nature portfolio

https://doi.org/10.1038/s43586-021-00038-x

Supplementary information

Single-molecule localization microscopy

In the format provided by the authors and unedited

Supplemental Table 1: Selected commercial reagents for SMLM experiments

This table lists examples of some recommended reagents for newcomers to SMLM. For multi-color imaging, see **Box 2**. For more comprehensive information and/or systematic comparisons of SMLM labeling techniques, see e.g. [1-5] and the protocols described in **Supplementary Note 1.**

Type of reagent	Reagent(s)	Supplier	Reference	Application
Primary antibodies, unlabeled (use with labeled secondary antibodies)	Rat anti-tubulin antibody (recognizes the α subunit of tubulin)	Bio-Rad	link	(*d*)STORM of microtubules
	Rabbit anti-nucleoporin Nup133	Abcam	link	(*d*)STORM of nuclear pores in human cells
Secondary antibodies conjugated to synthetic dyes (use with unlabeled primary antibodies)	Anti-rat antibody coupled to Alexa Fluor 647	ThermoFisher	link	*d*STORM
	Anti-rabbit antibody coupled to Alexa Fluor 647	Jackson ImmunoResearch	link	*d*STORM
Fluorescent protein plasmids	mEos3.2 plasmid	Addgene	link	PALM
	mMaple3 plasmid	Addgene	link	PALM
DNA-PAINT kits	DNA-conjugated secondary antibodies/nanobodies (docking strands)	Massive Photonics	link	DNA-PAINT
	Dye-conjugated DNA (imager strands)	Massive Photonics	link	DNA-PAINT
Direct labeling	Phalloidin conjugated to Alex Fluor 647	ThermoFisher	link	*d*STORM of actin
	MitoTracker Red	ThermoFisher	link	Live cell STORM of mitochondrial membranes
Photoswitching buffers	SAFe Reagents	Abbelight	link	(*d*)STORM
	Everspark	idylle	link	(*d*)STORM
Labeled samples	Cells with labeled microtubules, mitochondria, podosomes..	Abbelight	link	(*d*)STORM
Fiducial markers	TetraSpeck microspheres (100 nm diameter)	ThermoFisher	link	Correction of drift and/or chromatic aberrations

Supplementary Note 1: Selected experimental protocols for SMLM

The following is a non-exhaustive list of some recommended experimental protocols for SMLM.

- Van de Linde et al. (ref.[6]) : *d*STORM, live cells.
- Gould et al. (ref.[7]) : PALM.
- Schnitzbauer et al. (ref.[8]): DNA-PAINT, multicolor, quantification (qPAINT).
- Jimenez et al. (ref.[9]): *d*STORM, DNA-PAINT, multicolor, optimized sample preparation.
- Manley et al. (ref.[10]): spt-PALM.
- Kaplan et al. (ref. [11]): sample preparation optimized for *d*STORM in yeast.
- Davis et al. (ref.[12]): how to prepare coverslips to minimize impurities in SMLM

Supplemental References:

1 Turkowyd, B., Virant, D. & Endesfelder, U. From single molecules to life: microscopy at the nanoscale. *Anal Bioanal Chem* **408**, 6885-6911, doi:10.1007/s00216-016-9781-8 (2016).

2 Dempsey, G. T., Vaughan, J. C., Chen, K. H., Bates, M. & Zhuang, X. Evaluation of fluorophores for optimal performance in localization-based super-resolution imaging. *Nat Methods* **8**, 1027-1036, doi:10.1038/nmeth.1768 (2011).

3 Wang, S., Moffitt, J. R., Dempsey, G. T., Xie, X. S. & Zhuang, X. Characterization and development of photoactivatable fluorescent proteins for single-molecule-based superresolution imaging. *Proc Natl Acad Sci U S A* **111**, 8452-8457, doi:10.1073/pnas.1406593111 (2014).

4 Shim, S. H. *et al.* Super-resolution fluorescence imaging of organelles in live cells with photoswitchable membrane probes. *Proceedings of the National Academy of Sciences of the United States of America* **109**, 13978-13983, doi:10.1073/pnas.1201882109 (2012).

5 Jradi, F. M. & Lavis, L. D. Chemistry of Photosensitive Fluorophores for Single-Molecule Localization Microscopy. *ACS Chem Biol* **14**, 1077-1090, doi:10.1021/acschembio.9b00197 (2019).

6 van de Linde, S. *et al.* Direct stochastic optical reconstruction microscopy with standard fluorescent probes. *Nature protocols* **6**, 991-1009, doi:10.1038/nprot.2011.336 (2011).

7 Gould, T. J., Verkhusha, V. V. & Hess, S. T. Imaging biological structures with fluorescence photoactivation localization microscopy. *Nat Protoc* **4**, 291-308, doi:10.1038/nprot.2008.246 (2009).

8 Schnitzbauer, J., Strauss, M. T., Schlichthaerle, T., Schueder, F. & Jungmann, R. Super-resolution microscopy with DNA-PAINT. *Nat Protoc* **12**, 1198-1228, doi:10.1038/nprot.2017.024 (2017).

9 Jimenez, A., Friedl, K. & Leterrier, C. About samples, giving examples: Optimized Single Molecule Localization Microscopy. *Methods* **174**, 100-114, doi:10.1016/j.ymeth.2019.05.008 (2020).

10 Manley, S., Gillette, J. M. & Lippincott-Schwartz, J. Single-particle tracking photoactivated localization microscopy for mapping single-molecule dynamics. *Methods Enzymol* **475**, 109-120, doi:10.1016/S0076-6879(10)75005-9 (2010).
11 Kaplan, C. & Ewers, H. Optimized sample preparation for single-molecule localization-based superresolution microscopy in yeast. *Nat Protoc* **10**, 1007-1021, doi:10.1038/nprot.2015.060 (2015).
12 Davis, J. L., Dong, B., Sun, C. & Zhang, H. F. Method to identify and minimize artifacts induced by fluorescent impurities in single-molecule localization microscopy. *J Biomed Opt* **23**, 1-14, doi:10.1117/1.JBO.23.10.106501 (2018).