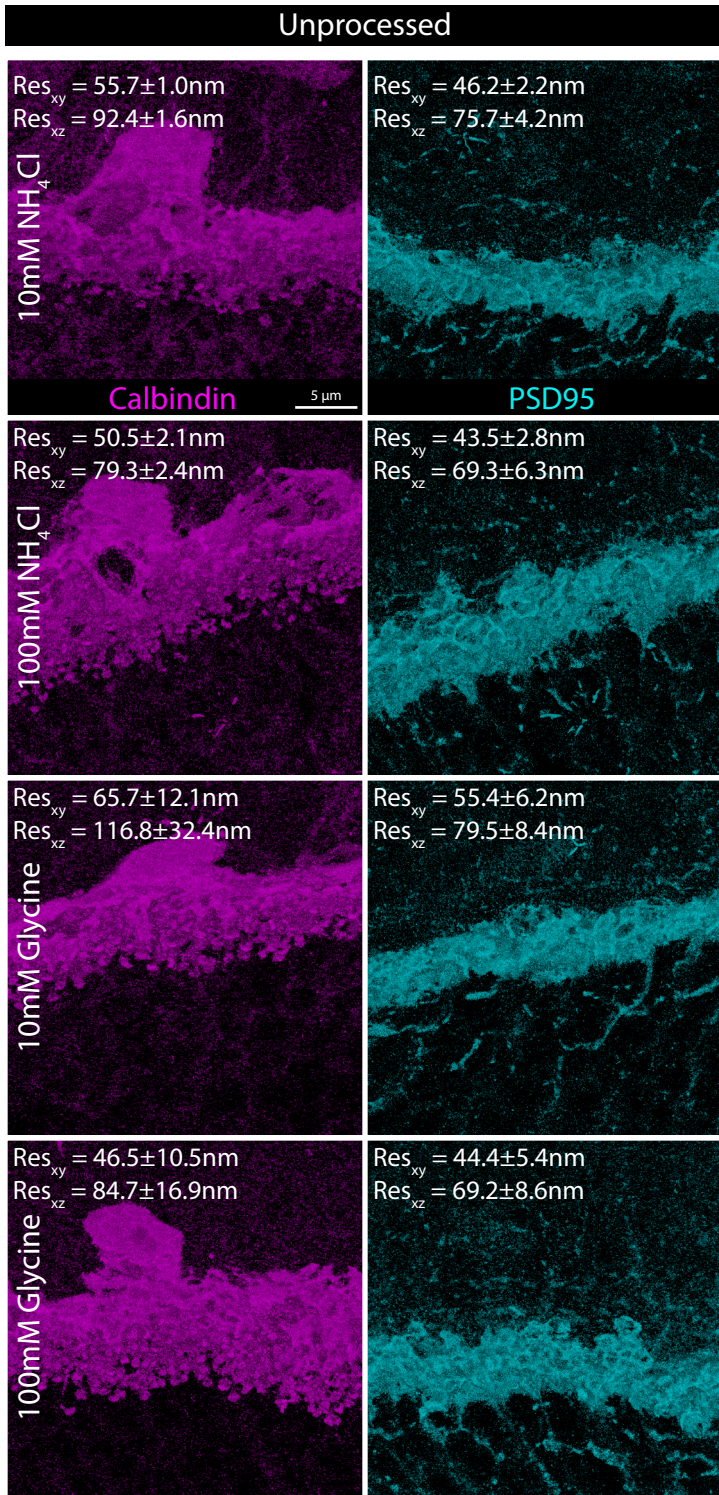
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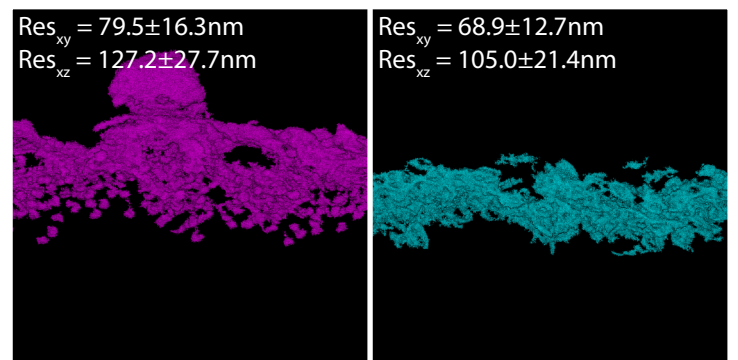
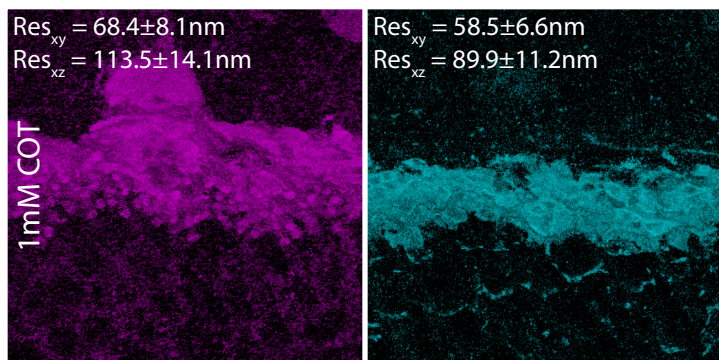
Tissue Preparation - Primary Fixation, Time



Staining - Secondary Quenching, Concentration & Reagent

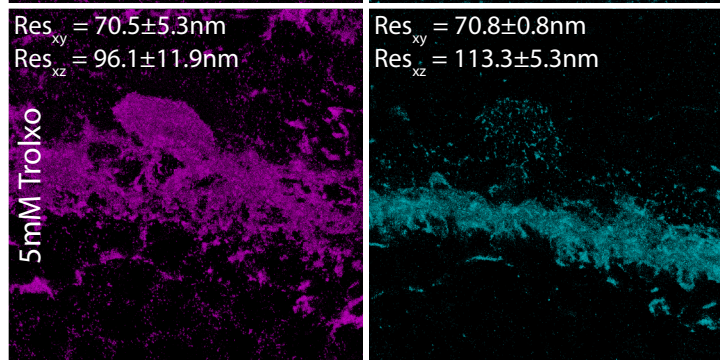
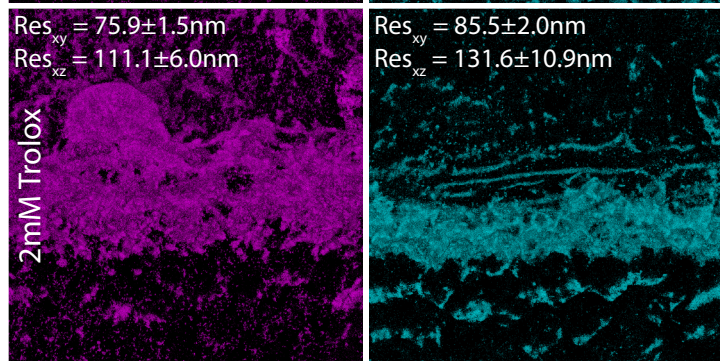
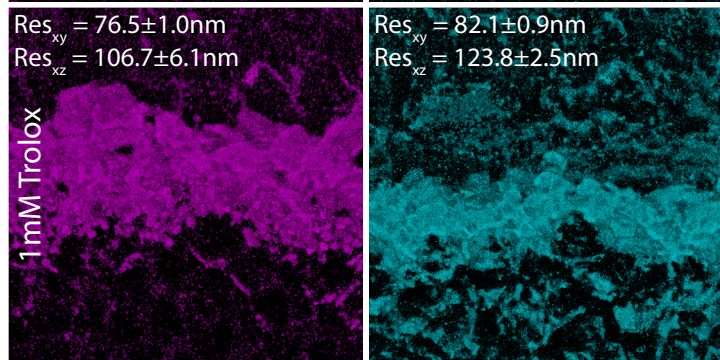
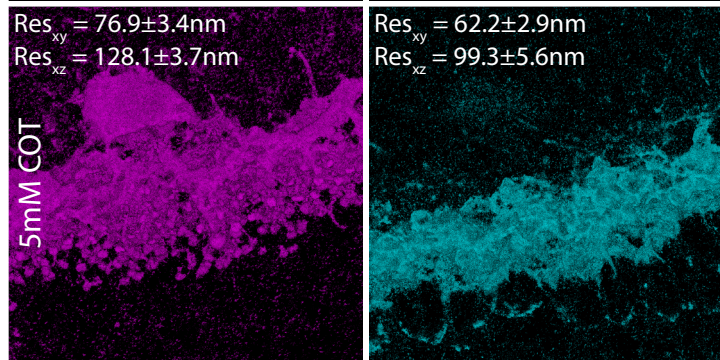
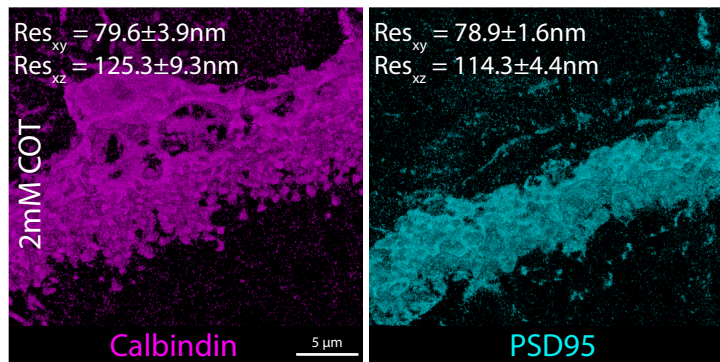


Imaging - Electron Sinks, Concentration & Reagent

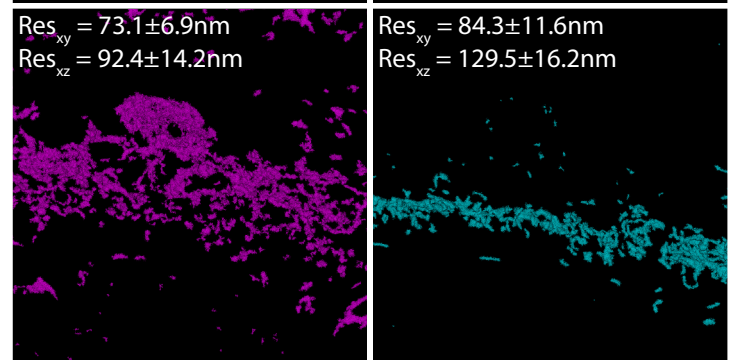
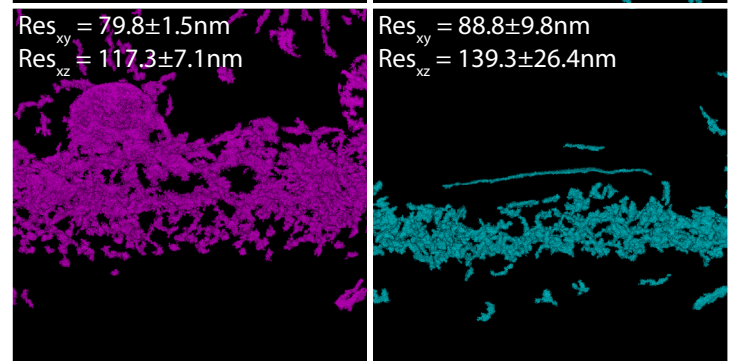
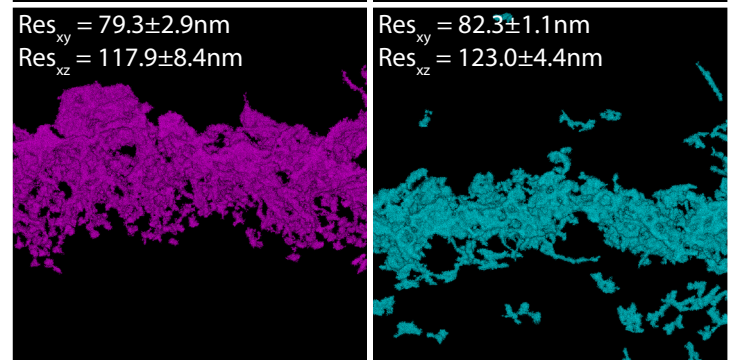
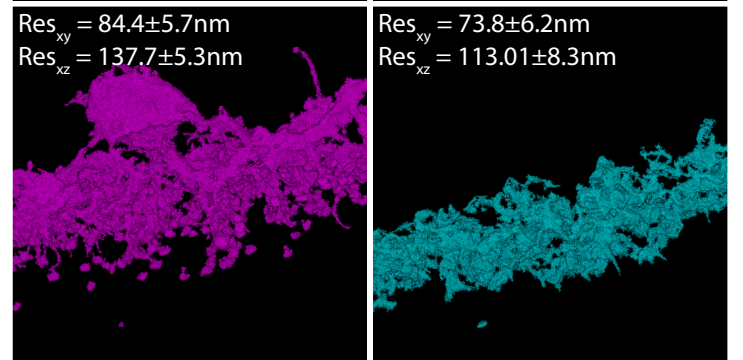
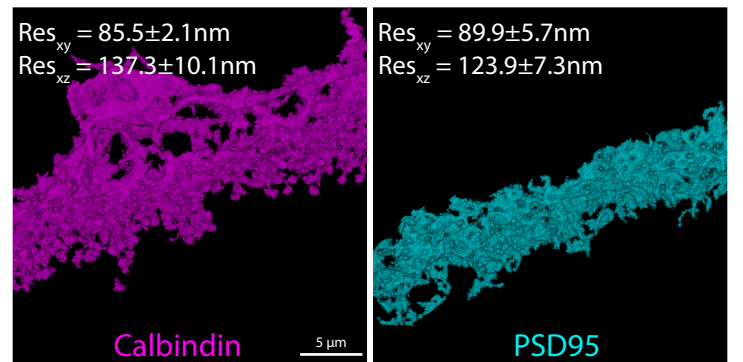


