ThermoFisher SCIENTIFIC

Engineering fluorescence for cell biology

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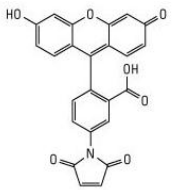
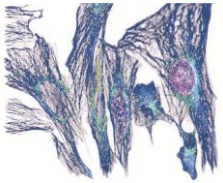
Research & Development
Protein & Cellular Analysis Business Unit

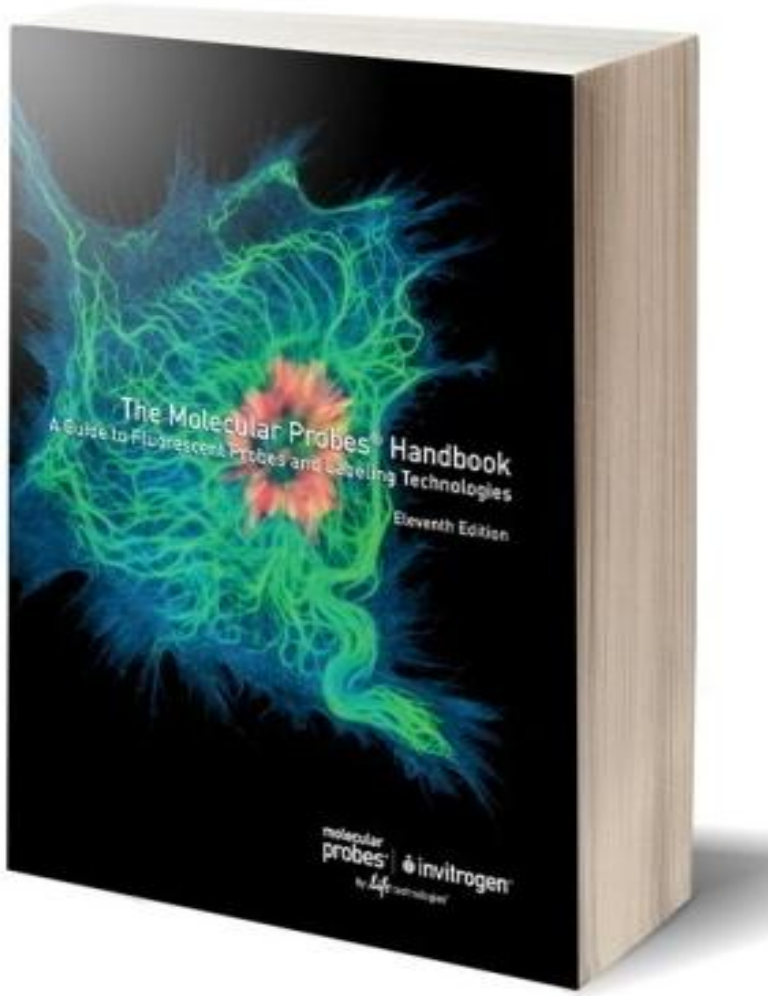
Centre Médical Universitaire, Geneva

Engineering fluorescence for cell biology





1975: *MPI founded in kitchen in St Paul MN, 5 products*
1978: *Moved to Texas, Texas Red dye*
1982: *Relocated to Junction City, OR. Fura-2, Indo*
1985: *First non-fluorescein green dye – BODIPY™*
1987: *50 dyes for membrane voltage, 3 dyes for DNA*
1992: *CellTracker ™ Green,*
1994: *MitoTracker ™ and LysoTracker ™ dyes*
1997: *Alexa Fluor ™ dyes and SYBR ™ stains*
1998: *Fluo-4 AM, SYPRO ™ Ruby Protein Gel Stain*
2002: *Zenon ™ Antibody Labeling Technology*
2003: *Invitrogen Corporation acquires Molecular Probes*
2005: *Qdot ™ nanocrystals, Qubit, SYBR ™ Greener*
2007: *Click-iT ™ chemistry; CellLight™ reagents, pHrodo™*
2009: *Countess ™ Automated Cell Counter*
2010: *Invitrogen and ABI merge=Life Technologies*
2011: *CellEvent™ and CellROX™ reagents*
2013: *Acquire AMG, EVOS Fl Auto launches*
2014: *Click-iT ™ PLUS*
Acquired by Thermo Fisher Scientific
2015: *CellInsight Cx7 HCS, ProLong Live, Image-iT ™ Hypoxia*



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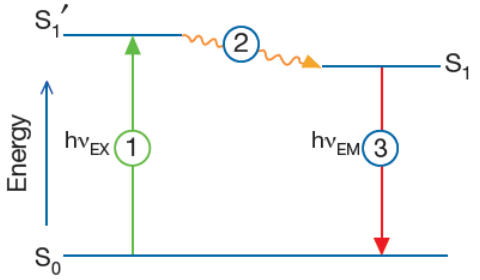
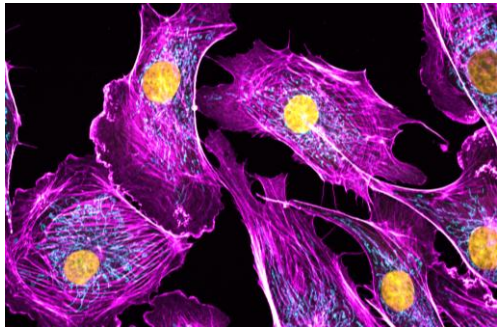
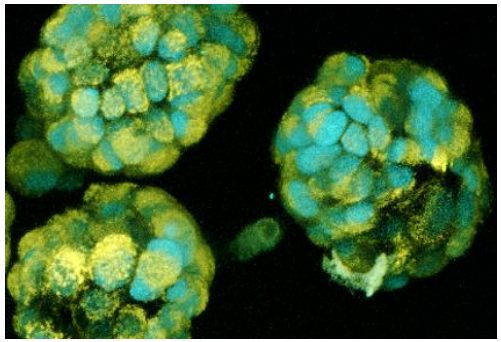
Engineering fluorescence for cell biology

- Content
  - Fluorescence
    - How fluorescence works
    - Fluorophores
  - Engineering fluorescence for cell biology
    - Markers: Cell Tracking, proliferation, organelles, autophagy, cytoskeletal dynamics, receptor-ligand, DNA damage, RNA & protein dynamics
    - Reporters: Calcium, ROS, pH, mitochondrial biology,
  - Advanced microscopy
    - SRM (SIM, STED, SMLM)







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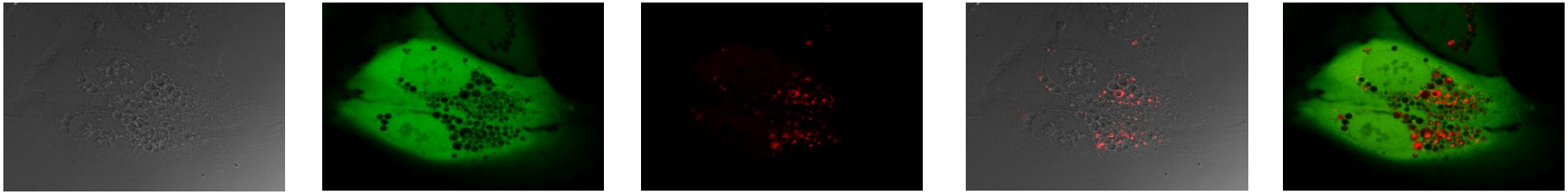
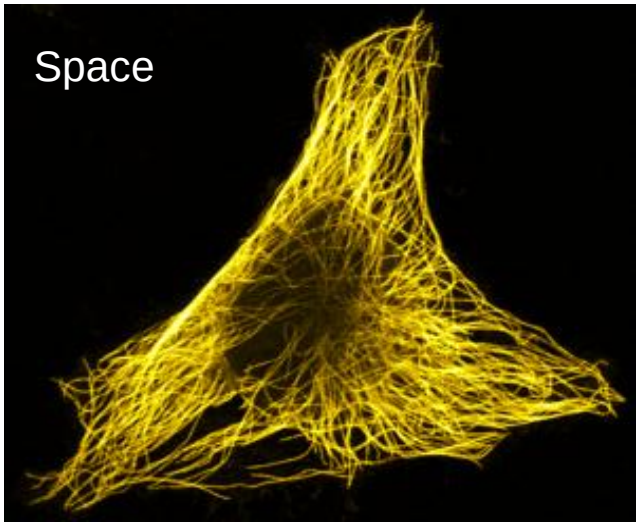
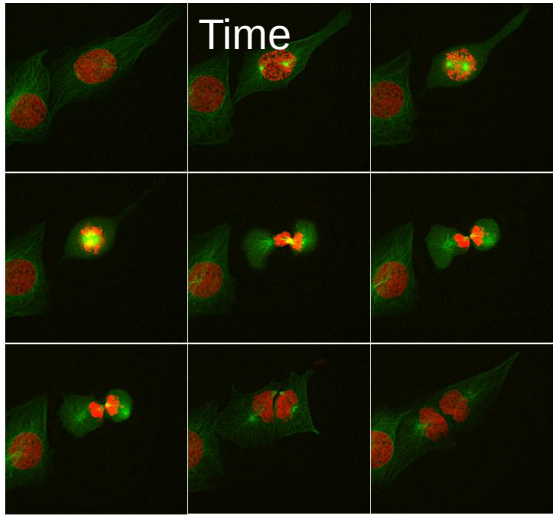
Fluorescence

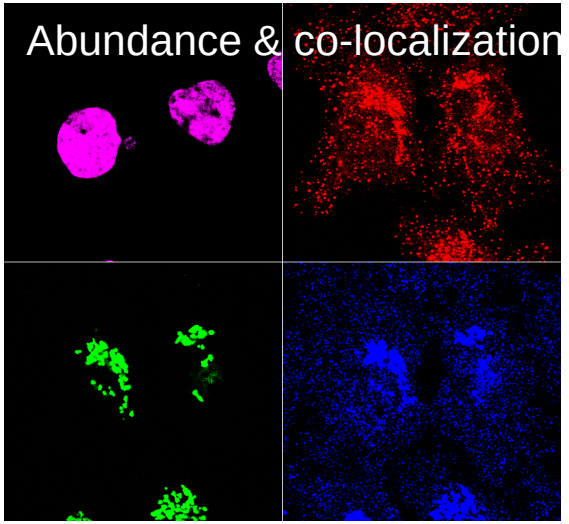
Studying biology in an intact system: Microscopy & Fluorescence

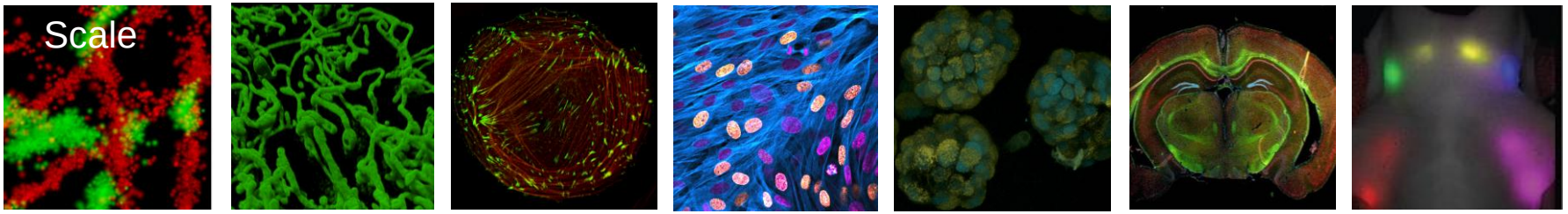


Markers of defined structures & processes









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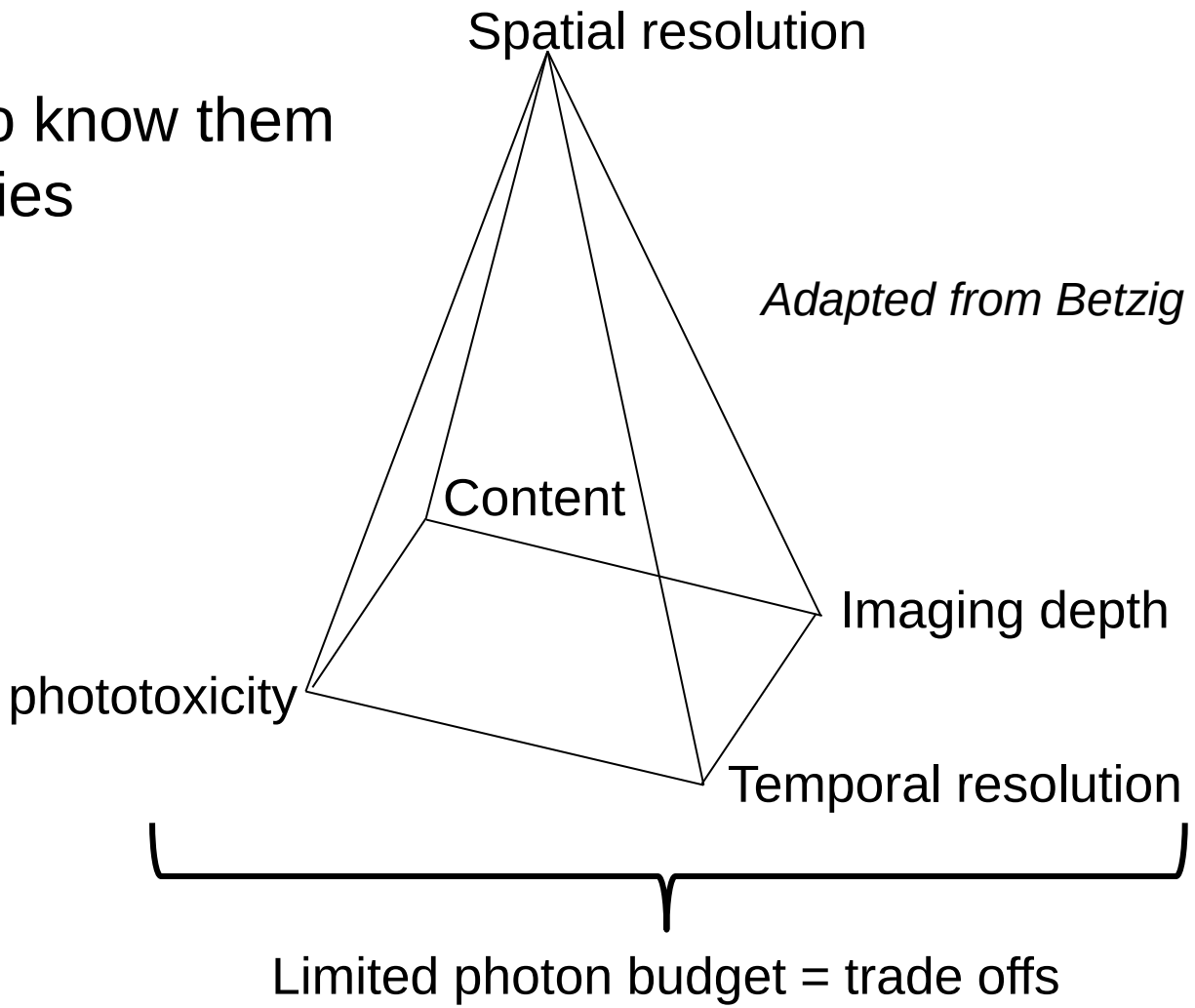
We need fluorescent molecules

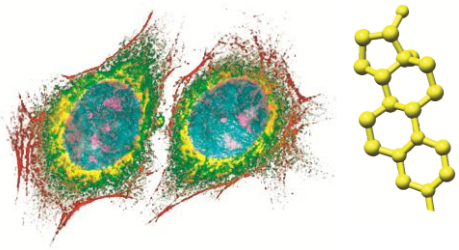
But we need to know them
& their properties



Adapted from Betzig et al

Fluorescent moieties

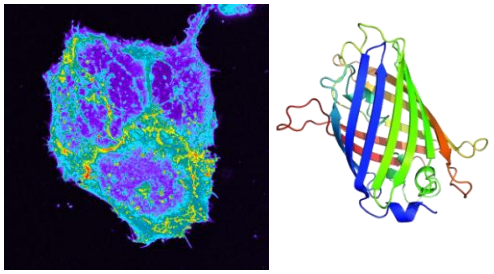
Organic dyes



- UV to Infrared
- Live and/or fixed cells
- Photolabile

- Antibody & conjugates
- Cell Tracking
- Biochemical, Ion & voltage indicators
- Organelle stains

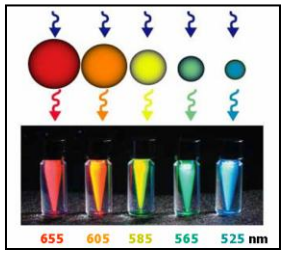
Fluorescent proteins

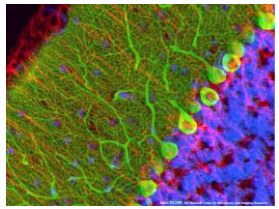


- Blue to Near-Infrared
- Live cells, fixable
- Photolabile

- SDM-function:location
- Functional "Biosensors"
- Organelle targeting

Nanocrystals





- Green to Infrared
- Live and/or fixed cells
- Very Photostable

- Single molecule
- In vivo imaging
- CLEM

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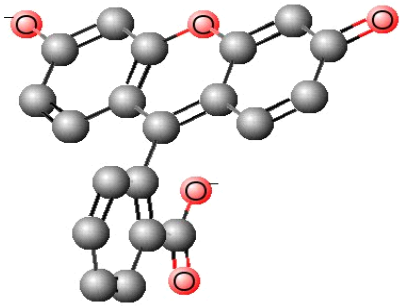
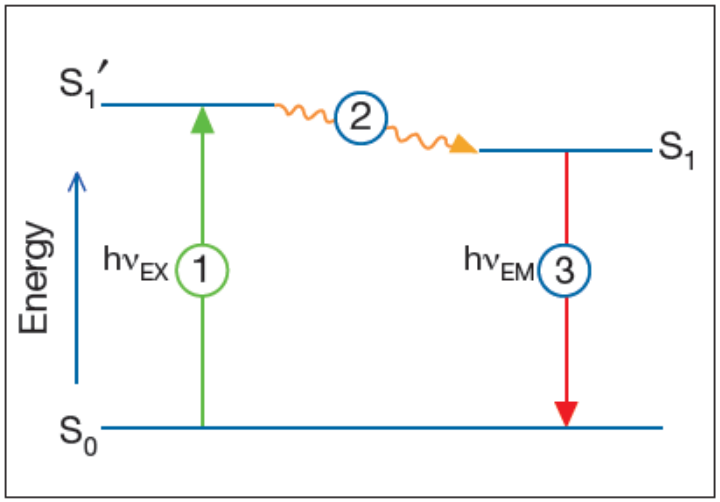
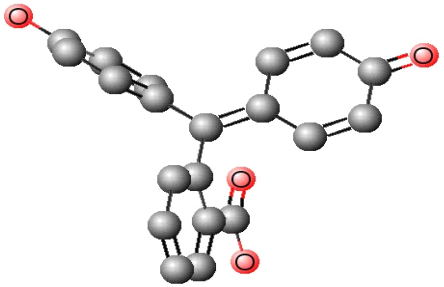
Fluorescence & Fluorophores



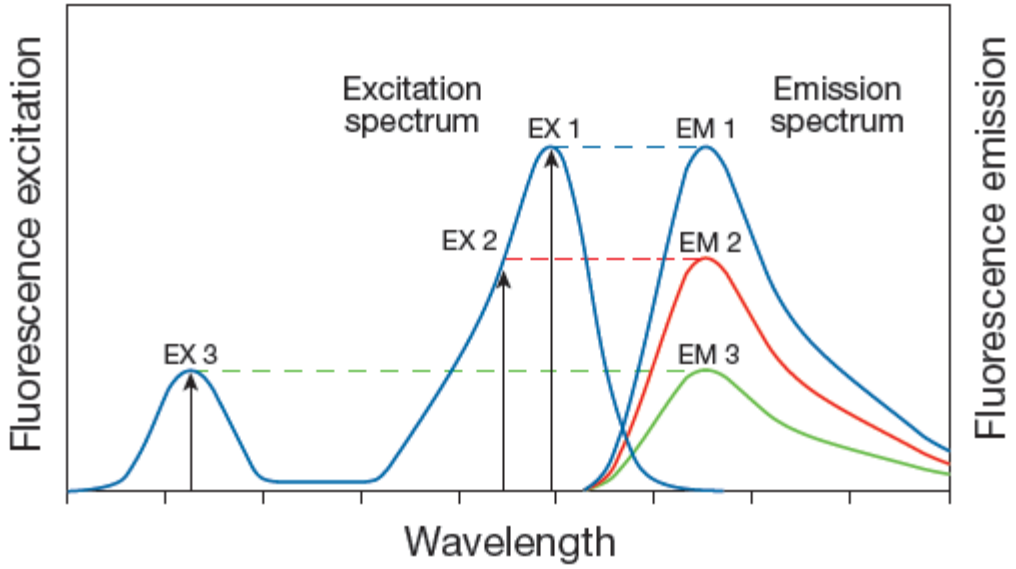
Fluorescein
(Fluorescent)



Brightness
1. Quantum yield
2. Extinction co efficient



Phenolphthalein
(Nonfluorescent)



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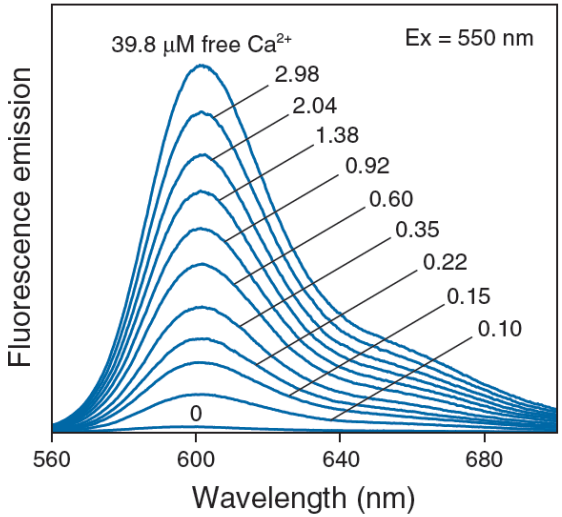
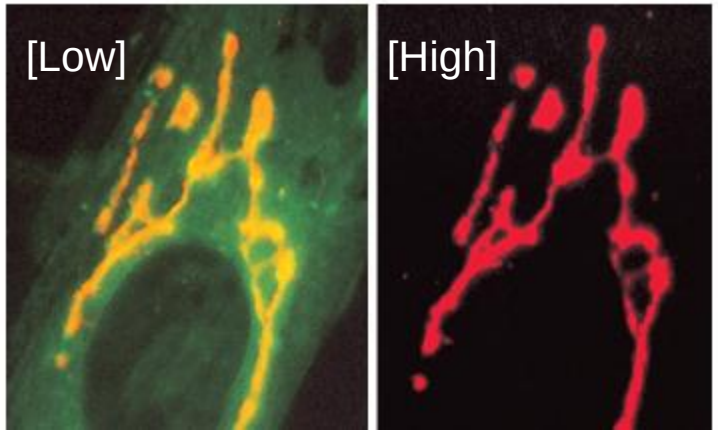
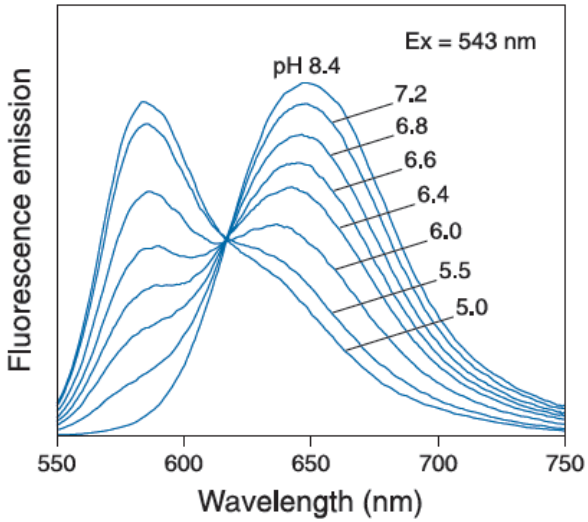
Fluorescence & Fluorophores: Changing properties

