Johns Hopkins Engineering

Methods in Neurobiology

Small Molecules Probes for Bioimaging

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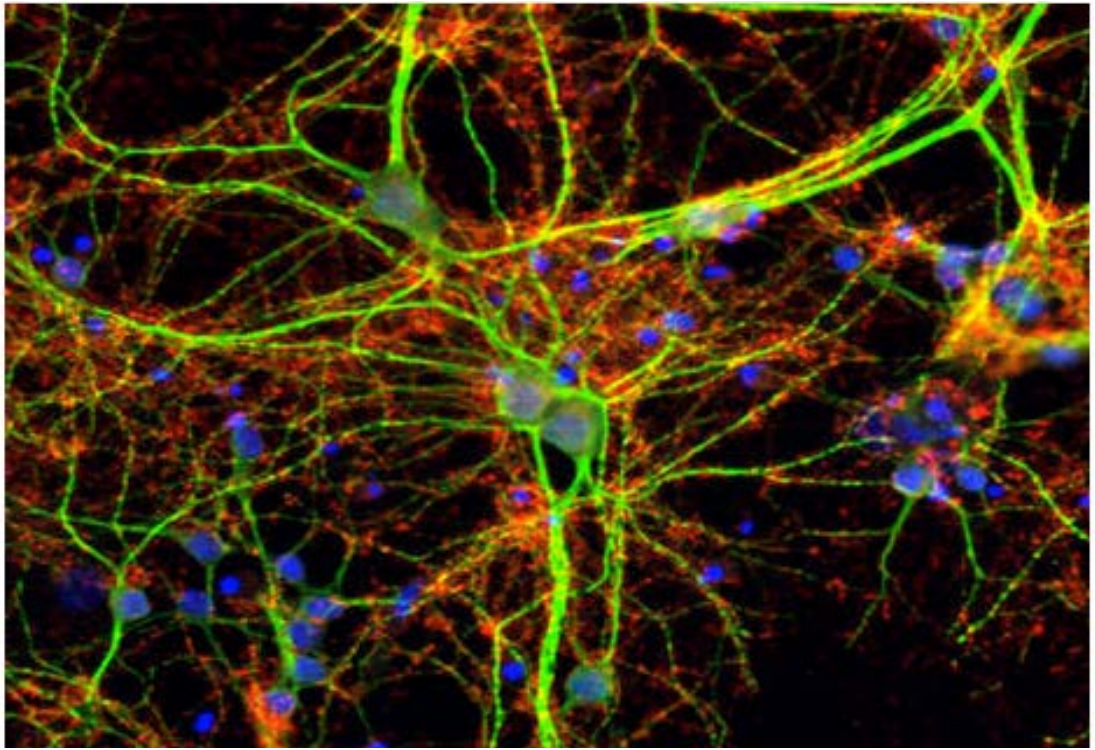
Imaging in Neurobiology



Small Molecule Fluorophores for Bioimaging

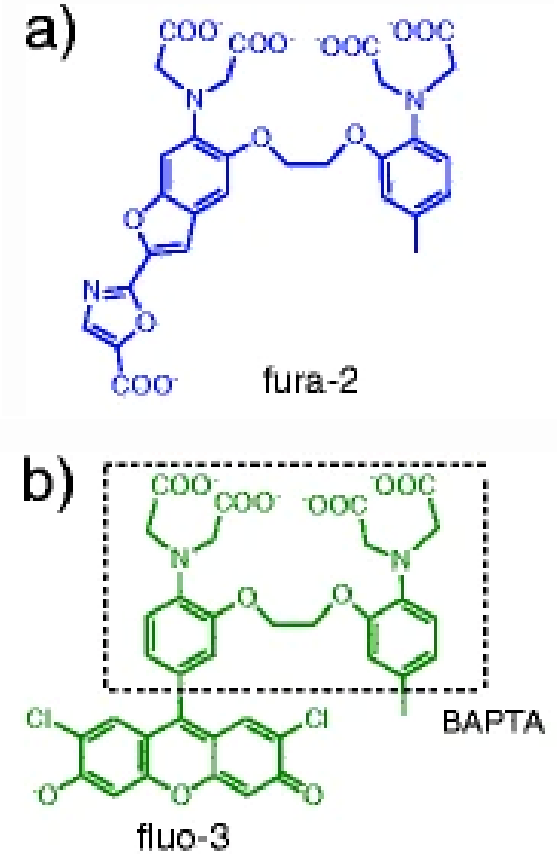
Target	Probes	Target	Proges
Ca^{2+}	Fura2, Indo1, Flu Calcium green, BODIPY, Oregon green BAPTA	Mitochondria	JC-1, MitoProbe, Mitotracker, DilC1,TMRM
K^{+}, Na^{2+}, Zn^{2+}, Cu^{+}	PBFI, TAC, CoroNA green, CoroNA red, TSQ, Bodipy	Lysosomes	Lisotracker, LysoSensor
pH	BCECF, pHrodo AM	Endoplasmic reticulum	ER tracker
Oxidative stress (ROS, H_2O_2, NO)	DCFH, H2DCFDA, dihydrorhodamine 123, Fluorescein	Cell viability/Proliferation	Calcein, Cell tracker, Film tracer, Click-iT EdU Alexa Fluor
Apoptosis, Chromatin condensation	Propidium iodide, Annexin, Tunel	Membrane potential	ANEP dyes, RH Dyes
Enzymes/Signal transduction	Small peptides fluorescent upon cleavage	.....	