Imaging - Electron Sinks, Concentration & Reagent

Unprocessed

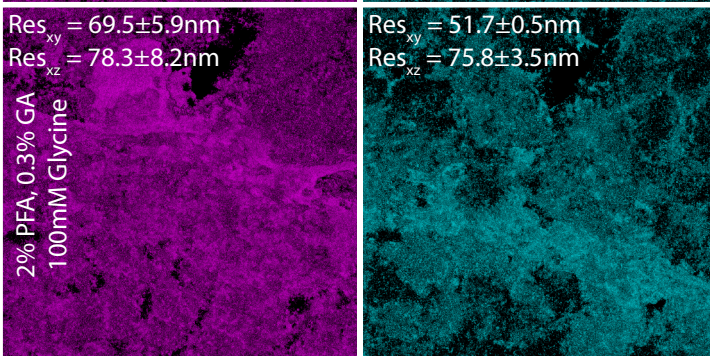
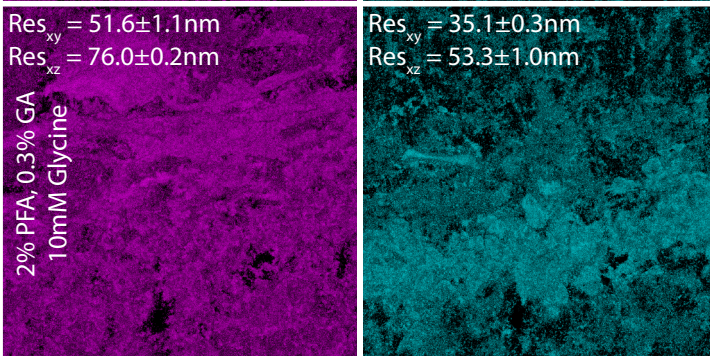
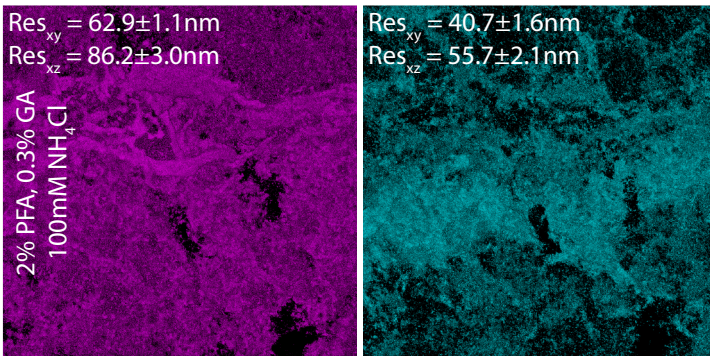
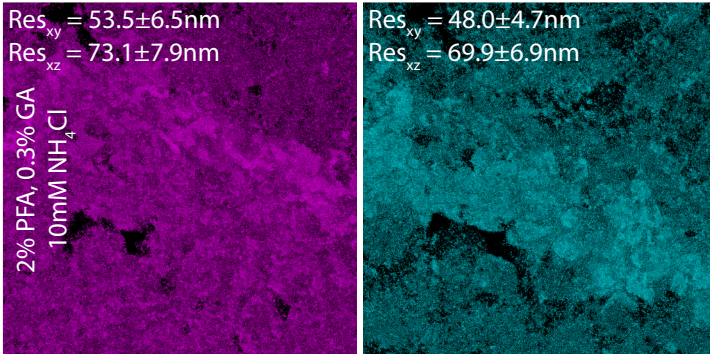
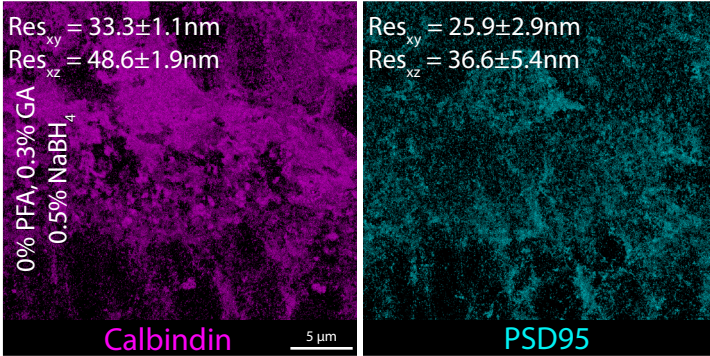


Processed

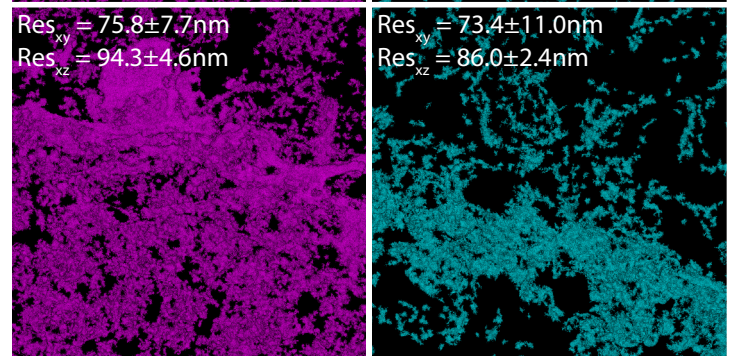
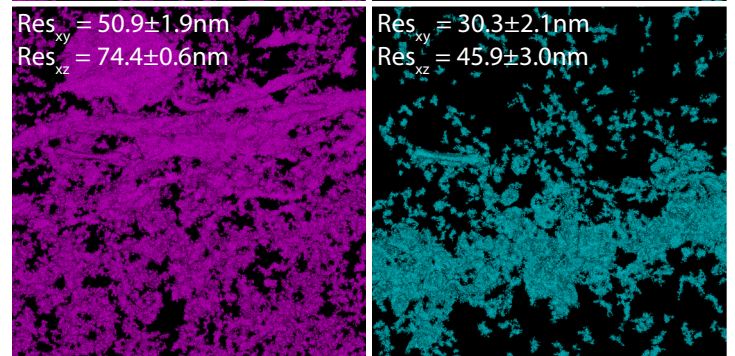
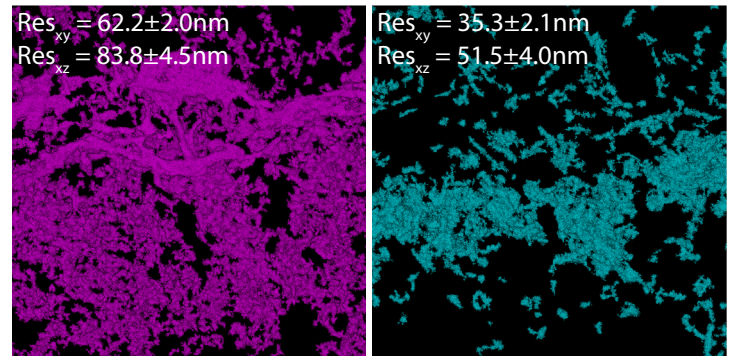
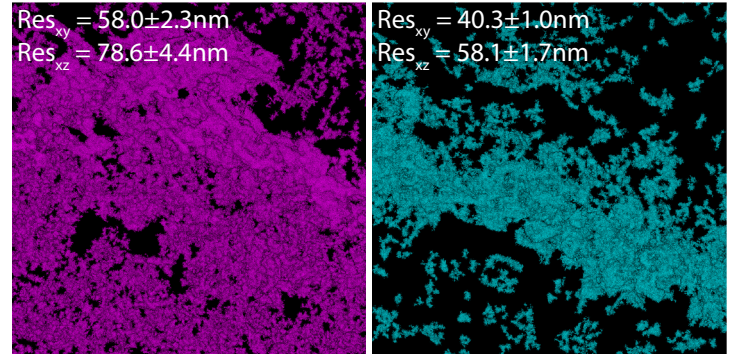
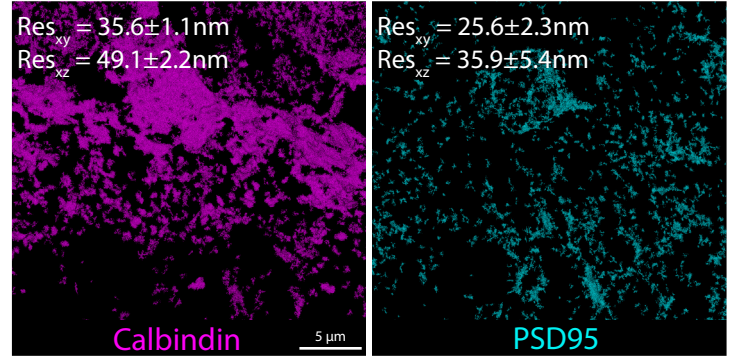


Tissue Preparation-Primary Fixation & Quenching, Reagent & Conc.

