

Highly-Multiplexed Tissue Imaging with Raman Dyes

 Lixue Shi¹, Mian Wei¹, Wei Min^{1,2}
¹Department of Chemistry, Columbia University ²Kavli Institute for Brain Science, Columbia University

Corresponding Author

 Wei Min
 wm2256@columbia.edu

Citation

 Shi, L., Wei, M., Min, W. Highly-Multiplexed Tissue Imaging with Raman Dyes. *J. Vis. Exp.* (182), e63547, doi:10.3791/63547 (2022).

Date Published

April 21, 2022

DOI

10.3791/63547

URL

jove.com/video/63547

Materials

Name	Company	Catalog Number	Comments
16% Paraformaldehyde, EM Grade	Electron Microscopy Sciences	15710	
α -tubulin	Abcam	ab18251	Primary antibodies
α -tubulin	BioLegend	625902	Primary antibodies
β -III-tubulin	BioLegend	657402	Primary antibodies
β -III-tubulin	Abcam	ab41489	Primary antibodies
β -tubulin	Abcam	ab131205	Primary antibodies
Agarose, low gelling temperature	Sigma Aldrich	A9414	For brain embedding
Anti- α -tubulin antibody produced in rabbit (α -tubulin)	Abcam	ab52866	Primary antibodies
Anti-Calbindin antibody produced in mouse (Calbindin)	Abcam	ab82812	Primary antibodies
Anti-GABA B receptor R2 antibody produced in guinea pig (GABA B receptor R2)	Millipore Sigma	AB2255	Primary antibodies
Anti-GFAP antibody produced in goat (GFAP)	Thermo Scientific	PA5-18598	Primary antibodies
Anti-Glucagon antibody produced in mouse (Glucagon)	Santa Cruz Biotechnology	sc-514592	Primary antibodies
Anti-insulin antibody produced in guinea pig (insulin)	DAKO	IR00261-2	Primary antibodies
Anti-MBP antibody produced in rat (MBP)	Abcam	ab7349	Primary antibodies
Anti-NeuN antibody produced in rabbit (NeuN)	Thermo Scientific	PA5-78639	Primary antibodies
Anti-Pancreatic polypeptide (PP) antibody produced in goat-Pancreatic polypeptide (PP)	Sigma Aldrich	SAB2500747	Primary antibodies
Anti-Pdx1 antibody produced in rabbit (Pdx1)	Millipore	06-1379	Primary antibodies
Anti-Somatostatin antibody produced in rat (Somatostatin)	Abcam	ab30788	Primary antibodies
Anti-Vimentin antibody produced in chicken (Vimentin)	Abcam	ab24525	Primary antibodies
Band-pass filter	KR Electronics	KR2724	8 MHz
BNC 50 Ohm Terminator	Mini Circuits	STRM-50	

