

Materials List for

# Real-Time, Two-Color Stimulated Raman Scattering Imaging of Mouse Brain for Tissue Diagnosis

Robert Espinoza<sup>\*1</sup>, Brian Wong<sup>\*1</sup>, Dan Fu<sup>1</sup>

<sup>1</sup>Department of Chemistry, University of Washington

\* These authors contributed equally

## Corresponding Author

Dan Fu  
danfu@uw.edu

## Citation

Espinoza, R., Wong, B., Fu, D. Real-Time, Two-Color Stimulated Raman Scattering Imaging of Mouse Brain for Tissue Diagnosis. *J. Vis. Exp.* (180), e63484, doi:10.3791/63484 (2022).

## Date Published

February 1, 2022

## DOI

10.3791/63484

## URL

jove.com/video/63484

## Materials

Name	Company	Catalog Number	Comments
100 mm Achromatic Lens	THORLABS	AC254-100-B	Broadband, 650 - 1,050 nm, achromatic lens focal length, 100 mm
20 MHz bandpass filter	Minicircuits	BBP-21.4+	Lumped LC Band Pass Filter, 19.2 - 23.6 MHz, 50 Ω
200 mm Achromatic Lens	THORLABS	AC254-200-B	Broadband, 650 - 1,050 nm, achromatic lens focal length, 200 mm
Achromatic Half Waveplate	Union Optic	WPA2210-650-1100-M25.4	Broadband half waveplate
Achromatic Quarter Waveplate	Union Optic	WPA4210-650-1100-M25.4	Broadband quarter waveplate
Beam Sampler	THORLABS	BSN11	10:90 Plate Beamsplitter
Dichroic Mirror	THORLABS	DMSP1000	Other dichroics with a center wavelength around 1,000 nm can be used.
DMSO (Dimethyl sulfoxide)	Sigma Aldrich	472301	Solvent for calibration of Raman shift. Other solvents with known Raman peaks can be used.
Electrooptic Amplitude Modulator	THORLABS	EO-AM-NR-C1	Two EOMs are needed for orthogonal modulation and dual-channel imaging. Resonant version is recommended so lower driving voltage can be used.
False H&E Staining Script	Matlab		<a href="https://github.com/TheFuGroup/HE_Staining">https://github.com/TheFuGroup/HE_Staining</a>
Fanout Buffer	PRL-414B	Pulse Research Lab	1:4 TTL/CMOS Fanout Buffer and Line Driver, for generating the EOM driving frequency and the reference to the lock-in
Fast Photodiode	THORLABS	DET10A2	Si Detector, 1 ns Rise Time
Frequency Divider	PRL-220A	Pulse Research Lab	TTL Freq. Divider (f/2, f/4, f/8, f/16), for generating 20MHz from the laser output.
Highly Dispersive Glass Rods	Union Optic	CYLROD01	High dispersion H-ZF52A Rod lens 120 mm, SF11 Rod lens 100 mm

Insight DS+	Newport		Laser system capable of outputting two synchronized pulsed lasers (one fixed beam at 1,040 nm and one tunable beam, ranging from 680-1,300 nm) with a repetition rate of 80 MHz.
Lock-in Amplifier	Liquid Instruments	Moku Lab	Lock-in amplifier to extract SRS signal from the photodiode. A Zurich Instrument HF2LI or similar instrument can be used as well.
Mirrors	THORLABS	BB05-E03-10	Broadband Dielectric Mirror, 750 - 1,100 nm. Silver mirrors can also be used.
Motorized Delay Stage	Zaber	X-DMQ12P-DE52	Delay stage for fine control of the temporal overlap of the pump and the Stokes lasers. Any other motorized stage should work.
Oil Immersion Condensor	Nikon	CSC1003	1.4 NA. Other condensers with NA>1.2 can be used.
Oscilloscope	Tektronix	TDS7054	Any other oscilloscope with 400 MHz bandwidth or higher should work.
Phase Shifter	SigaTek	SF50A2	For shifting the phase of the modulation frequency
Photodiode	Hamamatsu Corp	S3994-01	Silicon PIN diode with large area (10 x 10 cm <sup>2</sup> ). Other diodes with large area and low capacitance can be used.
Polarizing Beam Splitter	Union Optic	PBS9025-620-1000	Broadband polarizing beamsplitter
Refractive Index Database			refractiveindex.info
Retro-reflector	Edmund Optics	34-408	BBAR Right Angle Prism. Other prisms or retroreflector can be used.
RF Power Amplifier	Minicircuits	ZHL-1-2W+	Gain Block, 5 - 500 MHz, 50 Ω
Scan Mirrors	Cambridge Technologies	6215H	We used a 5mm mirror set with silver coating
ScanImage	Vidrio	ScanImage Basic	Laser scanning microscope control software
Shortpass Filter	THORLABS	FESH1000	25.0 mm Premium Shortpass Filter, Cut-Off Wavelength: 1,000 nm. For efficient suppression of the Stokes, two filters may be necessary.
Upright Microscope	Nikon	Eclipse FN1	Any other microscope frame can be used. If a laser scanning microscope is available, it can be used directly. Otherwise, a galvo scanner and scan lens needed to be added to the microscope.
Water Immersion Objective	Olympus	XLPLN25XWMP2	The multiphoton 25X Objective has a NA of 1.05. Other similar objectives can be used.