Small Molecule Fluorophores Features

Probe feautures
• Ratiometric or non ratiometric;
• Binding affinity for the target (0.1-10 times their K_d);
• Property changes upon binding the target;
• Membrane permeability (AM tail, live cells);
• Non toxic;
• Differ according to experiment and aim.

How to Measure Ca^{2+} in Cells

- Ca^{2+} indicators bind and interact only with freely diffusible Ca^{2+} ions.
- The majority of Ca^{2+} within cells is not free to diffuse but tightly bound to various cellular buffers.
- The ratio of bound to free Ca^{2+} varies from cell to cell as well as within the various compartments of the cell.
- (The bound to free ratio of Ca^{2+} within the endoplasmic reticulum is of the order of 10 to1).
- Chemical Ca^{2+} indicators themselves also act as Ca^{2+} buffers and can therefore impact both the levels and most noticeably, the kinetics of Ca^{2+} signaling within cells



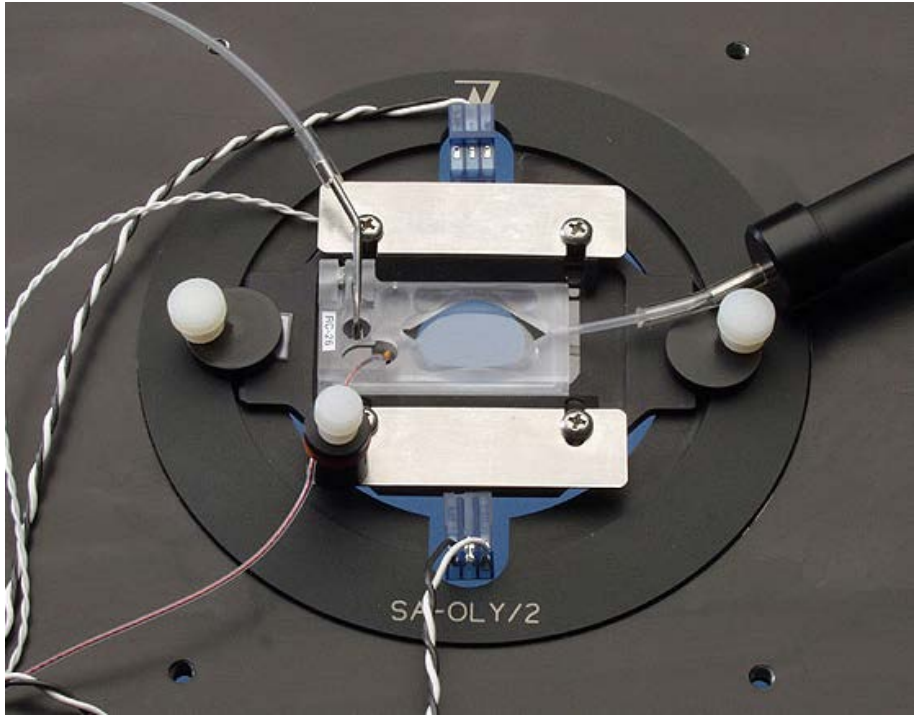
Measuring Ca^{2+} in Cells with Live Imaging

Ca^{2+} range in cells is between 50 nM to 50 µM.

High Affinity Calcium Indicators

Indicator	Kd for Ca^{2+} (nM)	Excitation (nm), emission (nm)	Notes
Calcium Green-1	190	490ex 531 em	single wavelength
Fluo-3	325	506 ex 526 em	single wavelength
Fluo-4	345	494 ex 516 em	single wavelength
Fura-2	145	363/335 ex 512 em	dual excitation/ single emission
Indo-1	230	488 ex 405/485 em	single excitation/dual emission
Oregon Green 488 Bapta-1	170	488 ex 520 em	single long wavelength
Fura-4F	0.77	336/366 ex, 511em	Ratiometric Exitation / Single emission
Fura-5F	0.40	336/363 ex, 512em	Ratiometric Exitation / Single emission
Calcium Crimson	185	590ex 615 em	single long wavelength
X-rhod-1	0.7	580 ex,602 em	Single excitation/emission

Examples of Equipment Necessary for Live Cell Imaging



References

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