Fluorophore	Excitation Maximum (nm)	Emission Maximum (nm)	Photons/Emission Event	Photoactivation	Buffer(s)	Reference	Comments
Synthetic Dyes							
488 nm Excitation							
Vybrant Dye Cycle Violet	369 (B), ~488 (G)	437 (B), ~510 (G)	2409 (1)	UV-Violet (405 nm)	Glycerol/OS (1)	1-3	Binds dsDNA. Low cytotoxicity and cell permeable. Does not require photoactivation but can be applied.
DAPI	364 (B), ~488 (G)	454 (B), ~510 (G)	--	UV-Violet (405 nm)	Glycerol/OS (4)	4	DNA minor groove binder. Cell impermeant.
Hoescht 33342	350 (B), ~488 (G)	461 (B), ~510 (G)	--	UV-Violet (405 nm)	Glycerol/OS (4)	4	DNA minor groove binder. Cell permeant.
Hoescht 33258	355 (B), ~488 (G)	465 (B), ~510 (G)	--	UV-Violet (405 nm)	Glycerol/OS (4)	4	DNA minor groove binder. Cell permeant.
SYTO-13	488	509	--	--	50mM MEA/OS (5)	5	DNA minor groove binder. Cell permeant. Also binds RNA.
YOYO-1	489	509	--	--	50mM MEA/OS (7)	6, 7	DNA minor groove binder. Cell impermeant. Also binds RNA. Dimeric.
YO-PRO-1	491	509	--	--	50mM MEA/OS (7)	7	DNA minor groove binder. Cell impermeant. Also binds RNA. Monomeric.
Alexa Fluor 488	495	519	1193 (8)	UV-Violet (405 nm)	10mM MEA/OS (8) 100mM MEA/OS (9)	8, 9, 44	High performance 488nm-excitable dye for STORM
Atto 488	501	523	1341 (8)	UV-Violet (405 nm)	10mM MEA/OS (8)	8, 9	Highest performance 488nm-excitable dye for STORM
Oregon Green	501	526	900 (10, Live)	UV-Violet (405 nm)	Live-OS	10	Recommend for live cell only (cell permeable), better green dyes for fixed work. BODIPY derivative.
Picogreen	502	524	(Live)	--	Live-OS+AA (11)	11	Binds dsDNA. Cell impermeant
Atto 520	516	538	868 (8), 1000 (12)	UV-Violet (405 nm)	143mM BME/OS (8) 100mM MEA/OS (12)	8, 12	Recommend Atto 488 or Alexa Fluor 488 instead, if possible
532 nm Excitation							
Alexa Fluor 532	532	552	--	--	100mM MEA/OS	13	
Atto 532	532	552	--	--	100mM MEA/OS	9, 14	

Fluorophore	Excitation Maximum (nm)	Emission Maximum (nm)	Photons/Emission Event	Photoactivation	Buffer(s)	Reference	Comments
561 nm Excitation							
CF 543	541	560	--	UV-Violet (405 nm)	10mM MEA/OS (15)	15	Needs further testing
TAMRA/TMR (Tetramethyl Rhodamine)	546	575	4884 (8) 1100 (10, Live)	--	10mM MEA/OS (8) Live-OS (10)	8, 10, 16	TMR conjugates (TMR Star) often used for SNAP-tag labeling
DiI	551	569	720	UV-Violet (405 nm)	Live-OS	17	Live cell stain for plasma membranes
MitoTracker Orange	554	576	--	UV-Violet (405 nm)	Live-OS	17	
Alexa Fluor 555	555	580	2500 (2)	UV-Violet (405 nm)	Glycerol/OS (2)	1, 2, 44	A good red dye for STORM, also works well in thiol+oxygen scavenger buffer well (unpublished).
CF 555	555	565	--	UV-Violet (405 nm)	10mM MEA/OS (15)	15	Needs further testing
DY-547	557	574	--	UV-Violet (405 nm)	10mM MEA/OS (15)	15	Needs further testing
Cy3B	559	570	1365 (8, MEA) 2057 (8, BME)	UV-Violet (405 nm)	10mM MEA/OS (8) 143mM BME/OS (8)	8, 15	Highest performing red dye for STORM.
CF 568	562	583	--	UV-Violet (405 nm)	10mM MEA/OS (15)	15	Very promising red alternative, but needs further testing.
FLIP-565	565	580	--	UV-Violet (405 nm)	Physiological Buffer or Culture Medium	18, 19	
LysoTracker Red	577	590	820	UV-Violet (405 nm)	Live-OS	17	Live cell stain for lysosomes. BODIPY derivative.
Alexa Fluor 568	578	603	2826 (8), 1700 (10, Live)	UV-Violet (405 nm)	10mM MEA/OS (8) Live-OS (10)	8, 10, 15	Good red dye for STORM
MitoTracker Red	578	599	790	UV-Violet (405 nm)	Live-OS	17	Specific for mitochondria, for live-cell imaging
ER-Tracker Red	587	615	820	UV-Violet (405 nm)	Live-OS	17	Specific for endoplasmic reticulum, for live-cell imaging
647 nm Excitation							
DY-634	635	658	--	--	100-200mM MEA/OS (20)	20	Needs further testing.
MitoTracker Deep Red	644	665	--	UV-Violet (405 nm)	Live-OS	17	Specific for mitochondria, for live-cell imaging
DiD	644	665	--	UV-Violet (405 nm)	Live-OS	17	Specific for plasma membranes, for live-cell imaging
SiR	645	661	630	--	Physiological Buffer or Culture Medium	21	Silicon rhodamine, doesn't require reducing buffer.
HMSiR	645	661	2,600	--	Physiological Buffer or Culture Medium	22	Higher performance silicon rhodamine derivative, doesn't require reducing buffer.
Cy5	649	670	4254 (8, MEA), 5873 (8, BME)	UV-Violet (405 nm)	10mM MEA/OS (8) 143mM BME/OS (8)	8, 23, 24	One of the highest performing dyes for STORM
Alexa Fluor 647	650	665	3823 (8, MEA), 5202 (8, BME), 2400 (25), 18050 (26)	UV-Violet (405 nm)	10mM MEA/OS (8) 143mM BME/OS (8) TCEP (25) Vectashield (26)	8, 15, 24, 25, 26, 44	Currently regarded as best dye for STORM
CF 647	650	665	--	--	10mM MEA/OS (15)	15	

