

## Live Cell Imaging

**Toxicity**

**Phototoxicity**

**Compartmentalization**

**Compatibility**

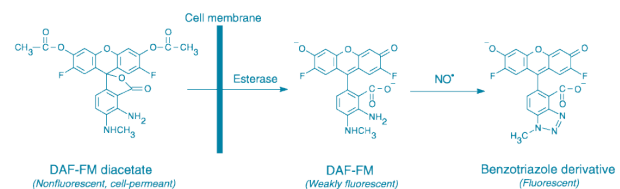
**Interference (buffer / sink)**

**Temporal Constraints**

**Permeability**

## Permeability

1. Endocytosis
2. Injection / infusion
3. Esterification  
acetate  
acetoxymethyl (AM)



## Ion Selective Dyes

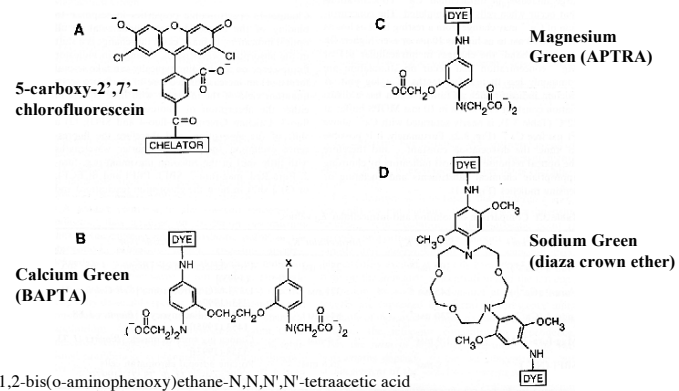
**Calcium / Magnesium / Zinc ions**

**Protons (pH)**

**Sodium & Potassium ions**

**Chloride / Halide ions**

## Ion Selective Dyes



## Ion Selective Dyes

Spectral compatibility

Stoichiometry

Specificity

Affinity ( $K_D$ )

Dynamic range

Permeability

Optical response

## Optical Response

1. Change (+/-) in Quantum Yield with little change in spectra  
e.g., fluo-3, Calcium Green, SPQ
2. Blue shift of absorption (excitation) spectrum with little change in maximum emission  
e.g., fura-2, Fura Red, SBFI, PBFI, BCECF
3. Blue shift in both absorption and emission spectra  
e.g., indo-1, SNARF & SNAFL

## Calcium Dyes - Issues

Qualitative vs quantitative (calibration)

