ADVANCED FUNCTIONAL MATERIALS

Supporting Information

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Time Resolved Cryo-Correlative Light and Electron Microscopy

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Supporting Information

#	Observation method	Publication	Temporal resolution	Lateral resolution	Axial resolution	Maintenance method	Sample size (x-y)	Sample size (z)	Biological observation
1	Fluorescence microscopy	[30]	9.6ms	~500nm	~um	Native	70x80µm	90µm	3D volumetric confocal FM of cellular dynamics
2	Super-resolution light microscopy (STED)	[72]	n/a	35nm	~100s of nm's	Native	n/a	n/a	n/a
2	Super-resolution LM (STED)	[21]	35ms	62nm	n/a	Native	1.8x2.5µm	n/a	Vesicle mobility in synaptic buttons
2	Super-resolution LM (PALM)	[73]	n/a	~10nm	n/a	Native	1x1µm or 4x4µm	n/a	Target proteins in thin sections of lysosomes and mitochondria
2	Super-resolution LM (PALM)	[31]	3s (entire image)	~10nm	n/a	Native or chemical	26x26µm	n/a	Focal adhesion protein paxillin labeled with Alexa Fluor 647
2	Super-resolution LM (PALM and SOFI)	[22]	<10s	~100nm	n/a	Native	~10x10µm	n/a	Dynamics of focal adhesions (local mean velocities)
2	Super-resolution LM (STORM)	[23]	3s	60nm	n/a	Native or chemical	~5x5µm	n/a	Imaging microtubule dynamics in living cells
2	Super-resolution LM (SIM)	[18]	3-4ms	~100nm	n/a	Native	~5x5µm (at highest fps)	~1µm	Membrane-membrane contact interactions, micro-tubule function.
2	Super-resolution LM (SIM)	[19]	~2ms	~60nm	~266nm	Native	~1x1µm (at highest fps)	n/a	Fusion pores in insulin-secreting cells
3	MINFLUX	[17]	0.4ms (single fluorophore)	~2nm	n/a	Native	30nm (ROI)	n/a	Motions of individual (macro)molecules
3	MINFLUX	[34]	0.1ms (single fluorophore) 40min (image)	1-3nm	1.6nm	Native	80x80µm	n/a	Single fluorophore tracking and diffusion of single labeled lipids in lipid-bilayer model membranes
4	Atomic force microscopy	[33]	~80ms	~2nm	n/a	Native	240x240nm	~140nm	n/a (Test with myosin V)
4	Atomic force microscopy	[74]	~>1s	<~nm?	n/a	Native	1x1µm	5nm	n/a (Au nanoparticles and gratings)
5	Soft X-ray imaging	[75]	n/a	20-50nm	n/a	Native	10x10µm		Non-live test plates
5	Soft X-ray imaging	[32]	~min	~100nm	~100nm	Native (initially alive)	~20x20µm	10µm	Chromosome motion in a live fission yeast zygote
6	Liquid cell EM	[76]	~1s (0.1s)	~0.08nm (0.02nm)	~10's of nm's	Native	~100x100nm[1]	~0.1-10nm	Viruses and Au nano-particles in kidney cells
6	Liquid cell EM	[26]	~3s	25nm	n/a	Native	12x15µm[2]	50nm	Investigation of the viability of. cells
a	Traditional cryo-CLEM	[35]	~1-10s	~<nm	~nm	Plunge freezing (FLM with DSMO)	~80x50µm[3]	~100nm[4]	Structural characterization of tunneling nanotubes

[1] From Figure 3. of publication

[2] Calculated from pixel size and imaging times

[3] From Figure 1. of publication

[4] (Blancard & Salin, 2017)

#	Observation method	Publication	Temporal resolution	Lateral resolution	Axial resolution	Maintenance method	Sample size (x-y)	Sample size (z)	Biological observation
b	Cryo-CLEM	[36]	~4s	~<nm	n/a	HPF, freeze substitution	n/a	~10-20um	Endocytic fusion and fission events
b	Cryo-CLEM	[61]	4 s	~nm's	~nm's	Plunge freezing (MAVIS)	~200x400µm[5]	~100nm[4]	TRF-DsRed transfected U2OS cells
c	LM (electrical trigger)	[64]	~100ms[6]	~10 nm	n/a	Trigger, chemical fixation	~500x500µm[3]	70µm	Structural changes after transmitter release, frog neuromuscular junction
c	LM (electrical trigger)	[65]	~5ms (+ freezing)	~10 nm	n/a	Trigger, freeze fracture	~50x100µm[7]	~2µm	Synaptic vesicle cytosis
c	Cryo-EM (light trigger)	[37]	~10ms	0.3nm[8]	n/a	Trigger, HPF	Ø6mm	~40nm (200µm)	Membrane trafficking of neuronal synapses
c	Cryo-EM (light trigger)	[66]	~20ms	~nm	n/a	Trigger, HPF	~1x1µm[9]	~150-200 µm	Cortical synapses in acute brain slices
c	Cryo-EM (light trigger)	[67]	~10ms	~nm	n/a	Trigger, HPF	Few µm	350µm	Membrane-Trafficking Events in Cultured Brain Slices
c	Cryo-EM (light trigger)	[68]	70ms[10]	~nm	n/a	Trigger, plunge freezing	n/a (autogrid)	~100nm[4]	Exploration of short-lived conformational states
c	Cryo-EM (chemical trigger)	[28]	~9.4ms	~23Å	n/a	Trigger, slam freezing	n/a (autogrid)	~100nm[4]	Bridge forming between ribosomal sub-units
c	Cryo-EM (chemical trigger)	[77]	~30ms	~3Å	n/a	Trigger, spray plunging	n/a (autogrid)	~100nm[4]	Growth of RaC filaments
d	Cryo-CLEM	[6]	~700ms	~7Å	n/a	Chemical fixation	1.4x1.4 µm	<300nm	Intraflagellar transport direction in chlamydomonas rehinhartii
e	Cryo-CLEM (Rapid cooling)	[38]	~1ms[11]	~80nm	n/a	Rapid-cryo-arrest on diamond, by jet	Ø5mm (diamond)	100µm	Molecular signaling system whose spatiotemporal organization is highly dynamic
f	Cryo-CLEM (Rapid cooling)	[53]	~5ms	~nm	n/a	Microfluidic freezing	~20x100µm	~20µm	n/a (Cryo-fixation test with C. elegans)

[5] From Figure 4. of publication

[6] Not including chemiucal fixation times

[7] Field of view size

[8] Hitachi H-7100 electron microscope

[9] From Figure 6. of publication

[10] 25ms light, 45ms freeze

[11] Calculated from cooling rate of 200.000°C/s