Fluorophore	Excitation Maximum (nm)	Emission Maximum (nm)	Photons/Emission Event	Photoactivation	Buffer(s)	Reference	Comments
DyLight 650	652	672	--	--	100-200mM MEA/OS (20)	20	
Atto 655	663	684	1105 (8), 1200 (10, Live)	UV-Violet (405 nm)	10mM MEA/OS (8) Live-OS (10) Culture Medium (26)	8, 10, 27, 28, 29	Oxazine dye, first demonstrated in live cells in conjunction with TMP tag
CF 660C	667	685	--	--	100-200mM MEA/OS (20)	20	
DY-678	674	694	--	--	10mM MEA/OS (15)	15	
Atto 680	680	700	1656 (8)	UV-Violet (405 nm)	10mM MEA/OS (8) Culture Medium (26)	8, 15, 27, 28, 29	Oxazine dye, similar to Atto 655
CF 680	681	698	--	--	10mM MEA/OS (15) 100-200mM MEA/OS (20)	15, 20	
Alexa Fluor 700	696	719	--	--	10mM MEA/OS (15) 100mM MEA/OS (29)	15, 30	
Atto 700	700	716	--	--	10mM MEA/OS (15)	15, 29	
750 nm Excitation							
Cy7	743	767	997	UV-Violet (405 nm)	143mM BME/OS (8)	8	
DiR	748	780	--	UV-Violet (405 nm)	Live-OS	17	
Alexa Fluor 750	749	775	703 (8), 2800 (25)	UV-Violet (405 nm)	143mM BME/OS (8) TCEP (25)	8, 25	Does not perform well without TCEP buffer system
DyLight 750	752	778	749	UV-Violet (405 nm)	143mM BME/OS (8)	8	
Fluorescent Proteins							
PA-FPs							
PA-GFP	504	517	313 (32, Live)	UV-Violet (405 nm)	Physiological Buffer or Culture Medium	31, 32	Recommended only for certain live cell and multicolor experiments as performance for STORM is relatively low.
PA-TagRFP	562	595	906 (32, Live)	UV-Violet (405 nm)	Physiological Buffer or Culture Medium	32, 33	Recommend higher performing green-red PC-FPs except for certain multicolor experiments.
PA-mCherry1	570	596	706 (32, Live)	UV-Violet (405 nm)	Physiological Buffer or Culture Medium	32, 34	Recommend higher performing green-red PC-FPs except for certain multicolor experiments.
PAmKate	586	628	--	UV-Violet (405 nm) or Blue (445 nm)	Physiological Buffer or Culture Medium	35	Only viable far-red FP for STORM.
PS-FPs							
Dronpa	503	518	262 (32, Live)	UV-Violet (405 nm)	Physiological Buffer or Culture Medium	31, 32	Reversibly switchable dark-green. We recommend PS-CFP2 instead for most SMLM applications.
mGeosM	503	514	248 (32, Live)	UV-Violet (405 nm)	Physiological Buffer or Culture Medium	32, 36	Better than Dronpa but higher duty cycle than PS-CFP2