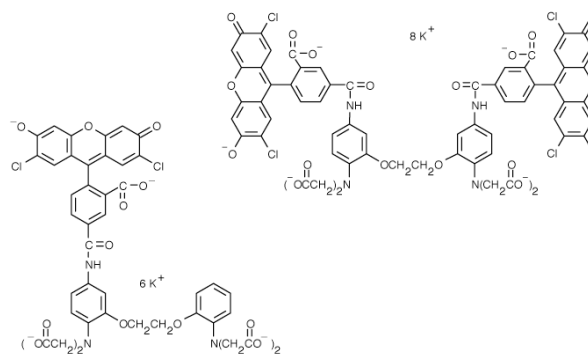
$K_D$  for calcium

Compartmentalization

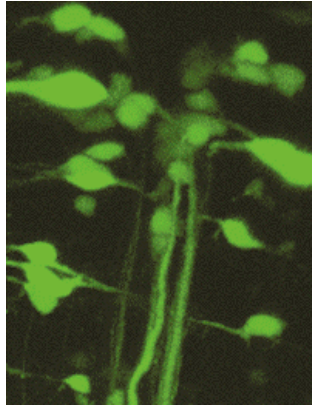
pH effects

Interaction with other ions

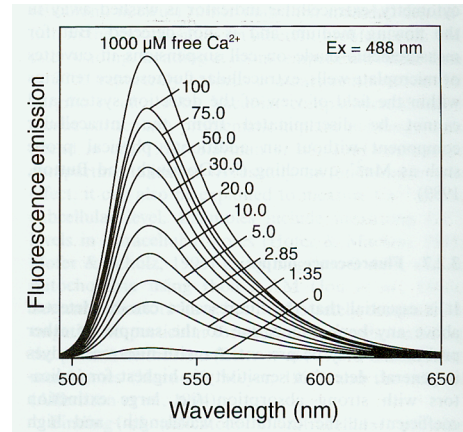
## Calcium Green



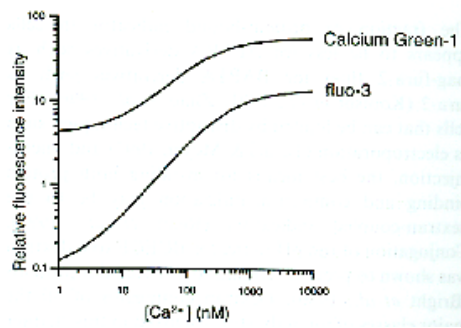
## Calcium Green



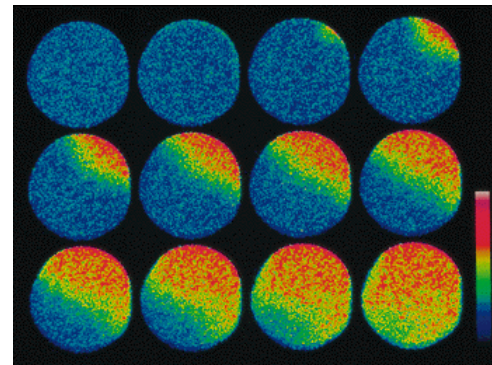
## Calcium Green



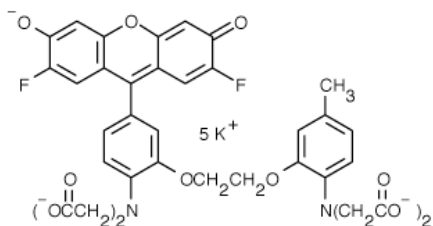
## Dynamic Range



## Calcium Green

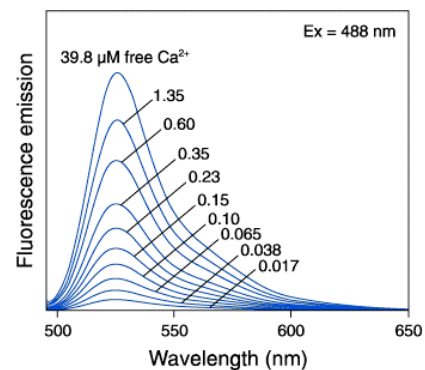


## Fluo Dyes

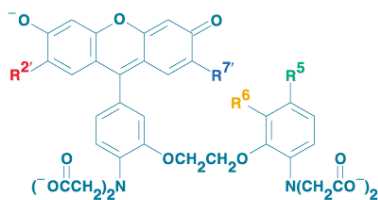


Fluo 4

## Fluo-3



## Fluo Calcium Indicators

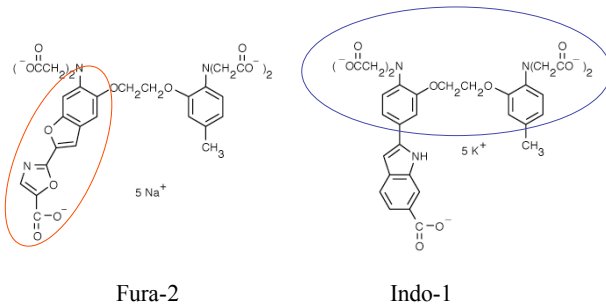


Indicator	$K_d(\text{Ca}^{2+})$	$R^{2'}$	$R^{7'}$	$R^5$	$R^6$
Fluo-3	0.39 $\mu\text{M}$	Cl	Cl	$\text{CH}_3$	H
Fluo-4	0.35 $\mu\text{M}$	F	F	$\text{CH}_3$	H
Fluo-5F	2.3 $\mu\text{M}$	F	F	F	H
Fluo-5N	90 $\mu\text{M}$	F	F	$\text{NO}_2$	H
Fluo-4FF	9.7 $\mu\text{M}$	F	F	F	F

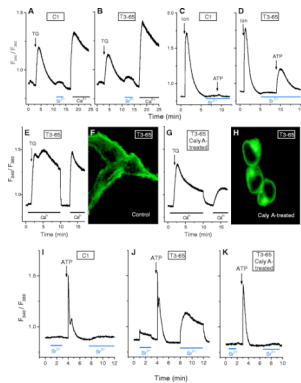
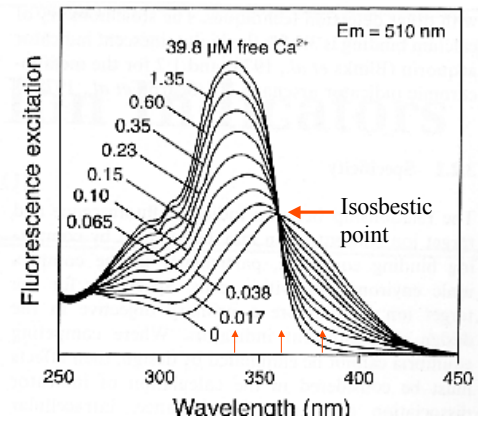
## Optical Response

1. Change (+/-) in Quantum Yield with little change in spectra  
e.g., fluo-3, Calcium Green, SPQ
2. Blue shift of absorption (excitation) spectrum with little change in maximum emission  
e.g., fura-2, Fura Red, SBFI, PBFI, BCECF
3. Blue shift in both absorption and emission spectra  
e.g., indo-1, SNARF & SNAFL

## Fura-2 & Indo-1



## Fura-2

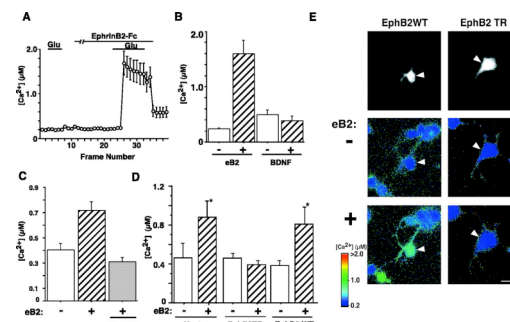


H. Ma et al., *Science* 287, 1647-1651 (2000)

Published by AAAS



## No Caption Found



M. A. Takasu et al., *Science* 295, 491-495 (2002)

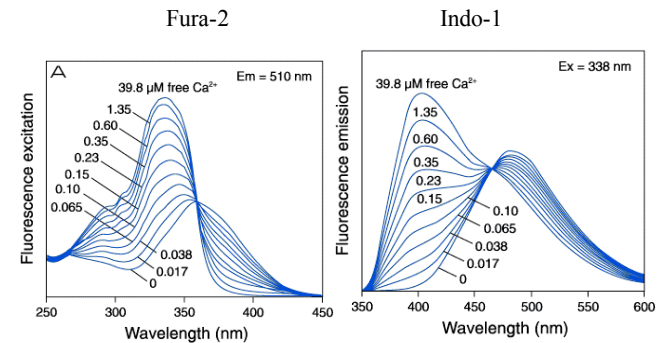
Published by AAAS



## Optical Response

1. Change (+/-) in Quantum Yield with little change in spectra  
e.g., fluo-3, Calcium Green, SPQ
2. Blue shift of absorption (excitation) spectrum with little change in maximum emission  
e.g., fura-2, Fura Red, SBFI, PBFI, BCECF
3. Blue shift in both absorption and emission spectra  
e.g., indo-1, SNARF & SNAFL

## Fura-2 & Indo-1

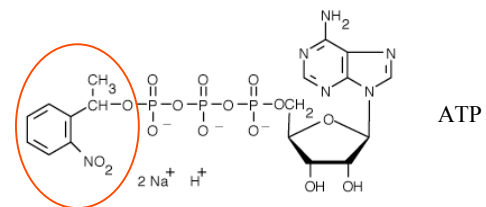
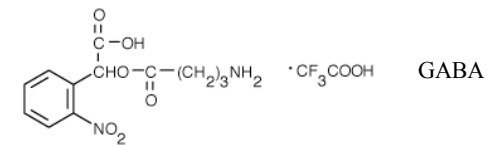


## Calcium indicators - affinity

Table 20.8 Comparison of *in vitro* and *in situ*  $K_d$  values for fluo-3, fura-2 and indo-1.

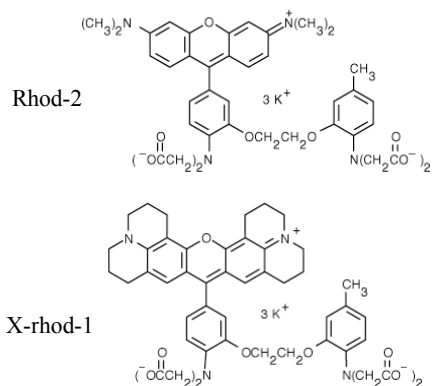
Indicator	$K_d$ <i>in vitro</i> *	$K_d$ <i>in situ</i> †	Cell/Tissue Type
fluo-3	390 nM	2570 nM	Frog skeletal muscle
fura-2	145 nM	371 nM	U373-MG astrocytoma cell
fura-2	145 nM	350 nM	Rabbit gastric gland
indo-1	230 nM	844 nM	Rabbit cardiac myocyte