Unprocessed

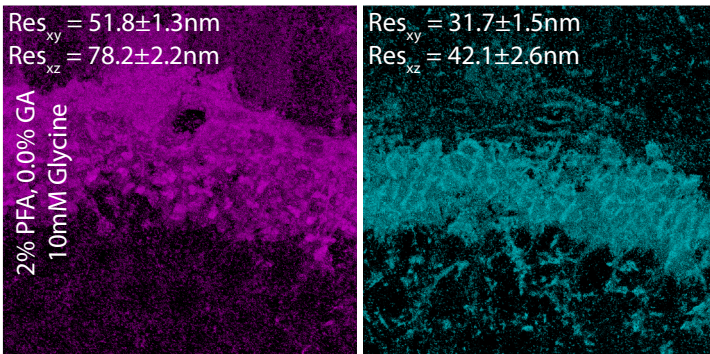
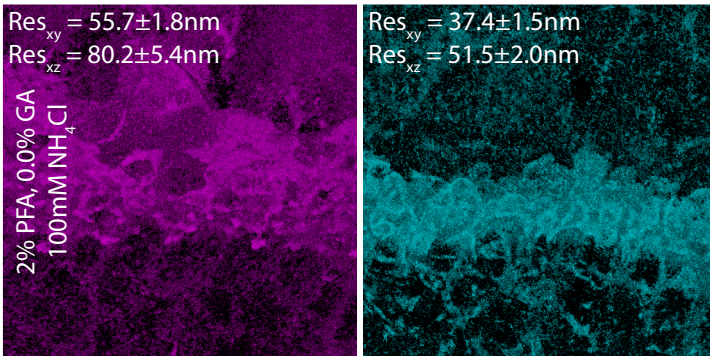
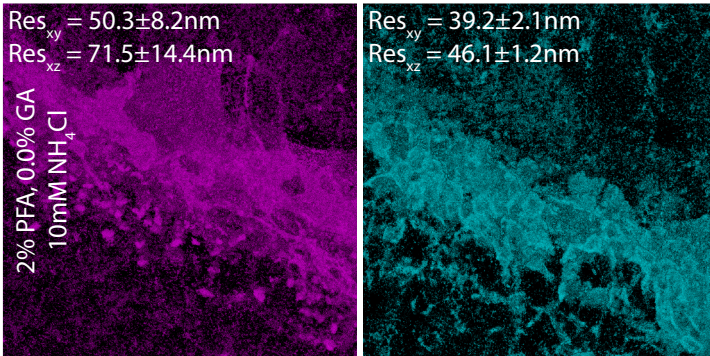
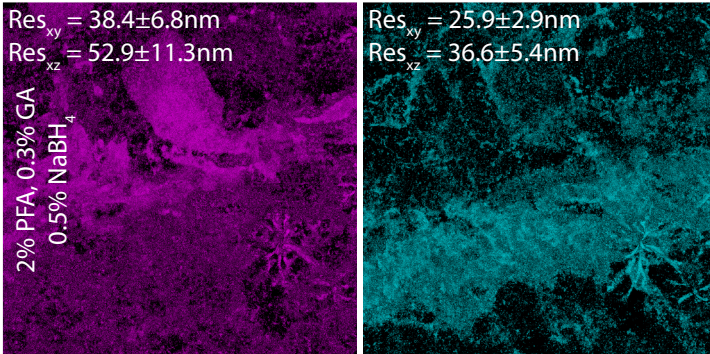
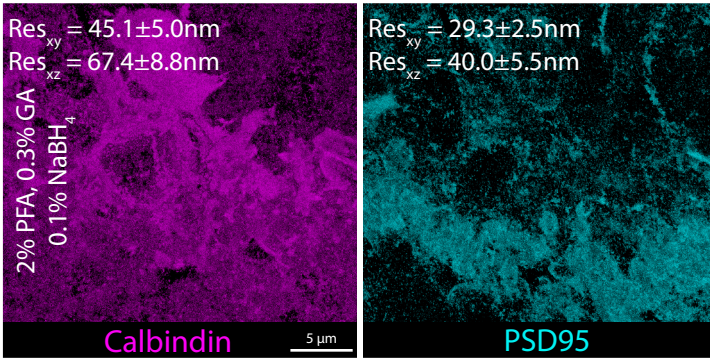


Processed

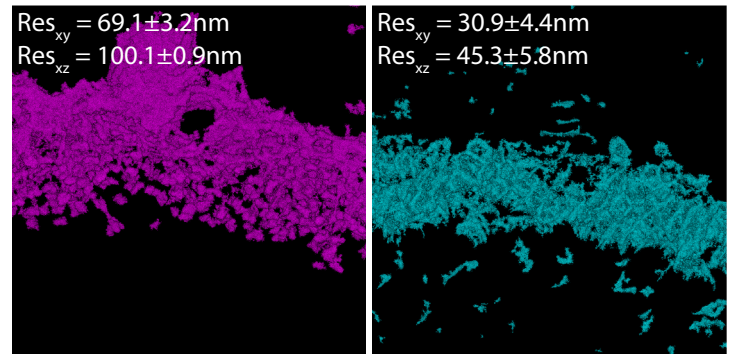
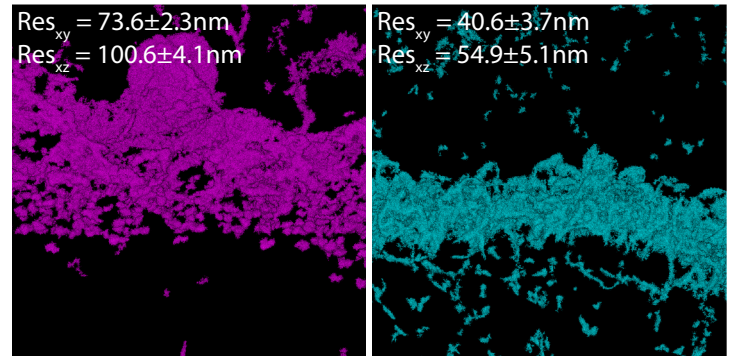
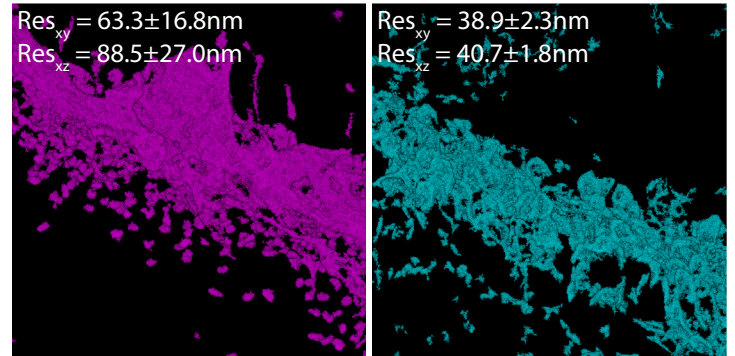
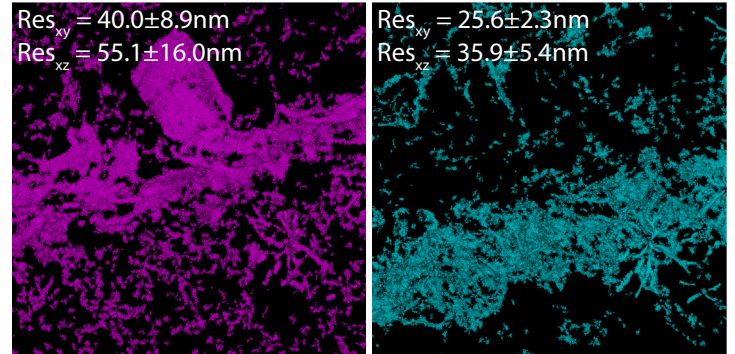
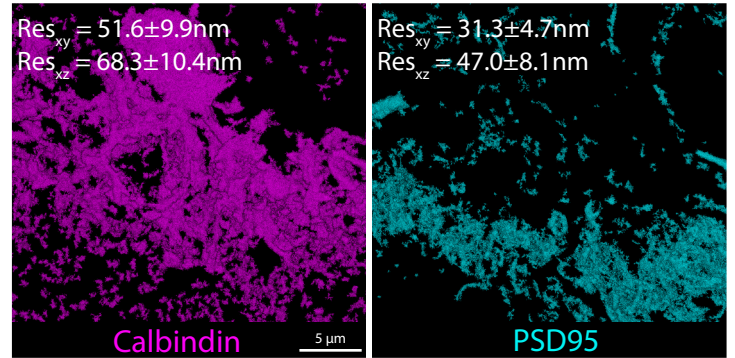


Tissue Preparation-Primary Fixation & Quenching, Reagent & Conc.

Unprocessed

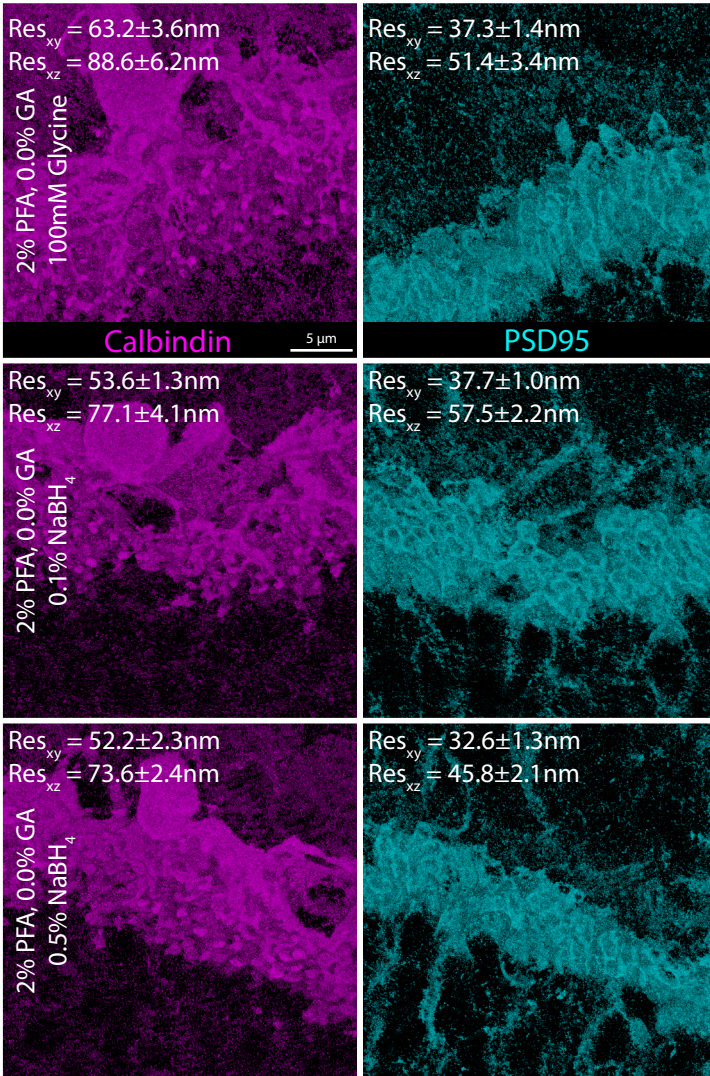


Processed

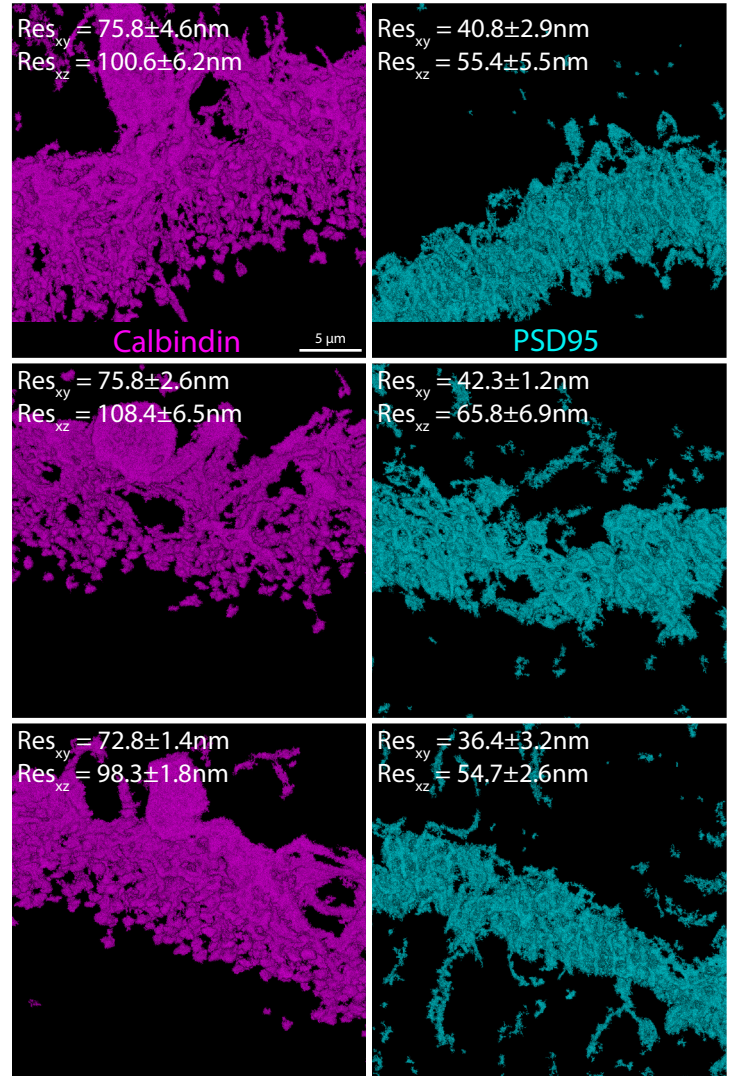


Tissue Preparation-Primary Fixation & Quenching, Reagent & Conc.

