Supplementary Information

Hybrid Microscopy: Enabling Inexpensive High-Performance Imaging through Combined Physical and Optical Magnifications

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Supplementary Table 1. Hydrogel solution recipe.

	Stock concentration	Amount added for plated cells and bacteria (µL)	Amount added for brain slices (µL)
Sodium acrylate	33% (w/w)	227	227
Acrylamide	50% (w/w)	50	50
N,N′-Methylenebisacrylamide	2% (w/w)	75	75
Sodium chloride	5 M	400	400
PBS	10x	100	100
4-hydroxy-2,2,6,6-tetramethylpiperidin-1-oxyl	1% (w/w)	0	10
Ammonium persulfate	10% (w/w)	20	20
Tetramethylethylenediamine	10% (v/v)	20	20
Deionized water		108	98
Final volume		1000	1000

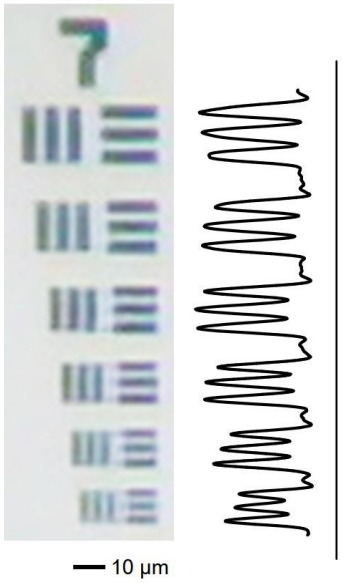
Supplementary Table 2. Cost analysis of one single sample preparation process at a working volume of 100 µL, which is sufficiently large for observation using the mini-microscope.

Product Name	Vendor	Product number	Price ($)	Amount	Unit	Amount used / single sample	Cost / single sample ($)
Antibody and DNA							
Primary antibody	Millipore	ab16901	285.00	250	µL	1	1.14
Second antibody	Jackson ImmunoResearch Laboratory	703-005-155	68.00	1000	µL	1	0.068
DNA, for antibody conjugation	IDT	N/A	118.00	665	nmol	0.067	0.012
DNA, tertiary linker 1	IDT	N/A	616.5	60.4	nmol	0.007	0.069
DNA, tertiary linker 2	IDT	N/A	616.5	72.6	nmol	0.007	0.057
Reagents for DNA antibody conjugation							
Desalting column	ThermoFisher	89882	122.00	25	EA	0.02*	0.098
Centrifugal concentrator	Sigma	Z614009	126.30	25	EA	0.01**	0.051
Centrifugal filter	Sigma	Z648043	133.00	24	EA	0.01**	0.055
Sulfo-S-4FB Crosslinker	Solulink	S-1008-010	265.00	10	mg	0.0004	0.009
S-HyNic Crosslinker	Solulink	S-1002-105	165.00	5	mg	0.00006	0.002
Chemicals for gelation							
Sodium acrylate	Sigma	408220	58.80	25	g	0.008	0.018
Acrylamide	Sigma	A9099	63.30	100	g	0.003	0.002
N,N′-Methylenebisacrylamide	Sigma	M7279	49.30	25	g	0.0002	0.0003
Ammonium Persulfate	Sigma	A3678	28.30	25	g	0.0002	0.0002
N,N,N′,N′-Tetramethylethylenediamine	Sigma	T7024	37.90	25	mL	0.0002	0.0003
4-Hydroxy-TEMPO	Sigma	176141	27.50	1	g	0.0001	0.003
Chemicals for immunostaining							
Dextran Sulfate	Millipore	S4030	138.00	100	mL	0.04	0.055
SSC	Life Technologies	15557	45.00	1000	mL	0.02	0.001
Yeast tRNA	Roche	10109495001	127.00	100	mg	0.2	0.254
Normal Donkey	Jackson	017-000-	20.00	2	mL	0.04	0.4

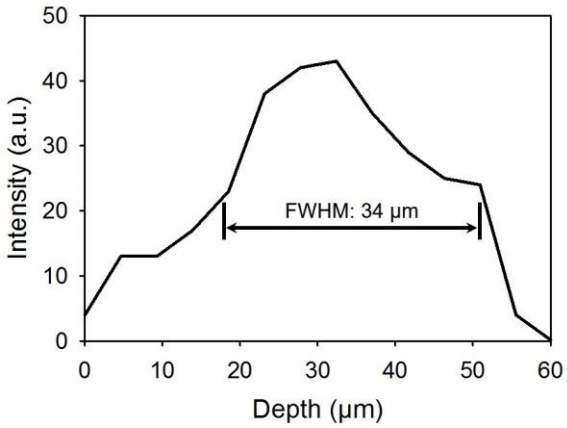
Serum	Immunoresearch	001					
Chemicals for digestion							
Proteinase K	New England Biolabs	P8107S	73.00	2	mL	0.001	0.037
Ethylenediaminetetraacetic acid	Sigma	EDS	21.40	100	g	0.00003	0.000006
Guanidine HCl	Sigma	G3272	34.90	25	g	0.0077	0.011
Tris-HCl, 1M pH 8.0	Life Technologies	AM9855	49.00	100	mL	0.005	0.0002
Chemicals for fixation							
Paraformaldehyde	Electron Microscopy Sciences	15710	26.00	100	mL	0.025	0.007
Triton X-100	Sigma	X100	35.60	100	mL	0.0004	0.0001
Glycine	Sigma	50046	19.20	50	g	0.0023	0.0009
PBS (10x)	Life Technologies	70011-044	40.00	500	mL	0.17	0.014
Total							**2.365**