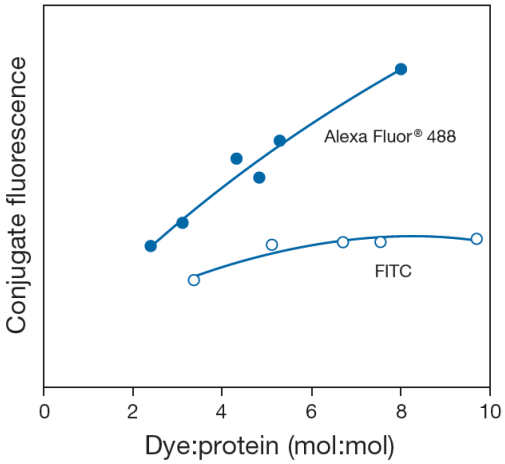


X-rhod-1



BODIPY™ FL C5-ceramide
J Cell Biol (1991)113:1267

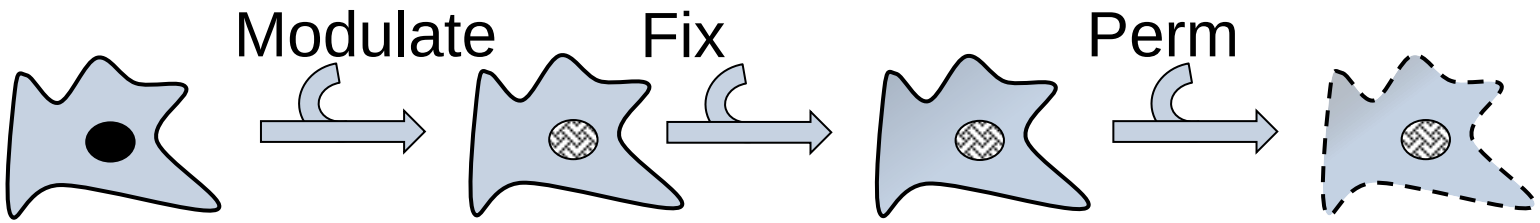




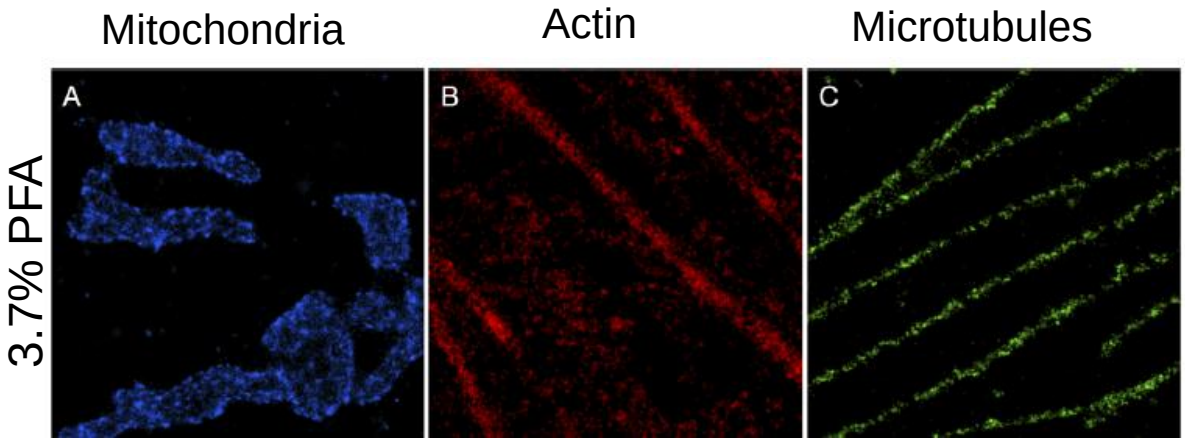
Fluorophore	EC	QY
FITC	78000	0.79
Alexa Fluor™ 488	71000	0.92

SNARF™-4F 5-(and 6-)carboxylic acid

Labeling & detection



- Most standard fix/perm have been used
- Need to optimize

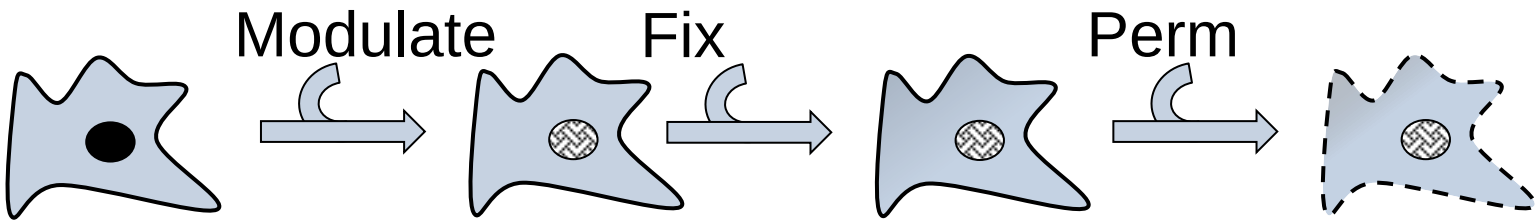


All three targets look good

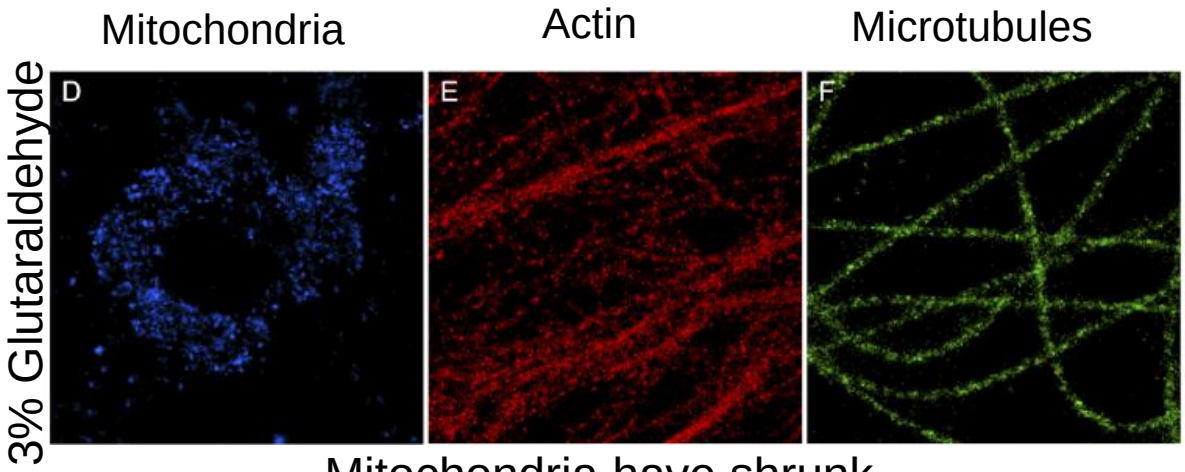
Whelan & Bell (2015) Sci Reports 5 7924

SMLM imaging using Alexa Fluor™ 647

Labeling & detection



- Most standard fix/perm have been used
- Need to optimize

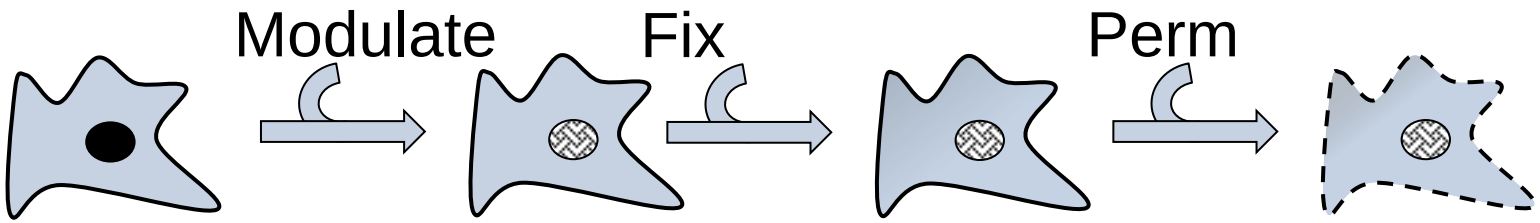


Mitochondria have shrunk

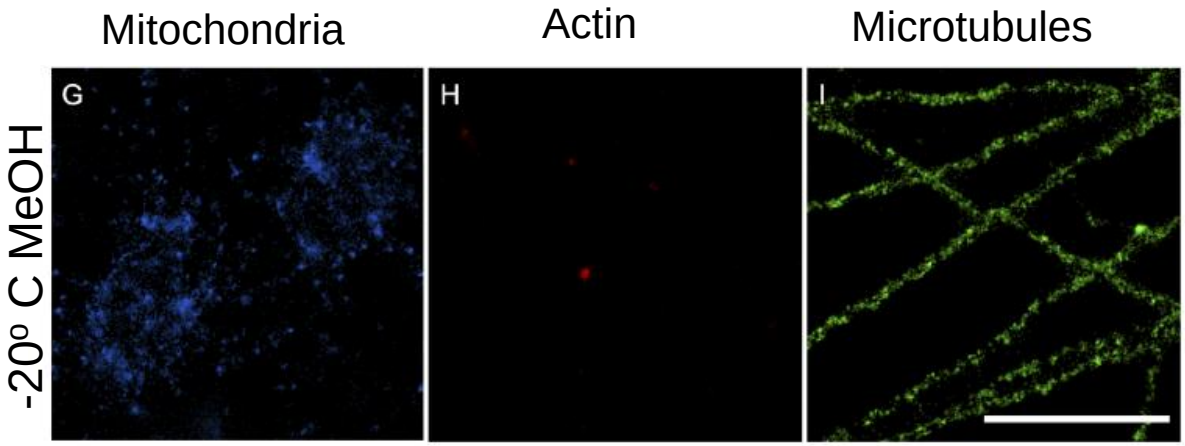
Whelan & Bell (2015) Sci Reports 5 7924

SMLM imaging using Alexa Fluor™ 647

Labeling & detection



- Most standard fix/perm have been used
- Need to optimize

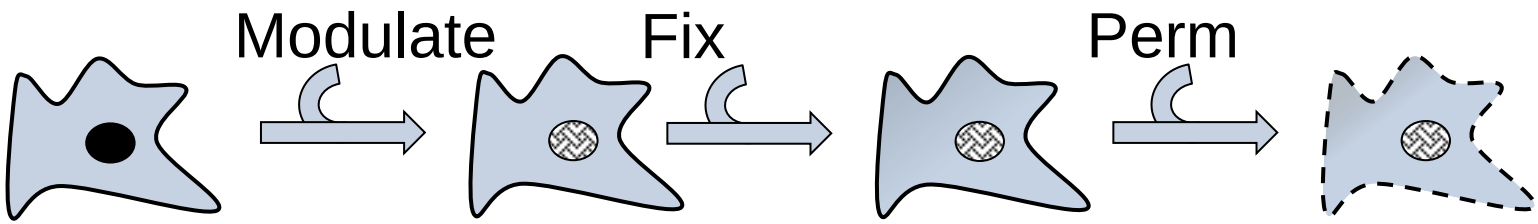


Actin labeling is lost

Whelan & Bell (2015) Sci Reports 5 7924

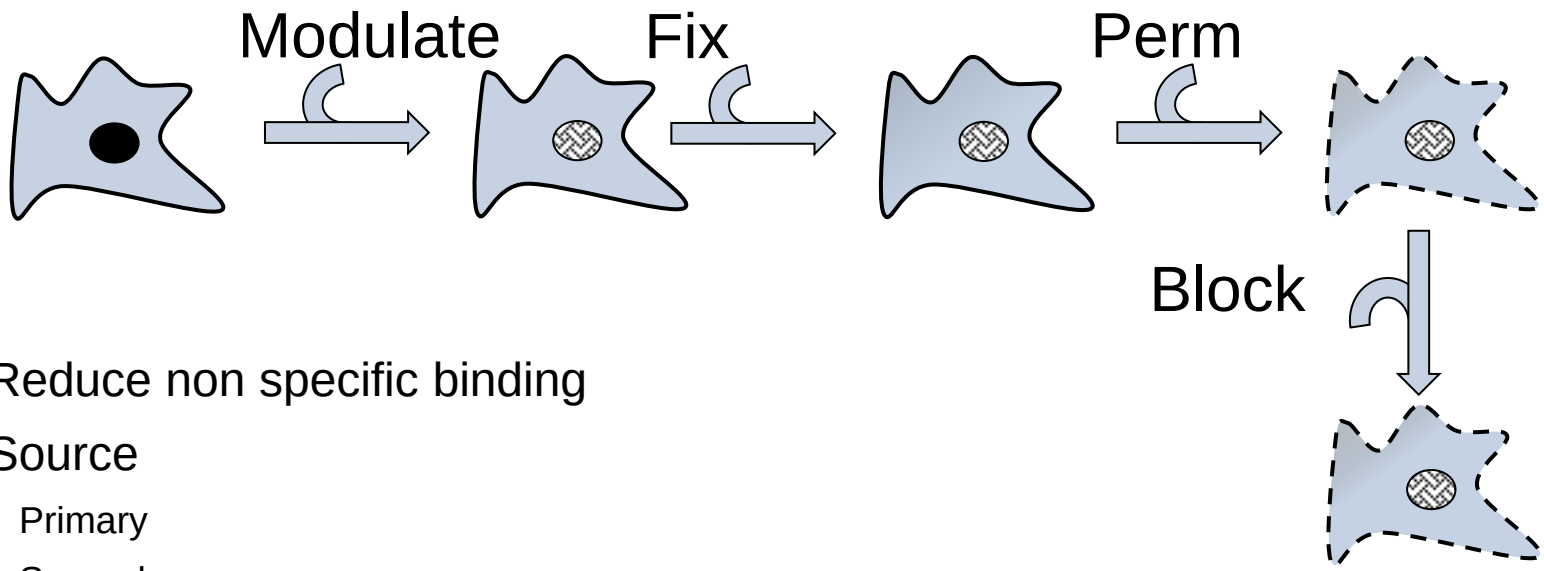
SMLM imaging using Alexa Fluor™ 647

Labeling & detection



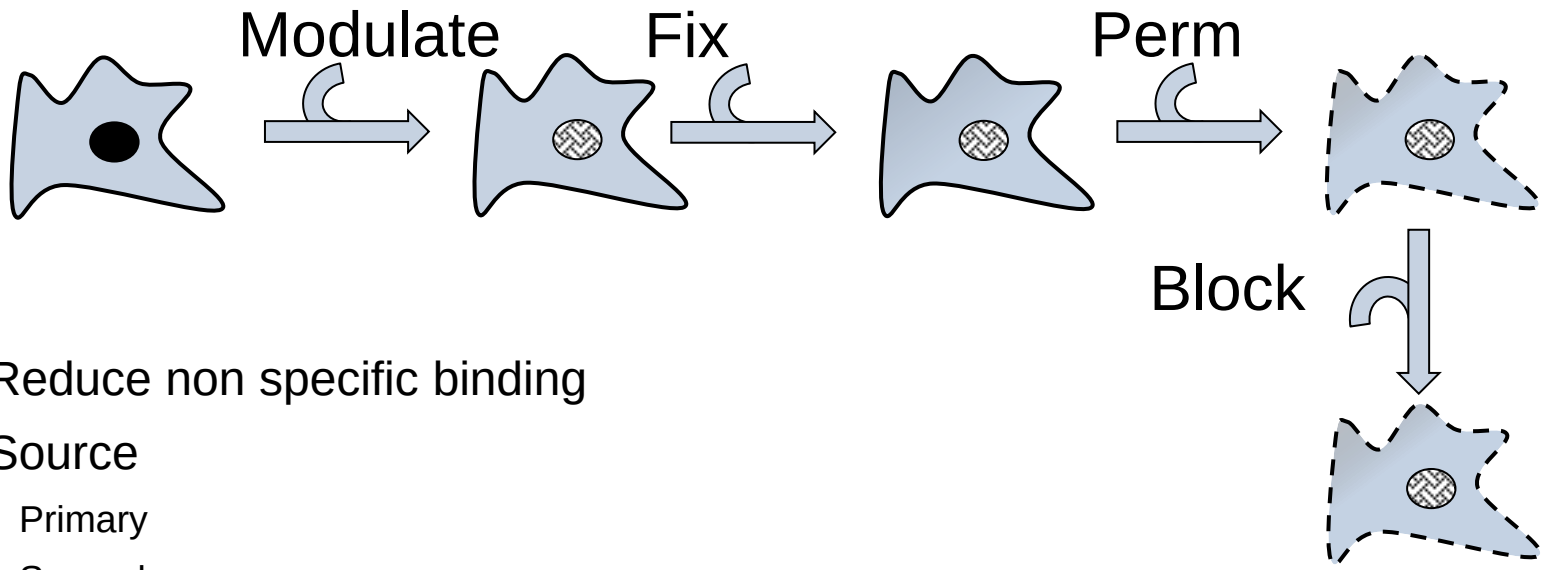
- Most standard fix/perm have been used
- Need to optimize
- If using Glutaraldehyde then auto fluorescence needs to be removed (with NaBH4)
- These references are excellent starting points:
  - Allen JR, Ross ST, Davidson MW (2013) *Phys Chem Chem Phys* 15:18771– 18783.
  - Halpern AR, Howard MD, Vaughan JC (2015) *Curr Protoc Chem Biol* 7:103–120.
  - Whelan DR, Bell TD (2015) *Sci Rep* 5:7924.

Labeling & detection



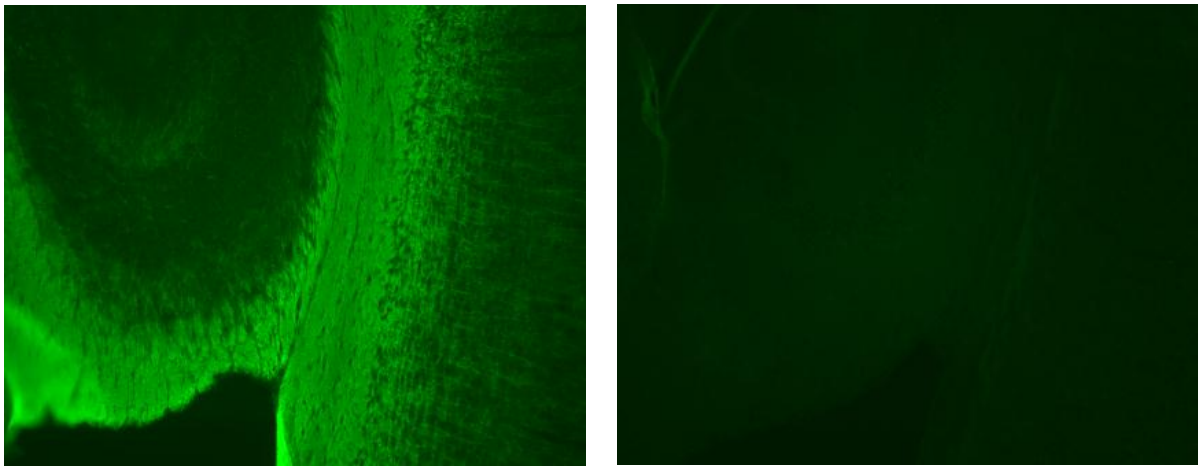
- Reduce non specific binding
- Source
  - Primary
  - Secondary
  - Tertiary
  - Fluorophore

Labeling & detection



- Reduce non specific binding
- Source
  - Primary
  - Secondary
  - Tertiary
  - Fluorophore
- Addition of serum
  - BSA/HINGS
  - Image-iT FX Signal enhancer

16um Mouse Hippocampus Section No primary with Alexa Fluor® 488 GAM



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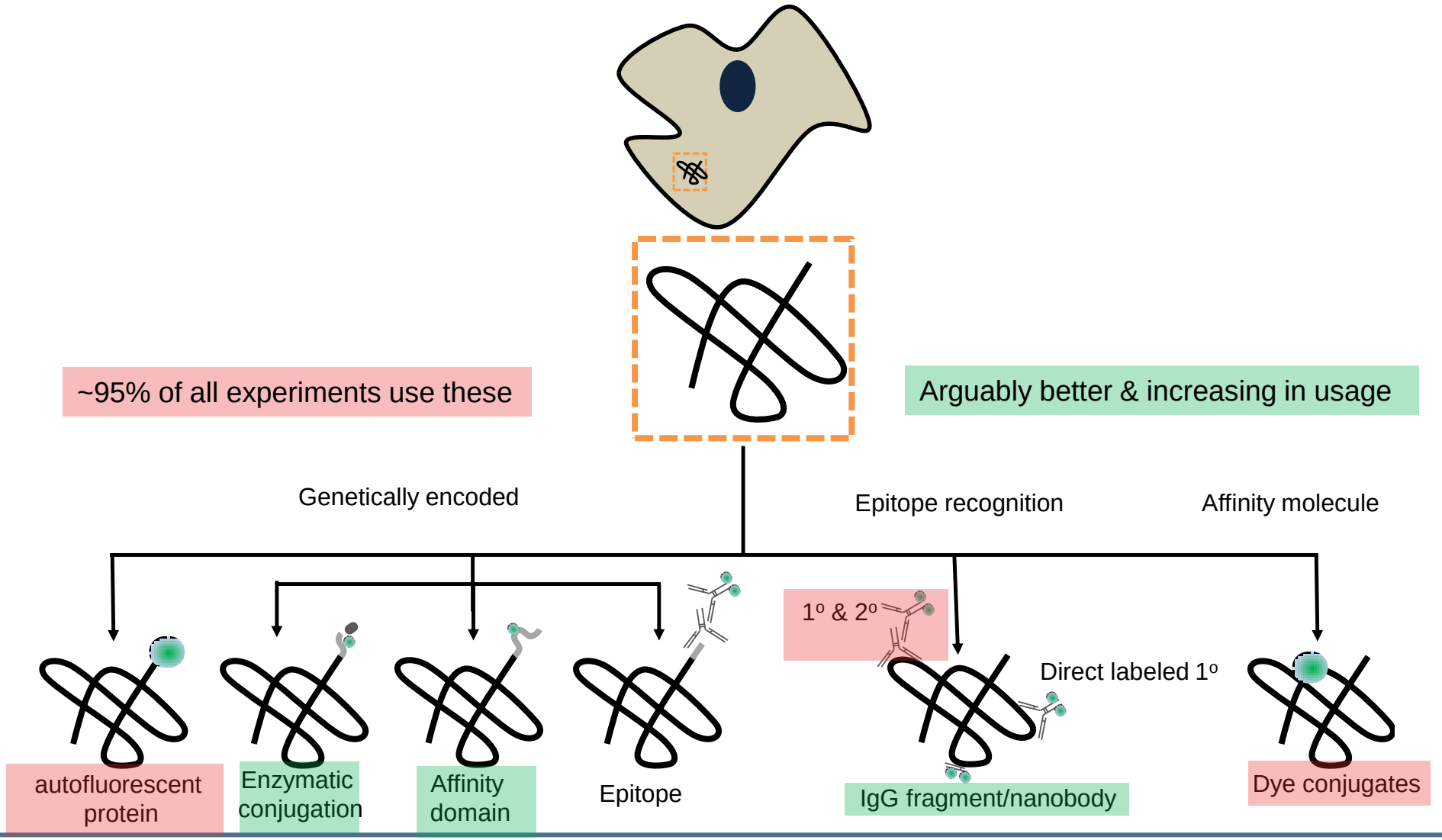
Ways to label & detect a molecule



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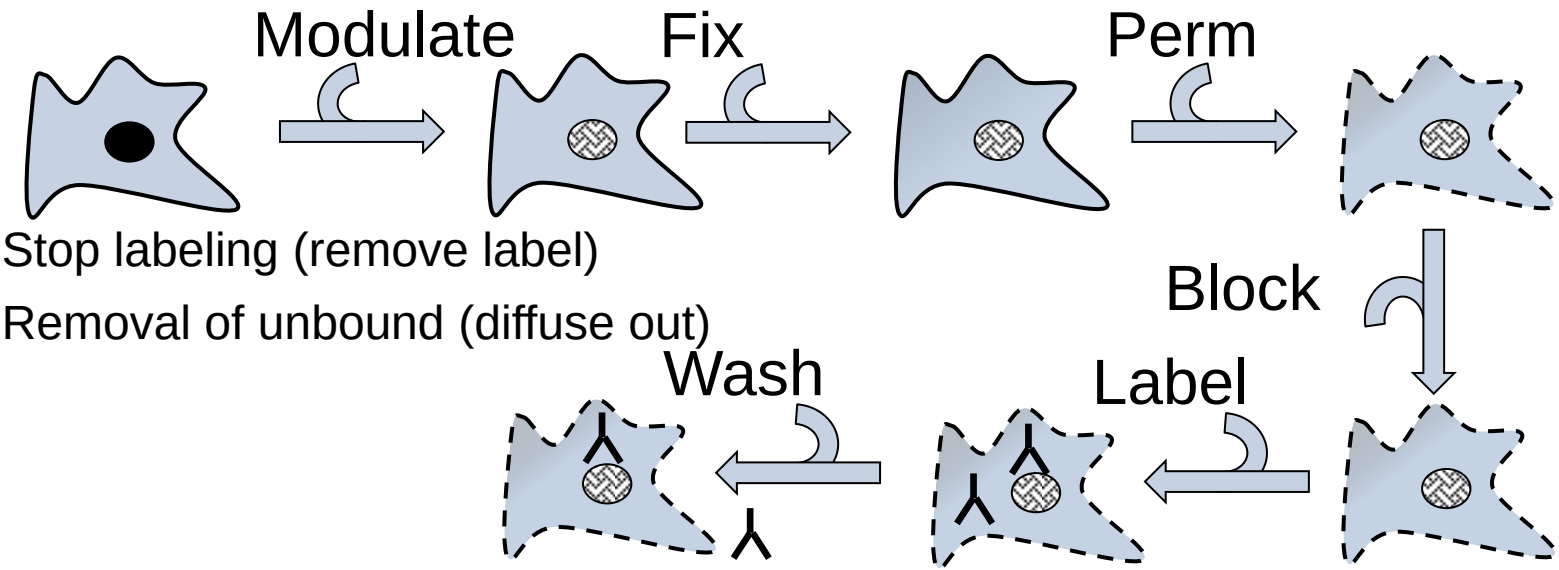
Labeling & detection



