

Materials List for

Single-Molecule Imaging of Lateral Mobility and Ion Channel Activity in Lipid Bilayers using Total Internal Reflection Fluorescence (TIRF) Microscopy

Shuo Wang¹, Stephan Nussberger¹

¹Biophysics Department, Institute of Biomaterials and Biomolecular Systems, University of Stuttgart

Corresponding Author

Stephan Nussberger

stephan.nussberger@bio.uni-stuttgart.de

Citation

Wang, S., Nussberger, S. Single-Molecule Imaging of Lateral Mobility and Ion Channel Activity in Lipid Bilayers using Total Internal Reflection Fluorescence (TIRF) Microscopy. *J. Vis. Exp.* (192), e64970, doi:10.3791/64970 (2023).

Date Published

February 17, 2023

DOI

10.3791/64970

URL

jove.com/video/64970

Materials

Name	Company	Catalog Number	Comments
1,2-diphytanoyl-sn-glycero-3-phosphocholine	Avanti Polar Lipids	850356C	
100x Oil objective Apochromat N.A. 1.49	Nikon	MRD01991	TIRF microscope
10x Hoffmann modulation contrast objective NA 0.25	Nikon		Microscope to assess DIB membrane formation
10x objective N.A. 0.25	Nikon	MRL00102	TIRF microscope
40x Hoffmann modulation contrast objective NA 0.55	Nikon		Microscope to assess DIB membrane formation
488 nm laser, 100 mW	Visitron		TIRF microscope
Adhesive tape			
Äkta pure	Cytiva		Protein purification system
Bradford assay kit, Pierce	Thermo Fisher	23236	
CaCl ₂	Roth	5239.2	
Chelax 100 resin	Biorad	143-2832	
Chloroform	Sigma-Aldrich	MC1024452500	
Dialysis cassettes Slide-A-Lyzer 20 k MWCO	Thermo Fisher	87735	
DIB chamber	Custom made		PMMA chamber for DIB membranes
Digital power meter and energy console	Thorlabs	PM100D	Laser power meter
Dimethyl sulfoxide	Roth	4720.1	
Double distilled H ₂ O			
Eclipse TS 100 Hoffmann modulation contrast microscope	Nikon		Microscope to assess DIB membrane formation
EDTA	Roth	8042.2	
EMCCD camera iXon Ultra 897	Andor		TIRF microscope
Ethanol	Sigma-Aldrich	32205-M	

Fixed angle rotor Ti70	Beckman Coulter		
Fluo-8, CalciFluor™	Santa Cruz Biotechnology	SC-362561	Ca ²⁺ -sensitive dye
French press cell disruption homogenizer	Igneus	Igneus 40000 psi	
GFP filter	AHF		Filter seeting used for excitation of DIB-membranes by epifluorescence with white light source
Glass capillaries	World Precision Instruments	4878	
Glass coverslips 40 mm x 24 mm x 0.13 mm	Roth	1870.2	
Glycerol	Roth	3783.2	
Hamilton syringe 10 mL	Roth	X033.1	
Hamilton syringe 100 mL	Roth	X049.1	
Hamilton syringe 500 mL	Roth	EY49.1	
Heating block	Eppendorf	Thermomixer comfort	
Heating plate	Minitube	HT200	
Hepes	Roth	9205.3	
Hexadecane	Sigma-Aldrich	296317	
His Trap HP 1 mL	Cytiva	29051021	Ni-NTA column
Imidazole	Sigma-Aldrich	1.04716.1000	
KCl	Honeywell	10314243	
KLM spin coater	Schaefer Tec	SCV-10	
List medical L/M-3P-A vertical pipette puller	Artisan Technology Group	57761-1	
Low melting point agarose	Sigma-Aldrich	A9414	
M8 Stereomicroscope	Wild		Stereomicroscope
Matlab	MathWorks	R2022a	
Methanol	Sigma-Aldrich	34860	
MicroFil pipette tips	World Precision Instruments	MF34G-5	
N ₂ gas			
NaCl	Roth	3957.1	
Nanoliter 2010 injector	World Precision Instruments	Nanoliter 2010	
n-dodecyl-b-D-maltoside	Glycon Biochemicals	D97002-C	
Ni-NTA agarose, non-crosslinked	Cube Biotech	124115393	Custom made
NIS-Elements AR software	Nikon	MQS31100/MQS42560/MQS42580/MQS42780/MQS41930	Imaging software
n-octyl-polyoxyethylene	Sigma-Aldrich	40530	
O ₂ gas			
Phenylmethylsulfonyl fluoride	Roth	6367.3	
Photodiode sensor Si, 400 - 1100 nm, 500 mW	Thorlabs	S130C	Sensor for laser power meter
Plasma cleaner	Diener Electronics	Zepto	
Preparative ultracentrifuge Optima	Beckman Coulter		
Quad-band TIRF-filter 446/523/600/677 HC	AHF		Filter setting used for excitation of DIB-membranes with 488 nm laser
Resource Q 1 mL	Cytiva	17117701	Anion exchange column
Silicon oil AR 20	Sigma-Aldrich	10836	
Sodium dodecyl sulfate	Roth	2326.2	
Super LoLux camera	JVC		Stereomicroscope
Thermoshaker	Gerhardt	THL 500/1	

Ti-E Fluorescence microscope	Nikon	MEA53100	
Tris-HCl	Sigma-Aldrich	9090.3	
Tryptone	Roth	8952.2	
Ultrasonic bath	Bandelin Sonorex	RK 100	
Vacuum pump	Vacuubrand	MD 4C NT	
White light source for epifluorescence illumination (100 W)	Nikon	MBF72655	TIRF microscope
Yeast extract	Roth	2363.2	
β -mercaptoethanol	Sigma-Aldrich	M3148	