[*] Two columns are used to conjugate 100 µL secondary antibody with DNA.

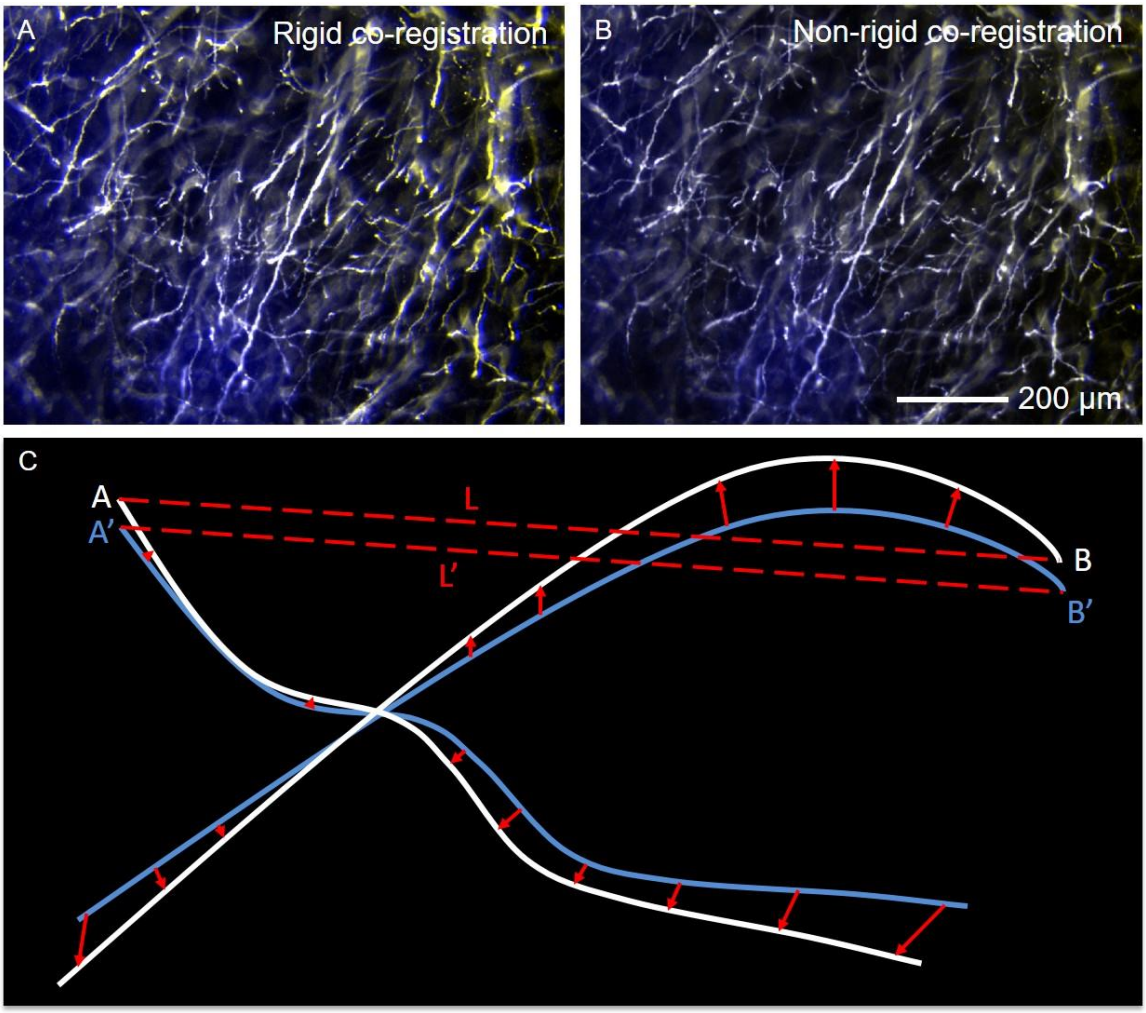
[**] One concentrator and filter are used to conjugate 100 µL secondary antibody with DNA.