## Photo-activated “Caged” compounds

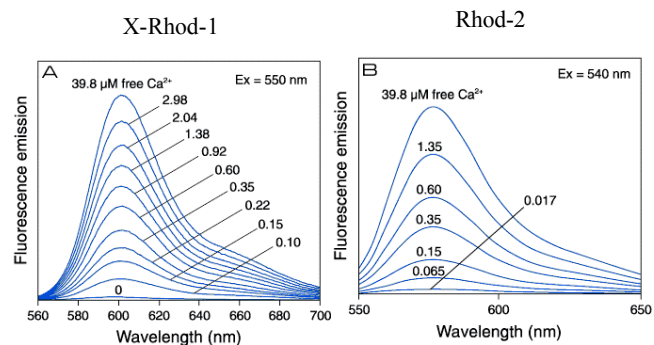


uncaging requires violet - UV irradiation

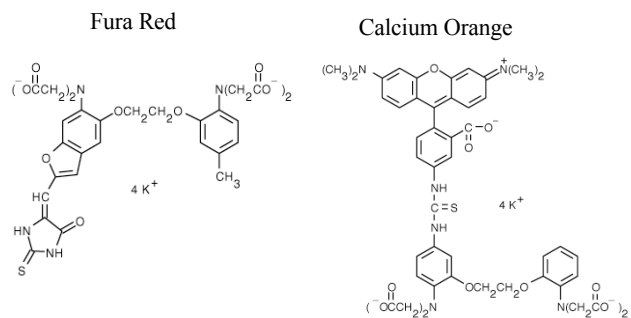
### Red calcium indicators



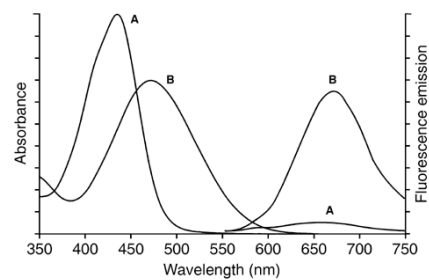
### Rhod-2 & X-Rhod-1



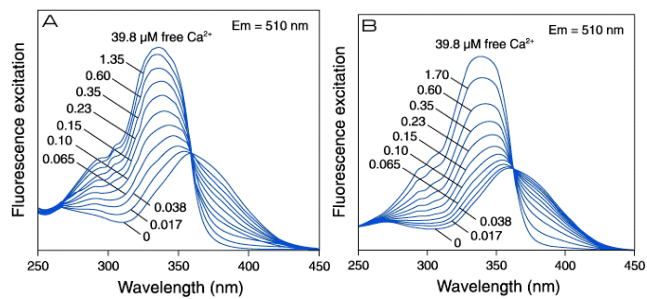
### Red calcium indicators



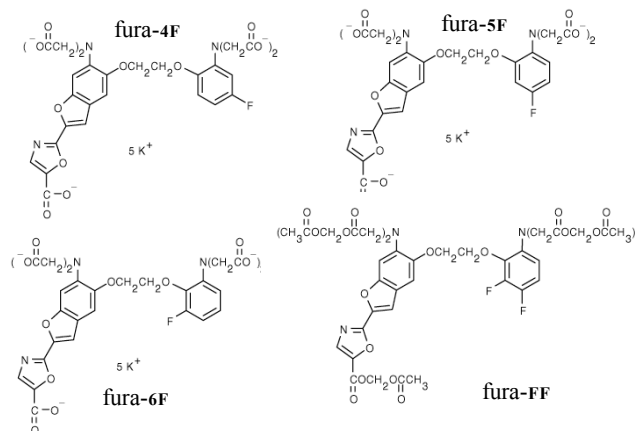
### Fura Red



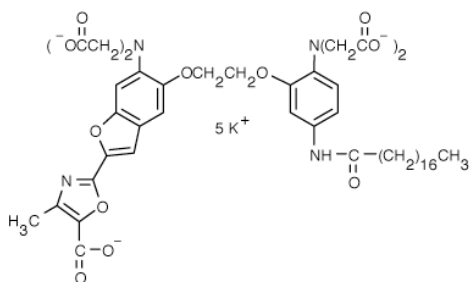
## Fura-2



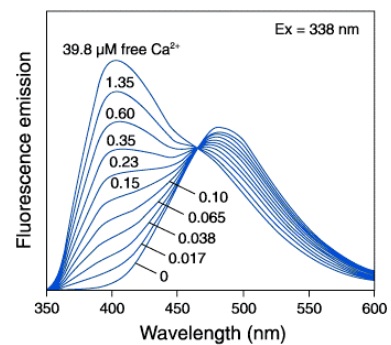
## More Furas



## Fura-2 C18

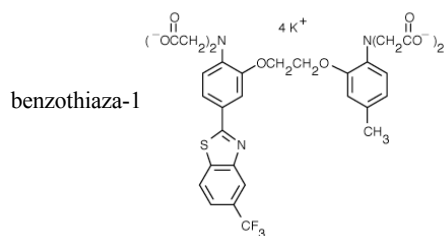
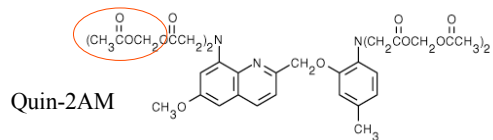


## Indo-1





## Other Ratiometric Dyes



## Ion Selective Dyes

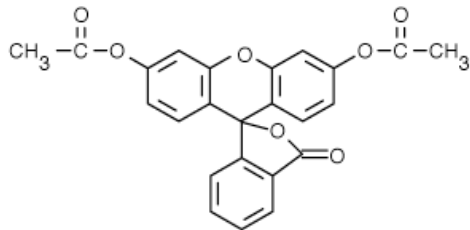
Calcium / Magnesium / Zinc ions

Protons (pH)

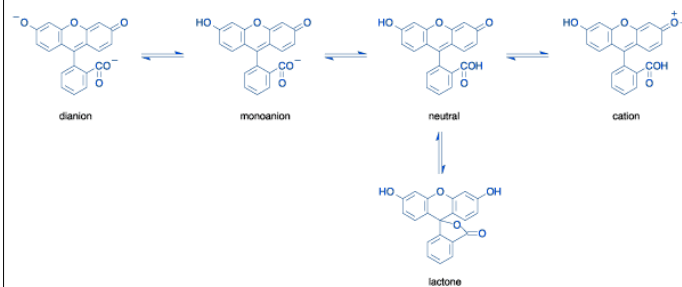
Sodium & Potassium ions

Chloride / Halide ions

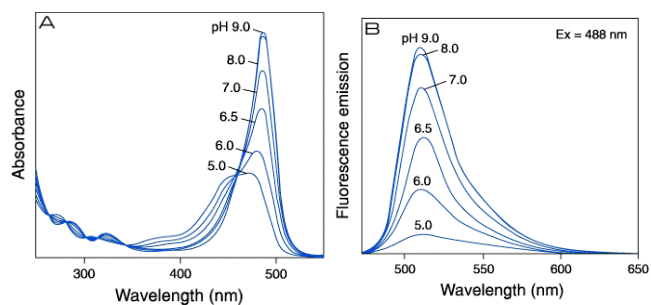
## pH Sensitive Dyes Fluorescein Diacetate (FDA)