- Stop labeling (remove label)
- Removal of unbound (diffuse out)

30s → 60s → 5min → 10min → 15 min (all in PBS)

Stop labeling
(serial dilution)

Removal of unbound
(diffuses out)

Whelan & Bell (2015) Sci Reports 5 7924

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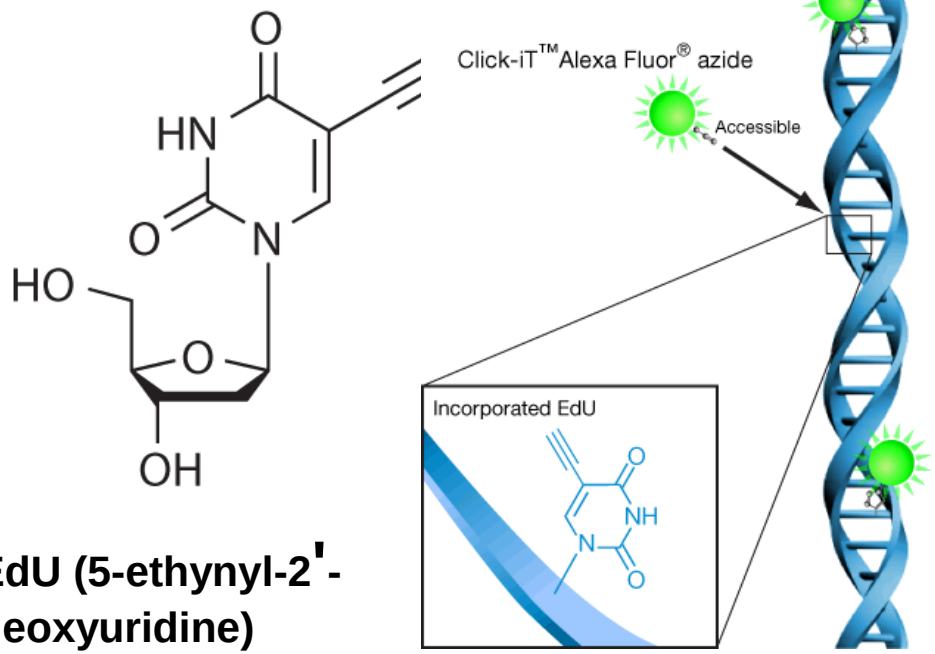
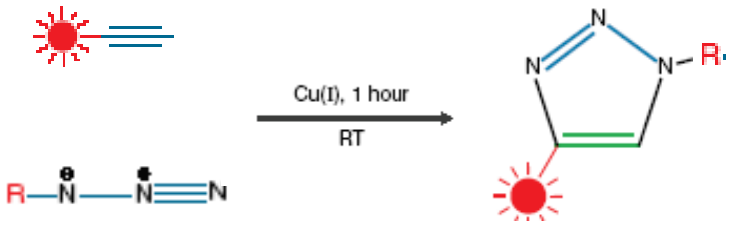
Engineering fluorescence for cell biology: Markers

Labeling proliferating cells with Click-iT™ reagents



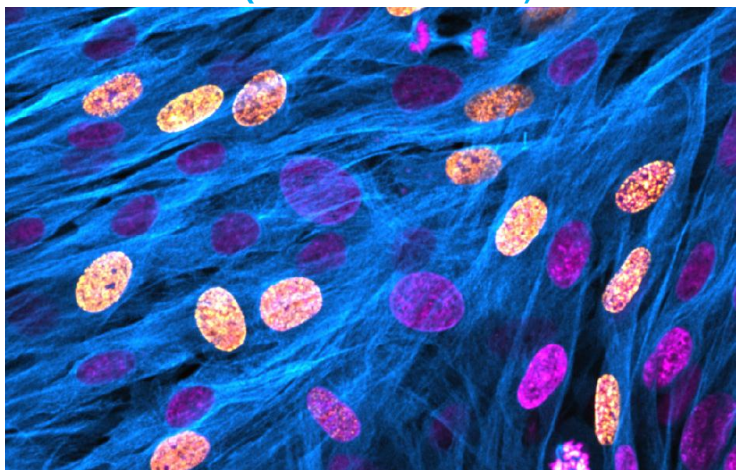
EdU (5-ethynyl-2'-deoxyuridine)

Buck et al (2006) Biotechniques

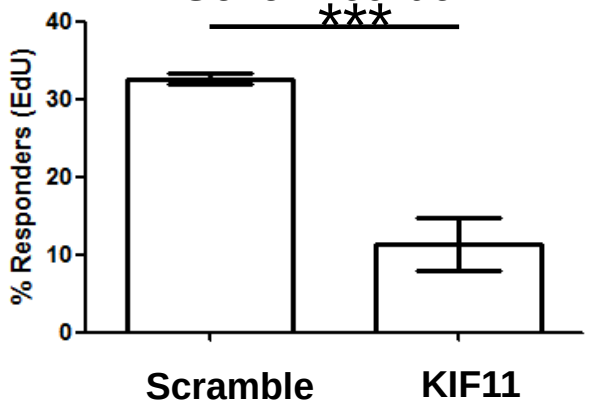


Copper catalyzed click reaction between an alkyne and an azide

Gibco™ HDFn cells **Hoechst 33342**
Click-iT™ EdU (Alexa Fluor™ 488)
Anti α-tubulin (Alexa Fluor™ 555)



Gene knockdown



Lipofectamine™ RNAiMAX
Silencer™ Select RNAi

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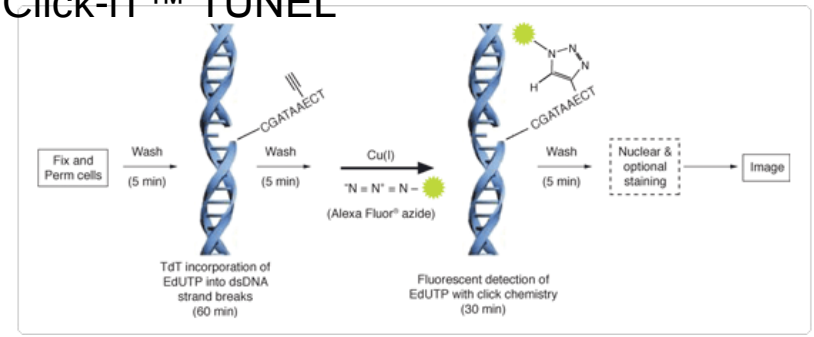
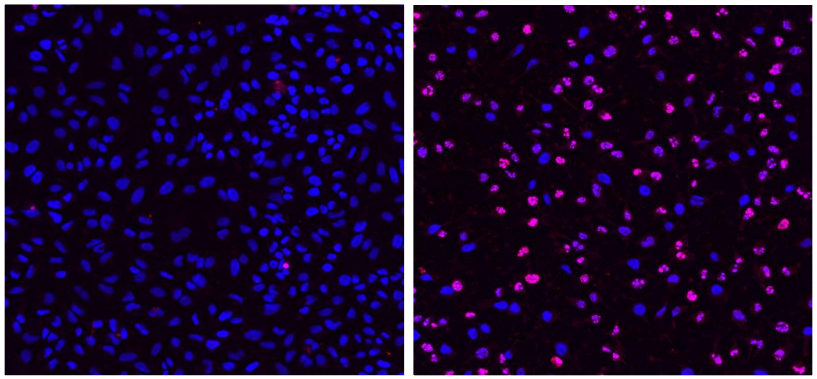
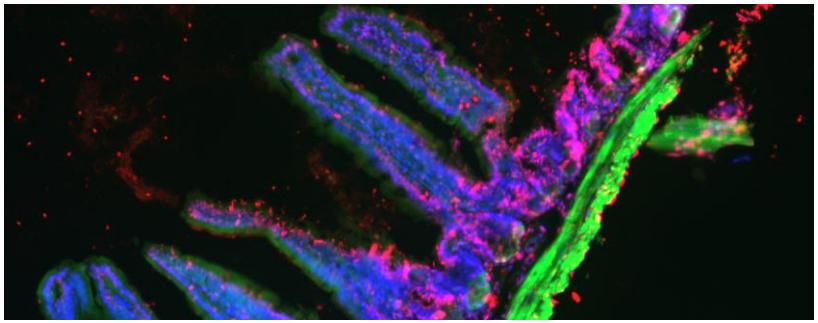
Other uses of Click-iT™

Click-iT™ TUNEL

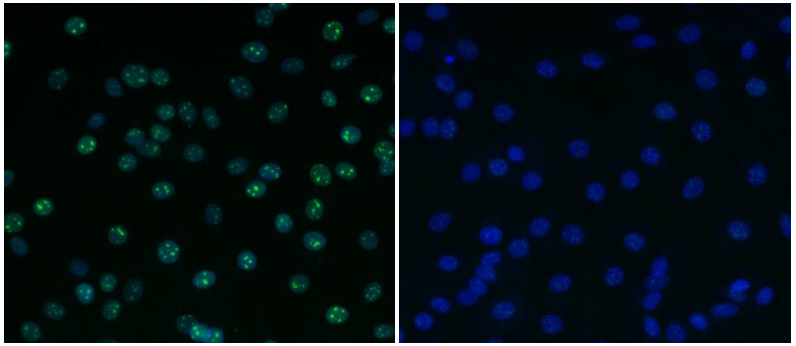


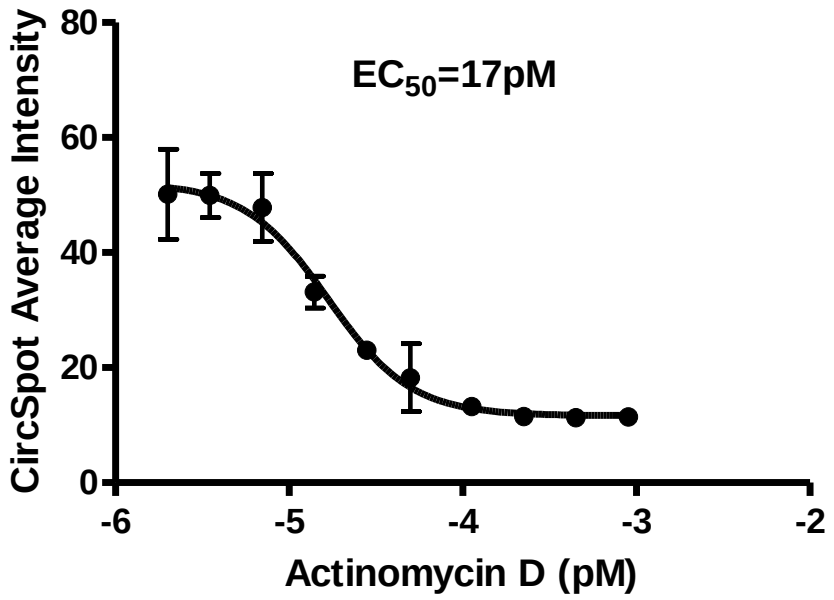




GFP TG mouse intestine, Hoechst & Click-iT™ TUNEL, Alexa Fluor™ 594

Click-iT™ RNA





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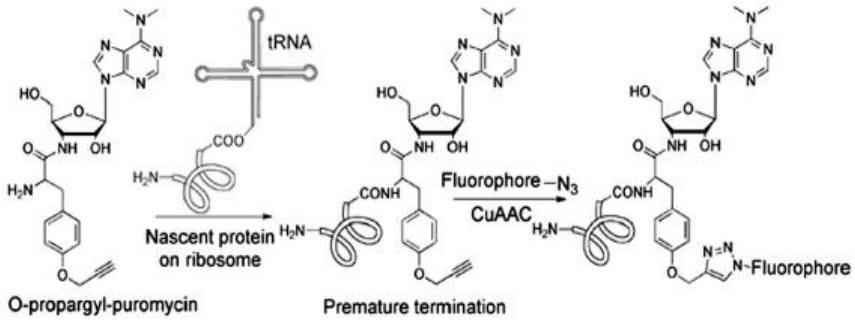
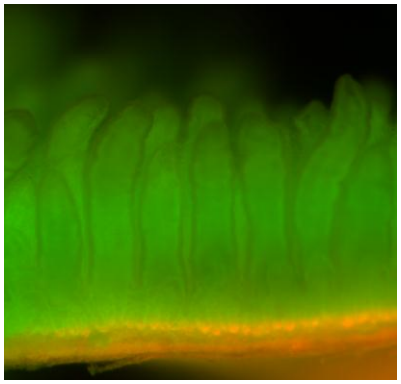
Click-iT™ probes for proteastasis

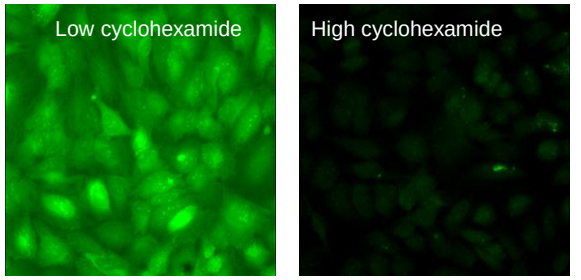


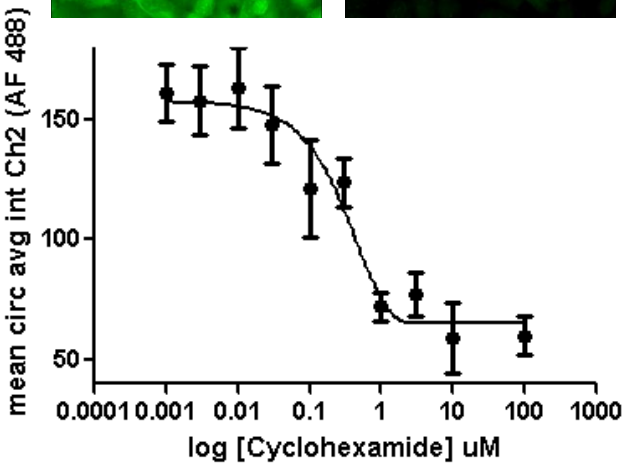
Liu et al (2012) PNAS 109 413–*418*

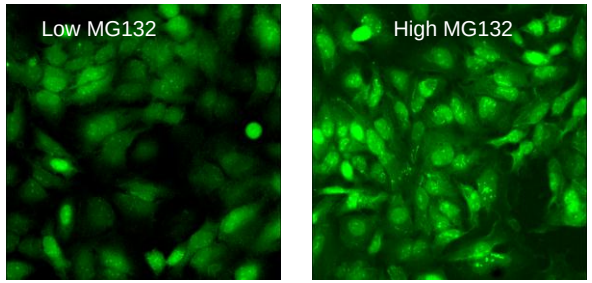


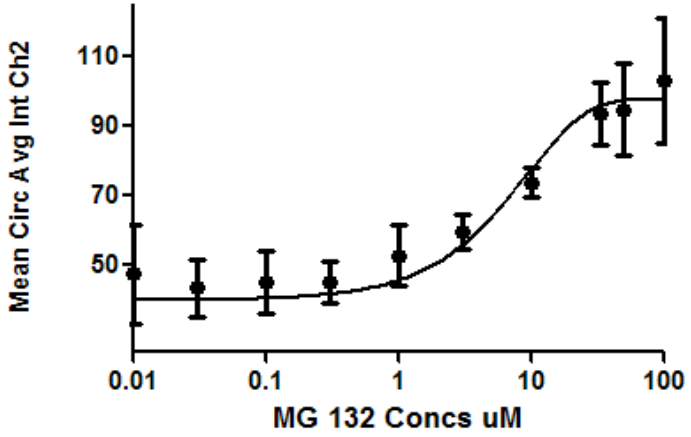
Mouse intestine
Total cell (Oregon Green®)
Click-iT™ OPP

Courtesy of Adrian Salic
Harvard medical school



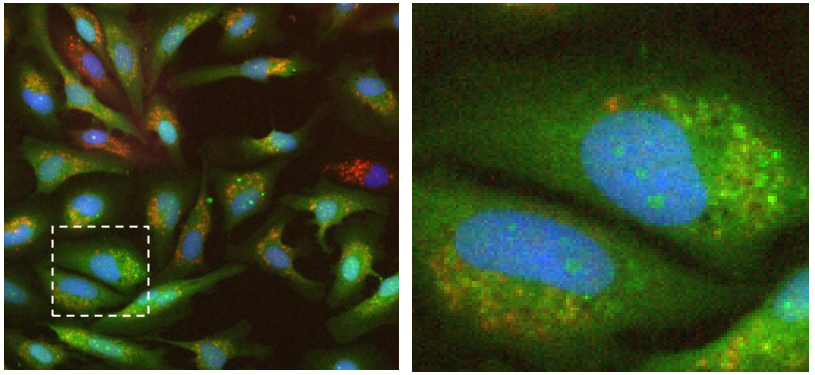




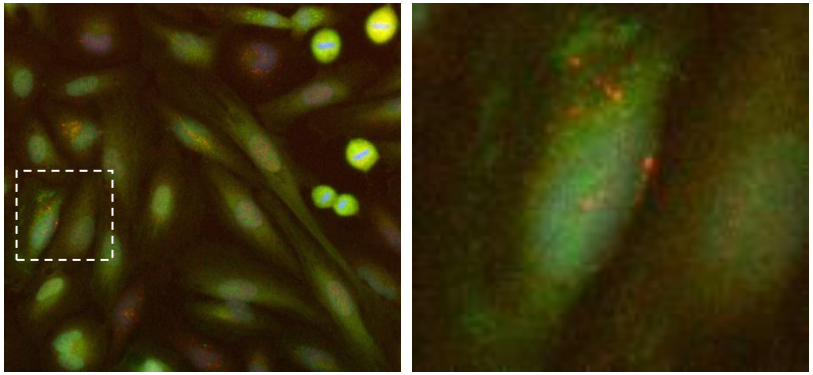


ThermoFisher SCIENTIFIC

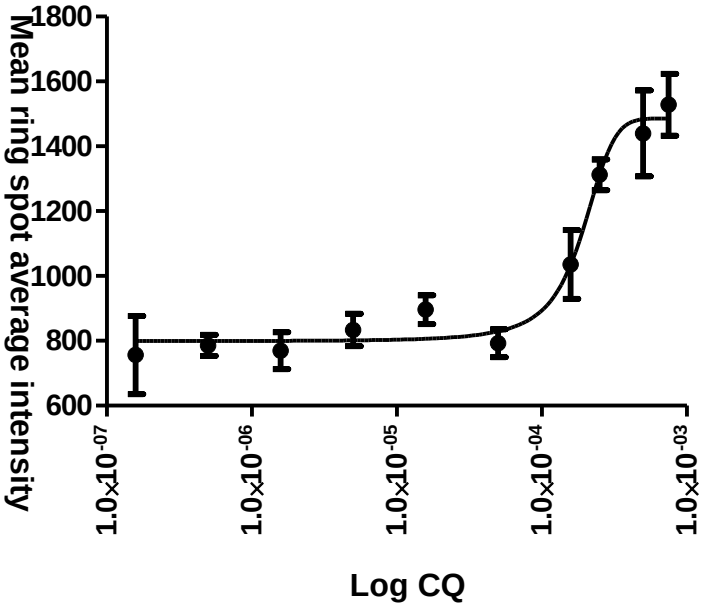
Click-iT™ probes for proteastasis

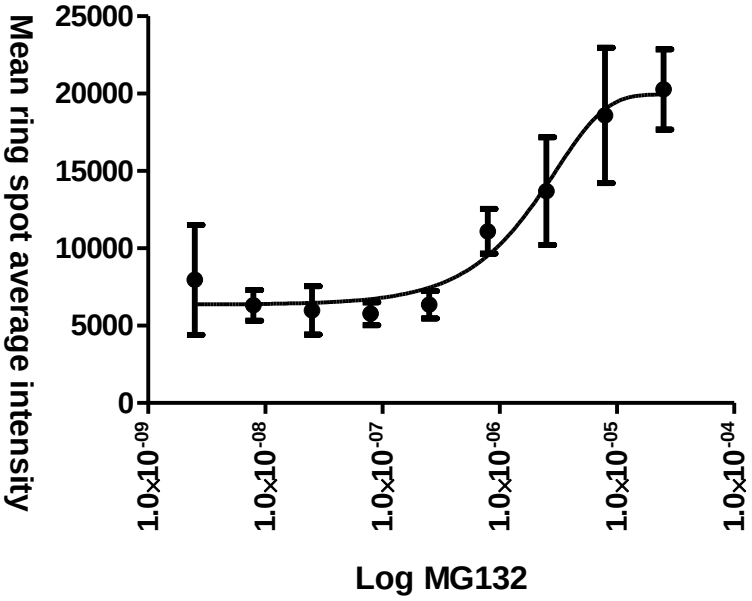


Hoechst/HPG (Azide 488)/Anti-LC3B: CQ treated



Hoechst/HPG (Azide 488)/Anti-LC3B: MG132 treated

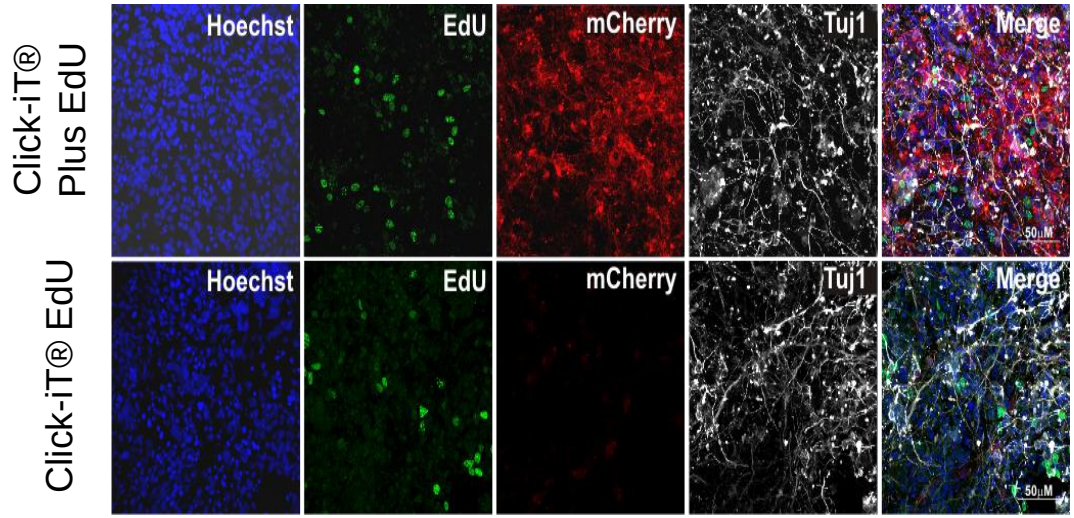




ThermoFisher SCIENTIFIC

Click-iT™ catalysis

Copper catalyzed click reaction between an alkyne and an azide

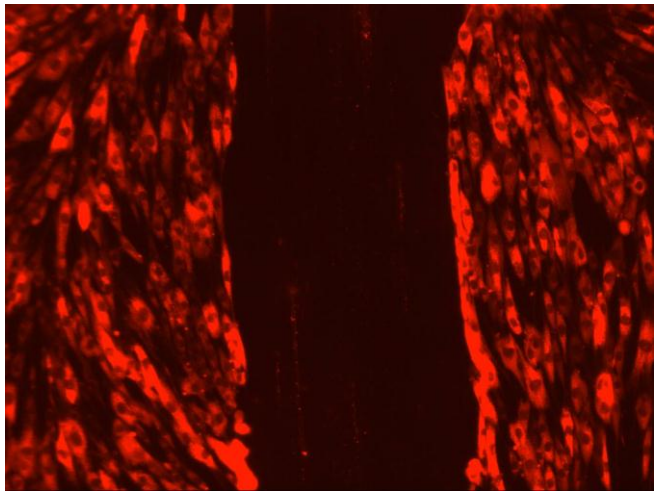


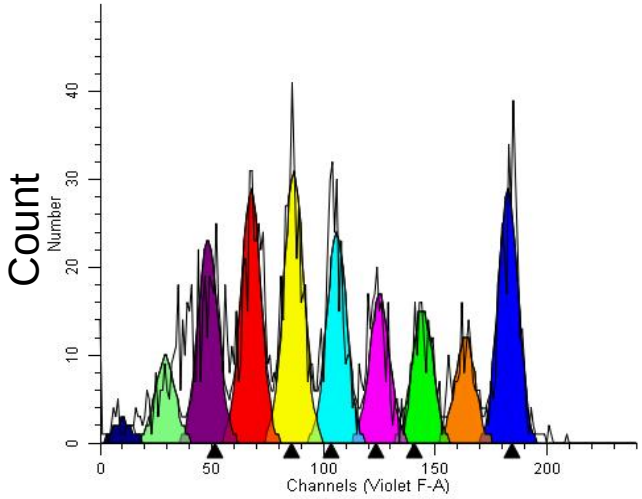
Uttamapinant et al (2012) Angew Chem Int Ed Engl.51: 5852–5856