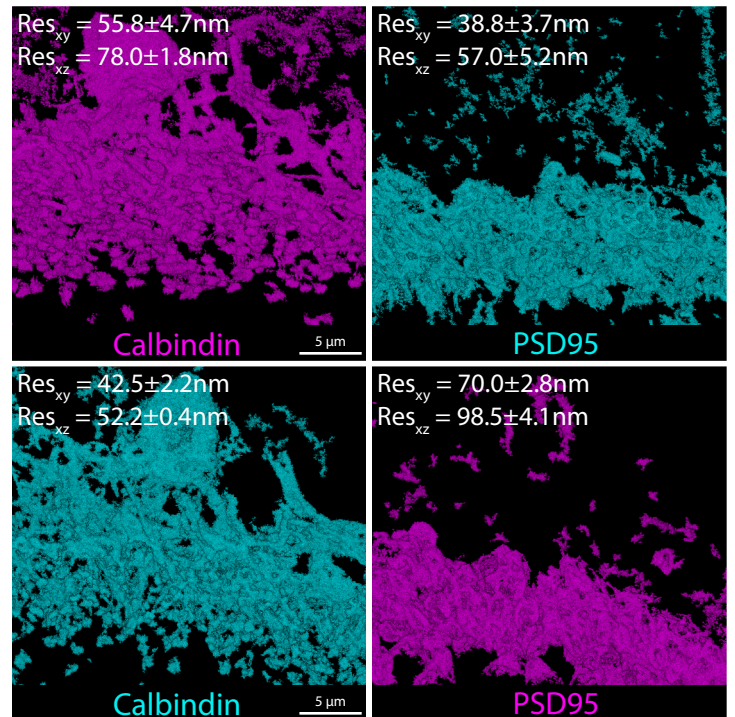
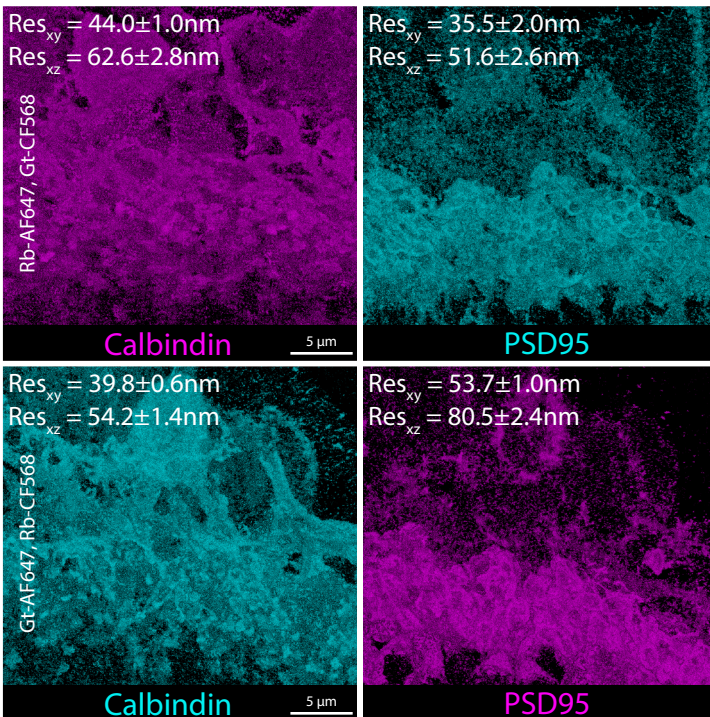
Unprocessed



Processed

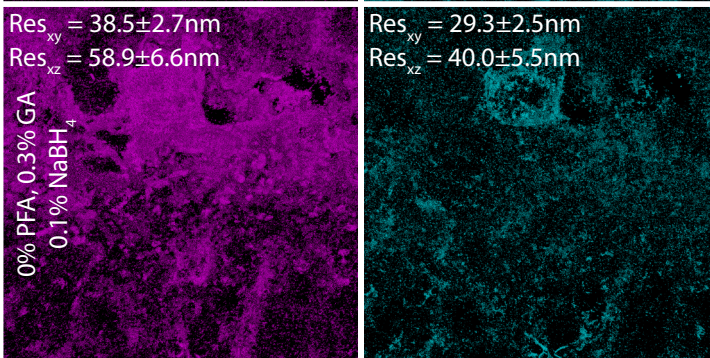
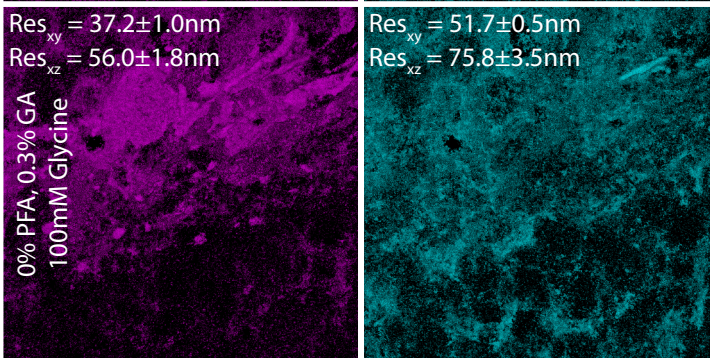
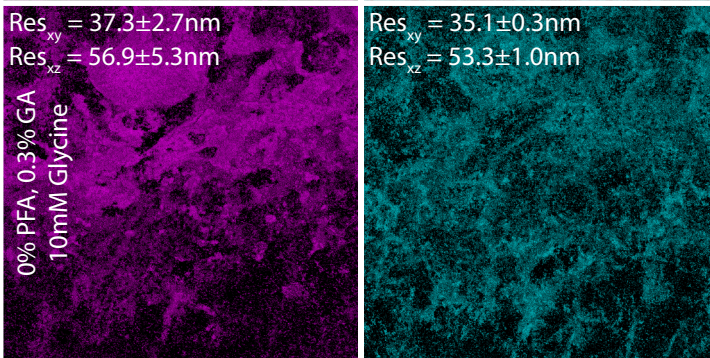
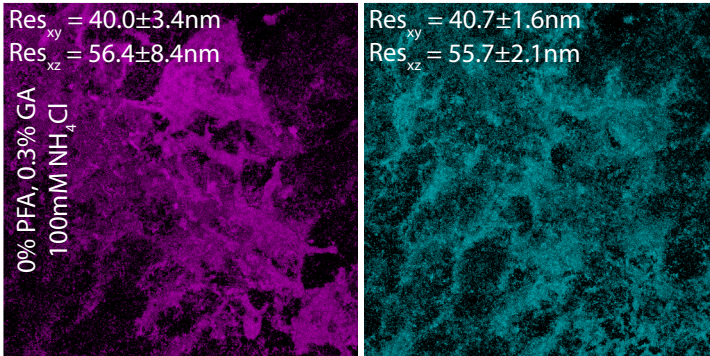
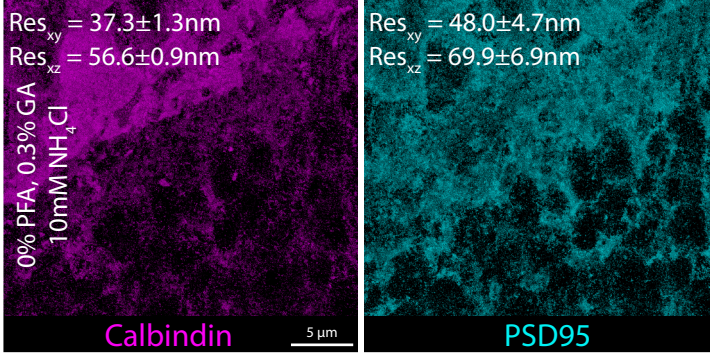


Staining - Secondary, Channel & Primary Target

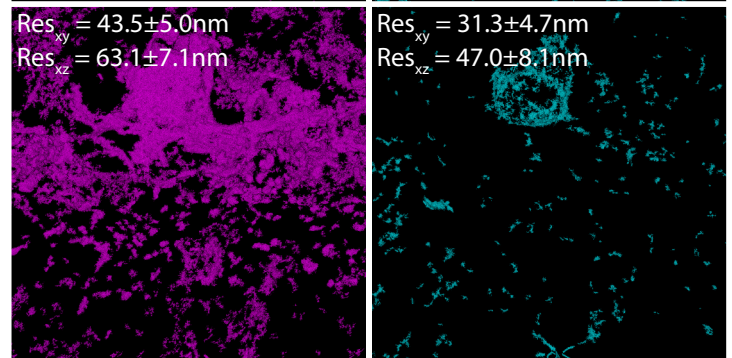
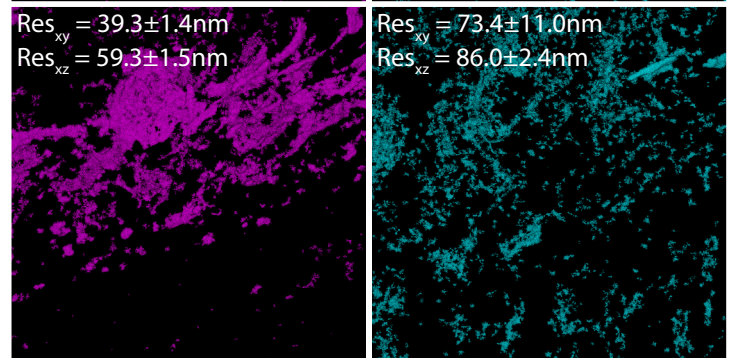
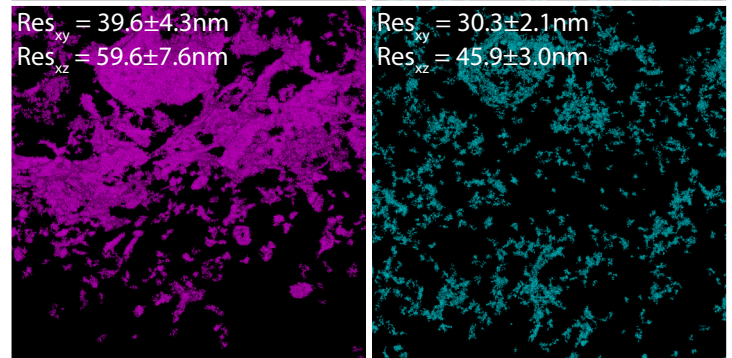
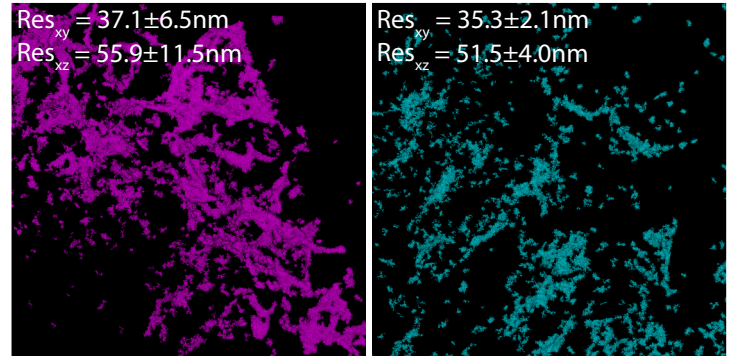
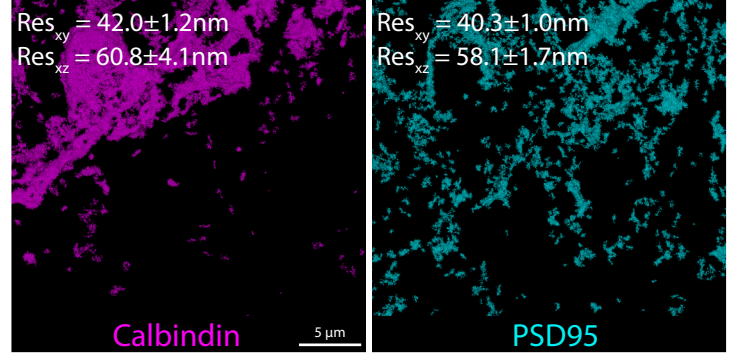


Tissue Preparation-Primary Fixation & Quenching, Reagent & Conc.

