

Use of Dual Optical Tweezers and Microfluidics for Single-Molecule Studies

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Materials

Name	Company	Catalog Number	Comments
100x objective	Leica	506318 or 506038	Oil immersion lenses; Imaging and optical trapping only; Plan APO objectives optimized for fluorescence imaging
10X Objective	Leica	506263	Used to locate laser beams spots during alignment; to find focus and X-Y position in flow cell
1 mm fluorescent beads	Bangs Labs	FSDG004	Used for trap performance, focal position determination
1 mm polystyrene beads	Bangs Labs	CPO1004	Used for trap performance evaluation and binding to biotinylated molecules
63x objective	Leica	506081	Used to locate laser beams spots during alignment and to find focus and X-y position in flow cell; can be used for optical trapping as it has an identical back aperture diameter to the 100X; oil immersion lens
Alignment laser	Lumentum	1100 series	10mW HeNe laser that is visible to the naked eye that is used to position optics
Beam alignment camera	Amscope	MU303	A simple, inexpensive and software controlled camera for imaging of the beam position
Camera control and Image capture software	Hamamatsu	HCIImage	Coordinates activities of the Lambda DG4 with the camera to facilitate rapid wavelength switching
Camera; Orca flash 4	Hamamatsu	c13440-20cu	CCD camera for imaging of single-molecule experiments
C-mount for the beam alignment camera	Spot imaging solutions	DE50CMT	Provides optimal positioning of the camera for imaging of laser beams during alignment
C-mount for the Orca Flash 4 camera			Has a retainer ring to hold an IR blocking filter in place. This eliminates reflected IR beam from the optical traps and facilitates clearer imaging of trapped objects.

Cy5 fluorescence filter cube	Semrock	cy5-404a-lsc-zero	Used in conjunction with Lambda DG4 to image Cy5 only
FITC-TxRed fluorescence filter cube	Semrock	fitc/txred-2x-b-000	Used in conjunction with Lambda DG4 to image FITC and TXRed
Fluidics tubing	Grace Bio	46004	PTFE tubing as an alternate to PEEK; works well on some flow cells. Can be used with PDMS flow cells or glass flow cells when Grace Bio fit tubing connectors are used
GFP fluorescence filter cube	Semrock	gfp-3035b-lsc-zero	Used in conjunction with Lambda DG4 to image GFP only
Glass flow cells	Translume	Custom	Clear flow channels for imaging (Fig. 2E)
Glass glue	Loctite	233841	Securely and easily bonds Nanoport assemblies to glass flow cells
Glass/PDMS sandwich flow cells	CIDRA Precision services	Custom design	Flow cells built according to your specifications; imaging channels are clear (Fig. 2C)
Hamilton Cleaning solution	Hamilton	18311	Gentle but efficient cleaning solution for glass flow cells; does not bubble when used carefully
Illumination system	Sutter Instrument	Lamda DG4	Discontinued so recommend Lambda 721
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Image analysis software	Media cybernetics	Image Pro Premiere	Analysis of images and single molecule tracking
Image analysis software	Fiji/NIH Image/Image J	Shareware	Analysis of images and single molecule tracking
Image display card	Melles Griot	06 DLA 001	Alternate product from Thorlabs: VRC5
Immersion oil	Zeiss	444960	Immersol 518 F fluorescence free
Laser beam alignment tools	Thor labs	FMP05/M; dgo5-1500-h1; BHM1	Used to ensure beams are horizontal and at the correct height
Laser beam viewer	Canadian Photonics labs	IR 3150	Used to image IR beam spots on mirrors and targets
Laser power meter	Thor labs		Measurement of laser output as well as trap strength
Laser safety glasses (HeNe)	Thor labs	LG7 or 8	Blocks >3 OD units of light of wavelengths >600 nm
Laser safety glasses (IR)	Thor labs	LG11	Blocks >7 OD units of light of wavelengths >1000 nm
MCherry fluorescence filter cube	Semrock	mcherry-a-lsc-zero	Used in conjunction with Lambda DG4 to image mcherry only
Microscope	Leica	DMIRE2	DIC port removed to accommodate Dichroic trapping/alignment mirror
Microscope control software	UCSF/shareware	uManager	Controls the microscope, permits focal alignment of objectives as well as stage control
Nanoport assembly	IDEX	N333	Connectors that are bonded to flow cells
Optical table support	Thor Labs	PA52502	Active isolation table support
Optics and lenses	Solar TII	Various	Interference mirrors, telescopes and lenses custom designed for the system
PDMS flow cells	ufluidix	Custom	Flow cells built according to your specifications; imaging channels are clear (Figs. 2B and D)

PEEK tubing	IDEX	1532	Provides excellent connection to flow cells and switching valves
Pinkel fluorescence filter cube	Semrock	If488/543/635-3x-a-000	Used in conjunction with Lambda DG4 to image multiple fluorophores rapidly
Press fit tubing connectors	GraceBio	46003	Clear silicone connector with adhesive that binds well to glass
Scanning mirrors	GSI Lumonics	VM500	Used to provide control of the second optical trap. GSI Lumonics no longer exists. Similar mirrors can be purchased from Cambridge Scientific
Stage	Leica		
Stage micrometer	Electron Microscopy Sciences	68042-08	Provides on screen ruler for positioning of the beam and system calibration
Switching valves	IDEX	V-101T	Control direction of fluid flow and eliminate introduction of bubbles into flow cells
Syringe and valve manifold	Machine shop	None	Custom built
Syringe pump	Harvard Apparatus	PHD 2000	Controls fluid flow through flow cells
Syringe pump software	Harvard Apparatus	70-6000	Flow control provides seamless, programmable control of fluid flow
Syringes	Hamilton	81320	Gas-tight, PTFE Luer Lock, glass barrels with Teflon-coated plungers
Table top	Thor Labs	T36H	Optical table top or breadboard
Trapping laser	Newport/Spectra Physics	J-series; BL106C	Nd:YAG laser; 1064 nm; 5W laser