AUGUSTA UNIVERSITY

Nikon

Cell Imaging
Core N-STORM
Sample Prep and Dye Tips

Determinants of Sample Quality

Probe Choice

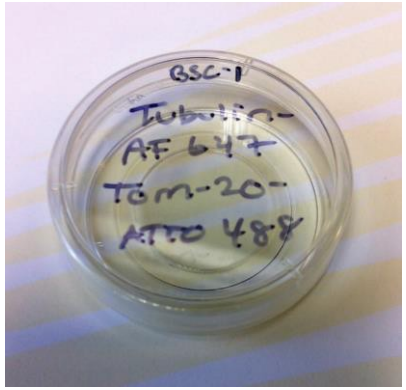
Dyes that work for N-STORM

Labeling Strategies

Fixation

Immunostaining

Don't forget to use glass-bottom (#1.5) dishes to hold STORM imaging buffer!



Sample Dish Manufacturers

MatTek

https://www.mattek.com/products/glass-bottom-dishes/

Willco Wells

https://willcowells.com/

Thermo

https://www.thermofisher.com/order/catalog/product/150680

STORM Sample Preparation Summary

Use glass-bottom (#1.5) dishes

Label with Alexa 647 and Atto 488

Block and wash samples thoroughly

Use MEA-containing imaging buffer and keep it fresh

Optimization is usually necessary for the best results!

Probe Choice

The most important considerations are….

High photon number

$$(s = s_{PSF})/N^{1/2}$$

High Localization Density

$$\text{Nyquist resolution} = \frac{2}{(\text{localization density})^{1/D}}$$

High Photostability

Longer imaging time

Laser Power

Dyes That Work for N-STORM

Dye	Excitation maximum (nm)[a]	Emission maximum (nm)[a]	Extinction (M^{-1} cm^{-1})[b]	Quantum yield[c]	Detected photons per switching event		Equilibrium on-off duty cycle (400–600 s)		Survival fraction after illumination for 400 s		Number of switching cycles (mean)	
					MEA	βME	MEA	βME	MEA	βME	MEA	βME
Blue-absorbing												
Atto 488	501	523	90,000	0.8	1,341	1,110	0.00065	0.0022	0.98	0.99	11	49
Alexa Fluor 488	495	519	71,000	0.92	1,193	427	0.00055	0.0017	0.94	1	16	139
Atto 520	516	538	110,000	0.9	1,231	868	0.0015	0.00061	0.92	0.86	9	17
Fluorescein	494	518	70,000	0.79	1,493	776	0.00032	0.00034	0.51	0.83	4	15
FITC	494	518	70,000	0.8	639	1,086	0.00041	0.00031	0.75	0.9	17	16
Cy2	489	506	150,000	0.12	6,241	4,583	0.00012	0.00045	0.12	0.19	0.4	0.7
Yellow-absorbing												
Cy3B	559	570	130,000	0.67	1,365	2,057	0.0003	0.0004	1	0.89	8	5
Alexa Fluor 568	578	603	91,300	0.69	2,826	1,686	0.00058	0.0027	0.58	0.99	7	52
TAMRA	546	575	90,430	0.2	4,884	2,025	0.0017	0.0049	0.85	0.99	10	59
Cy3	550	570	150,000	0.15	11,022	8,158	0.0001	0.0003	0.17	0.55	0.5	1.6
Cy3.5	581	596	150,000	0.15	4,968	8,028	0.0017	0.0005	0.89	0.61	5.7	3.3
Atto 565	563	592	120,000	0.9	19,714	13,294	0.00058	0.00037	0.17	0.26	4	5
Red-absorbing												
Alexa Fluor 647	650	665	239,000	0.33	3,823	5,202	0.0005	0.0012	0.83	0.73	14	26
Cy5	649	670	250,000	0.28	4,254	5,873	0.0004	0.0007	0.75	0.83	10	17
Atto 647	645	669	120,000	0.2	1,526	944	0.0021	0.0016	0.46	0.84	10	24
Atto 647N	644	669	150,000	0.65	3,254	4,433	0.0012	0.0035	0.24	0.65	9	39
Dyomics 654	654	675	220,000	–	3,653	3,014	0.0011	0.0018	0.79	0.64	20	19
Atto 655	663	684	125,000	0.3	1,105	657	0.0006	0.0011	0.65	0.78	17	22
Atto 680	680	700	125,000	0.3	1,656	987	0.0019	0.0024	0.65	0.91	8	27
Cy5.5	675	694	250,000	0.28	5,831	6,337	0.0069	0.0073	0.87	0.85	16	25
NIR-absorbing												
DyLight 750	752	778	220,000	–	712	749	0.0006	0.0002	0.55	0.58	5	6
Cy7	747	776	200,000	0.28	852	997	0.0003	0.0004	0.48	0.49	5	2.6
Alexa Fluor 750	749	775	240,000	0.12	437	703	0.00006	0.0001	0.36	0.68	1.5	6
Atto 740	740	764	120,000	0.1	779	463	0.00047	0.0014	0.31	0.96	3	14
Alexa Fluor 790	785	810	260,000	–	591	740	0.00049	0.0014	0.54	0.62	5	2.7
IRDye 800 CW	778	794	240,000	–	2,753	2,540	0.0018	0.038	0.6	1	3	127