Fluorophore	Excitation Maximum (nm)	Emission Maximum (nm)	Photons/Emission Event	Photoactivation	Buffer(s)	Reference	Comments
Dreiklang	515	529	700 (Live)	UV (365 nm) to activate and Violet (405 nm) to deactivate	Physiological Buffer or Culture Medium	37	Unique FP where 515 nm light elicits fluorescence, but can be activated or deactivated with two different wavebands.
mIrisFP	486 (G), 516 (R)	546 (G), 578 (R)	--	UV-Violet (405 nm) for dark-green and green-to-red. Blue (488 nm) for dark-red	Physiological Buffer or Culture Medium	38	Photoconvertable from green to red state, both green and red states reversibly photoswitchable to dark state. Demonstrated for combined SMLM+pulse chase experiments.
NijiFP	469 (G), 526 (R)	507 (G), 569 (R)	--	UV-Violet (405 nm) for dark-green and green-to-red. Blue (488 nm) for dark-red	Physiological Buffer or Culture Medium	39	Photoconvertable from green to red state, both green and red states reversibly photoswitchable to dark state.

PC-FPs

PS-CFP2	400 (C), 490 (G)	468 (C), 511 (G)	223 (32, Live)	UV-Violet (405 nm)	Physiological Buffer or Culture Medium	32, 40	PS-CFP2 is easily imaged without photo-activation and is a great candidate for multicolor imaging.
tdEos	506 (G), 569 (R)	516 (G), 581 (R)	1200 (10, Live) 774 (32)	UV-Violet (405 nm)	Live-OS (10) Physiological Buffer or Culture Medium	10, 31, 32	Tandem dimer so possible artifacts. Highest photon output in class.
mEos2	506 (G), 573 (R)	519 (G), 584 (R)	1200 (10, Live) 745 (32)	UV-Violet (405 nm)	Physiological Buffer or Culture Medium	10, 32, 41	Tends to dimerize, recommend mEos3.2 or mMaple3 instead.
mEos3.2	507 (G), 572 (R)	516 (G), 580 (R)	809 (32, Live)	UV-Violet (405 nm)	Physiological Buffer or Culture Medium	32, 42	Along with mMaple3, considered best of the green-red PC-FPs.
mEos4b	505 (G), 569 (R)	516 (G), 581 (R)	850	UV-Violet (405 nm)	Physiological Buffer or Culture Medium	43	Osmium Tetroxide resistant, engineered for correlative EM imaging
mMaple3	489 (G), 566 (R)	505 (G), 583 (R)	675 (32, Live)	UV-Violet (405 nm)	Physiological Buffer or Culture Medium	32	Most recently introduced PC-FP for SMLM, very high detectable FP: total FP ratio.
Dendra2	490 (G), 553 (R)	507 (G), 573 (R)	686 (32, Live)	UV-Violet (405 nm) or Blue (488 nm)	Physiological Buffer or Culture Medium	32	Blue light activation and good performance makes Dendra2 good candidate for live cell SMLM.

Imaging Buffers	Component 1	Component 2	Component 3	Component 4	Component 5	Total Volume
Fixed Cell Imaging						
MEA/OS (OS = GLOX or POC)	790 uL Buffer B 890 uL Buffer B 940 uL Buffer B 980 uL Buffer B	200 uL MEA Stock (200 mM) 100 uL MEA Stock (100mM) 50 uL MEA Stock (50 mM) 10 uL MEA Stock (10 mM)	10 uL GLOX Stock or POC Stock	-	-	1 mL
MEA/OS (OS = PCD)	790 uL Buffer C 890 uL Buffer C 940 uL Buffer C 980 uL Buffer C	200 uL MEA Stock (200 mM) 100 uL MEA Stock (100mM) 50 uL MEA Stock (50 mM) 10 uL MEA Stock (10 mM)	25 uL PCD Stock	-	-	1 mL
BME/OS (OS = GLOX or POC)	970 uL Buffer B 980 uL Buffer B 985 uL Buffer B 989 uL Buffer B	20 uL BME Stock (286 mM) 10 uL BME Stock (143 mM) 5 uL BME Stock (71.5 mM) 1 uL BME Stock (14.3 mM)	10 uL GLOX Stock or POC Stock	-	-	1 mL
BME/OS (OS = PCD)	920 uL Buffer C 930 uL Buffer C 935 uL Buffer C 939 uL Buffer C	20 uL BME Stock (286 mM) 10 uL BME Stock (143 mM) 5 uL BME Stock (71.5 mM) 1 uL BME Stock (14.3 mM)	25 uL PCD Stock	-	-	1 mL
TCEP	920 uL Buffer D	50 uL TCEP Stock (25 mM)	10 uL GLOX Stock	10 uL MV Stock (1 mM)	10 uL AA Stock (1 mM)	1 mL
Glycerol/OS (OS = GLOX or POC)	800 uL Buffer E	190 uL PBS	10 uL GLOX Stock	-	-	1 mL
OxEA	720 uL PBS (pH = 8-8.5)	50 uL MEA Stock	30 uL OxyFluor™	200 uL Sodium DL-lactate	-	1 mL
Live Cell Imaging						
Live-OS (OS = GLOX or POC) Optional MEA	990 uL Buffer F	10 uL GLOX Stock or POC Stock	OPTIONAL: 6 uL MEA stock (6 mM)	-	-	1 mL
Live-OS (OS = GLOX or POC) Optional BME	990 uL Buffer F	10 uL GLOX Stock or POC Stock	OPTIONAL: 5 uL BME stock (71.5 mM)	-	-	1 mL
Live-OS+AA (OS = GLOX or POC) Optional BME	980 uL Buffer F	10 uL GLOX Stock or POC Stock	OPTIONAL: 5 uL BME stock (71.5 mM)	10 uL AA Stock (1 mM)	-	1 mL