ThermoFisher SCIENTIFIC

Attaching dyes to cellular components: Cell tracking & tracing

CellTracker™ Deep Red





CellTrace™ Violet Fluorescence

Attaching dyes to cellular components: Cell tracking & tracing

CellTracker™ Deep Red

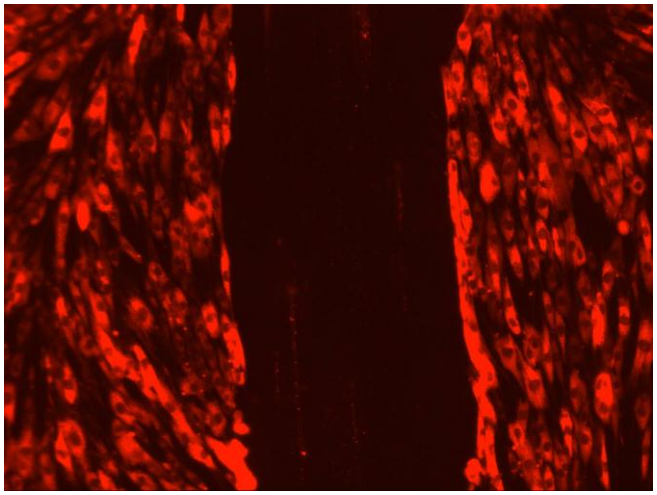
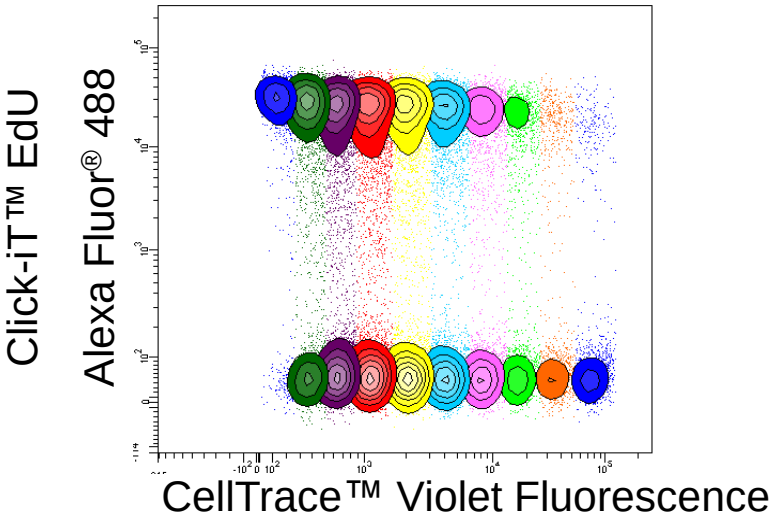
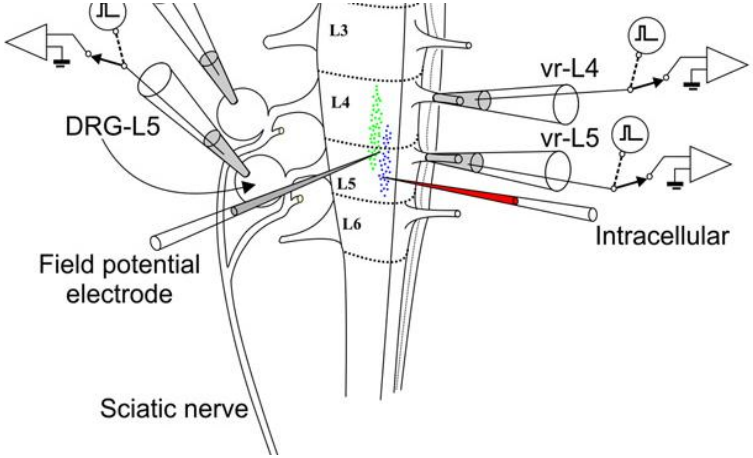


Image courtesy of
GMentis & M O'Donovan
NINDS/NIH, Bethesda, MD

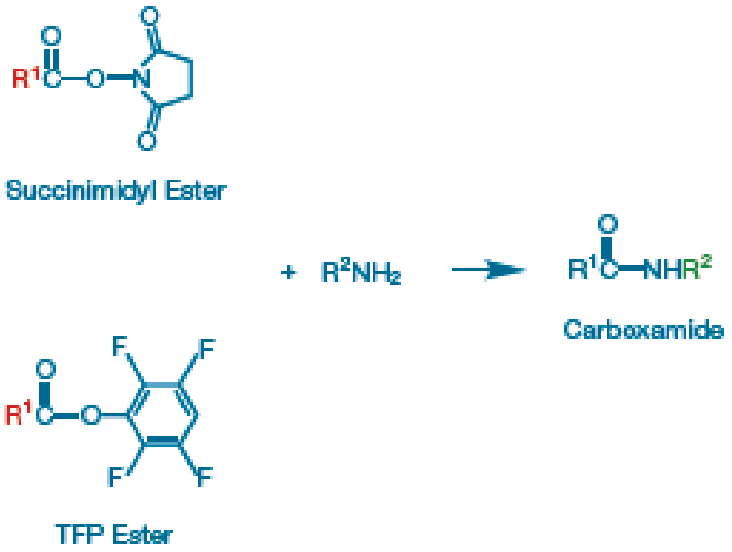


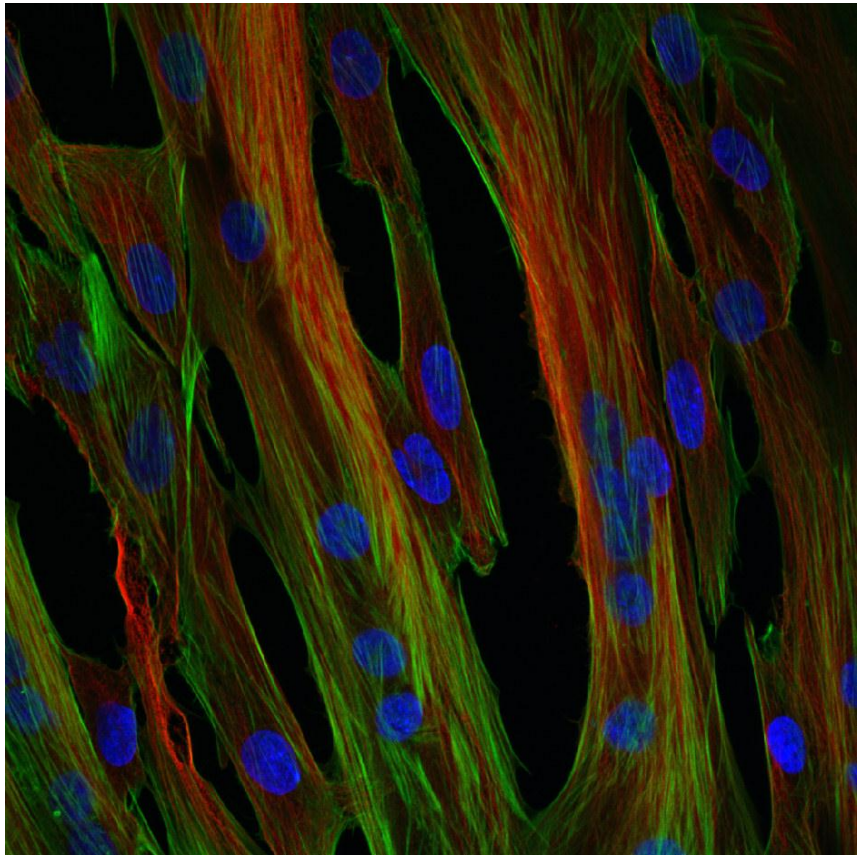


Schneider et al (2009) J Neurosci. 29(15): 4719–4735

ThermoFisher SCIENTIFIC

Biological conjugates: NHS ester coupling





Gibco™ Human Skeletal muscle cells
DAPI, Alexa Fluor™ 488 phalloidin
& anti-α-tubulin (Alexa Fluor™ 555)

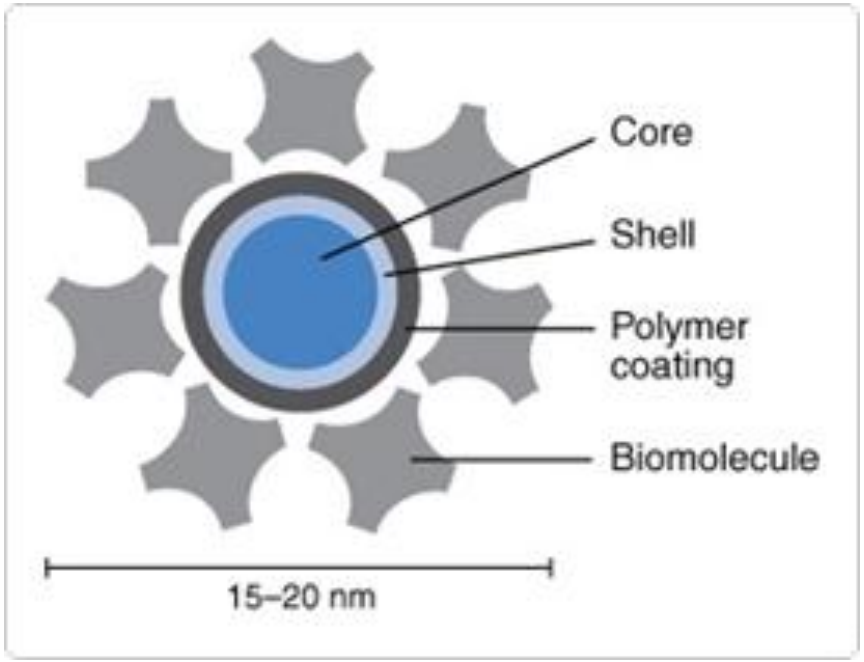
Qdot™ conjugates: Real-time Receptor Ligand Tracking

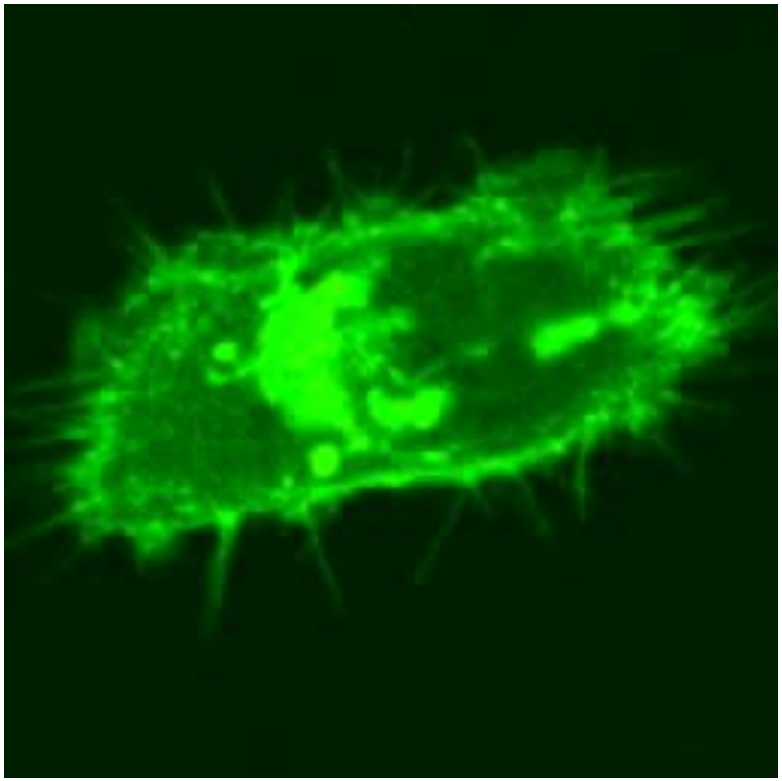
EGF Receptor Internalization

Qdot™ 605-EGF conjugate (erbB1)

EGF receptor (erbB3-Citrine)

Qdot™



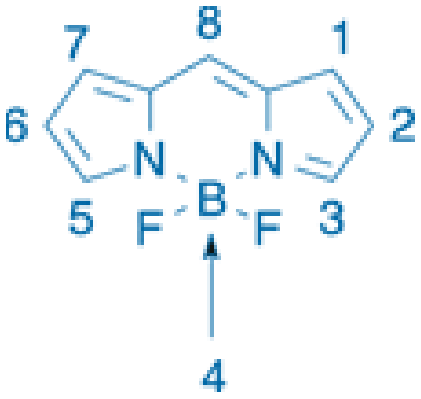


4.5 sec/frame; 100 frames
Sequential confocal scans

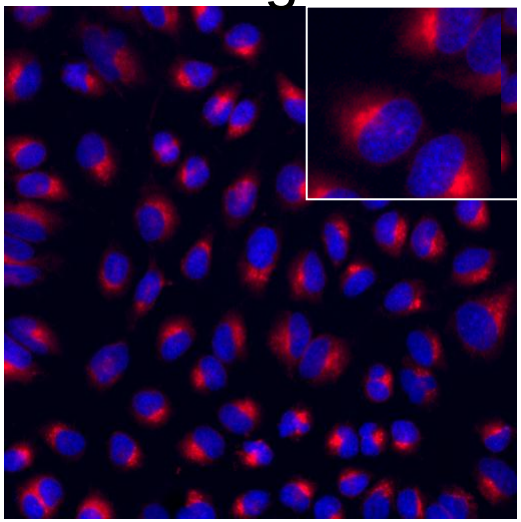
Lidke, D. e*t. al.* Nature Biotechnology 22 (20), 2004

ThermoFisher SCIENTIFIC

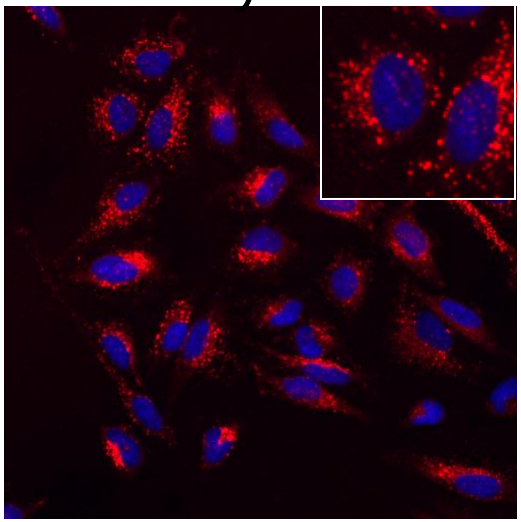
Imaging structures in live cells: fluorescent dyes



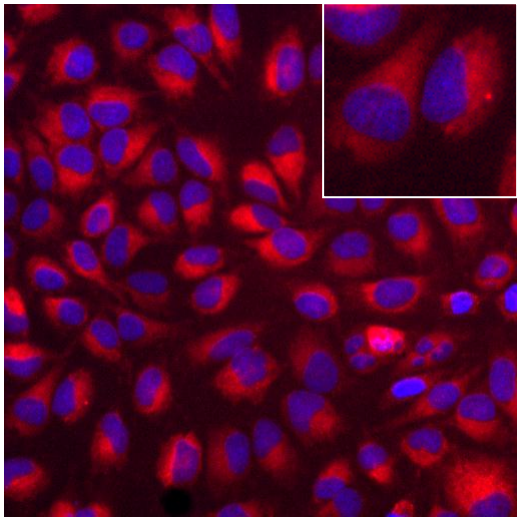
Golgi



Lysosomes

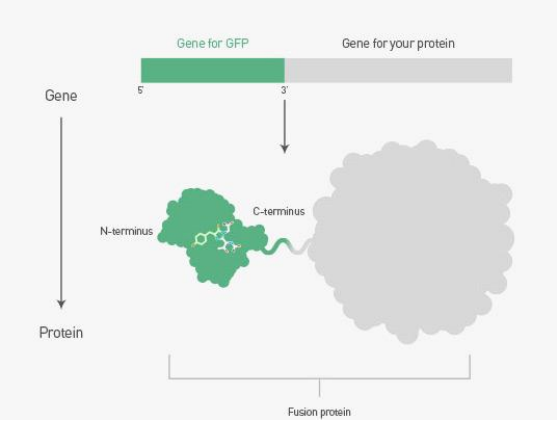


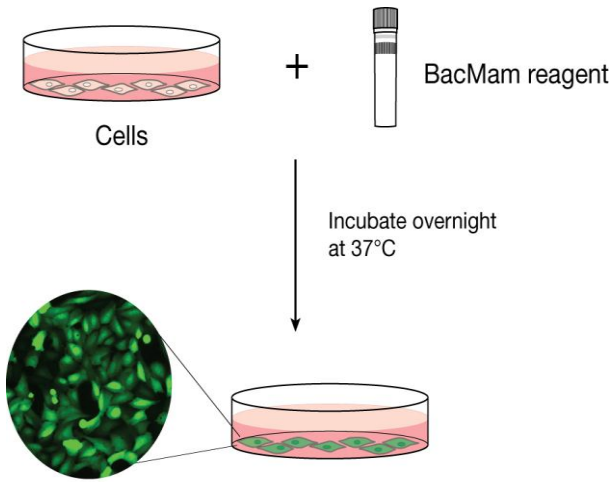
ER

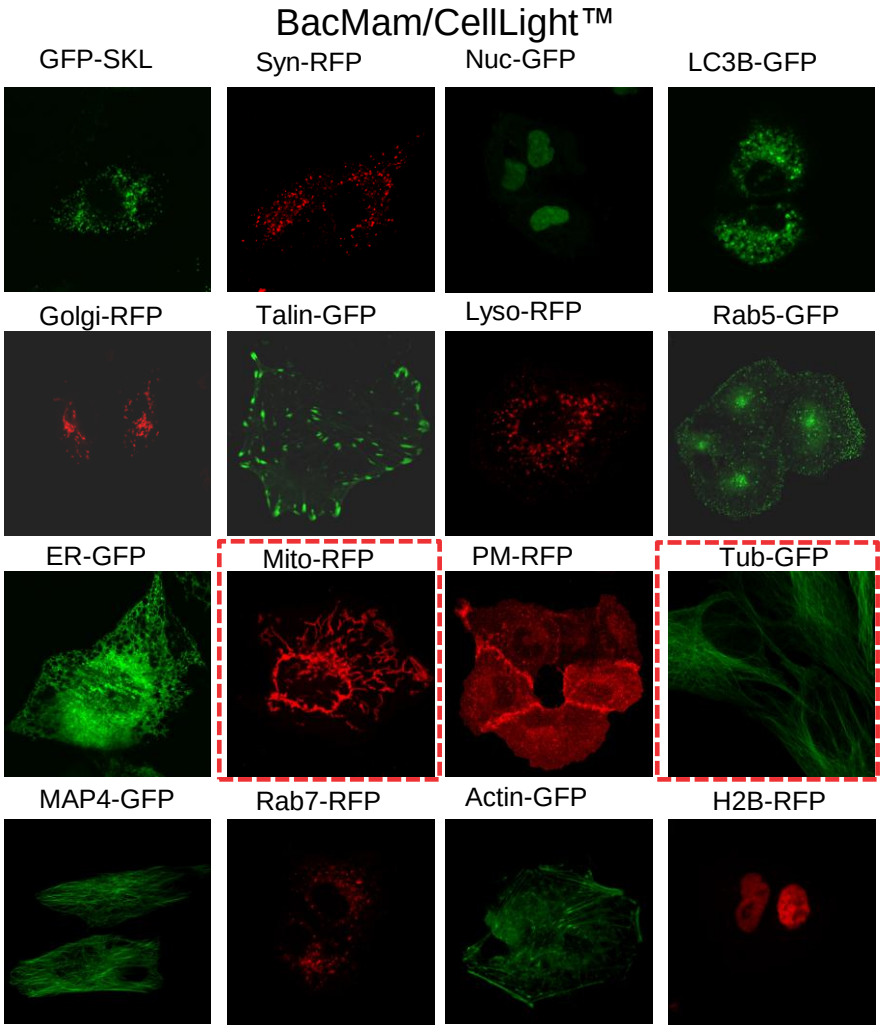


For more info see Kilgore, Dolman and Davidson (2014) Curr Prot Cytom, Chapter 12

Imaging structures in live cells: fluorescent proteins







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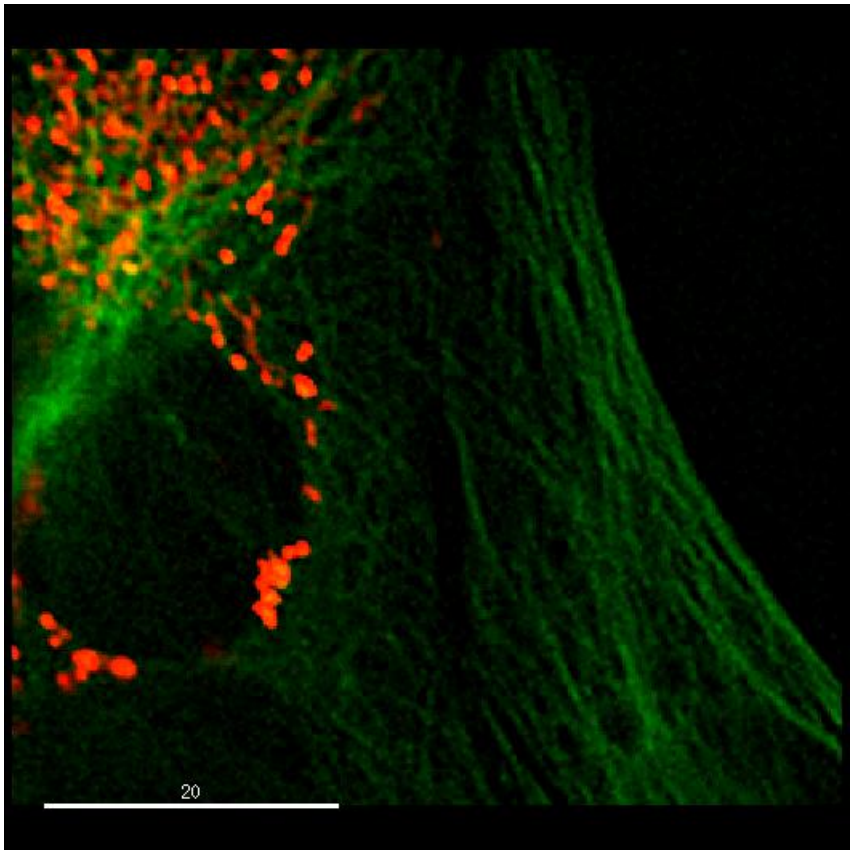
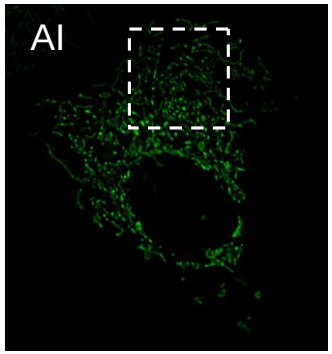
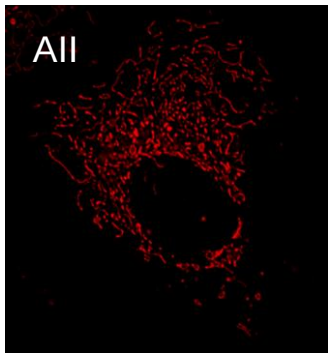
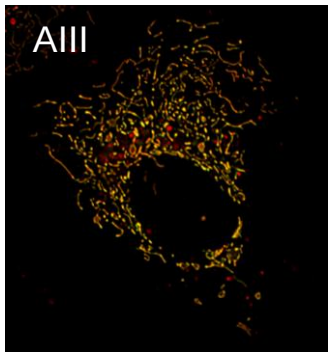
Mitochondrial transport