Dempsey et al., 2011

Secondaries and Sources for STORM

STORM Secondary Antibodies	Sources
Alexa647	Life Technologies, Jackson
Cy5	Jackson
Alexa568	Life Technologies
Cy3B	GE
Atto488	Rockland, Sigma

Fixation/Labeling Strategies

The goals of fixation are to preserve ultrastructure and ability of antibodies to bind.

Fixatives

Methanol – solvent (lipids) and coagulant (proteins)

Aldehydes – cross-linkers that create bridges between molecules

The best fixatives and concentrations are protein dependent.

3% PFA and 0.1% glutaraldehyde is good starting point.

Tips for STORM Sample Preparation

- Compare performance of antibodies from multiple sources.
- Optimize fixation (fixative concentration, permeabilization, etc.) to maximize structural preservation and antibody binding.
- Minimize background signal levels by titrating primary antibody.
- Block with heat-treated sterile filtered blocking serum.
- Don’t skip on the washing steps and use 1% blocking serum to remove antibodies AT EVERY STEP.
- Lock secondary antibodies in place with post-staining fixation.
- Remove residues with Tween 80 wash.

N-STORM Imaging Buffer Types

Several types of imaging buffers are outlined in his section. In general, imaging buffer containing 2- mercaptoethanol is recommended for dyes Alexa Fluor 647 and Cy3B. However, if ATTO 488 or Alexa Fluor 568 dyes are being used, MEA containing imaging buffer is preferable. When the experiment involved dual staining with Alexa Fluor 647 and ATTO 488 or Alexa Fluor 568, MEA- containing imaging buffer is recommended. Please refer to Dempsey et al (2011) for more details.

Recommended Reagants

2-mercaptoethanol #63689-100ML-F
Cysteamine (**MEA**) #30070-50G
Glucose **Ox**idase from Aspergillus niger-Type VII, lyophilized powder, ≥ 100,000 units/g solid #G2133-250KU
Catalase from bovine liver-lyophilized powder, ≥ 10,000 units/mg protein #C40-100MG
(above reagents from Sigma-Aldrich)

1M Tris pH 8.0
1N HCl
NaCl

Solutions

- **Buffer A**: 10 mM Tris (pH 8.0) + 50 mM NaCl
- **Buffer B**: 50 mM Tris (pH 8.0) + 10 mM NaCl + 10% Glucose
- **GLOX** solution (250 µl): 14 mg Glucose Oxidase + 50 µl Catalase (17 mg/ml) + 200 µl Buffer A Vortex to dissolve Glucose Oxidase Spin down at 14,000 rpm; only use supernatant; store at 4°C for up to 2 weeks, in case of reusing, spin down at 14,000 rpm again
- 1M **MEA**: 77 mg MEA + 1.0 ml 0.25N HCL; store at 4°C for up to 1 month

STORM Imaging MEA Buffer
Just before imaging mix on ice 7 µl GLOX and 70 µl 1M MEA to 620 µl Buffer B in a 1.5 ml Eppendorf tube and vortex gently to mix.

STORM Imaging 2-mercaptoethanol Buffer
Just before imaging mix on ice combine 7 µl GLOX, 7 µl 2-mercaptoethanol and 690 µl Buffer B.

Add sufficient imaging buffer in the well: for example 700 µl per well of 8-well Lab-Tek® II chambered cover glass. The buffer depletes rapidly and needs to be refreshed.

References and Recommended Reading

Dempsey, G.T., Vaughan, J.C., Chen, K.H., Bates, M., Zhuang, X. (2011) Evaluation of fluorophores for optimal performance in localization-based super-resolution imaging. Nature Methods, 8 (12): 1027-36.

Jones, S.A., Shim, S-H., He, J., Zhuang, X. (2011) Fast three-dimensional super-resolution imaging of live cells. Nature Methods, 8 (6): 499-505.

Shim, S-H., Xia, C., Zhong, G., Babcock, H.P., Vaughan, J.C., Huang, B., Wang, X., Xu, C., Bi, G-Q., Zhuang, X. (2012) Super-resolution fluorescence imaging of organelles in live cells with photoswitchable membrane probes. PNAS, 109 (35): 13978-83.