Unprocessed

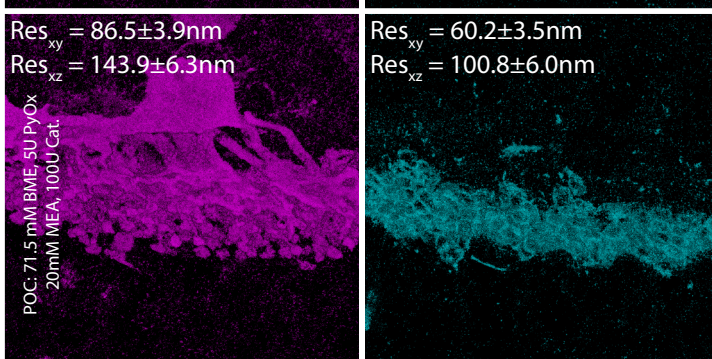
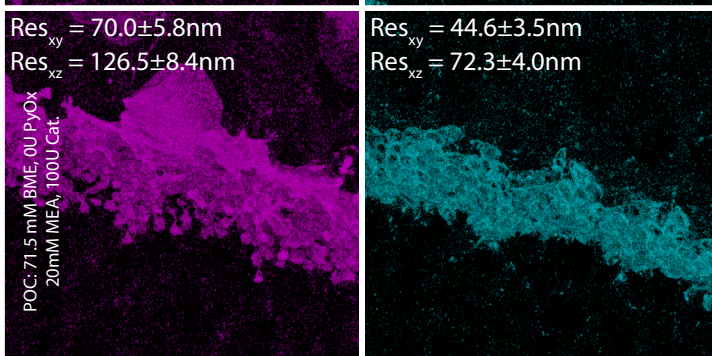
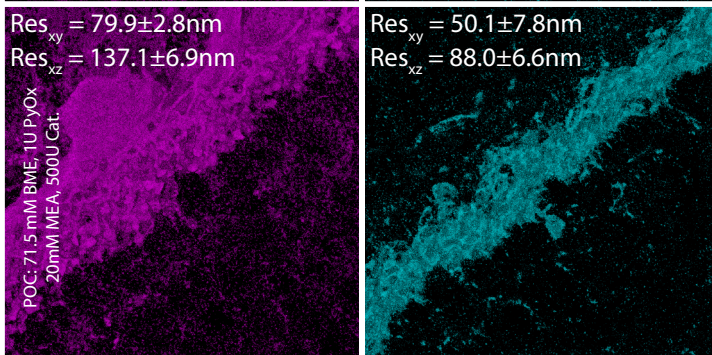
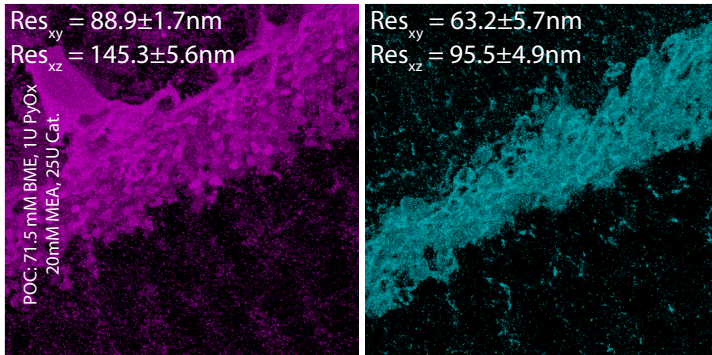
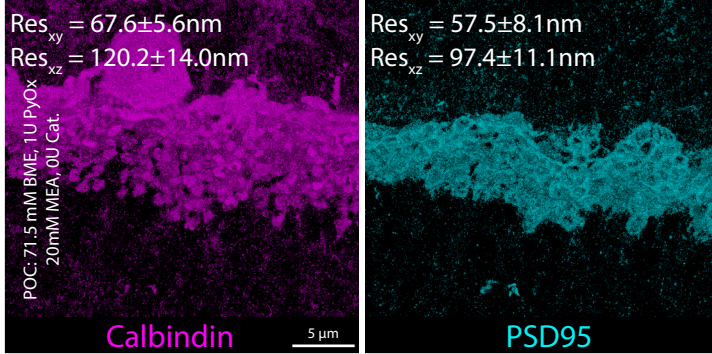


Processed

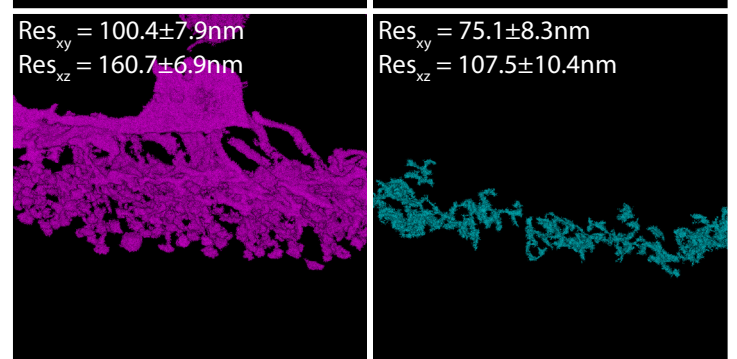
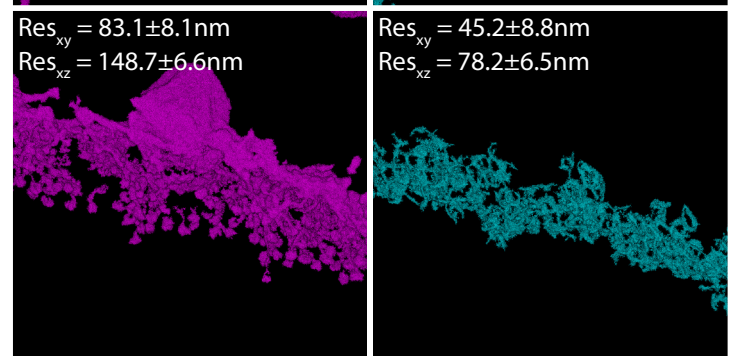
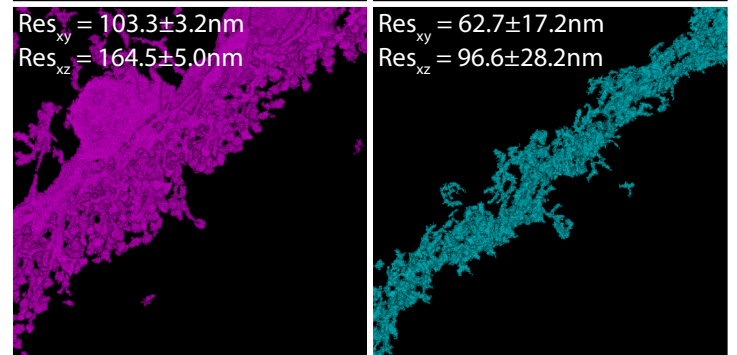
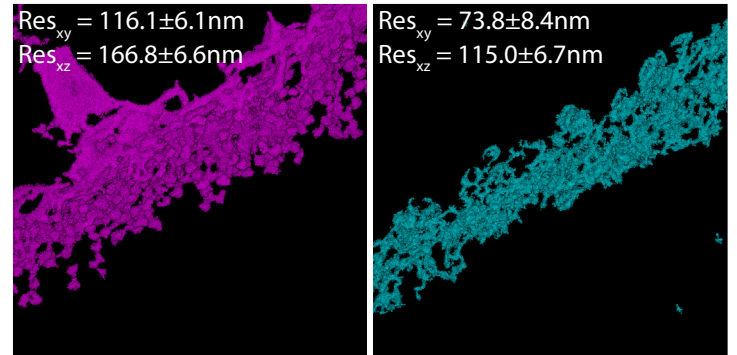
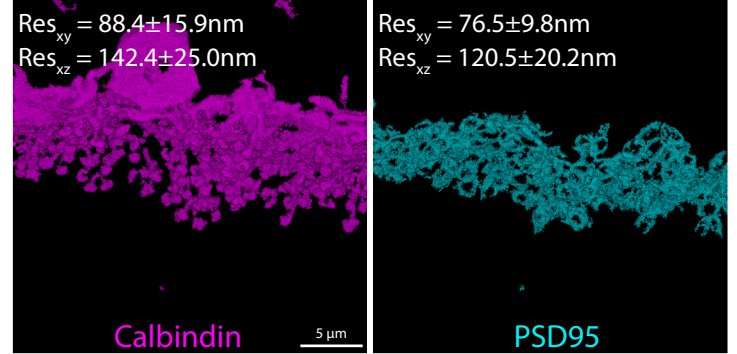