BNC cable	Thorlabs	2249-C	Coaxial Cable, BNC Male / Male
Broadband dielectric mirror	Thorlabs	BB1-E03	750 - 1100 nm
C57BL/6J mice	Jackson Laboratory	000664	
Centrifuge			
Condenser	Olympus		oil immersion, 1.4 N.A.
Cytokeratin 18	Abcam	ab7797	Primary antibodies
Cytokeratin 18	Abcam	ab24561	Primary antibodies
DC power supply	TopWard	6302D	Bias voltage is 64 V
Dichroic mount	Thorlabs	KM100CL	Kinematic Mount for up to 1.3" (33 mm) Tall Rectangular Optics, Left Handed
Donkey anti-Chicken IgY (H+L)	Jackson ImmunoResearch	703-005-155	Secondary antibodies for MARS conjugation
Donkey anti-Goat IgG (H+L)	Jackson ImmunoResearch	705-005-147	Secondary antibodies for MARS conjugation
Donkey anti-Guinea Pig IgG (H+L)	Jackson ImmunoResearch	706-005-148	Secondary antibodies for MARS conjugation
Donkey anti-Mouse IgG (H+L)	Jackson ImmunoResearch	715-005-151	Secondary antibodies for MARS conjugation
Donkey anti-Rabbit IgG (H+L)	Jackson ImmunoResearch	711-005-152	Secondary antibodies for MARS conjugation
Donkey anti-Rat IgG (H+L)	Jackson ImmunoResearch	712-005-153	Secondary antibodies for MARS conjugation
Donkey anti-Sheep IgG (H+L)	Jackson ImmunoResearch	713-005-147	Secondary antibodies for MARS conjugation
DPBS	Fisher Scientific	14-190-250	
EpCAM	Abcam	ab71916	Primary antibodies
Ethanol	Sigma Aldrich	443611	
Fast-speed look-in amplifier	Zurich Instruments	HF2LI	DC - 50 MHz
FFPE Kidney Sample	USBiomax	HuFPT072	
Fibrillarin	Abcam	ab5821	Primary antibodies
Giantin	Abcam	ab24586	Primary antibodies
Glucagon	Santa Cruz Biotechnology	sc-514592	Primary antibodies
H2B	Abcam	ab1790	Primary antibodies
HeLa	ATCC	ATCC CCL-2	
High O.D. bandpass filter	Chroma Technology	ET890/220m	Filter the Stokes beam and transmit the pump beam
Hydrophobic pen	Fisher Scientific	NC1384846	
Insulin	ThermoFisher	701265	Primary antibodies
Integrated SRS laser system	Applied Physics & Electronics, Inc.	picoEMERALD	picoEMERALD provides an output pulse train at 1,064 nm with 6-ps pulse width and 80-MHz repetition rate, which serves as the Stokes beam. The frequency doubled beam at 532 nm is used to synchronously seed a picosecond optical parametric oscillator (OPO) to produce a mode-locked pulse train with five~6 ps pulse width (the idler beam of the OPO is blocked with an interferometric filter). The output wavelength of the OPO is tunable from 720–950 nm, which serves as the pump beam. The intensity of the 1,064-nm Stokes beam is modulated sinusoidally by a built-in EOM at 8 MHz with a modulation

			depth of more than 90%. The pump beam is spatially overlapped with the Stokes beam by using a dichroic mirror inside picoEMERALD. The temporal overlap between pump and Stokes pulse trains is achieved with a built-in delay stage and optimized by the SRS signal of pure D2O at the microscope.
Inverted laser-scanning microscope	Olympus	FV1200MPE	
Kinematic mirror mount	Thorlabs	POLARIS-K1-2AH	2 Low-Profile Hex Adjusters
Lectin from Triticum vulgare (wheat)	Sigma Aldrich	L0636-5 mg	
Long-pass dichroic beam splitter	Semrock	Di02-R980-25x36	980 nm laser BrightLine single-edge laser-flat dichroic beamsplitter
MAP2	BioLegend	801810	Primary antibodies
Microscopy imaging software	Olympus	FluoView	
NanoQuant Plate	Tecan		For absorbance-based, small volume analyses in a plate reader.
Normal donkey serum	Jackson ImmunoResearch	017-000-121	
NucBlue Fixed Cell ReadyProbes Reagent (DAPI)	Thermo Scientific	R37606	
Nunc 4-Well Dishes	Fisher Scientific	12-566-300	
Objective lens	Olympus	XLPlan N	x25, 1.05-NA, MP, working distance = 2 mm
Paint brush			
Periscope assembly	Thorlabs	RS99	includes the top and bottom units, Ø1" post, and clamping fork.
pH meter			
Plate reader	Tecan	Infinite 200 PRO	An easy-to-use multimode plate reader. Absorbance measurement capabilities over a spectral range of 230–1000 nm.
ProLong Gold antifade reagent	Thermo Scientific	P36930	
PSD95	Invitrogen	51-6900	Primary antibodies
Sephadex G-25 Medium	GE Life Sciences	17-0033-01	gel filtration resin for desalting and buffer exchange
Shielded box with BNC connectors	Pomona Electronics	2902	Aluminum Box With Cover, BNC Female/Female
Si photodiode	Thorlabs	FDS1010	350–1100 nm, 10 mm x 10 mm Active Area
Synapsin 2	ThermoFisher	OSS00073G	Primary antibodies
Tissue Path Superfrost Plus Gold Slides	Fisher Scientific	22-035813	Adhesive slide to attract and chemically bond fresh or formalin-fixed tissue sections firmly to the slide surface (tissue binding glass slides)
Triton X-100	Fisher Scientific	BP151-500	
Vibratome	Leica	VT1000	
Vimentin	Abcam	ab8069	Primary antibodies
Xylenes	Sigma Aldrich	214736	