Stock Solutions	Composition	Storage	Notes
Dilution/Storage Buffers			
Buffer A	10 mM Tris (pH 8) + 50 mM NaCl	Room Temperature, long term	
Buffer B	50 mM Tris (pH 8) +10 mM NaCl + 10% glucose	Room Temperature or 4degC, long term	Recommend testing 10-200 mM Tris, higher pH may help due to OS-induced pH drop.
Buffer C	50 mM Tris (pH 8) +10 mM NaCl	Room Temperature or 4degC, long term	
Buffer D	200 mM Tris (pH 9) + 5% glucose	Room Temperature or 4degC, long term	
Buffer E	120 mg/mL Glucose in Glycerol	4degC	
Buffer F	L-15 Medium + 2-10% glucose	4degC, protected from light, ~1 month	For live imaging, can substitute for other growth medium + 75 mM HEPES. Use phenol red-free media.
Buffer G	100 mM Tris (pH 8) + 50mM KCl + 1mM EDTA + 50% glycerol	Room Temperature or 4degC, long term	For storage of PCD.
Reducing and Oxidizing Reagent Stock Solutions			
MEA Stock (1 M Mercaptoethylamine)	77 mg MEA + 1.0 mL 0.25N HCl	4degC for ~2 weeks	100x Stock Solution
BME Stock (14.3 M Beta-mercaptoethanol)	Provided by supplier as ~14.3 M stock.	4degC, long term	100x Stock Solution
TCEP Stock (0.5 M tris(2-carboxyethyl)phosphine)	Provided by suppliers as 0.5 M ampoules.	4degC, use same day ampoule opened.	20x Stock Solution
MV Stock (0.1 M methyl viologen)	25.7 mg MV + 1.0 mL ddH2O	4degC for ~2 weeks	100x Stock Solution
AA Stock (0.1 M ascorbic acid)	17.6 mg AA + 1.0 mL ddH2O	4degC for ~1 month	100x Stock Solution
COT Stock (cyclooctatetraene)	20.8 mg COT + 1.0 mL DMSO	-20degC	Buffer additive for improved Alexa Fluor 647 photon statistics. 100x stock solution.
Oxygen-Scavenging System Stock Solutions			
Glucose Stock	45% (w/v) solution		
Catalase Stock	17 mg/mL Catalase in dH2O	4degC for ~1 month	For use in GLOX and POC Stock solutions.
GLOX Stock	56 mg/mL Glucose Oxidase + 3.4 mg/mL Catalase Stock in Dilution Buffer A	4degC for ~2 weeks	Thoroughly vortex contents and centrifuge at max speed, harvest supernatant for use.
POC Stock	112 mg/mL Pyranose Oxidase + 3.4 mg/mL catalase in Dilution Buffer A	4degC for ~2 weeks	Thoroughly vortex contents and centrifuge at max speed, harvest supernatant for use.
PCD Stock	1.4 mg/mL Protocatechuate 3,4-Dioxygenase in Dilution Buffer G	-20degC	
PCA Stock	15.4 mg/mL Protocatechuic Acid in ddH2O (adjust pH to 9.0 with 10 N NaOH)	4degC	
Sodium DL-lactate	60% (w/w) solution	4degC	
OxyFluor™	Unknown	-20degC	Do not agitate vigorously or exceed warming temperature of 37degC

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