Imaging & Analysis - Imaging Buffer, Pyranose Oxidase Buffer

Unprocessed

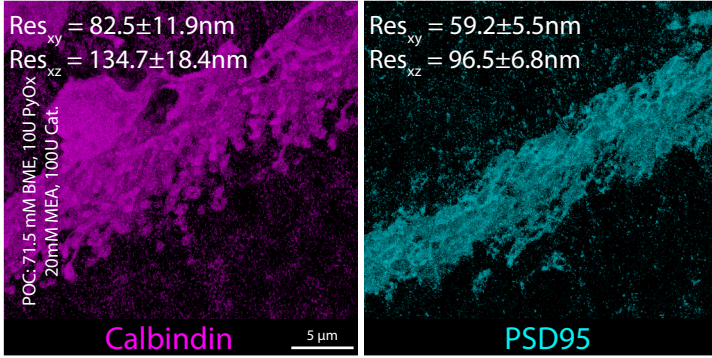


Processed

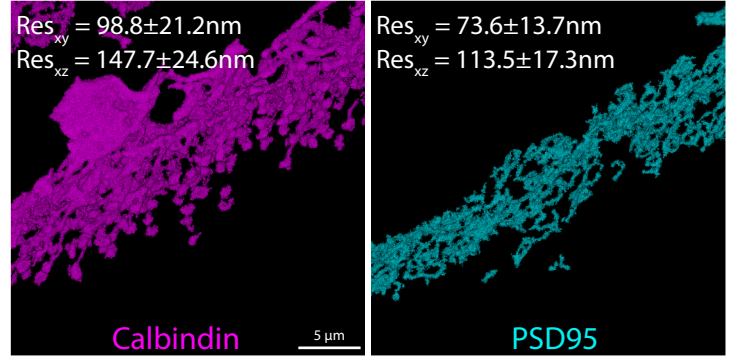


Imaging & Analysis - Imaging Buffer, Pyranose Oxidase Buffer

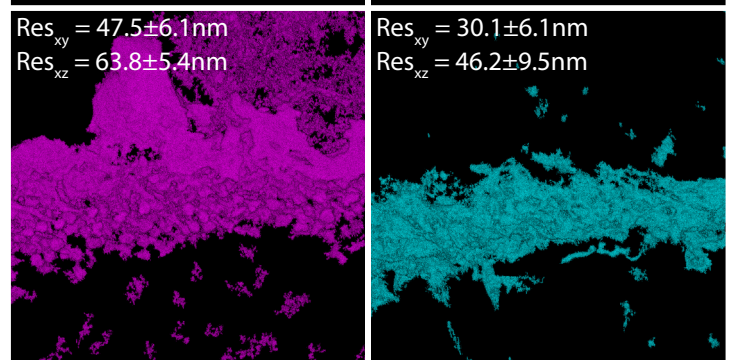
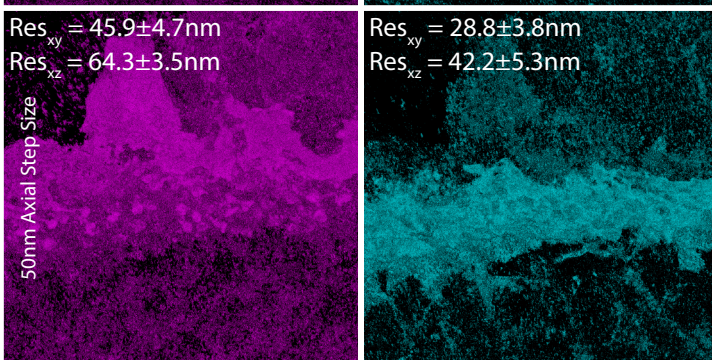
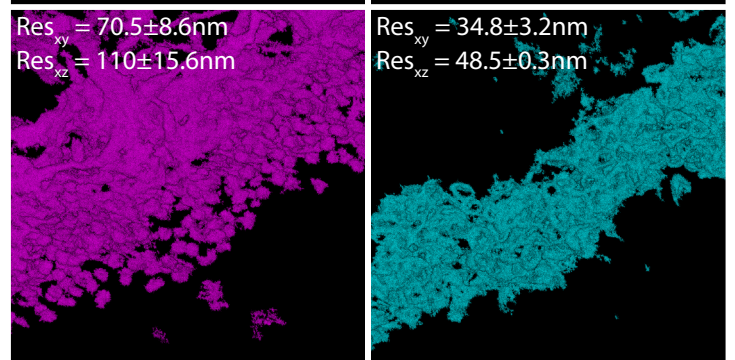
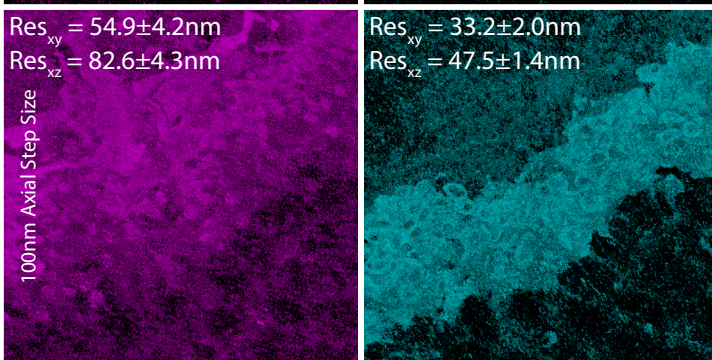
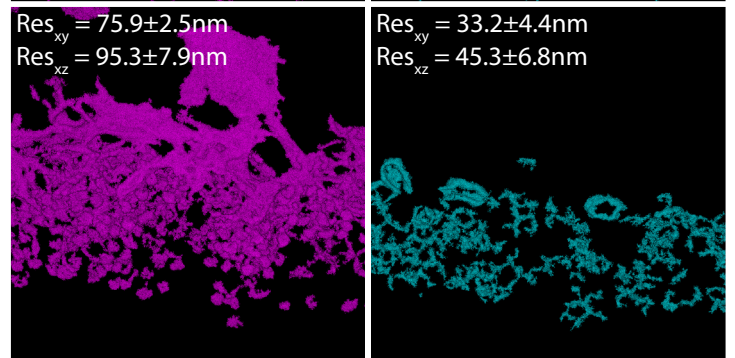
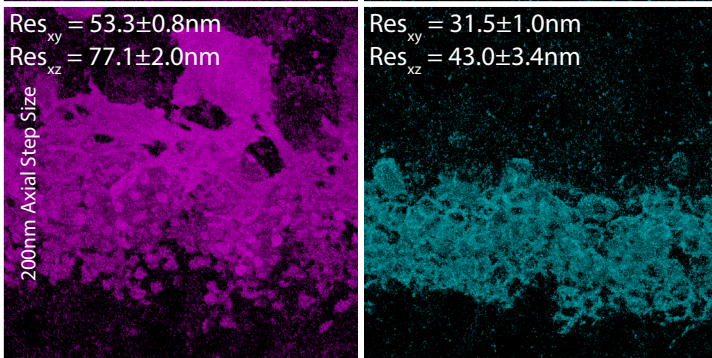
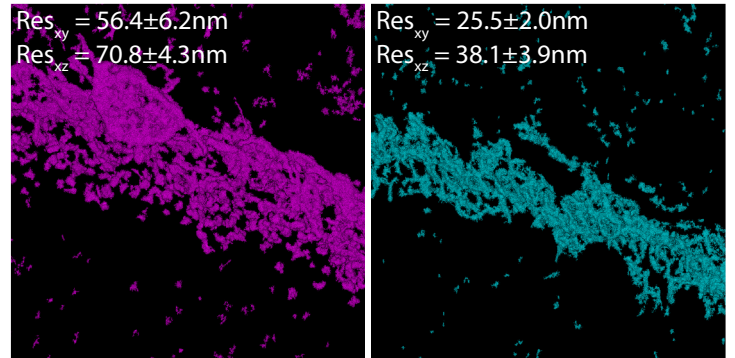
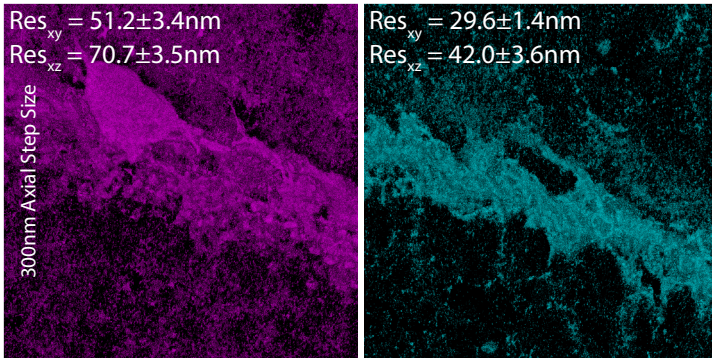
Unprocessed