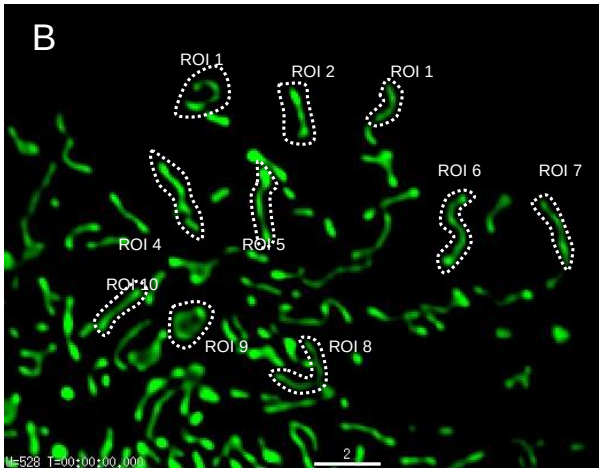


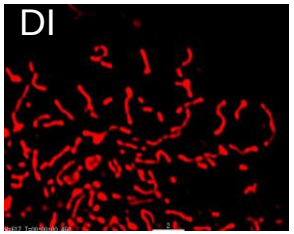
Pairing environmentally sensitive and insensitive probes

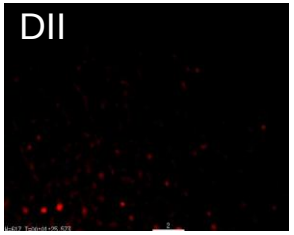


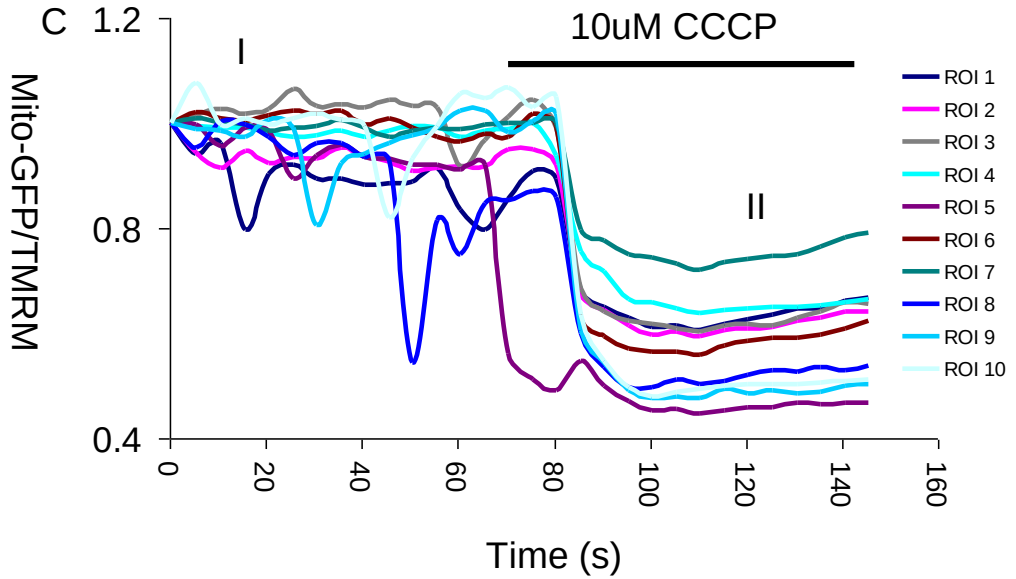




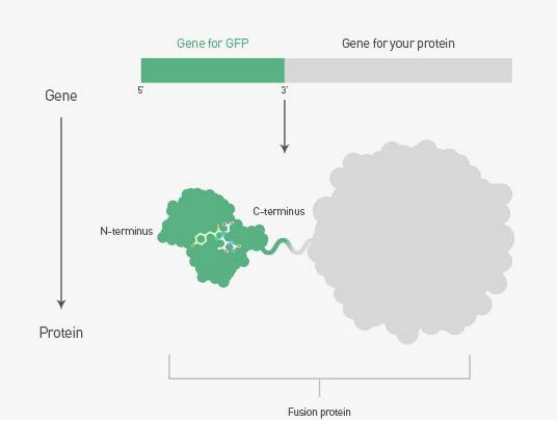


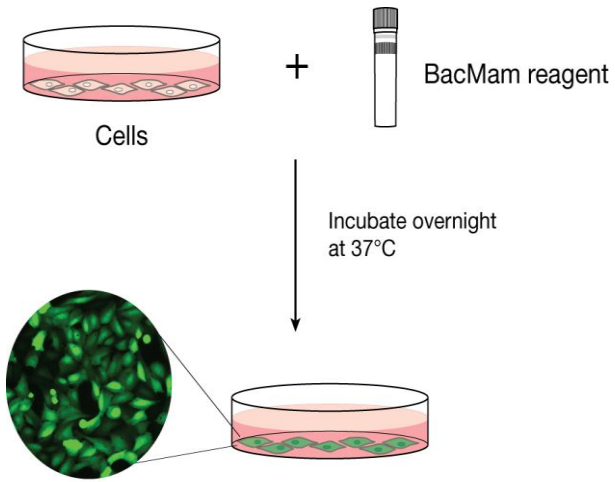




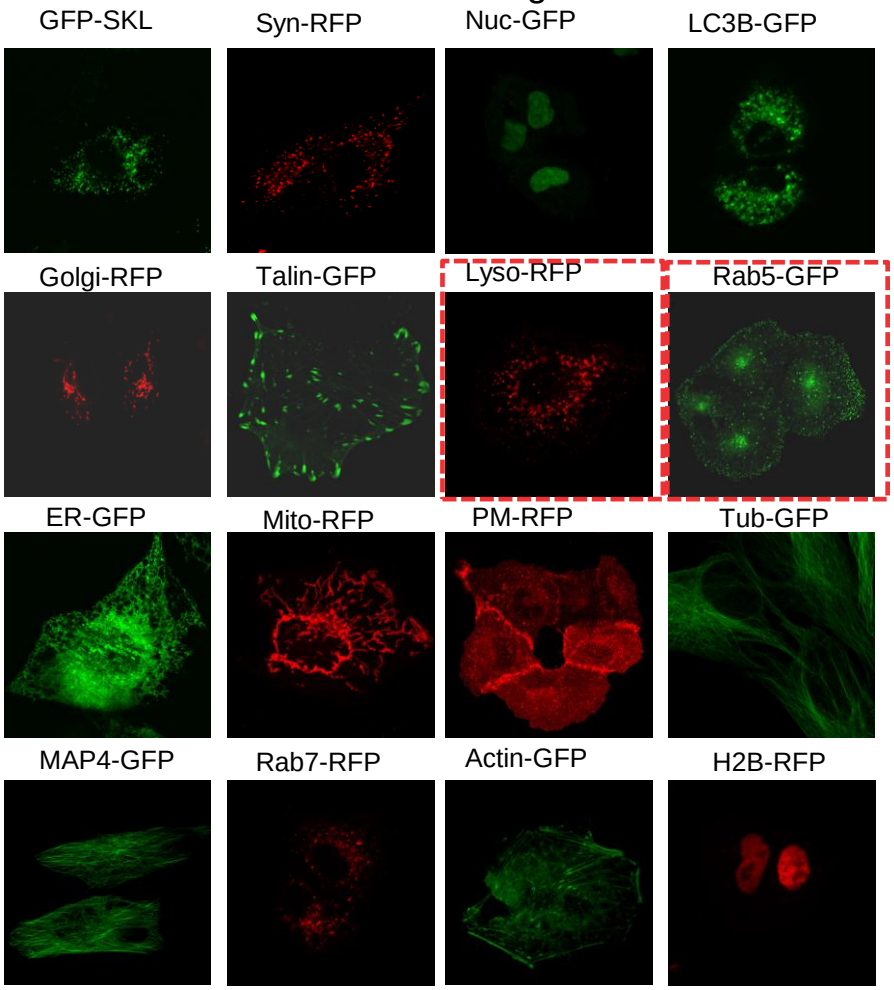


Imaging structures in live cells: Endosomes & lysosomes



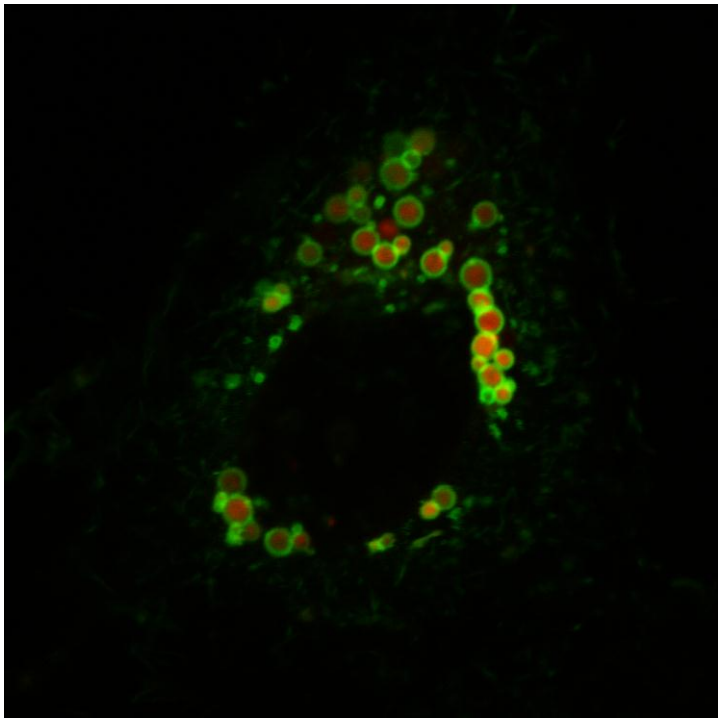


BacMam/CellLight™



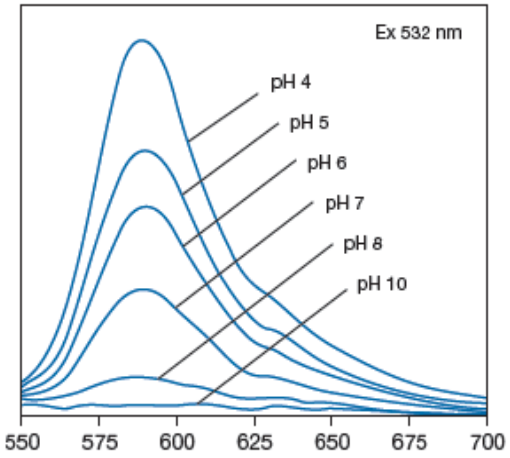
ThermoFisher SCIENTIFIC

Imaging structures in live cells: Endosomes & lysosomes

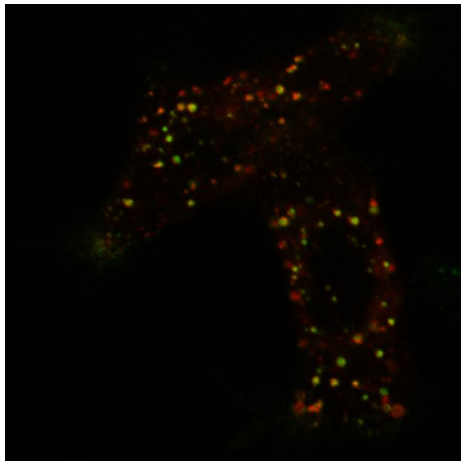


CellLight™ Lyso-GFP
LysoTracker™ Deep Red

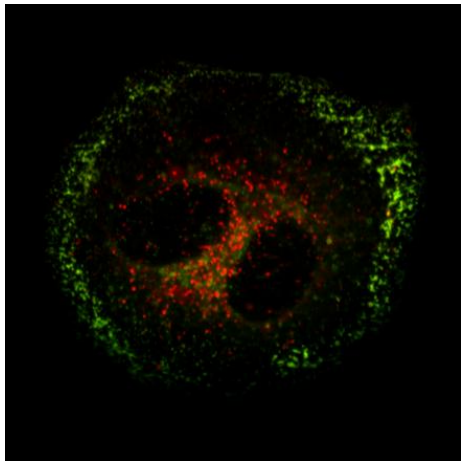
pHrodo™



CellLight™ Early Endosomes RFP
EGF-pHrodo™ green

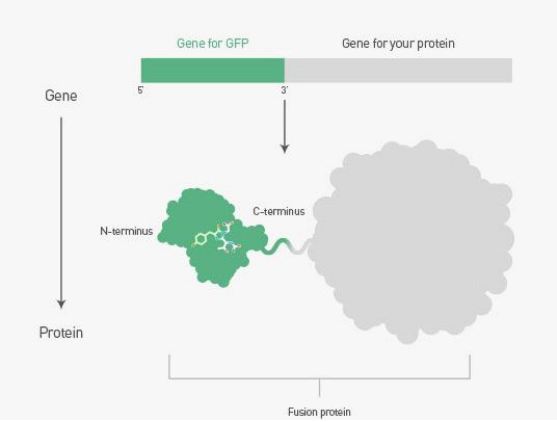


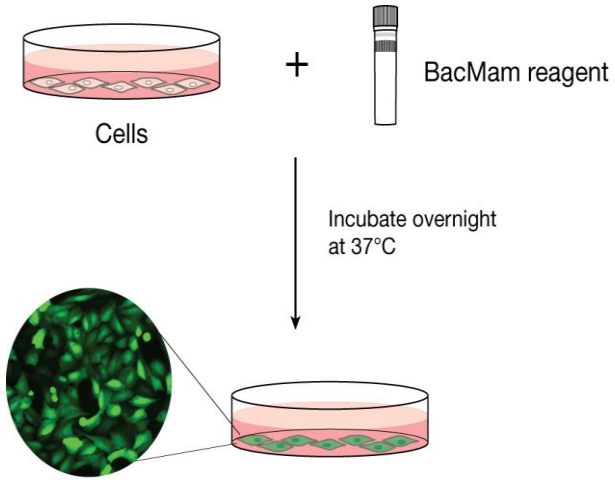
CellLight™ Early endosomes GFP
pHrodo™ red Transferrin