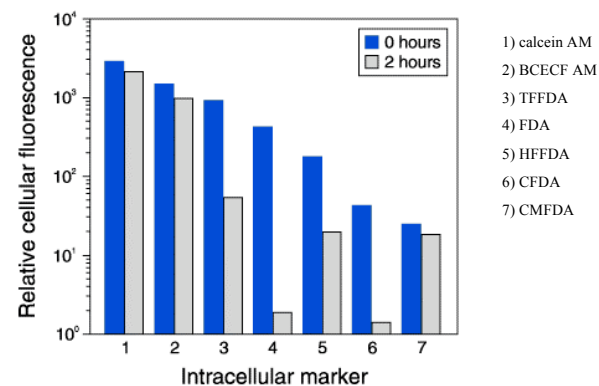
## Protonation of FDA



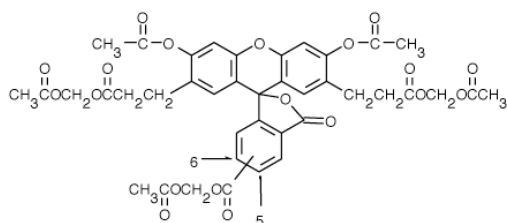
## Fluorescein Diacetate



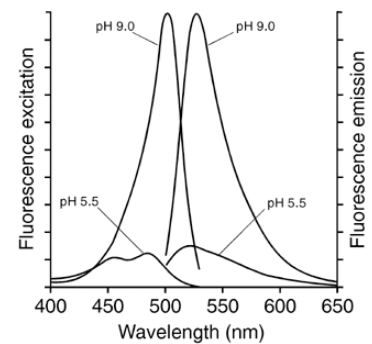
## Permeability of pH-sensitive dyes



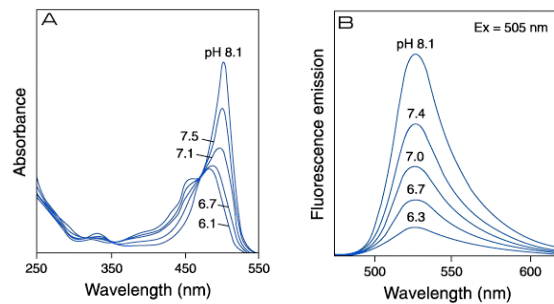
## BCECF-AM



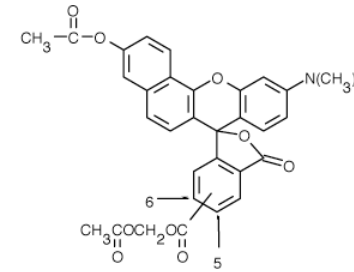
## BCECF-AM Fluorescence Spectrum



## BCECF-AM Spectra



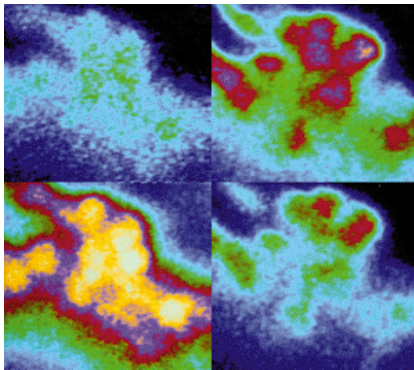
## SNARF & SNAFL



Carboxy-SNAFL-1 AM

SemiNAphthoRhodaFluors & SemiNAphthoFLuoresceins

## Indo / SNARF-1

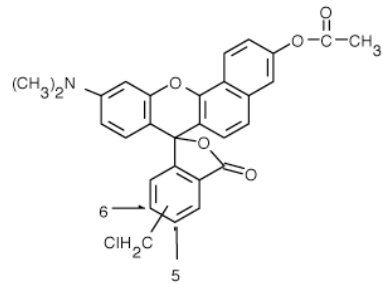


Fluorescence from the dual-emission  $\text{Ca}^{2+}$  indicator, indo-1 AM, is shown at 405 and 475 nm (left panels). Fluorescence from the dual-emission pH indicator, SNARF-1 AM, is shown at 575 and 640 nm (right panels)

## Applications of pH selective dyes

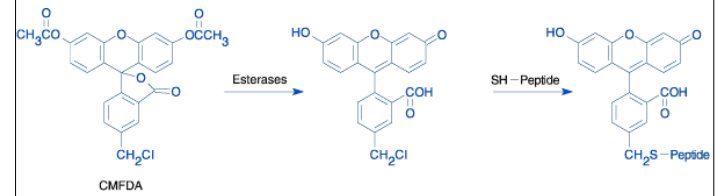
- $\text{Cl}^-/\text{HCO}_3^-$  exchange
- $\text{K}^+/\text{H}^+$  exchange
- Lactate transport and metabolism
- $\text{Na}^+/\text{H}^+$  exchange
- $\text{Na}^+/\text{Ca}^{2+}$  exchange
- $\text{NH}_4^+$  transport
- Apoptosis
- Cytotoxicity
- Multidrug resistance
- Cell volume changes
- Cytosolic pH regulation in osteoblasts and osteoclasts
- pH in lateral intercellular spaces of epithelial cell monolayers and interstitial spaces of normal and neoplastic tissue
- Phagocytosis

## Reactive Dyes



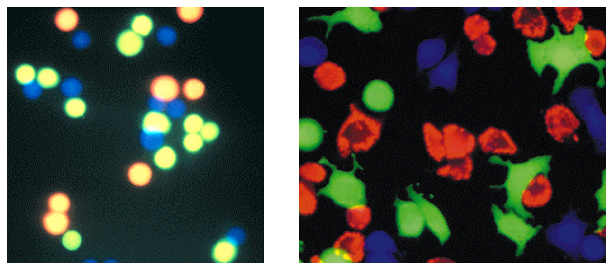
Chloromethyl-SNARF-1

## Reactive Dyes



Chloromethylfluorescein Diacetate

## “Cell Tracker” Dyes



## Ion Selective Dyes

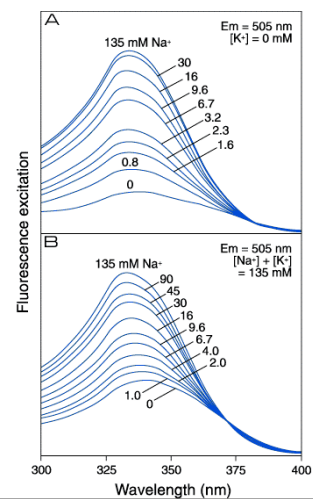
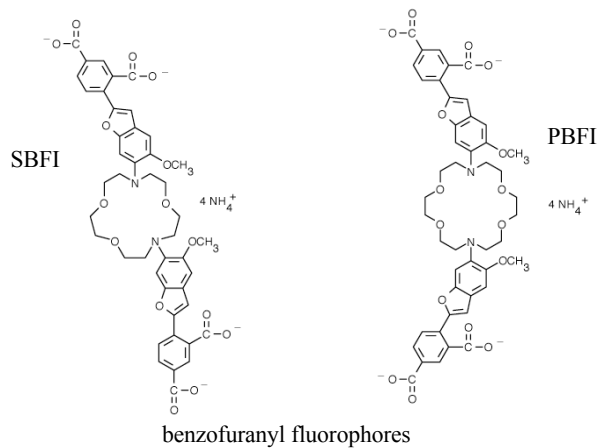
**Calcium / Magnesium / Zinc ions**