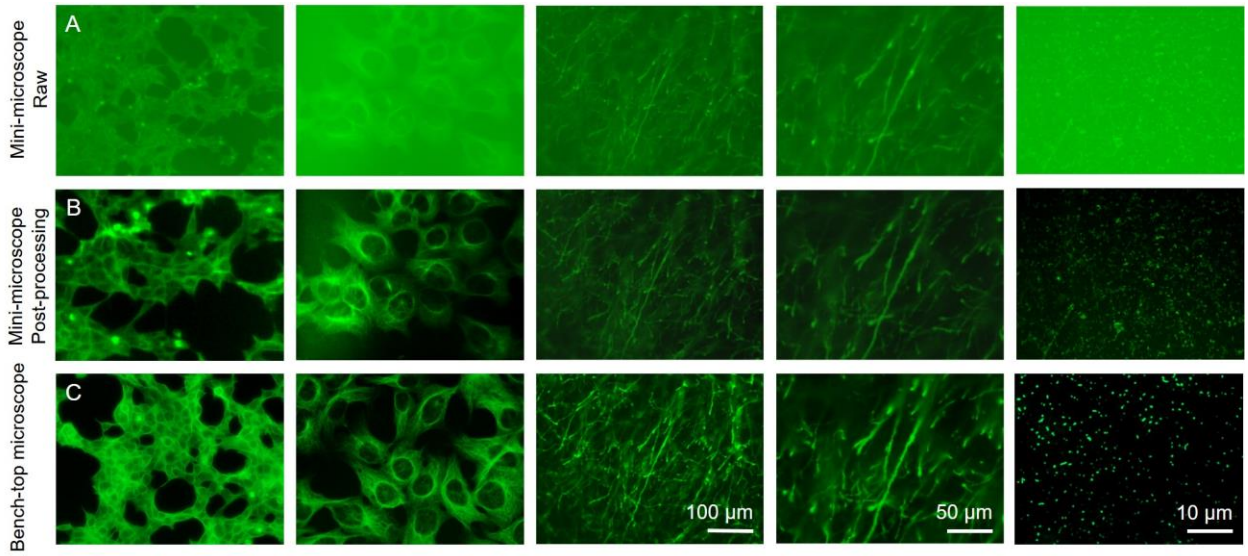
Supplementary Figure 1: Resolution determination of mini-microscope. Mini-microscope image of Group 7 resolution target and line profiles showing clear separation of the peaks between adjacent lines. The thinnest line width at the bottom is 2.19 µm.



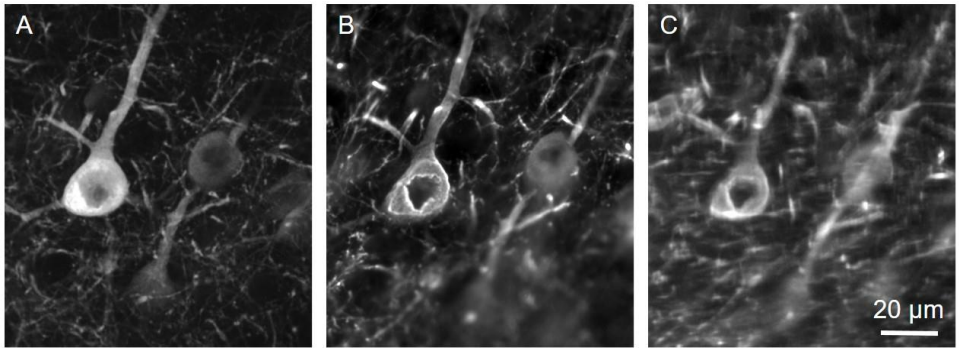
Supplementary Figure 2: Measurement of the PSF of the mini-microscope. The axial resolution of the mini-microscope was characterized to be approximately 34 μm based on the FWHM.



Supplementary Figure 3: Principle of RMS error quantification. (**A**,**B**) Rigid and non-rigid co-registration of ExMM and ExM at 10X magnification (see Fig. 4A,B). (**C**) The blue lines, representing structures in the ExMM image, are mapped to the white lines, representing the structures in the ExM image, via the vector field depicted by black arrows. Measurement L' along the line segment A'B' in the ExM image is mapped to measurement L along the line segment AB in the ExMM image. The ExM error is calculated as |L-L'|; i.e. the difference between the deformation vectors AA' and BB'. The generated deformation field generated can then be used to calculate the RMS error between all extracted features in the ExMM and ExM images.



Supplementary Figure 4: Comparison between mini-microscope and benchtop microscope images. (**A**,**B**) Raw and processed images obtained with the mini-microscope, respectively, and (**C**) raw images obtained with the benchtop microscope.



Supplementary Figure 5: Comparison among confocal, benchtop, and mini-microscope images. (**A**) Confocal image pre-expansion; NA=1.15 (water-immersion objective, 40X). (**B**) Benchtop microscope image post-expansion; objective: 10X, NA=0.25. (**C**) ExMM image at 10X (NA~0.32).