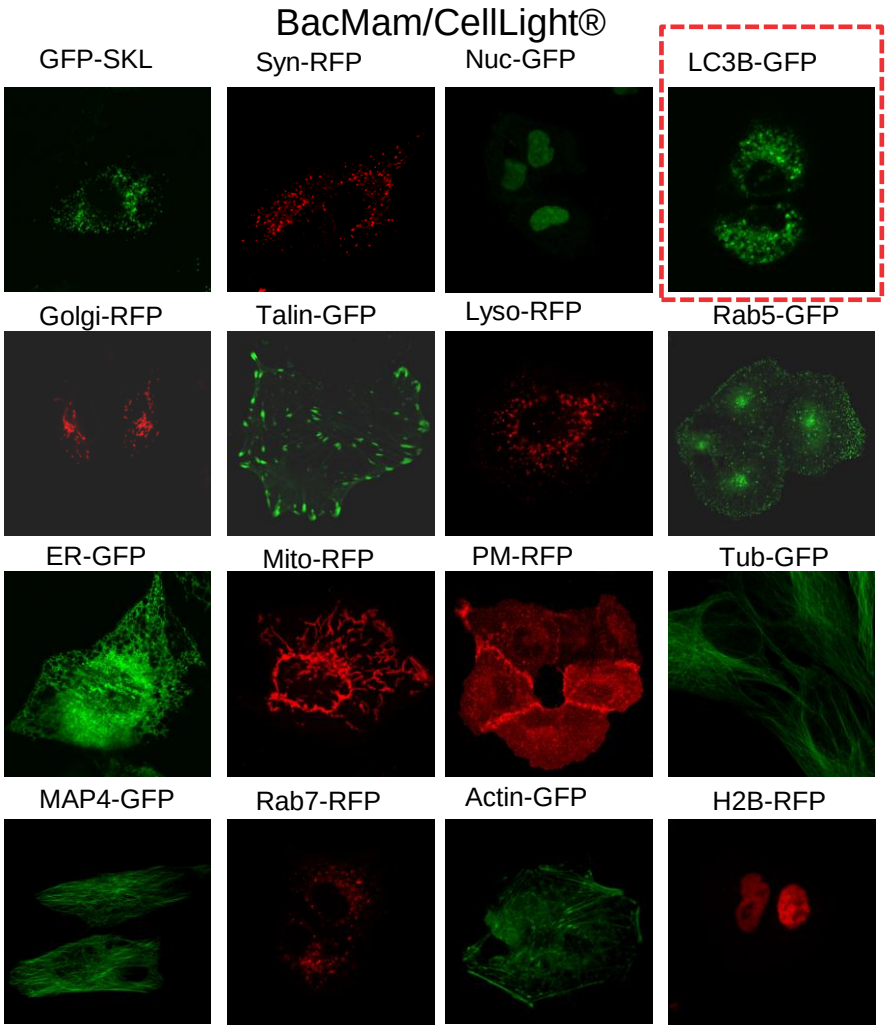


ThermoFisher SCIENTIFIC

Imaging structures in live cells: fluorescent proteins

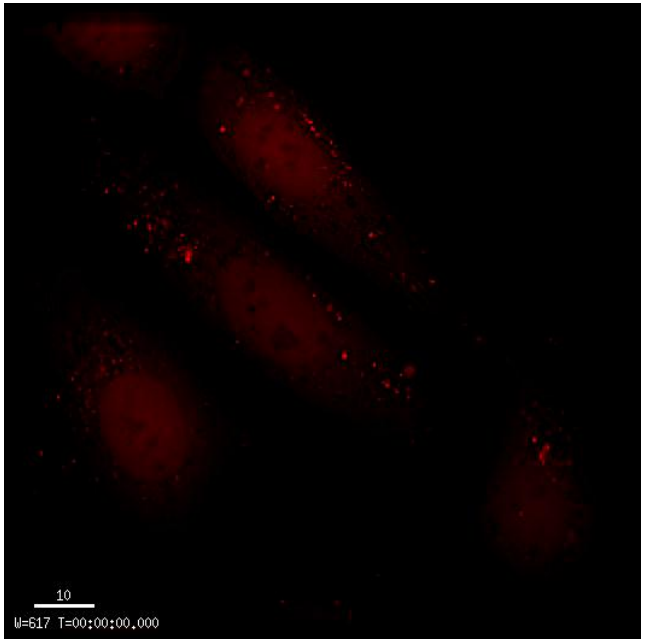




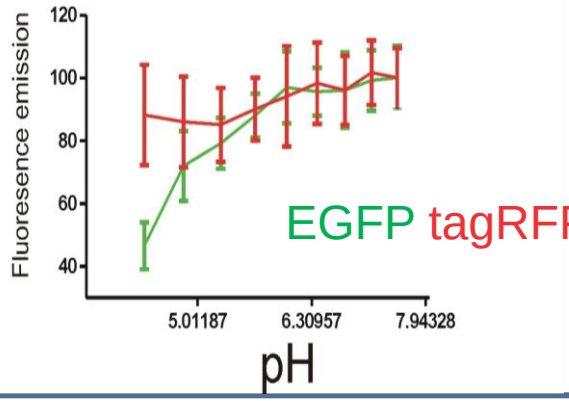


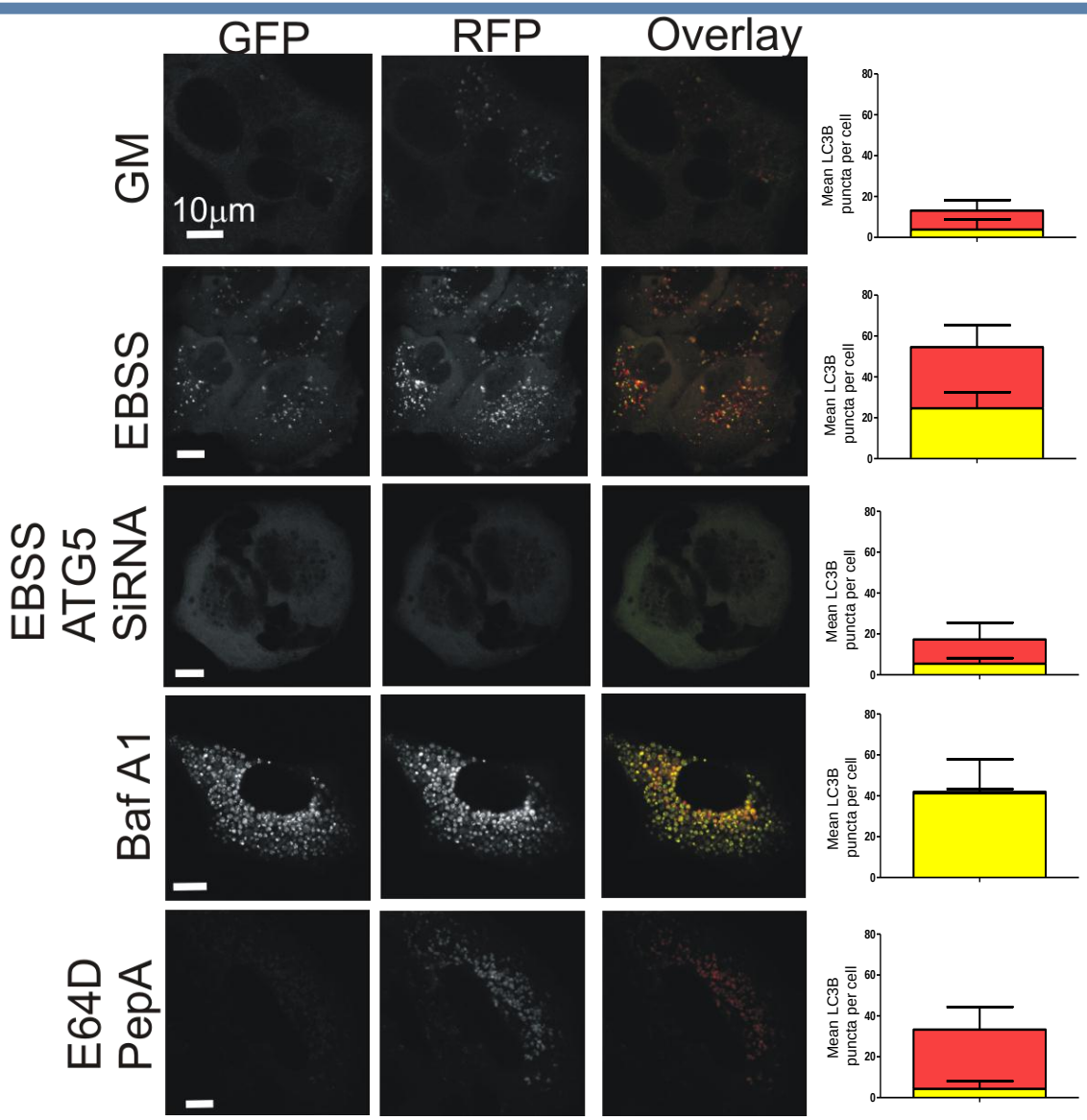
ThermoFisher SCIENTIFIC

Imaging structures in live cells: Autophagy



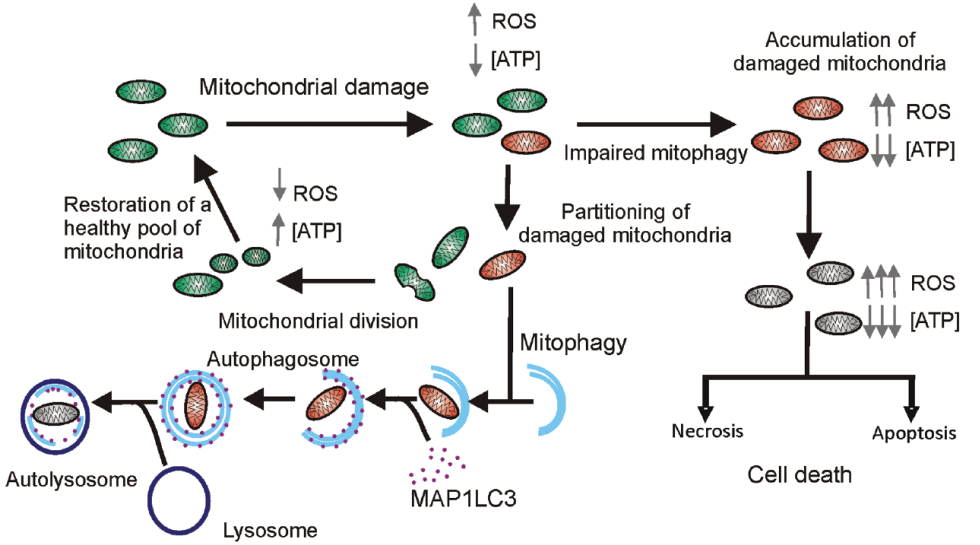
Premo™ tandem RFP-GFP-LC3B

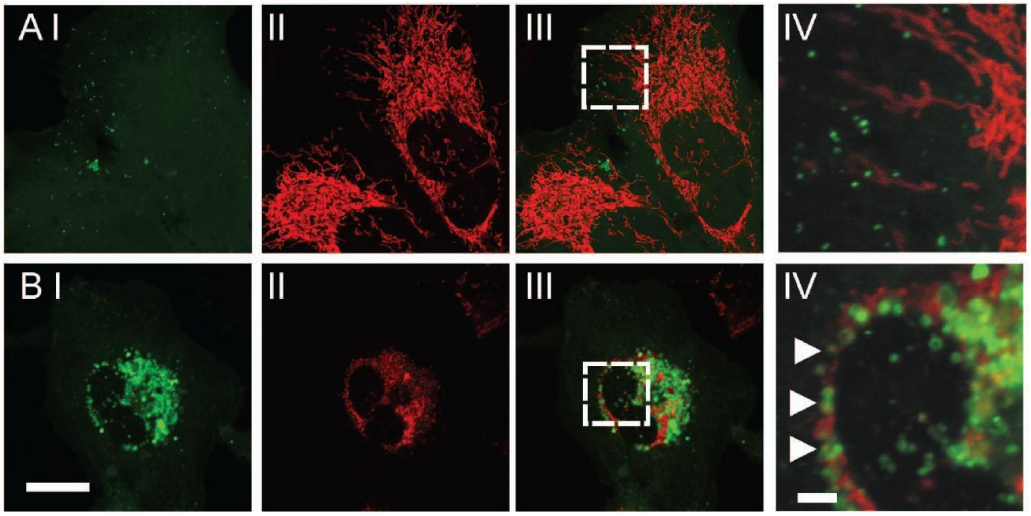


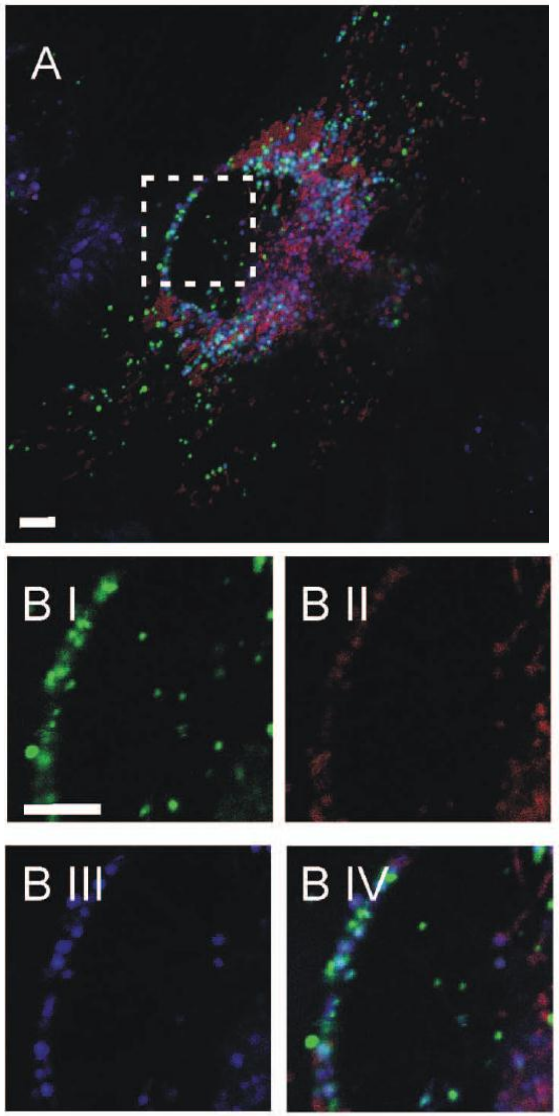


ThermoFisher SCIENTIFIC

Mitophagy

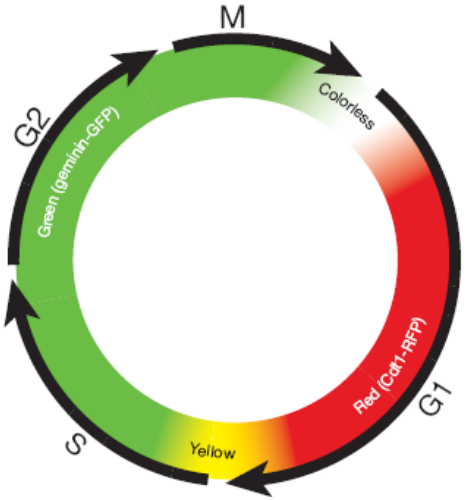




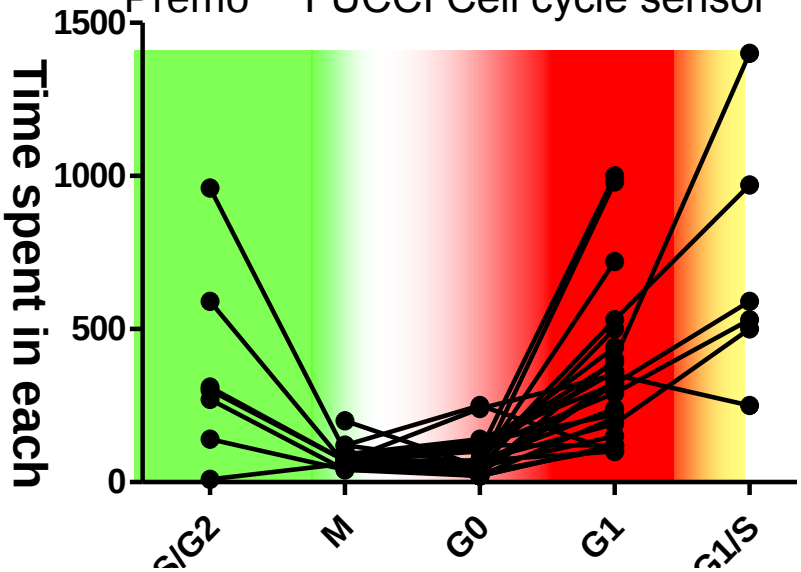


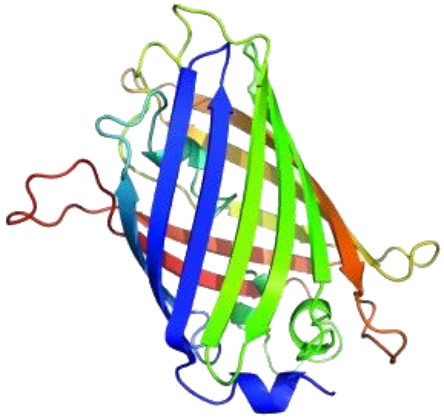
Dolman et al (2013) Autophagy 9 11

Other sensors in BacMam

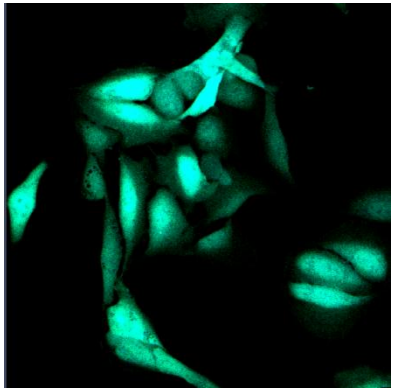


Premo™ FUCCI Cell cycle sensor

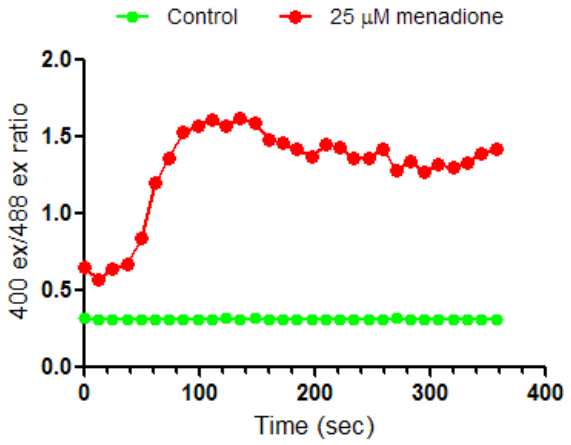




Ex: 405/485. Em: 520



Premo™ Ro-GFP GRX1



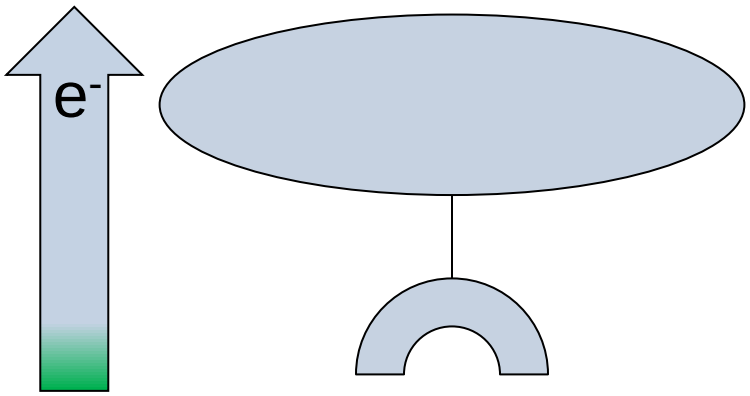
ThermoFisher SCIENTIFIC

ThermoFisher SCIENTIFIC

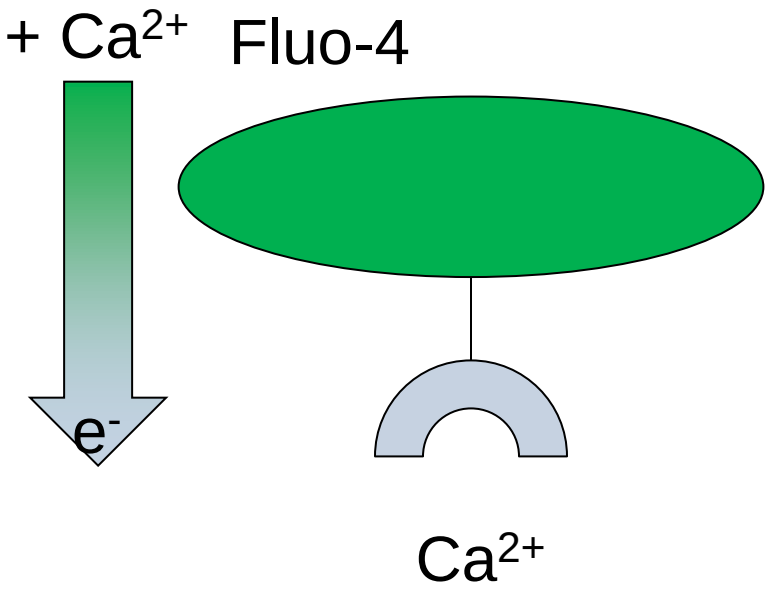
Engineering fluorescence for cell biology: Reporters

Fluorogenic reporters: Calcium dyes

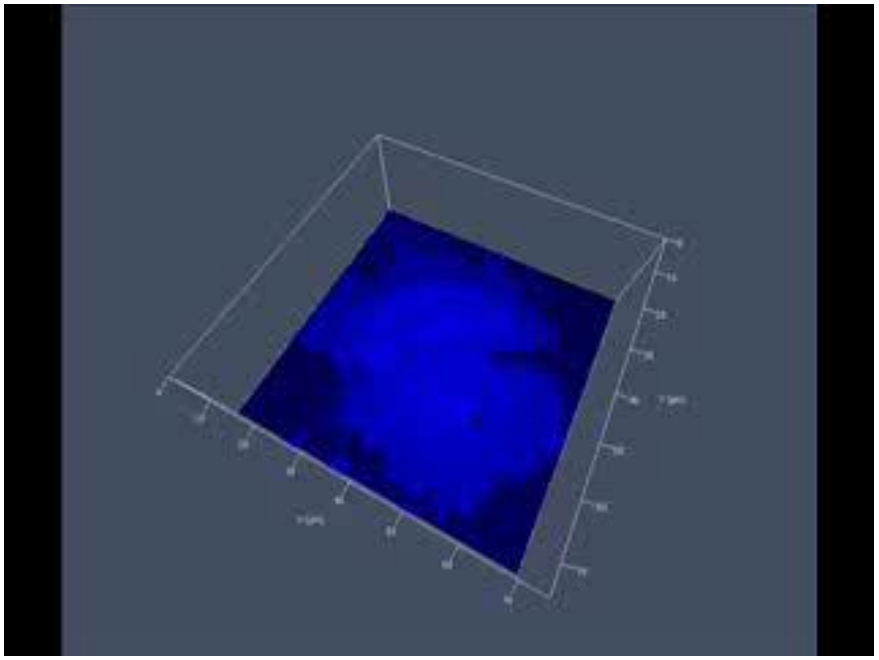
- Ca^{2+} Fluo-4



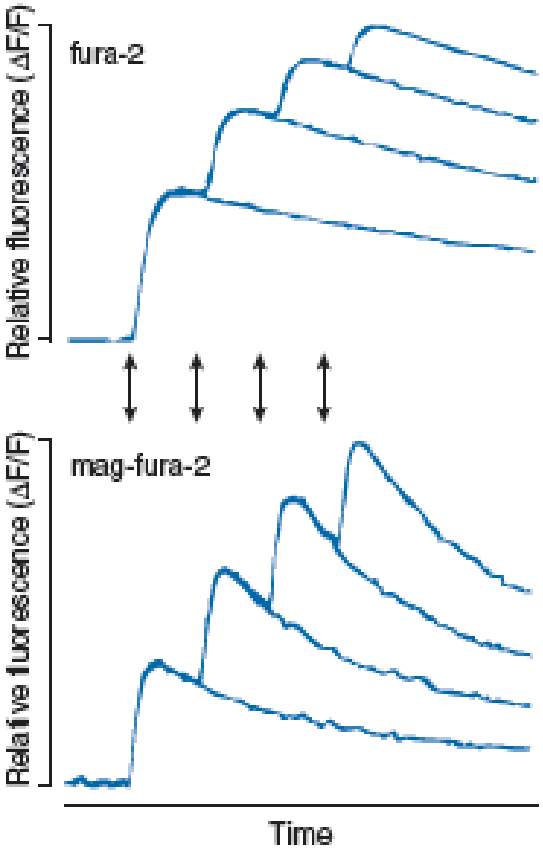
Fluorogenic reporters: Calcium dyes



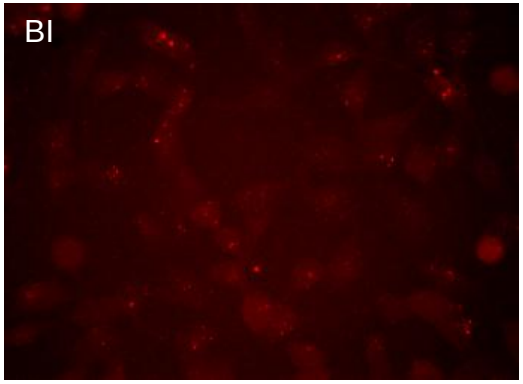
HeLa cell loaded with 2.5μM Fluo-4 AM

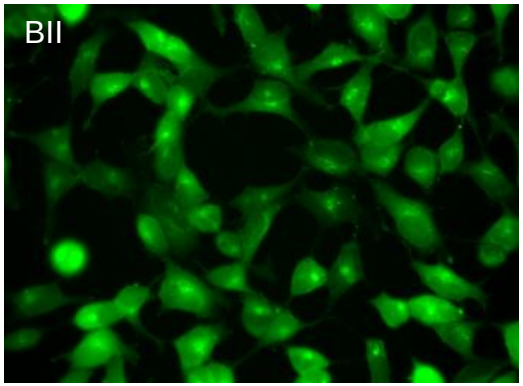


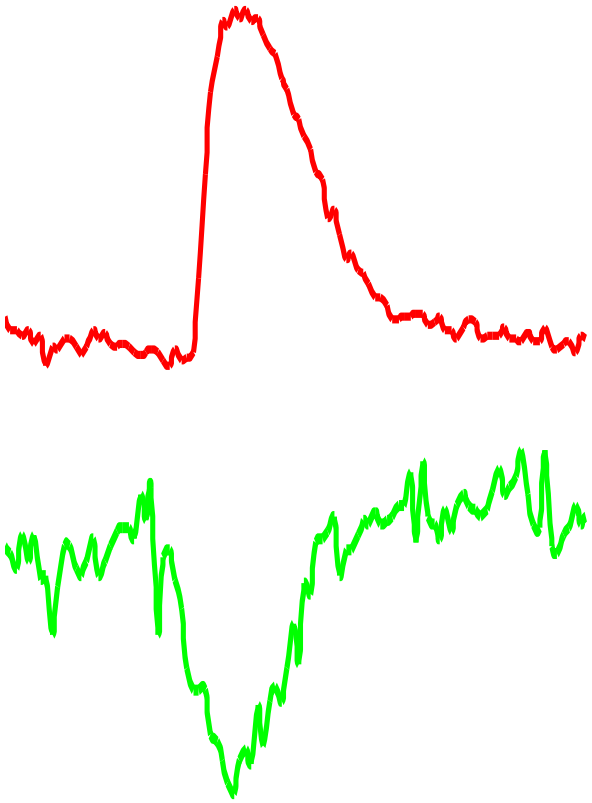
Fluorogenic reporters: Calcium dyes, Δ kd



Rhod-3 & Mag Fluo-4

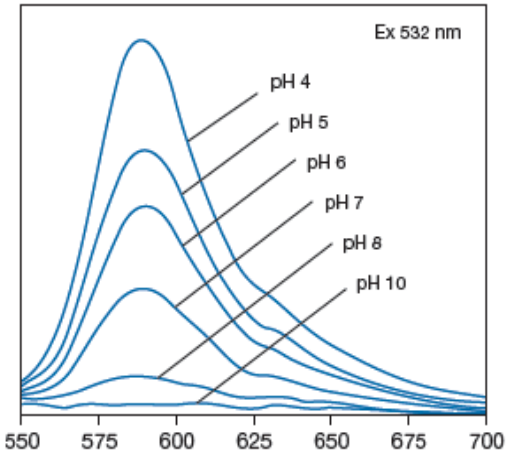


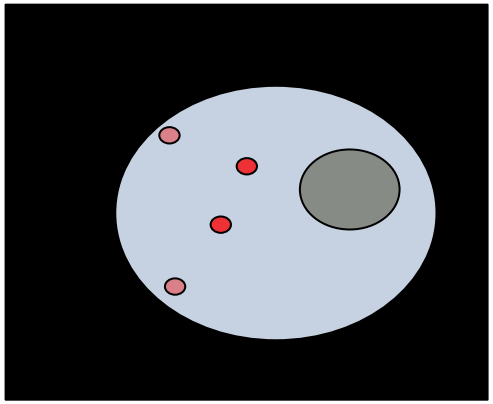




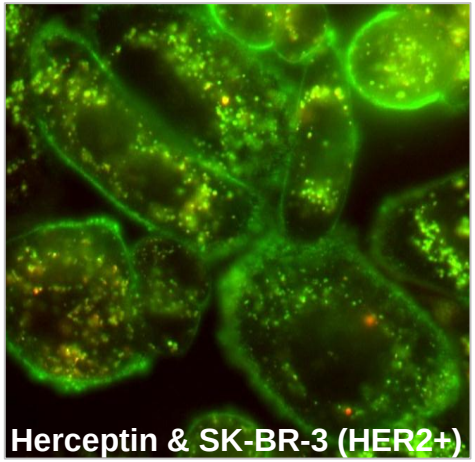
Fluorogenic probes: pH dyes for endocytosis

pHrodo™

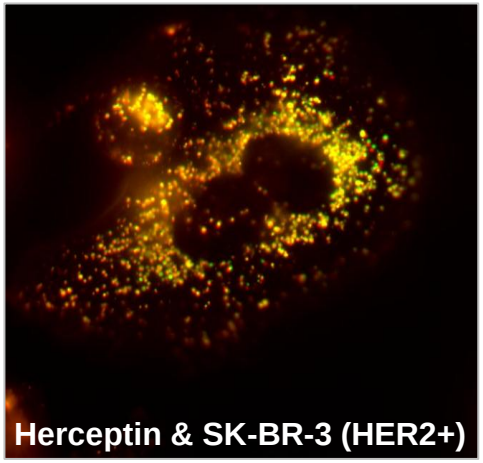




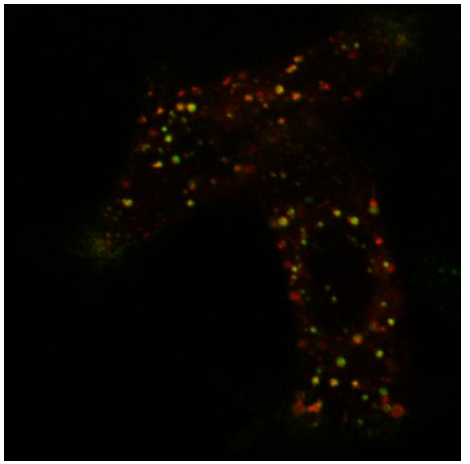
Alexa Fluor™ 594 Zenon™
LysoTracker™ Deep Red



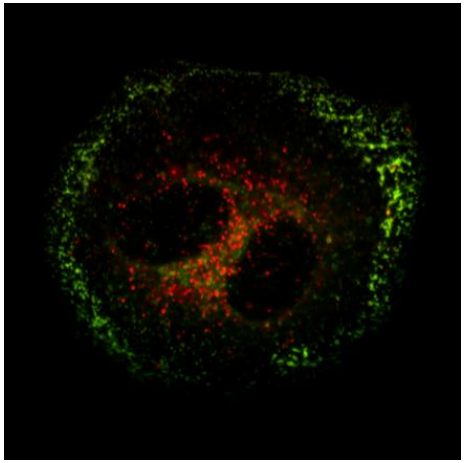
pHrodo™ Red Zenon® -
LysoTracker™ Deep Red



