

Materials List for:

Excitation-Scanning Hyperspectral Imaging Microscopy to Efficiently Discriminate Fluorescence Signals

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URL: <https://www.jove.com/video/59448>

DOI: [doi:10.3791/59448](https://doi.org/10.3791/59448)

Materials

Name	Company	Catalog Number	Comments
Airway Smooth Muscle Cells	National Disease Research Interchange (NDRI)	Isolated from human lung tissues obtained from NDRI	Highly autofluorescent, calcium sensitive cells
Automated Shutter	Thorlabs Inc.	SHB1	Remote-controllable shutter to minimize photobleaching
Automated Stage	Prior Scientific	H177P1T4	Remote-controllable stage for automated multiple field of view or stitched image collection.
Automated Stage Controller (XY)	Prior Scientific	Proscan III (H31XYZ-ES)	For interfacing automated stage with computer and joystick
Buffer	Made in-house	Made in-house	145 mM NaCl, 4 mM KCl, 20 mM HEPES, 10 mM D-glucose, 1 mM MgCl ₂ , and 1mM CaCl ₂ , at pH 7.3
Cell Chamber	ThermoFisher Scientific	Attolfluor Cell Chamber, A7816	Coverslip holder composed of surgical stainless steel and a rubber O-ring to seal in media and prevent sample and/or objective contamination
Excitation Filters	Semrock Inc.	TBP01-378/16	Center wavelength range (340-378 nm), Bandwidth (Minimum 16 nm, nominal FWHM 20 nm), Refractive index (1.88)
	Semrock Inc.	TBP01-402/16	Center wavelength range (360-400 nm), Bandwidth (Minimum 16 nm, nominal FWHM 20 nm), Refractive index (1.8)
	Semrock Inc.	TBP01-449/15	Center wavelength range (400-448.8 nm), Bandwidth (Minimum 15 nm, nominal FWHM 20 nm), Refractive index (1.8)
	Semrock Inc.	TBP01-501/15	Center wavelength range (448.8-501.5 nm), Bandwidth (Minimum 15 nm, nominal FWHM 20 nm), Refractive index (1.84)
	Semrock Inc.	TBP01-561/14	Center wavelength range (501.5-561 nm), Bandwidth (Minimum 14 nm, nominal FWHM 20 nm), Refractive index (1.83)
Fluorescence Filter Cube Dichroic Beamsplitter	Semrock Inc.	FF495-Di03	Separates excitation and emission light at 495 nm (>98% reflection between 350-488 nm,>93% transmission between 502-950 nm), Filter effective index (1.78)

Fluorescence Filter Cube Longpass Filter	Semrock Inc.	FF01 496/LP-25	Allows passage of light longer than 496 nm (>93% average transmission between 503.2-1100 nm), Refractive index (1.86)
GCaMP Probe	Addgene	G-CaMP3; Plasmid #22692	A single-wavelength GCaMP2-based genetically encoded calcium indicator
Integrating Sphere	Ocean Optics	FOIS-1	Used for accurate measurement of wide-angle illumination
Inverted Fluorescence Microscope	Nikon Instruments	TE2000	Inverted microscopes allow direct excitation of sample without the need to penetrate layers of media and/or tissue.
Mitotracker Green FM	ThermoFisher Scientific	M7514	Labels mitochondria
NIST-Traceable Calibration Lamp	Ocean Optics	LS-1-CAL-INT	A lamp with a known spectrum for use as a standard
NIST-Traceable Fluorescein	ThermoFisher Scientific	F36915	For verifying appropriate spectral response of the system
NucBlue	ThermoFisher Scientific	R37605	Labels cell nuclei
Objective (10X)	Nikon Instruments	Plan Apo λ 10X/0.45 ∞ /0.17 MRD00105	Useful for large fields of view
Objective (20X)	Nikon Instruments	Plan Apo λ 20X/0.75 ∞ /0.17 MRD00205	Most often used for tissue samples
Objective (60X)	Nikon Instruments	Plan Apo VC 60X/1.2 Wl ∞ /0.15-0.18 WD 0.27	Most often used for cell samples
sCMOS Camera	Photometrics	Prime 95B (Rev A8-062802018)	For acquiring high-sensitivity digital images
Spectrometer	Ocean Optics	QE65000	Used to measure spectral output of excitation-scanning spectral system
Tunable Filter Changer	Sutter Instrument	Lambda VF-5	Motorized unit for automated excitation filter tuning/switching
Xenon Arc Lamp	Sunoptic Technologies	Titan 300HP Lightsource	Light source with relatively uniform spectral output