Processed

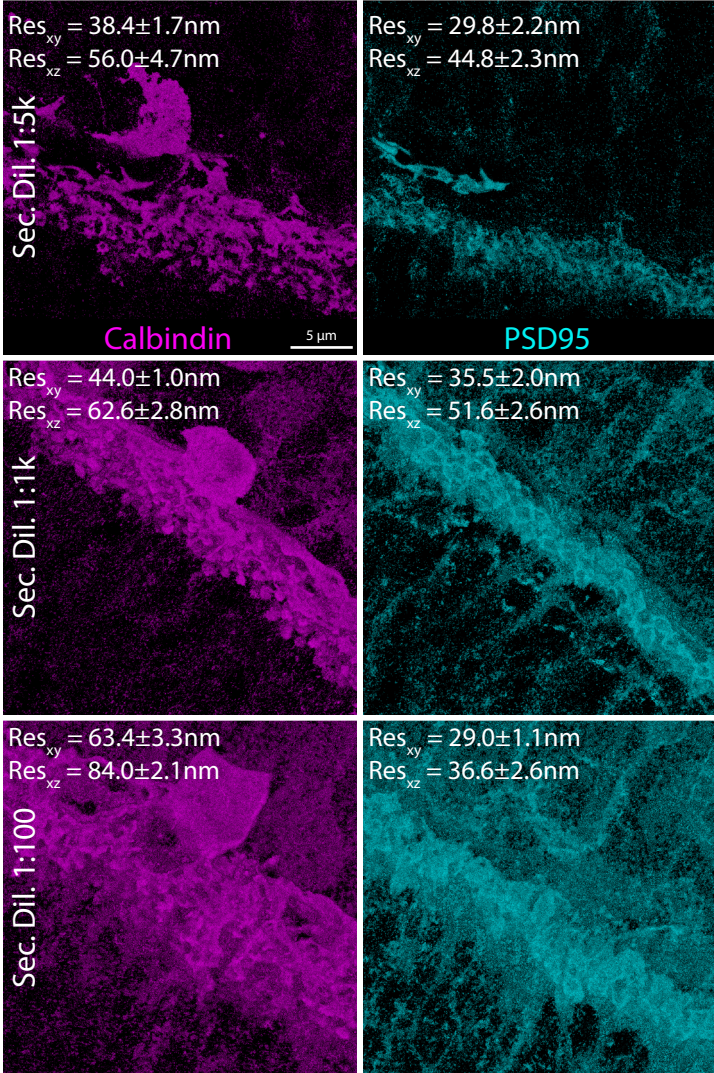


Imaging & Analysis - Image Acquisition, Step Size

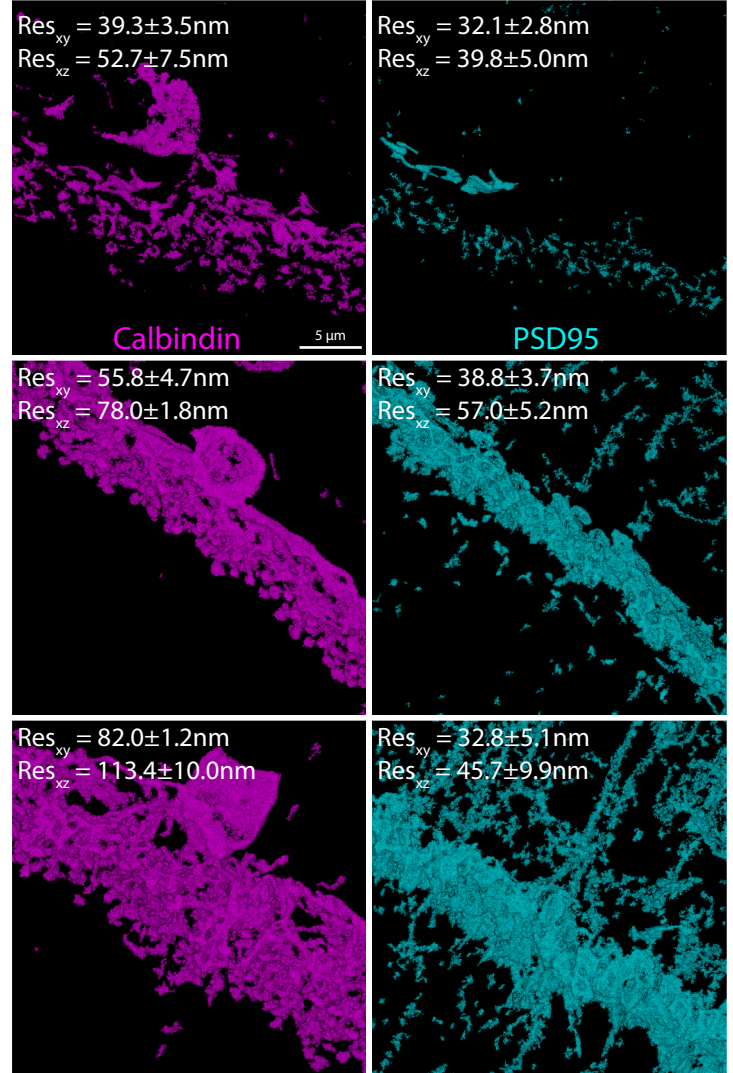


Staining- Secondary, Labeling Density

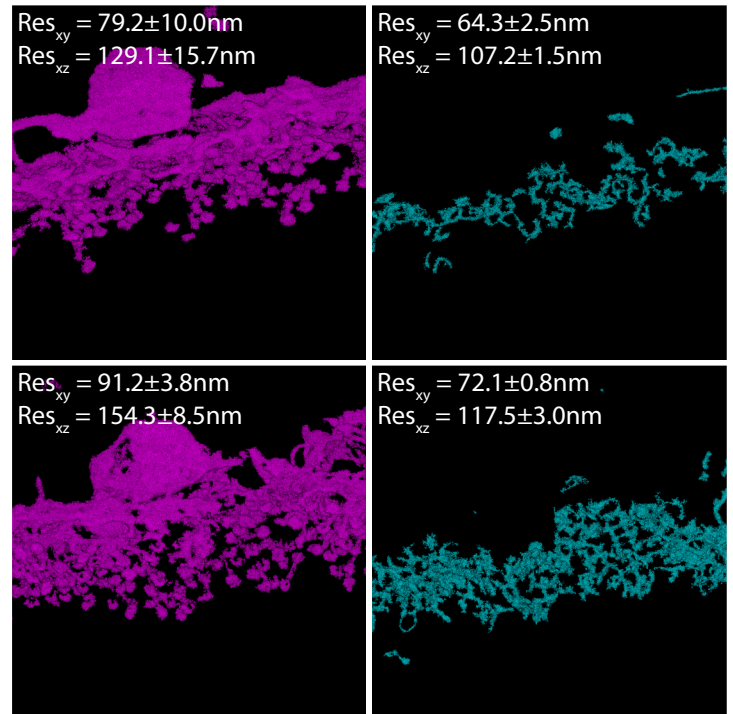
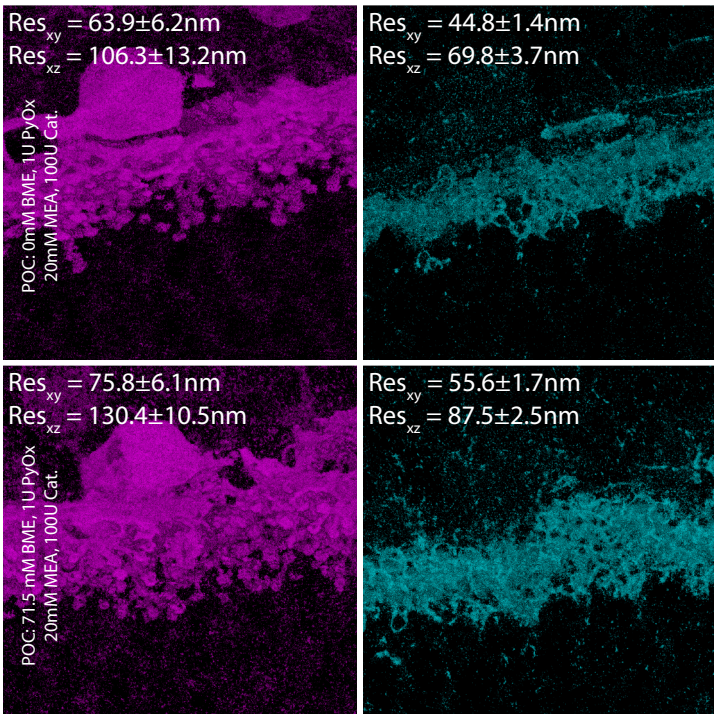
Unprocessed



Processed

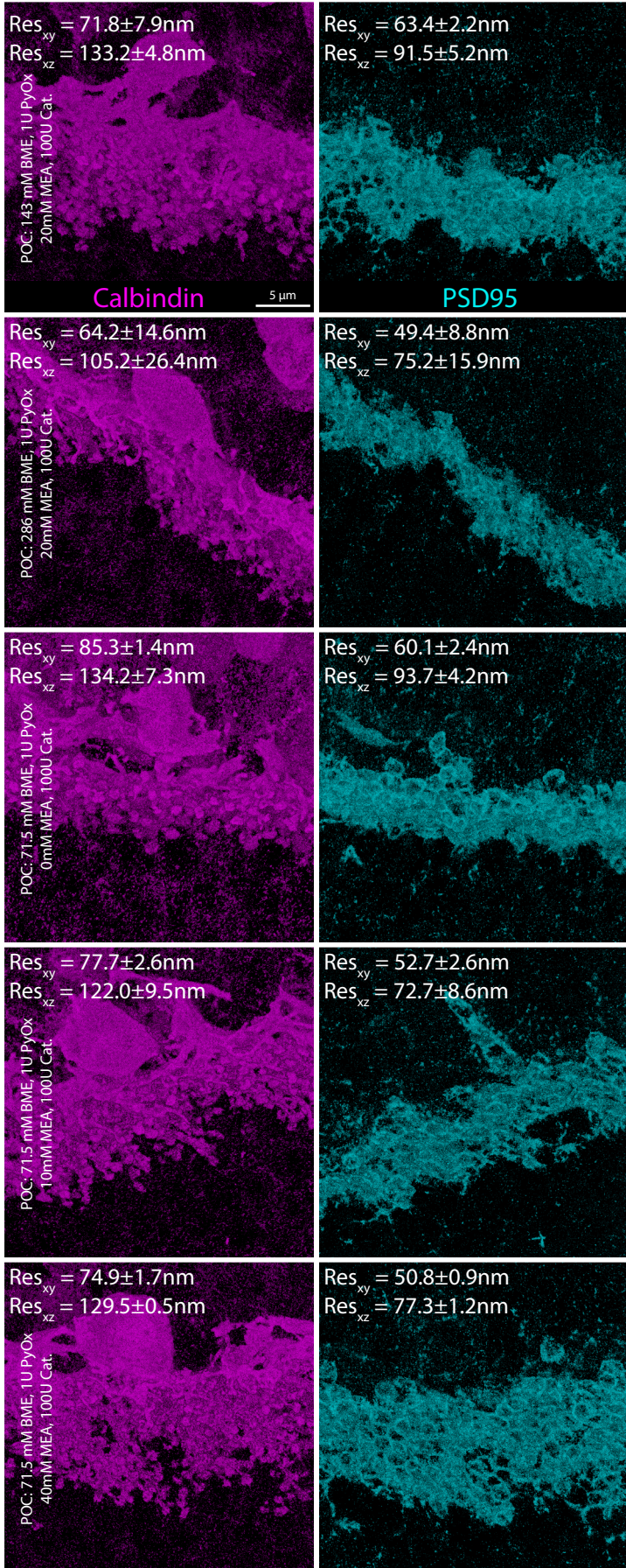


Imaging & Analysis - Imaging Buffer, Pyranose Oxidase Buffer

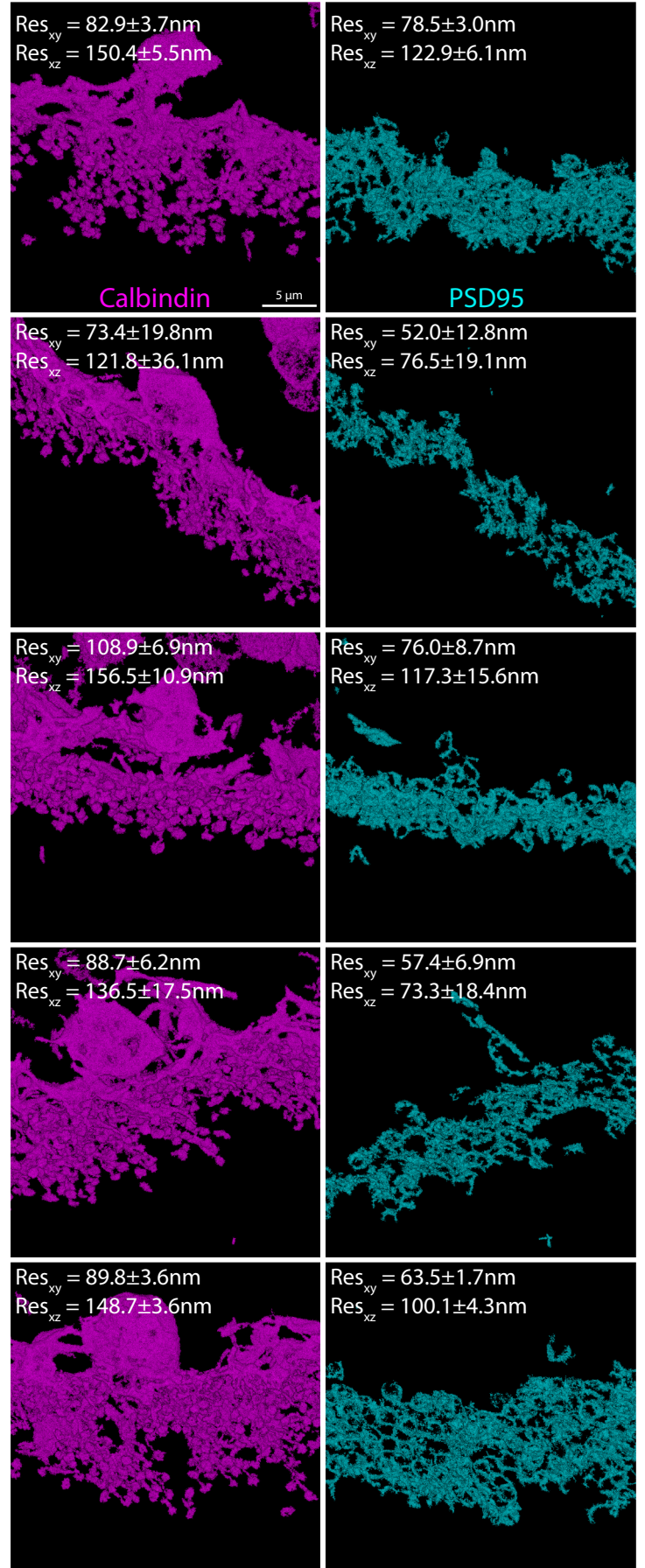


Imaging & Analysis - Imaging Buffer, Pyranose Oxidase Buffer

Unprocessed



Processed



Data S1. Sample and imaging conditions affect image quality and metrics, related to Figure 4.

Representative images of conditions are shown for each channel of a given condition. Unprocessed images are shown alongside their processed counterparts, as well as the resolutions that were measured for each condition. Each row shows a different condition tested. Calbindin-labeled horizontal cells are shown in magenta, and PSD95-labeled rod terminals in cyan, unless otherwise noted. Scale bars = 5 μm .

Supplemental Table 1: Primary antibodies used.