CellLight™ Early Endosomes RFP
EGF-pHrodo™ green

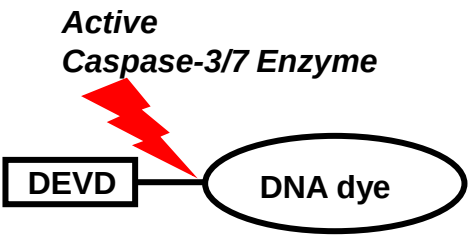


CellLight® Early endosomes GFP
pHrodo™ red Transferrin

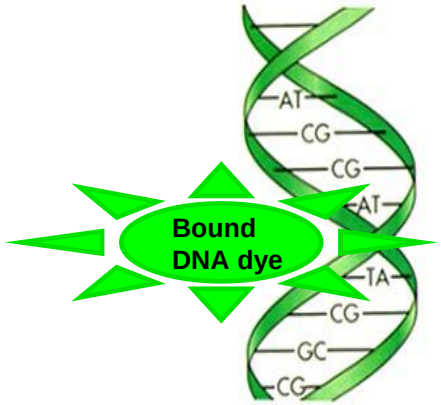


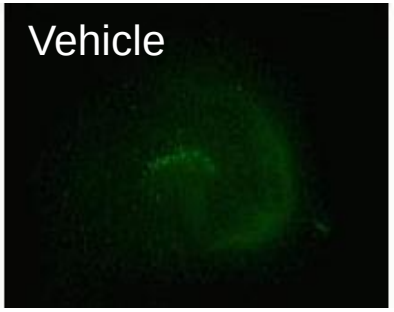
ThermoFisher SCIENTIFIC

Fluorogenic probes: caspase sensors

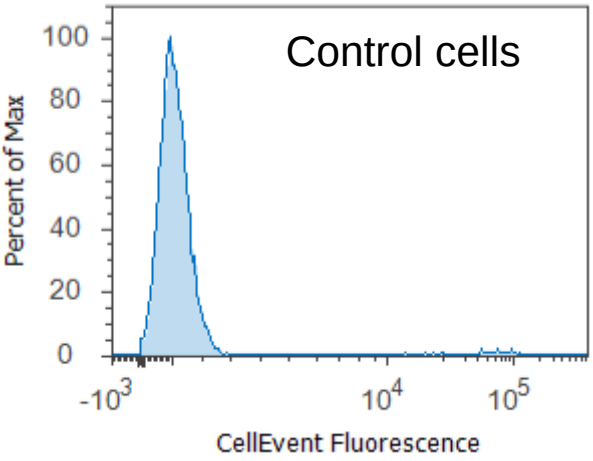


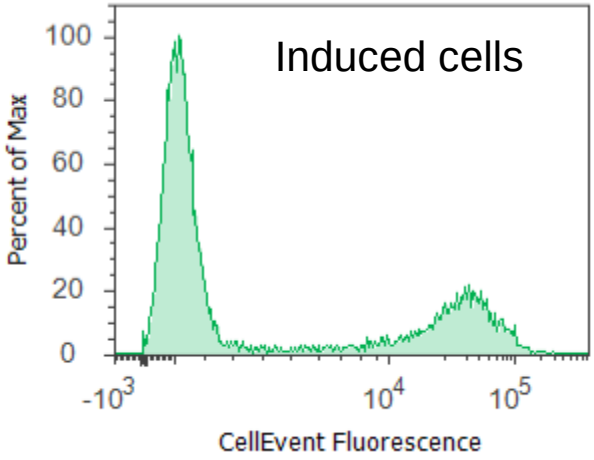
Non-fluorescent
No DNA binding



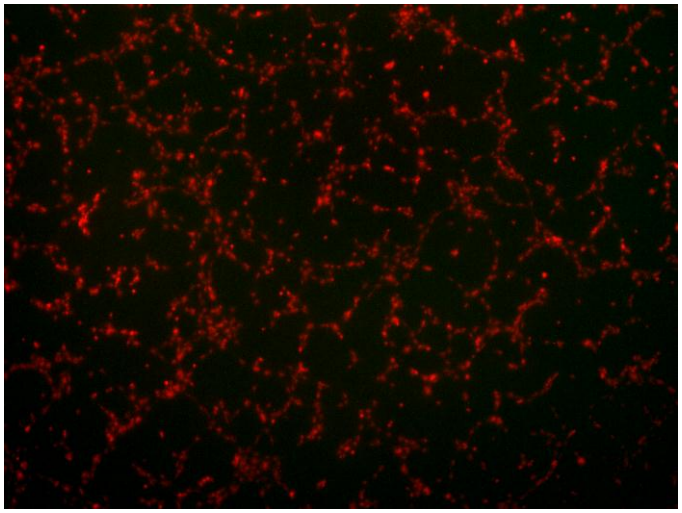


Courtesy of Brosnan & Bickler, UCSF





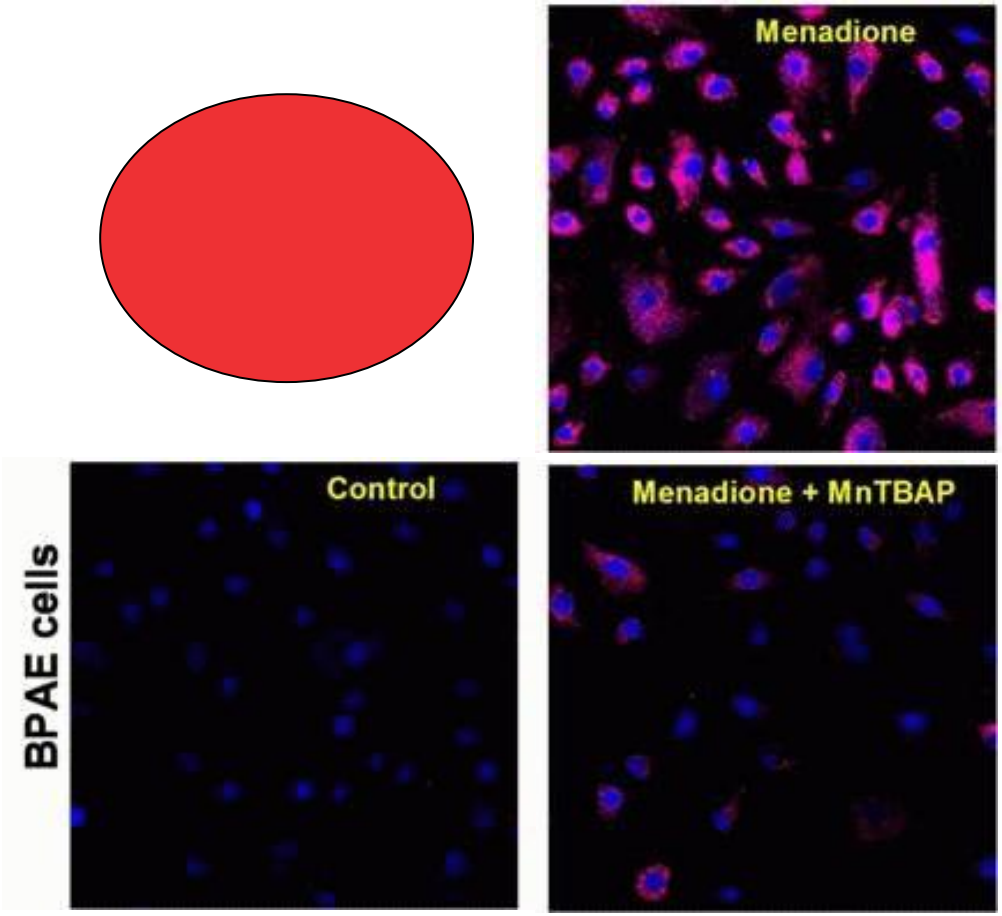
CellTracker™ & CellEvent™



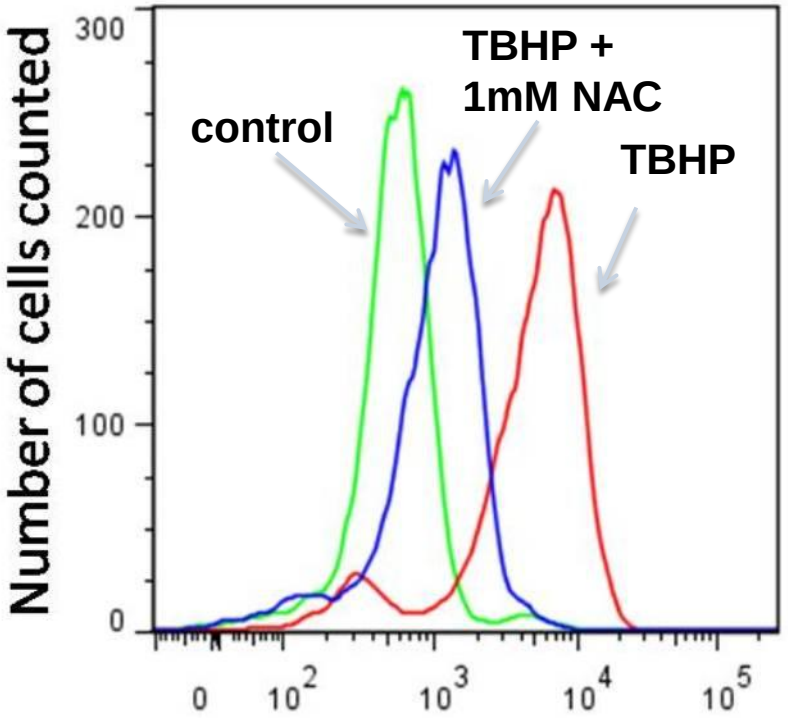
Gibco™ HUVEC cells

ThermoFisher SCIENTIFIC

Fluorogenic probes: ROS sensors



CellROX™ Deep Red



CellROX™ Deep Red

ThermoFisher SCIENTIFIC

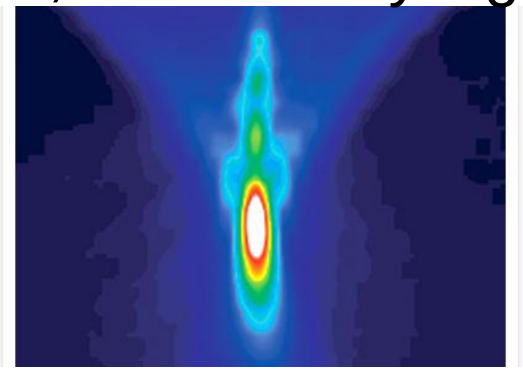
Advanced microscopy

Resolution limit

Fluorophores are small

What is the diffraction limit & how do you bypass it

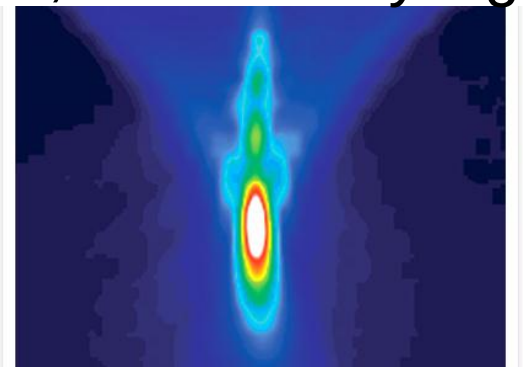
PSF, Abbe & Rayleigh



$$Abbe\ Resolution_{x,y} = \lambda/2NA$$
$$Rayleigh\ Resolution_{x,y} = 0.61\lambda/NA$$

What is the diffraction limit & how do you bypass it

PSF, Abbe & Rayleigh



$$Abbe\ Resolution_{x,y} = \lambda/2NA$$
$$Rayleigh\ Resolution_{x,y} = 0.61\lambda/NA$$

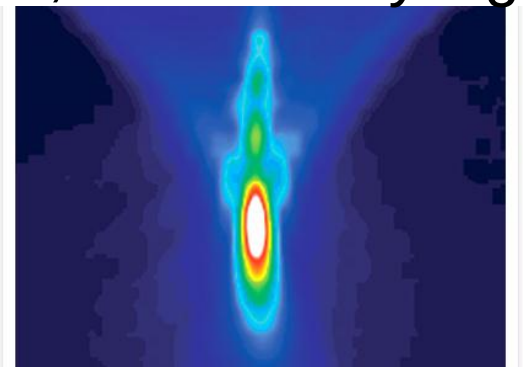
Objective/NA	d_0 (µm)	D_0 (µm)	n
0.5x / 0.15	2.2	11.2	1786
10x / 0.30	1.1	11.2	1786
20x / 0.50	0.7	13.4	1493
40x / 0.75	0.45	17.9	1117
40x / 1.30 (oil)	0.26	10.3	1942
63x / 1.40 (oil)	0.24	15.1	1325
100x / 1.30 (oil)	0.26	25.8	775

Resolution for Selected Objectives (550nm)

http://zeiss-campus.magnet.fsu.edu/articles/basics/resolution.html

What is the diffraction limit & how do you bypass it

PSF, Abbe & Rayleigh



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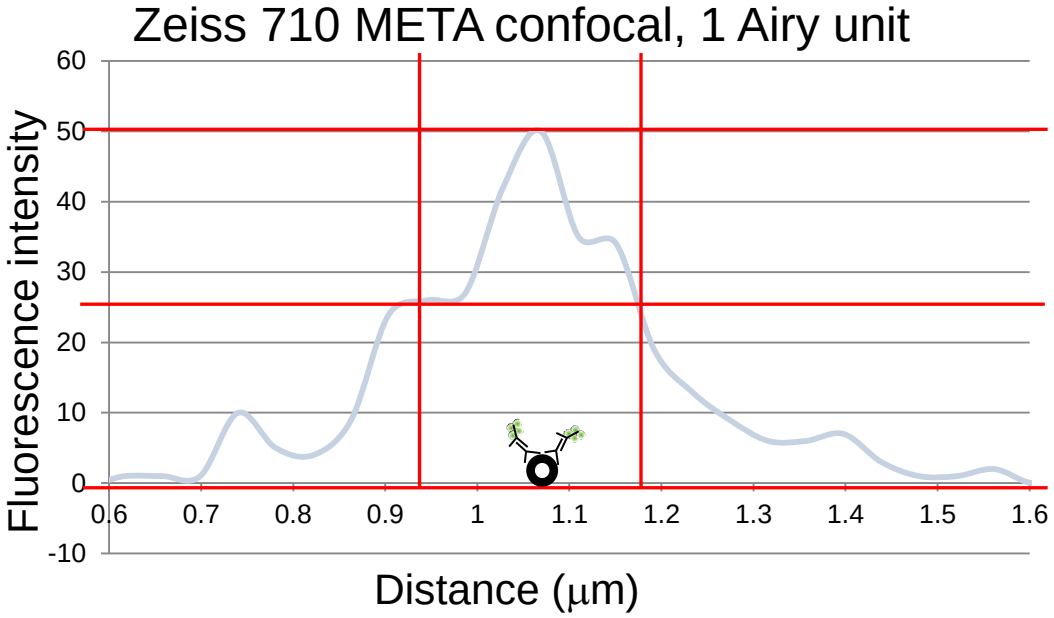
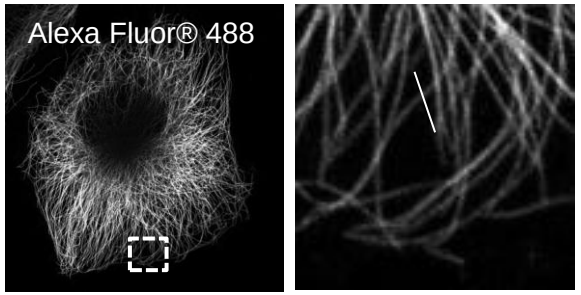


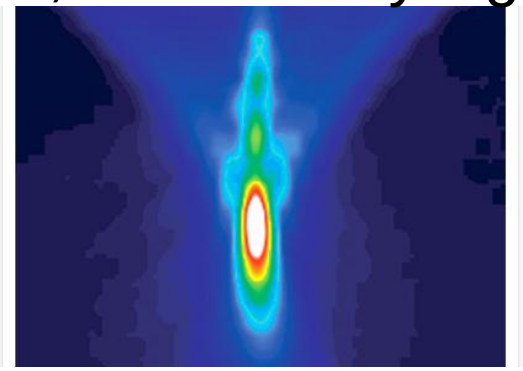


Microtubules are 25nm
Appear ~200nm

R=0.61 (519/1.4)
R=226nm

What is the diffraction limit & how do you bypass it

PSF, Abbe & Rayleigh



$$Abbe\ Resolution_{x,y} = \lambda/2NA$$
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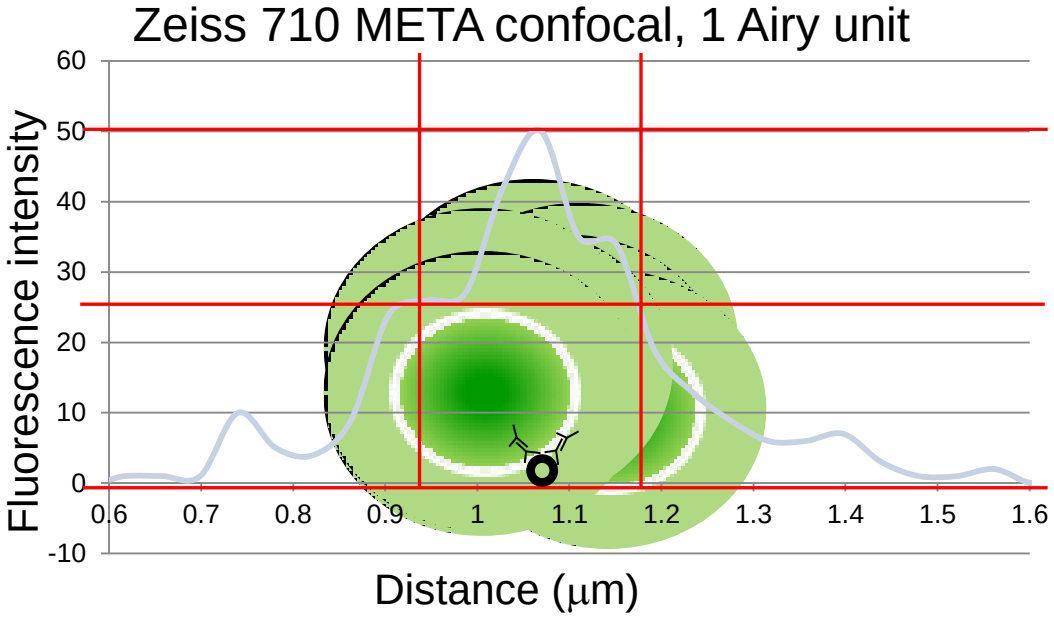

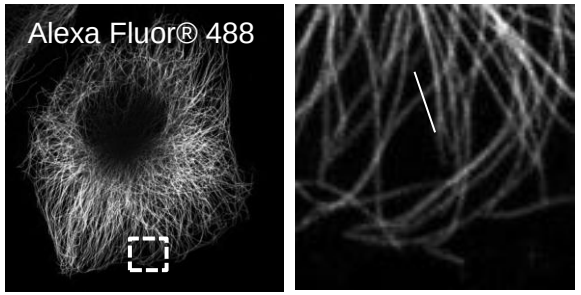

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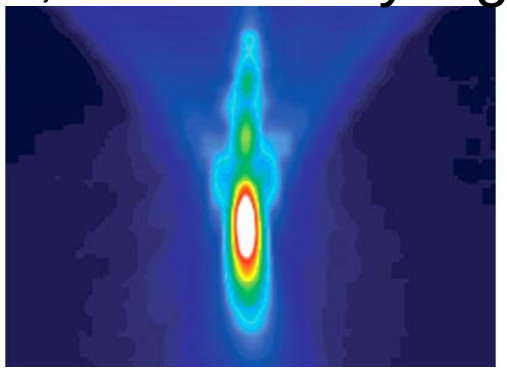
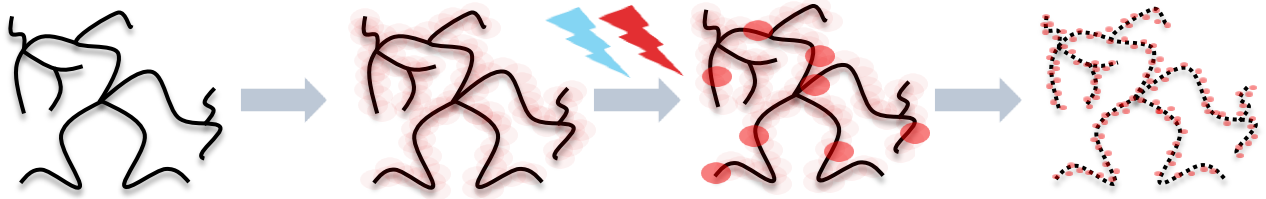
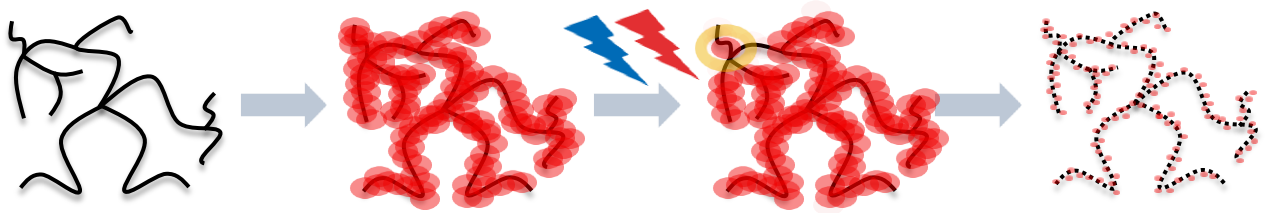


Bypass diffraction

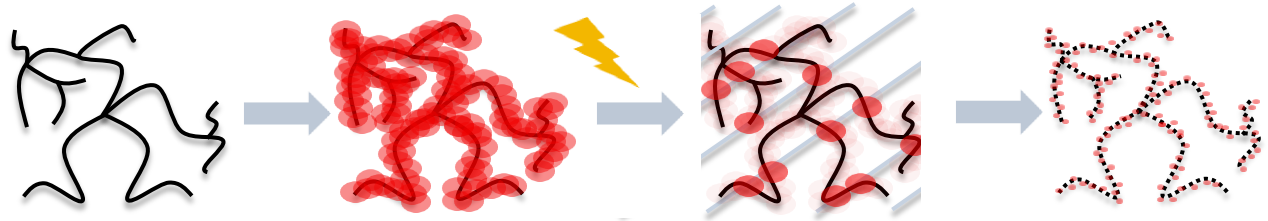
www.thermofisher.com/srm



SMLM
(STORM, dSTORM, PALM, GSDIM)

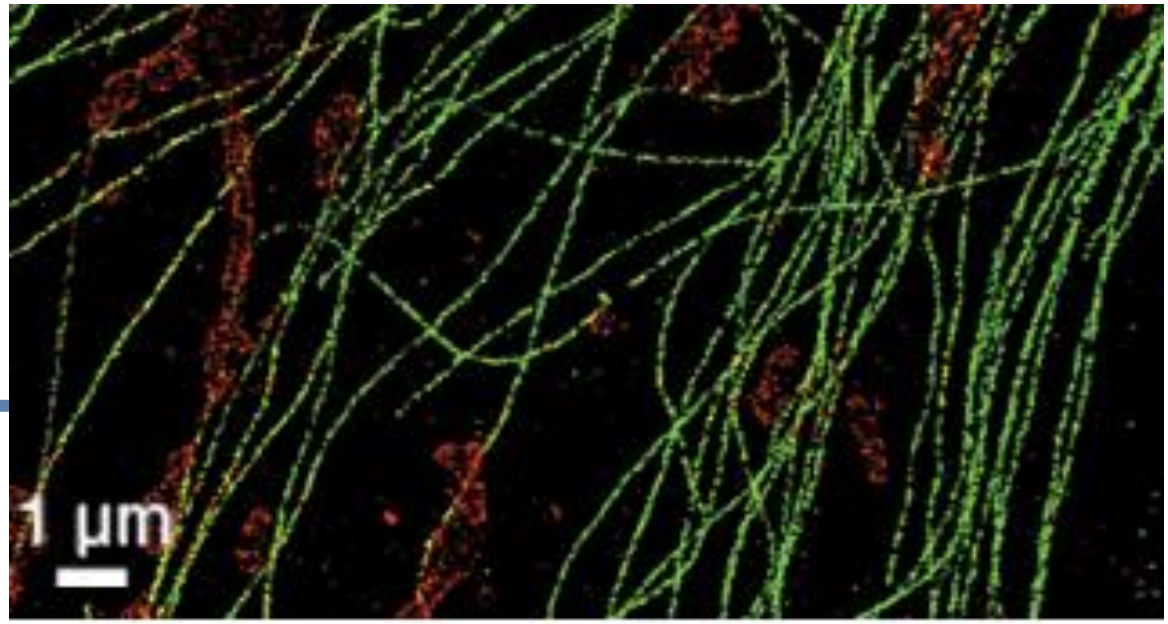


STED



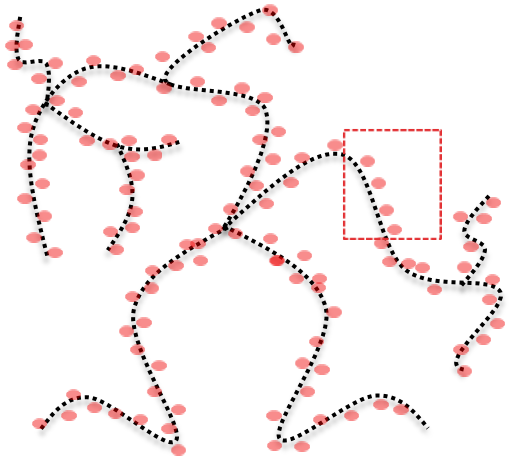
SIM
(Lattice light sheet)

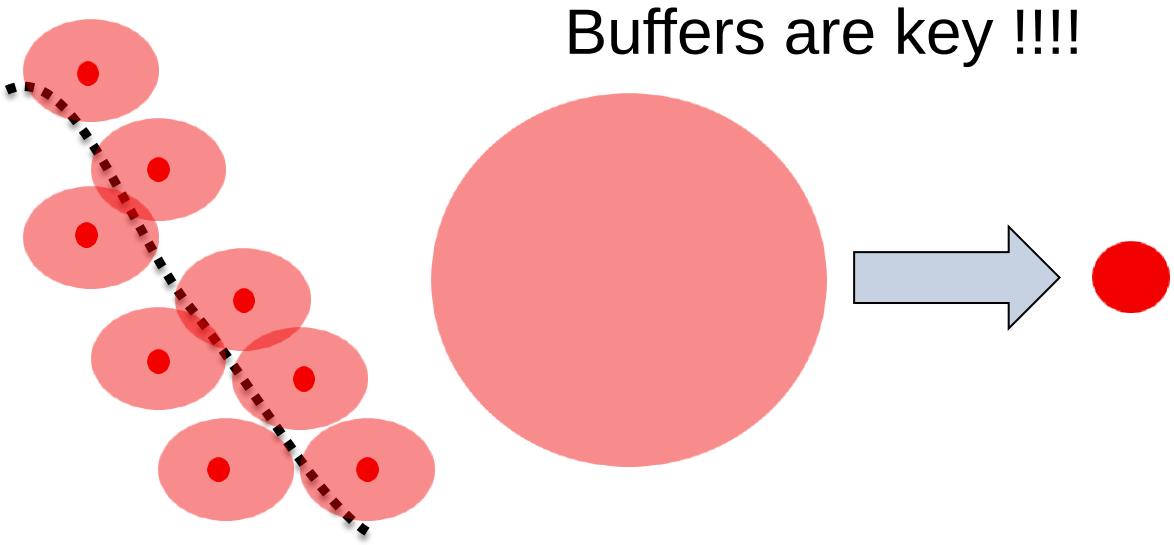
SMLM



STORM with dye pairs Alexa Fluor® 405/Alexa Fluor® 647 and Cy®3/Alexa Fluor® 647. Xiaowei Zhuang, Harvard,

How SMLM techniques build a picture of the cell





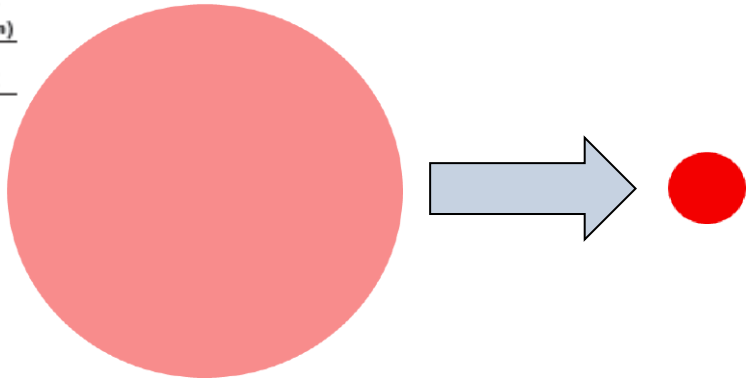
- Buffers facilitate controlled & efficient switching
- A good dye switches in an efficient and controlled manner
- What is a good dye?