**Protons (pH)**

**Sodium & Potassium ions**

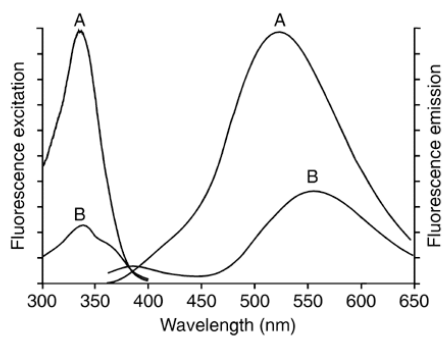
**Chloride / Halide ions**

## Sodium & Potassium Selective Dyes

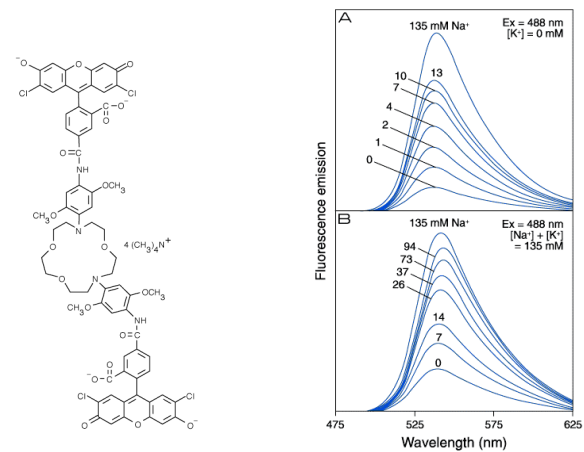


## SBFI Excitation Spectra

## SBFI Fluorescence Spectrum



## Sodium Green



## Ion Selective Dyes

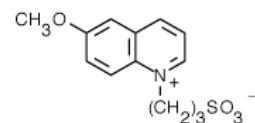
Calcium / Magnesium / Zinc ions

Protons (pH)

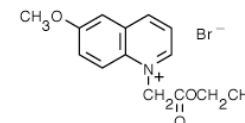
Sodium & Potassium ions

Chloride / Halide ions

## Halide Sensitive Dyes



6-methoxy-*N*-(3-sulfopropyl)quinolinium, inner salt (SPQ)



*N*-(ethoxycarbonylmethyl)-6-methoxyquinolinium bromide (MQAE)

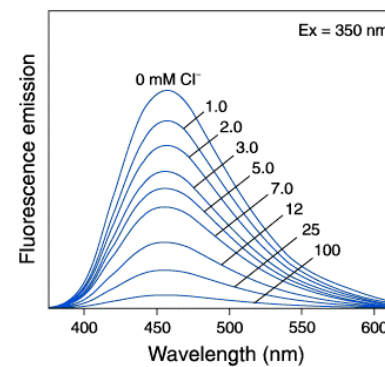
## Stern–Volmer constant

Stern–Volmer constant ( $K_{SV}$ )

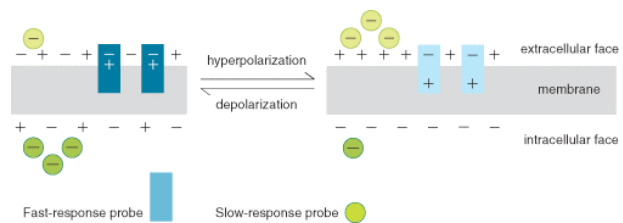
the reciprocal of the ion concentration that produces 50% of maximum quenching.

For SPQ,  $K_{SV}$  is reported to be  $118 \text{ M}^{-1}$  in aqueous solution and  $12 \text{ M}^{-1}$  inside cells.

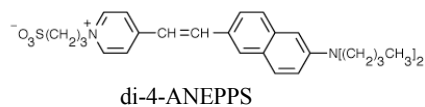
## MQAE Spectra



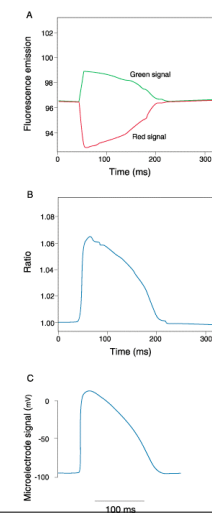
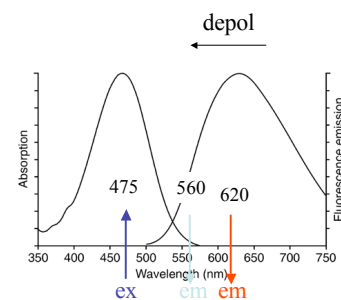
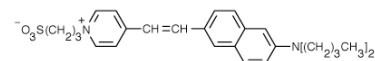
## Potentiometric Dyes



### ANEP Dyes (AminoNaphthylEthenylPyridinium)

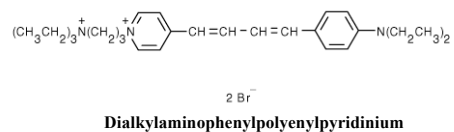


### di-4-ANEPPS

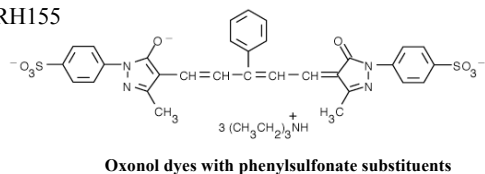


## Rina Hildesheim (RH) Family of Potentiometric Dyes

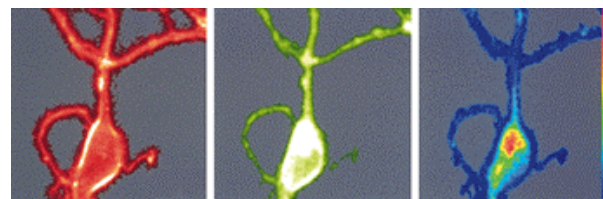
### RH414



### RH155

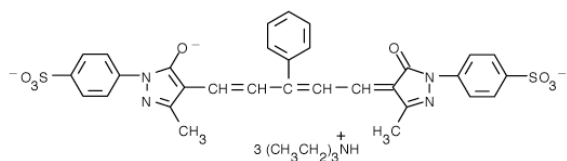


## RH414



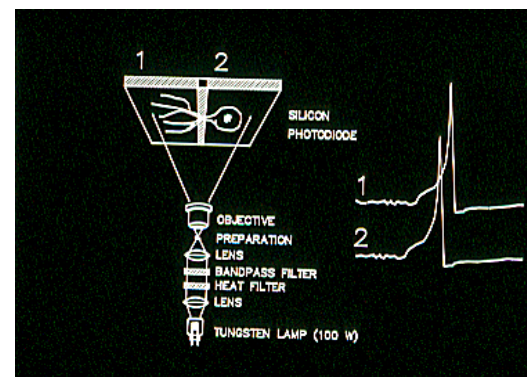
Cultured olfactory bulb neuron stained with RH 414 and (-)-DM-BODIPY dihydropyridine. Left: image at >580 nm; Middle: image at 510–580 nm; Right: ratio of the middle image divided by the left image. Images were acquired with a Leica confocal laser scanning microscope