Antigen	Labeling specificity	Source	Dilution (confocal)	Dilution (RAIN-STORM)
Calbindin D-28K	Horizontal cells, subsets of amacrine cells, and retinal ganglion cells	Swant, Cat# CB38a, RRID: AB_10000340	1:5000	1:1000
CD31	Blood vessels, endothelial cells	Fisher, Cat# BDB5500274, RRID: AB_393571	1:200	1:50
Cone arrestin	Cone photoreceptors	Millipore, Cat# AB15282, RRID: AB_11210270	1:2000	1:1000
Collagen IV	Blood vessels	Millipore, Cat# AB769, RRID: AB_92262	1:1000	1:500
Connexin 43	Pericyte gap junctions	Sigma, Cat#C6219, RRID: AB_476857	1:1000	1:500
Desmin	Pericytes	Thermo Fisher, Cat# MA513259, RRID: AB_11000611	1:500	1:500
Dystrophin	Photoreceptor synapses	Abcam, Cat# ab15277, RRID: AB_301813	1:200	1:100
Glutamine synthetase (GS)	Muller glia	BD Biosciences, Cat# 610517, RRID: AB_397879	1:1000	1:500
GFAP	Astrocytes	Sigma, Cat# G3893, RRID: AB_477010	1:500	1:500
Iba1	Microglia	Abcam, Cat# ab5076, RRID: AB_2224402	1:500	1:500
Islet1	ON bipolar cells, starburst amacrine cells, subset of retinal ganglion cells	R&D system, Cat# AF1837, RRID: AB_2126324	1:2000	1:1000
NG2	Pericytes	Abcam, Cat# ab129051, RRID: AB_2877152	1:1000	1:500
PKC α	Rod bipolar cells	Abcam, Cat# ab31, RRID: AB_303507	1:500	1:500
PSD95	Photoreceptor terminals	Abcam, Cat# ab12093, RRID: AB_298846	1:500	1:500
RIBEYE	Ribbon synapses	Synaptic system, Cat#192103, RRID: AB_2086775	1:500	1:500
Secretagogin (SCGN)	Cone bipolar cells	BioVendor, Cat# RD181120100, RRID: AB_2034060	1:1000	1:500
Tau	Microtubule-associated protein	Proteintech, Cat#66499-1-Ig, RRID: AB_2881863	1:1000	1:500
α -Tubulin	α -Tubulin protein	Sigma, Cat#T5168-.2ML, RRID: AB_477579	1:1000	1:500
Tomm20	Mitochondria	Abcam, Cat# ab78547, RRID: AB_2043078	1:1000	1:500
Tyrosine hydroxylase (TH)	Dopaminergic amacrine cell subset	EMD Millipore, Cat# AB1542, RRID: AB_90755	1:2000	1:500
Vesicular glutamate transporter 1 (VGlut1)	Photoreceptor ribbon synapses	Abcam, Cat# ab77822, RRID: AB_2187677	1:500	1:250
Vesicular glutamate transporter 3 (VGlut3)	Subset of amacrine cells	Millipore, Cat#AB5421, RRID: AB_2187832	1:1000	1:500

Supplemental Table 2: Summary of condition variations tested for RAIN-STORM, Related to Figure 1.

Stage	Parameter	Variations	Specifics	Total Localizations Acquired	Background Localizations	AF647 XY Res	CF568 XY Res		
Tissue Preparation	Primary Fixation	Conc./Temp	1% PFA, RT, 60min	8213357	2254413	55.7234	39.7570		
			2% PFA, RT, 60 min	8970285	2398174	50.3231	34.2027		
			4% PFA, RT, 60 min	9146043	3144002	60.1768	36.6071		
			4% PFA, 4C, 60 min	6779812	2732175	56.5597	35.5531		
			0% PFA, 0.3% GA, RT, 60 min	4914685	3059107	38.5014	27.1380		
		Type	2% PFA, 0.3% GA, RT, 60 min	5030786	3181862	45.1080	29.3187		
			4% PFA, RT, 30 min	8642310	3722096	54.3354	31.3137		
			4% PFA, RT, 120 min	10108757	3498390	48.5256	32.5775		
			10mM Glycine	9761923	2841048	51.7930	31.7254		
			100mM Glycine	12209377	3525533	63.2148	37.2768		
	Primary Quenching	Conc./Type	10mM NH3Cl	9751623	3043674	50.2599	39.2483		
			100mM NH3Cl	11649151	3425099	55.7147	37.4105		
			0.1% NaBH4	10066425	2222857	53.5908	37.7183		
			0.5% NaBH4	11155141	2673369	52.2041	32.6448		
			Embedding Method	Thickness	10µm	9146043	3144002	60.1768	36.6071
			20µm	3775049	2494498	63.0147	66.6873		
	Staining	Blocking Buffer	Serum	1% NDS	12554580	3953868	59.1086	29.2762	
				3% NDS	14772243	4423472	58.0771	29.6001	
				5% NDS	10276383	3368744	53.7408	30.4973	
				10% NDS	9029693	2928266	69.5874	34.9191	
15% NDS				13108963	3920780	48.8157	32.4246		
0.1% Triton				9958189	3309736	57.0414	25.8641		
Permeabilizer Type			0.2% Triton	13145415	4107653	60.7598	35.5746		
			0.5% Triton	9849749	3040177	57.1852	35.4691		
			1.0% Triton	10455550	3467400	50.3823	30.9056		
			2.0% Triton	10179749	3443009	50.8491	27.7015		
			0.1% Saponin	7443945	3638982	43.9059	26.9680		
			0.3% Saponin	6084083	2769395	48.9074	33.9253		
			Permeabilizer Type	0.5% Saponin	6343524	2916079	64.1917	41.0107	
				1.0% Saponin	7614606	3039943	50.7969	32.2521	
				2.0% Saponin	6171508	2849209	71.0758	39.7984	
				Time	10m Block	6165974	2671417	41.6942	37.3808
				Temp/Time	4°C, 30 min	5067694	2089764	45.6442	35.7788
					4°C, 120 min	5032729	2042575	45.9341	35.4185
Temp/Time			RT, 30 min	5802683	2586804	43.9987	42.6722		
			RT, 120 min	5932099	2392430	48.0725	34.6311		
Primary Antibody			Protein Targets	Cabeladin					
				CAR					
				PKCa					
				Rbeye					
				PSD95					
				Desmin					
				Iba1					
				SCGN					
				Dystrophin					
				GS					
		GFAP							
		Islat1							
		Connexin43							
		a-Tubulin							
		Tom20							
		CD31							
		Collagen IV							
		VGLU1							
		VGLU3							
		Tau							
		NC2							
		AF647 1:100		6073156	1867141	65.7862			
		AF647 1:1000		3739319	1475533	43.9605	N/A		
		AF647 1:5000		1188206	478033	38.4452			
		CF568 1:100		6320942	2239627		30.8011		
		CF568 1:1000		3430498	1453105	N/A	35.4525		
		CF568 1:5000		855764	445694		29.7523		
		Secondary Antibody		Fluorophore/Conc.	1% PFA, 10 min	6260957	2373438	46.1414	33.8572
					2% PFA, 10 min	6750477	2604387	48.1056	32.2951
					4% PFA, 10 min	8659132	3367457	54.9271	36.2804
1% PFA, 30 min			5374777		2091335	55.9734	33.1415		
2% PFA, 30 min			7270814		2700660	54.2458	42.6215		
Conc./Time			4% PFA, 30 min	8267553	2996594	47.6018	35.7969		
			10mM Glycine	5330971	1691966	65.6889	55.4278		
			100mM Glycine	5636215	1918352	46.5097	44.4271		
			10mM NH3Cl	4791254	2077605	55.9535	46.1726		
			100mM NH3Cl	8210985	1932665	52.5240	43.5233		
GLOX		Catalase	0mM BME	13030311	3553994	78.36503	61.88567		
			71.5mM BME	10612112	2538832	70.96677	46.30657		
			143mM BME	11190641	2723165	88.47950	68.97667		
			286mM BME	9332914	2006797	73.10220	72.54603		
			0U Catalase	7104374	1453545	77.87603	71.28623		
			20U Catalase	7137281	2047426	72.29090	76.41330		
			100U Catalase	9595769	2075109	84.14287	65.44140		
			500U Catalase	10195843	2537068	71.02247	64.64657		
			Glucose Ox.	0U Glu. Ox.	7104374	1453545	77.87603	71.28623	
				10U Glu. Ox.	7137281	2047426	72.29090	76.41330	
		20U Glu. Ox.		9595769	2075109	84.14287	65.44140		
		40U Glu. Ox.		10195843	2537068	71.02247	64.64657		
		0mM MEA		10036933	2362114	82.48007	67.48763		
		10mM MEA		11484612	2540791	74.56673	75.99387		
		20mM MEA		8917762	2307084	71.94410	66.66033		
		40mM MEA		9962371	2736120	73.49553	75.60517		
		0mM BME		6344927	2172067	63.94573	44.79663		
		71.5mM BME		6632177	2110500	75.79637	55.62803		
		PPT	BME	143mM BME	6865185	1935796	71.76753	63.42270	
				286mM BME	6990130	2025152	64.24200	49.40647	
				0U PCD	1878393	1100339	63.68107	42.81973	
				-0.09U PCD	3218723	1402939	68.00433	55.09720	
				-0.17U PCD	3178465	1258718	71.34050	60.59277	
			PCD	-0.35U PCD	2138172	1068920	53.55533	44.69093	
				0mM MEA	4388805	1545313	53.09887	54.86183	
				10mM MEA	4151177	1465681	73.34697	68.12620	
				20mM MEA	3063824	1336807	70.74163	56.03840	
				40mM MEA	2834421	1417678	58.42780	54.92740	
		PCA	MEA	0mM PCA	1501576	99725	62.61477	45.58707	
				1mM PCA	4425433	1679832	74.27553	74.12297	
2mM PCA				3473750	1608543	72.83673	55.05123		
4mM PCA				2099282	1104718	72.42687	63.55333		
0mM BME				6344927	2172067	63.9457	44.7966		
POC			BME	71.5mM BME	6632177	2110500	75.7964	55.6280	
				143mM BME	6865185	1935796	71.7675	63.4227	
				286mM BME	6990130	2025152	64.2420	49.4065	
				0U Py. Ox.	6160037	1606622	69.9593	44.6356	
				1U Py. Ox.	7348113	1753847	85.9037	59.7095	
		Pyranose Ox.	5U Py. Ox.	6057926	1699933	86.5024	60.1876		
			10U Py. Ox.	7268535	2161980	82.4528	59.1651		
			0mM MEA	8338811	2172407	85.3317	60.0705		
			10mM MEA	6045768	1873840	77.7011	52.7179		
			20mM MEA	6072488	2293750	73.6791	55.3963		
Electron Sinks	Conc./Type	40mM MEA	6479168	1771027	74.9191	50.7731			
		0U Catalase	6053582	1916488	67.6008	57.5033			
		20U Catalase	6486951	2078212	88.8967	68.1553			
		100U Catalase	7462499	2963145	83.4481	59.2703			
		500U Catalase	8097364	2179157	79.8785	50.1370			
	Conc./Type	1mM Trolox	10297129	2172781	76.4754	82.0630			
		2mM Trolox	5733708	1578968	75.8681	85.4778			
		5mM Trolox	2270131	862326	70.5364	70.7546			
		1mM COT	7249814	2213086	68.4071	53.4930			
		2mM COT	8668903	1677820	79.6458	78.9307			
5mM COT	7412183	1543031	76.8966	62.1923					
Optimized RAIN-STORM				12804183.3333	7082472.0000	61.1141	31.2234		
2% PFA 4C 60 min, 100mM Gly 4C 60min, 5% NDS, 0.5% Triton, 60min RT Block, 1.500 Ph DI 12h, 1:100 Sec Di 60min, 4% PFA 30min, 100mM NH4Cl 30min, 1U Py. Ox, 143mM BME, 200U Catalase, 40mM MEA, 2mM COT, 200nm Step size, 250f, 3 cycle, 0.16µm, 25p									
85000					1.50E+07	localizations			
20					90	nm			
Abbreviations									
RT	room temperature								
U	Units								