

Simultaneous Interference Reflection and Total Internal Reflection Fluorescence Microscopy for Imaging Dynamic Microtubules and Associated Proteins

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Materials

Name	Company	Catalog Number	Comments
10/90 (R/T) beam splitter	Thorlabs	BSN10R	Plane beam splitter used in the excitation beam path
90/10 (R/T) beam splitter	Thorlabs	BSX10R	Plane beam splitter used in the image splitter
Anti-biotin antibody	Sigma-Aldrich	B3640	Used to functionalize surface for bonding biotinylated microtubules
ATP	Sigma-Aldrich	FLAAS	Used for preparing the motility buffer
Band-pass filter	Newport	HPM535-50	Hard-coated band-pass filter is used in image splitter to image GFP signal
Biotinylated tubulin	Cytoskeleton, Inc.	T333P-A	Used to bind microtubule seeds to the surface of the flow channel
Casein	Sigma-Aldrich	C8654	Casein is used to block nonspecific interactions
Catalase	Sigma-Aldrich	C9322	Used for preparing the oxygen scavenger solution
Desiccator chamber	Southern Labware	55207	Desiccator is used for degassing the resin
DTT	Sigma-Aldrich	D0632	Used for preparing the oxygen scavenger solution
EGTA	Sigma-Aldrich	E4378	Used for preparing the BRB80 buffer
Glucose	Sigma-Aldrich	G7528	Used for preparing the oxygen scavenger solution
Glucose oxidase	Sigma-Aldrich	G7016	Used for preparing the oxygen scavenger solution
GMPCPP nucleotides	Jena Bioscience	NU-405L	Used for the polymerization of stabilized microtubules
Image splitter	Teledyne-Photometrics Imaging	OptoSplit II	An image splitter is used to split the images spatially. When buying, make sure about the compatibility with the microscope
ImageJ2	NIH		ImageJ2 is used for image analysis

Kinesin			prepared in house (see references in text)
LDPE tubing	Thomas Scientific	9565S22	Non-toxic, lower density polyethylene micro bore tubing is used for fluid transfers
LED light source	Lumencor	Lumencor sola light engine	Used for IRM imaging
Long-pass filters	Thorlabs	FELH0600	Hard-coated long pass filters. One is used as an excitation filter, other is used in image splitter to image IRM signal
Magnesium chloride	Sigma-Aldrich	63068	Used for preparing the BRB80 buffer
Microscope objective heater	okolab	H401-T-DUAL-BL	Used to keep sample temperature constant via heating the objective
Microscope	Nikon	Ti-Eclipse	An inverted microscope that is used in the experiments
Na-PIPES	Sigma-Aldrich	P2949	Used for preparing the BRB80 buffer
Nikon CFI Apochromat TIRF 100XC Oil objective	Nikon	MRD01991	The imaging objective has 1.49 numerical aperture
PDMS and curing agent	Electron Microscopy Sciences	Sylgard 184 (24236-10)	Used for constructing the flow channels
PDMS puncher	World Precision Instruments LLC	504529	Used to punch hole into the PDMS
Plasma cleaner	Harrick Plasma	DPC-32G	The air plasma is used to remove organic contamination from the PDMS surface
Poloxamer 407 (commercial name Pluronic F-127)	Sigma-aldrich	P2443	Used for channel surface passivation to minimize nonspecific binding
Sodium hydroxide	Sigma-Aldrich	567530	Used for preparing the BRB80 buffer
Stabilized microtubules			prepared in house (see references in text)
Table-top ultracentrifuge	Beckman Coulter	340400	Used to spin down microtubule seeds
TetraSpeck beads	ThermoFisher Scientific	T7279	Used as a reference for aligning images
Zyla 4.2 camera	Andor	Zyla 4.2	Scientific CMOS camera with specifications: 2048 x 2048 pixels (6.5 μ m pixel size) with quantum efficiency of 72% and 16 bit dynamic range