### Potentiometric Dyes: RH155

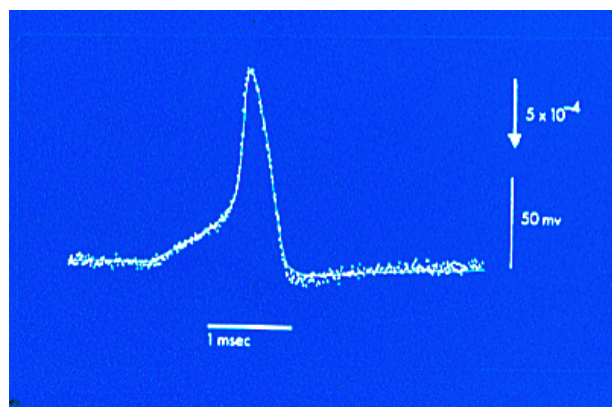


RH 155 is nonfluorescent; its potentiometric response is detected using absorption changes at approximately 720 nm.

### Photodiode Array Recording

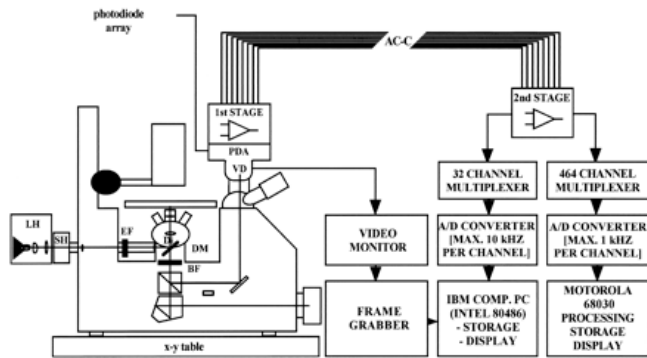


### RH155 A fast potentiometric dye

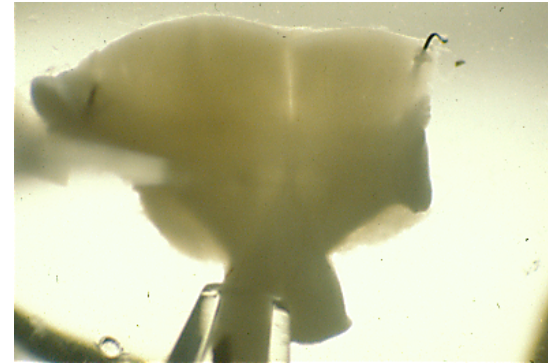




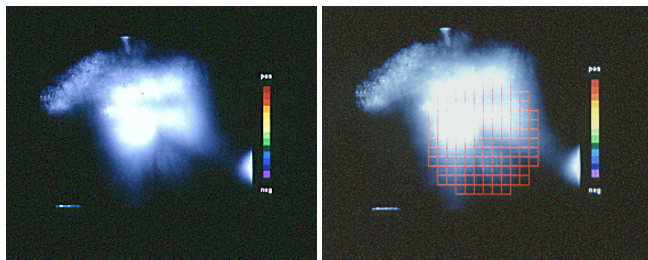
### PDA Recording Setup



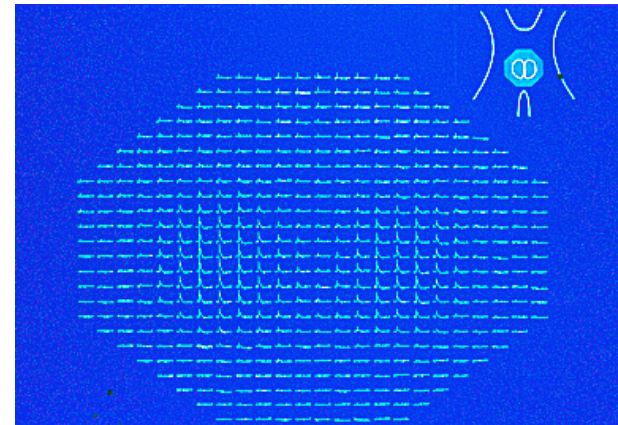
### SCN Slice Preparation



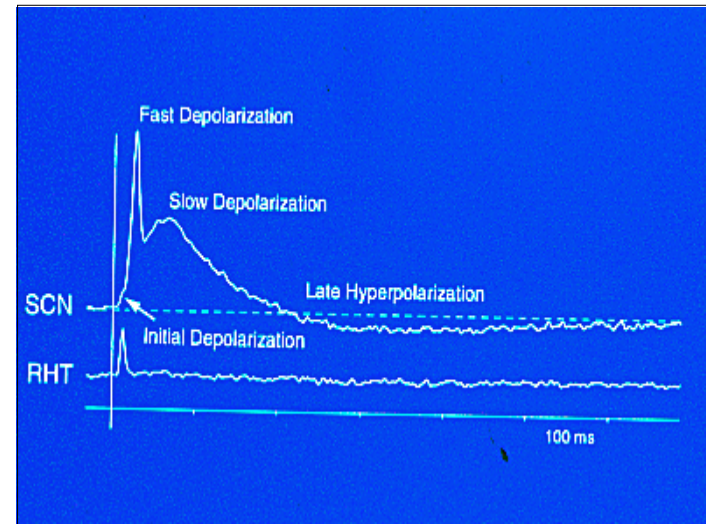
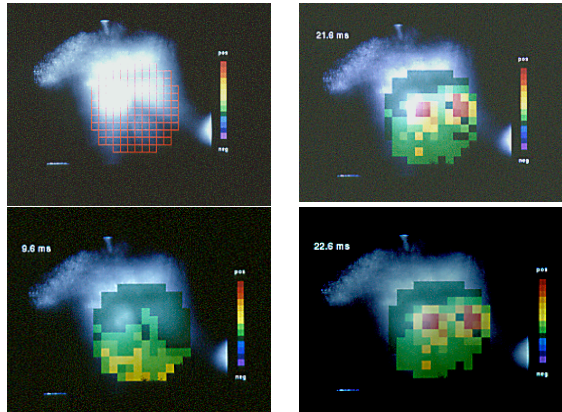
### PDA Recording of SCN Activity