What makes a good dye

Table 1 | Summary of switching properties of the 26 dyes tested in this study

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

Excitation wavelength, dichroic mirrors and emission filters used for characterization and imaging for each spectral range were 488 nm, T495LP (Chroma) and ET535/50m (Chroma) for blue-absorbing dyes; 561 nm, Di01-R561 (Semrock) and FF01-617/73-25 (Semrock) for yellow-absorbing dyes; 647 nm, Z660DCXRU (Chroma) and ET700/75m (Chroma) for red-absorbing dyes; 752 nm, Q770DCXR (Chroma) and HQ800/60m (Chroma) for NIR-absorbing dyes, respectively. Dye-switching properties are reported in the presence of GLOX and 10 mM MEA as well as GLOX and 140 mM βME.
[a]Excitation and emission peak wavelengths from dye spectra. [b]Extinction coefficients from the dye manufacturers. [c]Quantum yields from either the dye manufacturer when known or from the McNamara 2007 fluorophore data tables. –, quantum yield values not available from dye manufacturer or McNamara data tables.



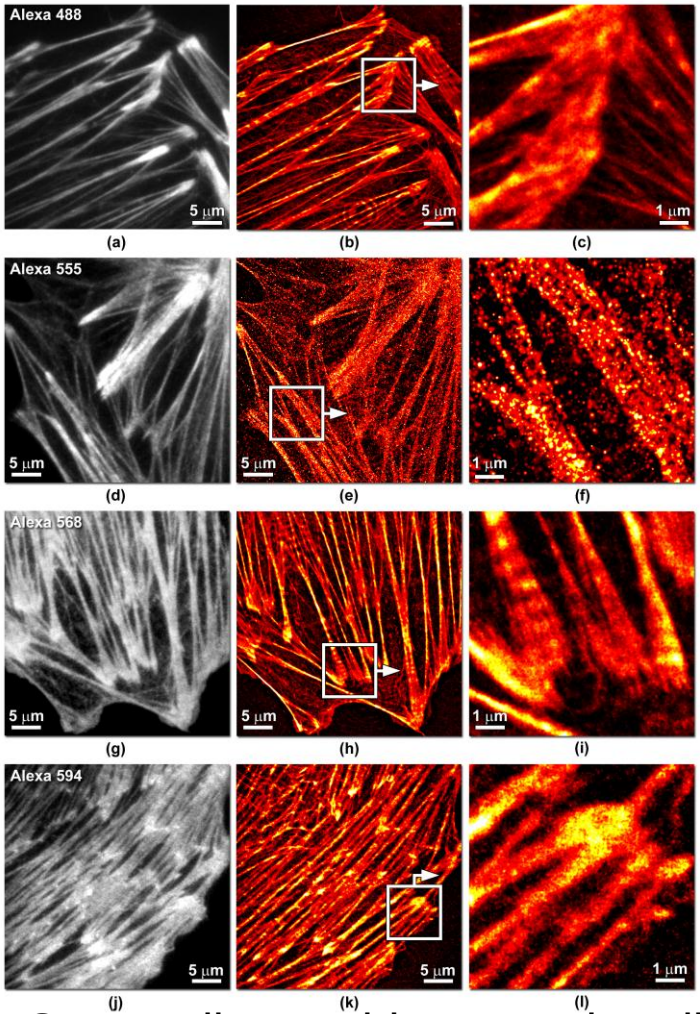
Desired properties

- ✓High # photon
- ✓Low duty cycle (off)
- ✓High survival fraction
- ✓High # cycles

From Dempsey et al (2012) Nature Methods 8 1027-1036

Understand performance: SMLM

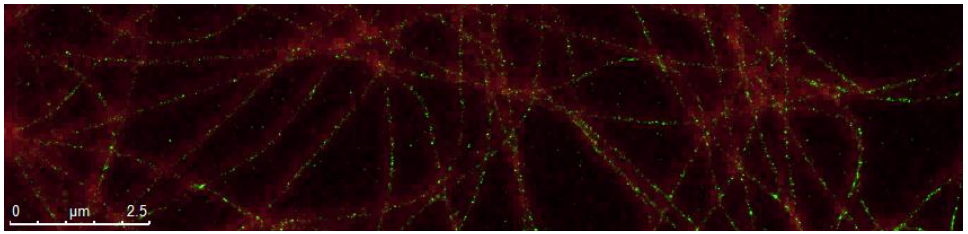
Alexa Fluor™ Phalloidin



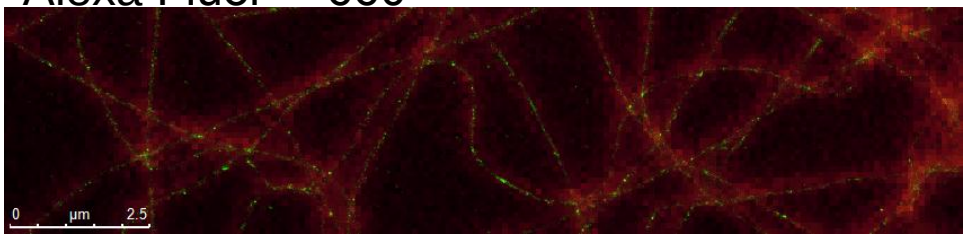
dSTORM, Mike Davidson & John Allen FSU

Anti α tubulin

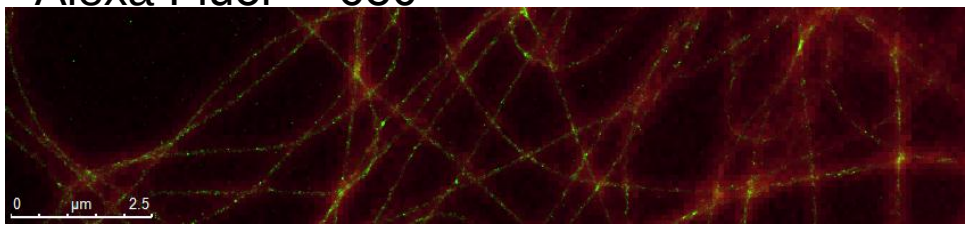
Alexa Fluor™ 568



Alexa Fluor™ 660



Alexa Fluor™ 680



GSDIM, Jana Doehner, Zurich

Dye photoswitching tables

IOP PUBLISHING JOURNAL OF OPTICS
J. Opt. 15 (2013) 094001 (15pp) doi:10.1088/2040-8978/15/9/094001

Single molecule localization microscopy for superresolution

John R Allen[1], Stephen T Ross[2] and Michael W Davidson[1]

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[2] Nikon Instruments, Incorporated, 1300 Walt Whitman Road, Melville, NY 11747, USA

Single Molecule Localization Microscopy for Superresolution

					growth medium			
			10 mM MEA	GLOX	TN	8		Dempsey *et al* 2011
			140 mM BME	GLOX	TN	8		Dempsey *et al* 2011
			10-100 mM MEA or GSH	N/A	PBS	7.4		van de Linde *et al* 2009
Rhodamine 6G	526	556	200 mM MEA	N/A	Concentrated dye solution	10.2	Experiments only performed on unbound dye	van de Linde *et al* 2011a
ATTO 532	532	553	100 mM MEA	N/A	Concentrated dye solution	9.3	Experiments only performed on unbound dye	van de Linde *et al* 2011a
Alexa Fluor 532	532	552	100 mM MEA	N/A	PBS	7.4		Heilemann *et al* 2009
Dy 530	535	556	100 mM MEA	N/A	Concentrated dye solution	9.3	Experiments only performed on unbound dye	van de Linde *et al* 2011a
TAMRA	546	575	10 mM MEA	GLOX	TN	8		Dempsey *et al* 2011
			140 mM BME	GLOX	TN	8		Dempsey *et al* 2011
DiI	549	565	N/A	0.5 mg mL^{-1} glucose oxidase, 40 µg mL^{-1} catalase, 2% (w/v) glucose	phenol red-free growth medium	7.4	Cell-permeant membrane-specific dye for live cell imaging	Shim *et al* 2012
Cy3	550	570	10 mM MEA	GLOX	TN	8		Dempsey *et al* 2011
			140 mM BME	GLOX	TN	8		Dempsey *et al* 2011
MitoTracker Orange	551	576	N/A	0.5 mg mL^{-1} glucose oxidase, 40 µg mL^{-1} catalase, 2% (w/v) glucose	phenol red-free growth medium	7.4	Cell-permeant mitochondria-specific dye for live cell imaging	Shim *et al* 2012
Dy547-SNAP	557	574	100 mM GSH (Optional)	GLOX Optional	phenol red-free growth medium	7.2-7.6	Cell-permeable tag for live cell imaging	Benke *et al* 2012
Cy3B	559	570	10 mM MEA	GLOX	TN	8	High performance for a red fluorescent dye	Dempsey *et al* 2011
			140 mM BME	GLOX	TN	8	High performance for a red fluorescent dye	Dempsey *et al* 2011
SNAP-Cell TMR-Star	554	580	N/A	N/A	phenol-red free growth medium	N/A	Cell-permeable tag for live cell imaging	Klein *et al* 2011
TMR-SNAP, HALO	554	580	100 mM GSH (Optional)	GLOX	phenol red-free growth medium	7.2-7.6	Cell-permeable tag for live cell imaging	Benke *et al* 2012

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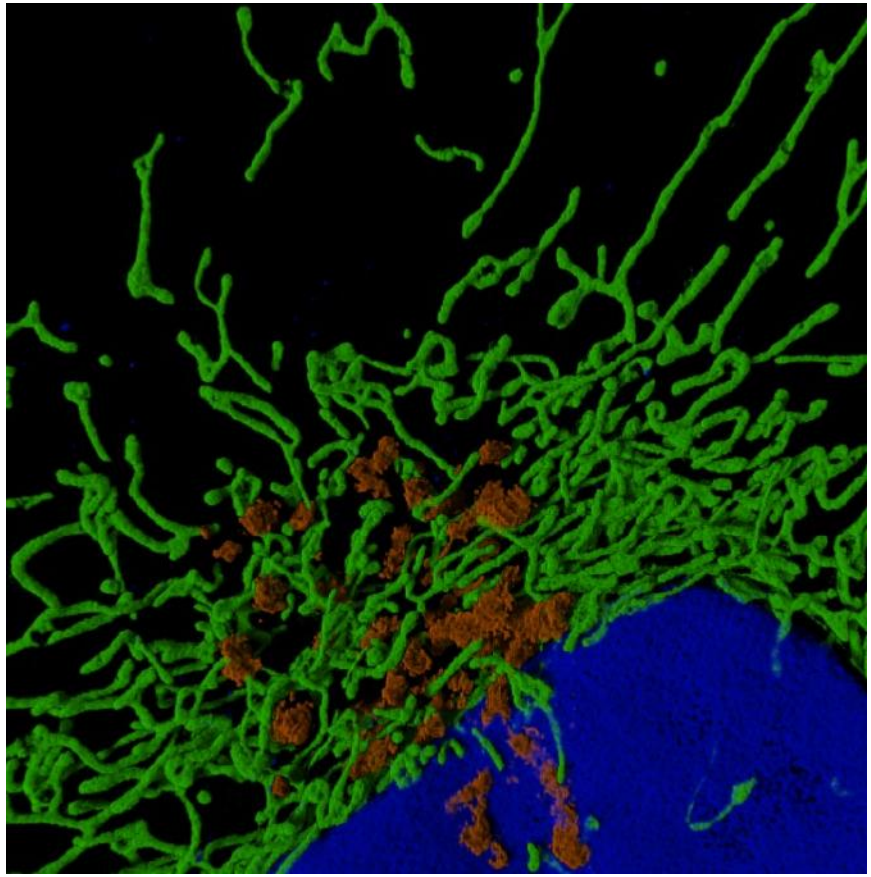
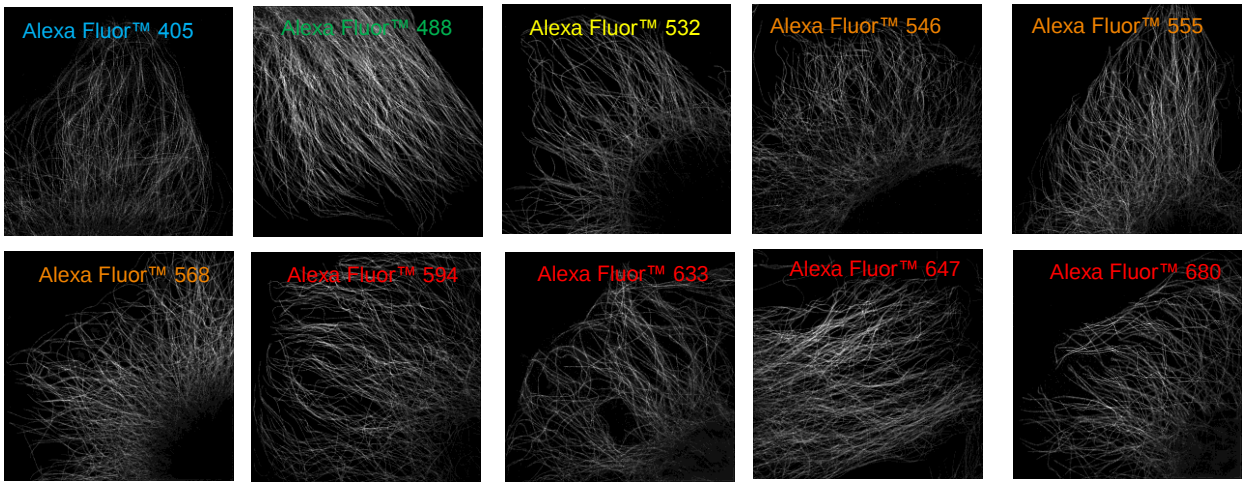
SIM

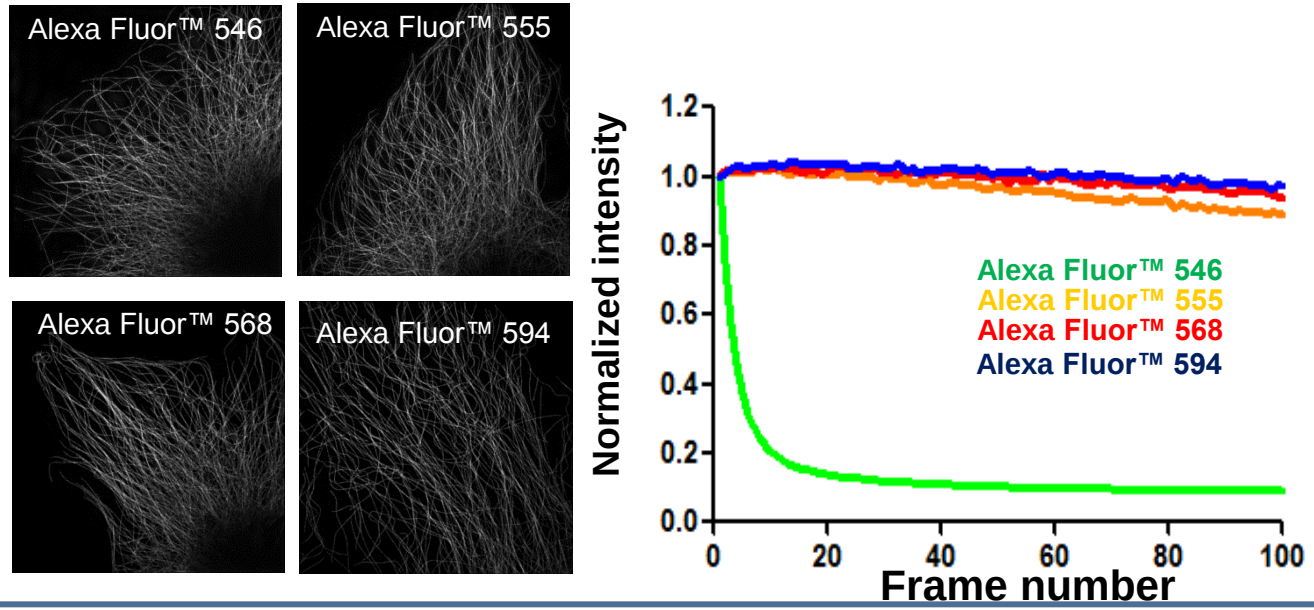


SIM imaging of Hoechst 33342, CellLight® Mito-GFP & CellLight® Golgi-RFP
Courtesy of Ian Clements, GE Healthcare

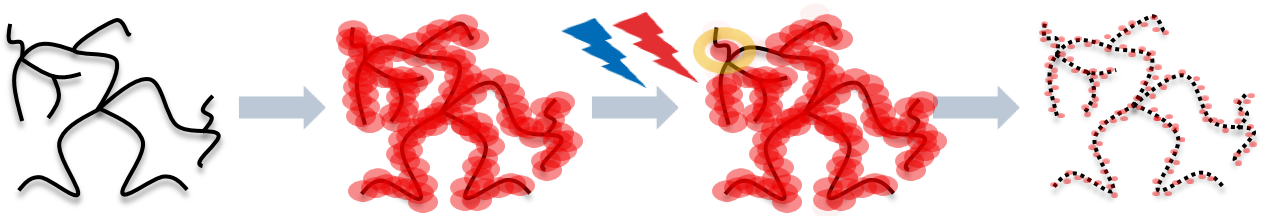
For SIM see wide field observations

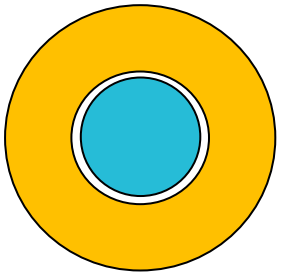


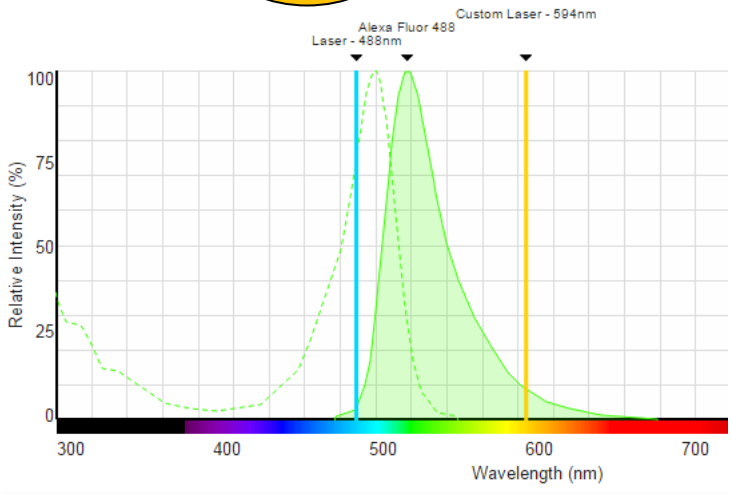
Talley Lambert & Jennifer Waters, Harvard

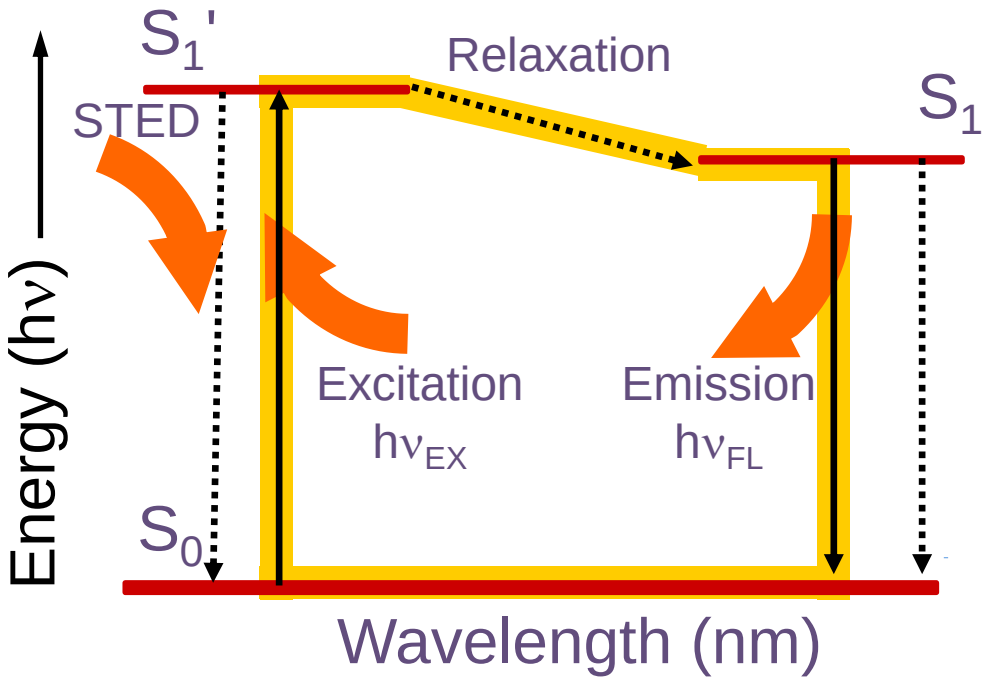


Stimulated emission depletion (STED) microscopy

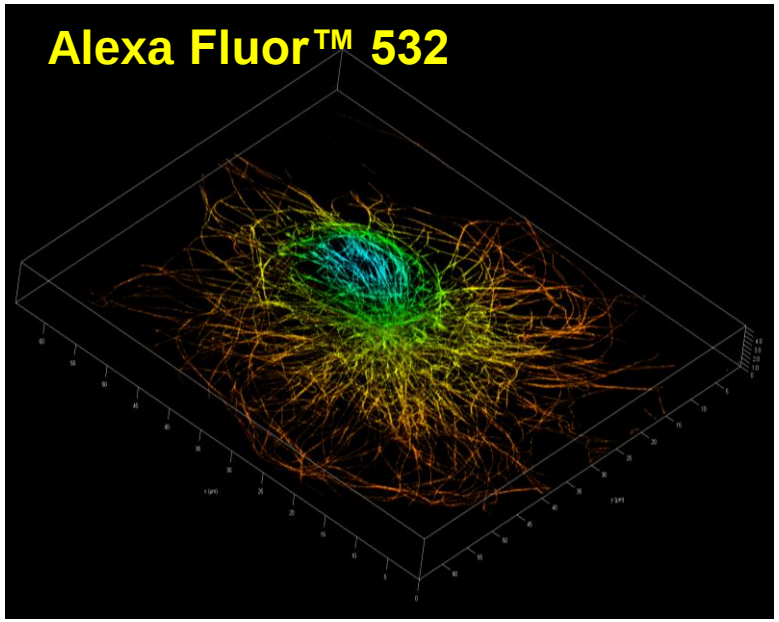




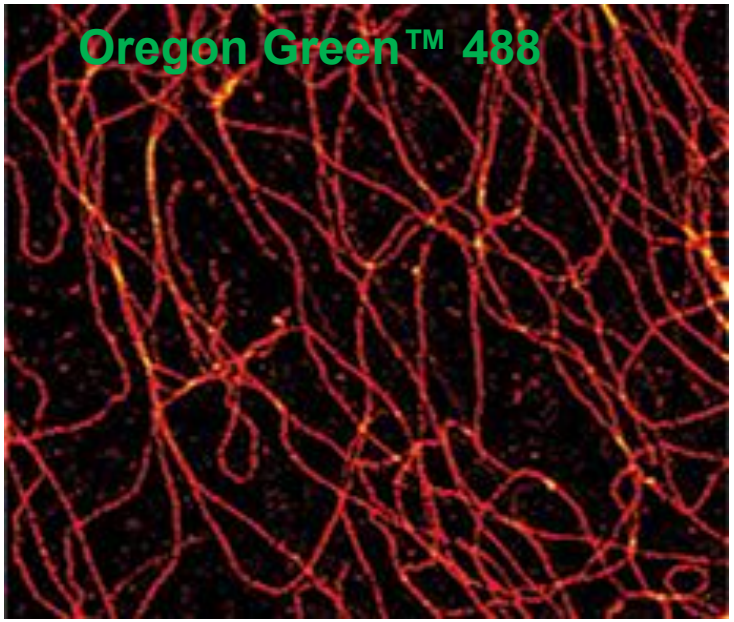




Understand performance: STED



CW STED, Jana Doehner, Zurich



STED, Leica Microsystems, Germany