

Materials List for:

Preparation of Segmented Microtubules to Study Motions Driven by the Disassembling Microtubule Ends

Vladimir A. Volkov^{1,2}, Anatoly V. Zaytsev³, Ekaterina L. Grishchuk³

¹Center for Theoretical Problems of Physicochemical Pharmacology, Russian Academy of Sciences

²Federal Research Center of Pediatric Hematology, Oncology and Immunology, Moscow, Russia

³Physiology Department, Perelman School of Medicine, University of Pennsylvania

Correspondence to: Ekaterina L. Grishchuk at gegate@mail.med.upenn.edu

URL: <https://www.jove.com/video/51150>

DOI: [doi:10.3791/51150](https://doi.org/10.3791/51150)

Materials

Name	Company	Catalog Number	Comments
Table 1. Microscopy and other equipment.			
Microscope	Zeiss Nikon	Axio Imager 2 Eclipse Ti	other microscope models capable of DIC and epifluorescence-imaging can be used
Objective	Zeiss Nikon	420490-9900-000 CFI Apo 100x Oil 1.49	100X, DIC, 1.3-1.49 NA
Objective heater	Biophtechs	150803, 150819-19	
Fluorescent filter cube	Chroma	49004 or 49008 41017 or 49020	optimized for Rhodamine fluorescence optimized for GFP fluorescence
Acquisition software	freeware MicroManager Molecular Devices	not applicable MetaMorph 7.5	http://valelab.ucsf.edu/~MM/MMwiki/ other software can be used to acquire images and for a particle tracking
EMCCD camera	Andor	iXon3, DU-897E-cs0-#BV	Highly sensitive EMCCD camera
Trapping laser	IPG Photonics	YLR-10-1064-LP	1,064 nm laser, 10 W
Fluorescence excitation lasers	Coherent, Inc. Coherent, Inc.	Sapphire 488 LP Sapphire 552 LP	excitation of green fluorophores excitation of red fluorophores
Plasma Cleaner	Harrick Plasma	PDC-001	
Commercial flow chambers	Warner Instruments	RC-20 or RC-30	
Perfusion pump	Cole Palmer Harvard Apparatus	Masterflex 77120-52 Pico Plus	Both pumps provide the required rate of liquid flow but a peristaltic pump may pulse at very slow speed. The flow with a syringe pump is more consistent for a wide range of rates but this pump has inertia.
Table 2. Microscopy chamber preparation.			
Modified microscope slides for reusable chambers	Precision Glassblowing of Colorado	Custom order www.precisionglassblowing.com	Sonic slots in slides using schematics in Figure 1
Polyethylene tubing	Intramedic	427410	I.D. 0.58 mm, O.D. 0.965 mm; use these tubes to connect assembled chamber to the pump and waste container
Polyethylene tubing	Intramedic	427400	I.D. 0.28 mm, O.D. 0.61 mm; use these tubes to make the reusable chamber
Regular microscope slides	VWR	48312-003	Other similar slides can be used

Coverslips	VWR	48393-150, 48366-067	Other similar coverslips can be used
Silicon sealant	World Precision Instruments	KIT, SILICON SEALANT 5 MIN CURE	
Epoxy glue	Loctite	83082	
Cyanoacrylate adhesive	Scotch 3M	AD114	Or cyanoacrylate adhesive from other manufacturers

Table 3. Coverslips cleaning and coating.

Molecular Sieves, Grade 564	Macron	4490-04	
Coverglass Staining Jar	Ted Pella, Inc.	21036	
Coverslip Ceramic Holder	Thomas Scientific	8542e40	
PlusOne Repel Silane	GE Healthcare Biosciences	17-1332-01	
Pluronic F-127	Sigma-Aldrich	P2443	
Anti-digoxigenin AB	Roche Applied Science	11093274910	

Table 4. Preparation of seeds and segmented microtubules.

Tubulin	purified from cow brains Cytoskeleton, Inc	T238P	For purification protocols see ⁴⁹⁻⁵¹ Unlabeled porcine tubulin
Labeled tubulin	Cytoskeleton, Inc Invitrogen Invitrogen	TL590M C1171 (Rhodamine) A-2952 (Digoxigenin)	Rhodamine-labeled porcine tubulin Tubulin can be labeled with any amine-reactive dye as in reference ⁵² .
GMPCPP	Jena Biosciences	NU-405	Aliquot and store at -70 °C
VALAP	Vaseline, lanolin, and paraffin at 1:1:2 by mass		see reference ⁹