### PDA Recording of SCN Activity

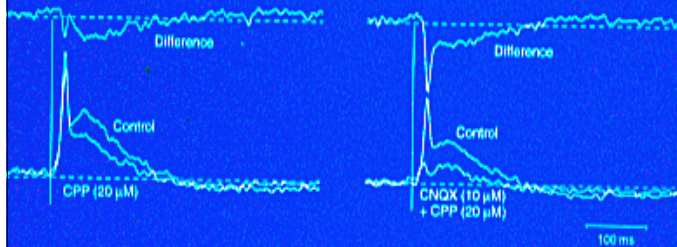


### PDA Recording of SCN Activity

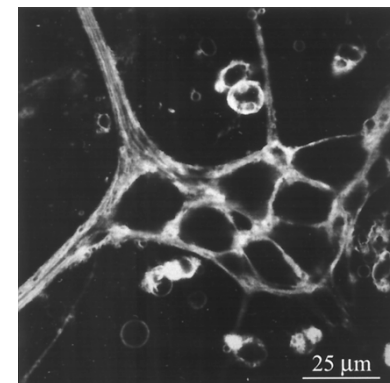


### EAA Pharmacology of Evoked Responses

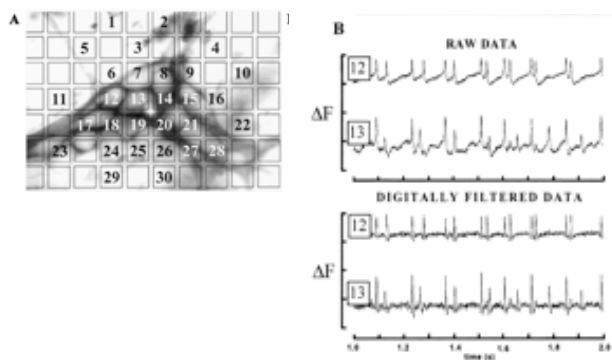
#### Excitatory Amino Acid Antagonists



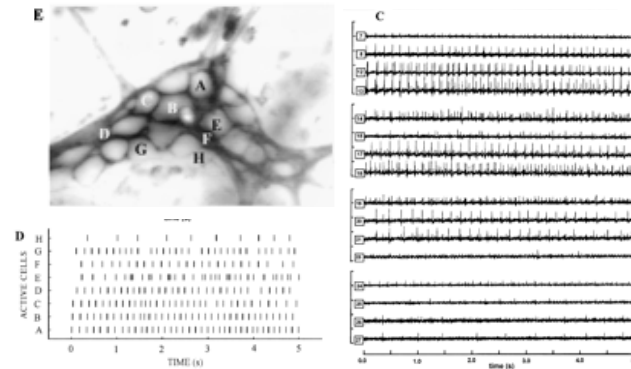
### Rat Enteric Plexus



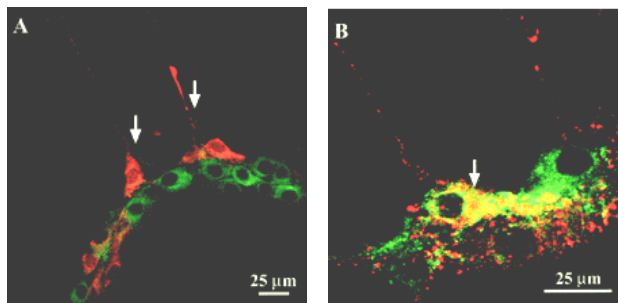
### Obaid et al., 1999



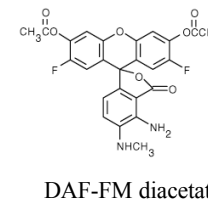
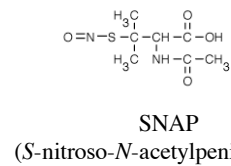
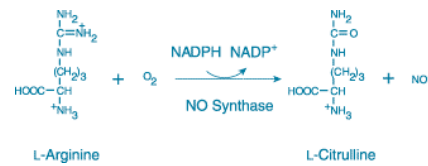
### Obade et al., 1999



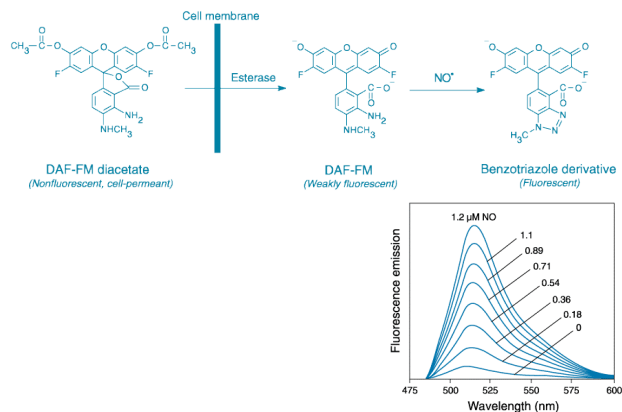
### Obade et al., 1999



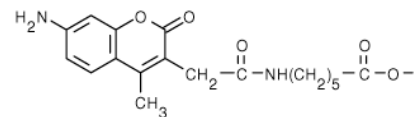
### Cell Signaling - NO



## Cell Signaling - NO



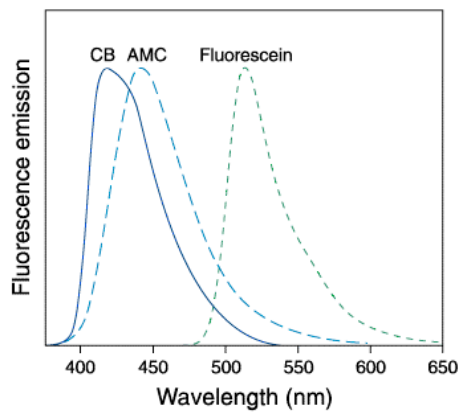
## Coumarin Fluorophores



6-((7-amino-4-methylcoumarin-3-acetyl)amino)hexanoic acid

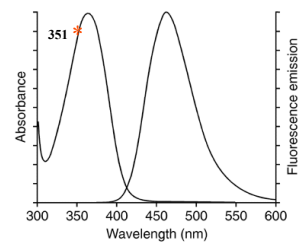
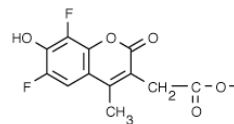
AMCA-X

## Coumarin Fluorophores

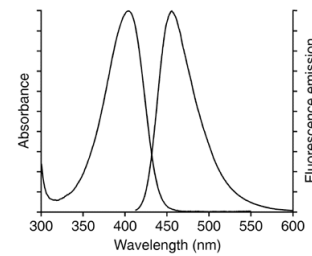
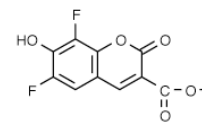


## Coumarin Fluorophores

Marina Blue

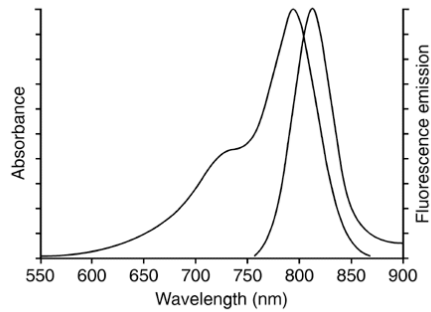


Pacific Blue

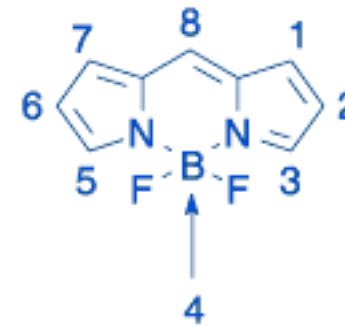


## Infrared Fluorophores

Laser Pro IR 790

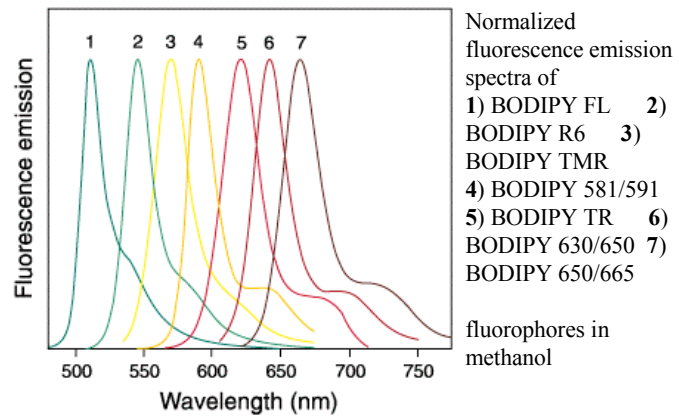


## BODIPY fluorophores

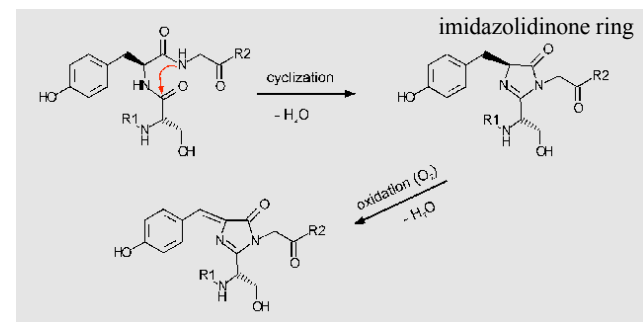


4,4-difluoro-4-bora-3a,4a-diaza-s-indacene

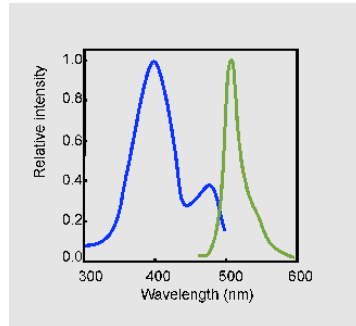
## BODIPY fluorophores



## Green Fluorescent Protein

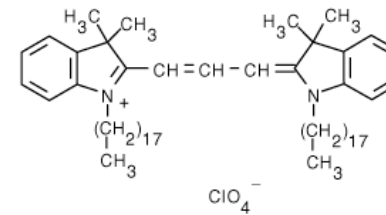


### Green Fluorescent Protein



The excitation spectrum of native GFP from *A. victoria* (blue) has two excitation maxima at 395 nm and at 470 nm. The fluorescence emission spectrum (green) has a peak at 509 nm and a shoulder at 540 nm.

### DiI Family

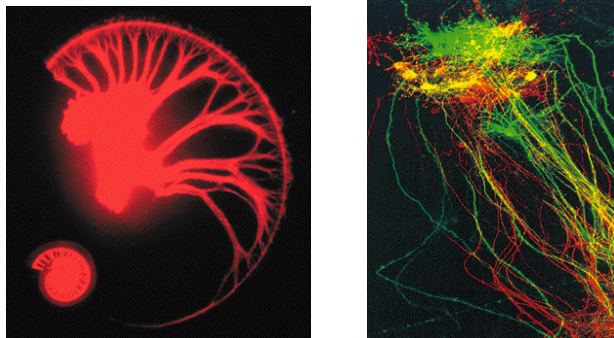


1,1'-diiodo-3,3',3'-tetramethylindocarbocyanine perchlorate ('DiI'; DiI<sub>18</sub>(3))

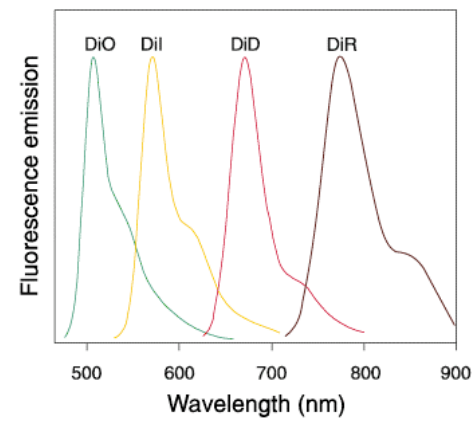
Tract tracing in fixed tissue

Remarkably stable – up to 2 years in vitro

### DiI images

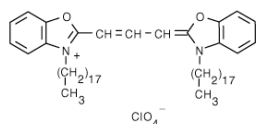


### Fluorescence Spectra for DiXs



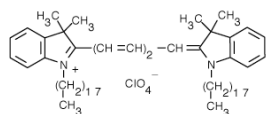
## DiI Family

DiO

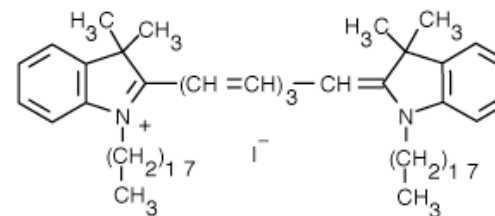


3,3'-dioctadecyloxacarbo-cyanine perchlorate ('DiO'; DiOC<sub>18</sub>(3))

DiD

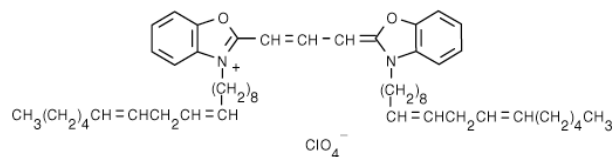


1,1'-dioctadecyl-3,3,3',3'-tetramethylindodicarbocyanine perchlorate ('DiD' oil; DiIC<sub>18</sub>(5) oil)



1,1'-dioctadecyl-3,3,3',3'-tetramethylindotricarbocyanine iodide ('DiR'; DiIC<sub>18</sub>(7))

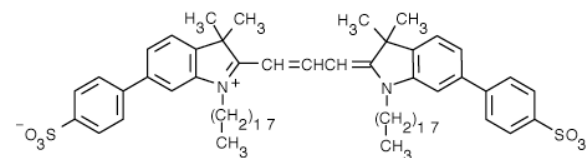
## Fast DiO



3,3'-dilinoleyloxacarbo-cyanine perchlorate (FAST DiO solid; DiO δ<sup>9,12</sup>-C<sub>18</sub>(3), ClO<sub>4</sub><sup>-</sup>)

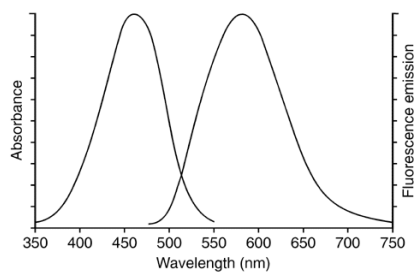
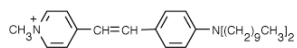
Diunsaturated linoleyl (C<sub>18:2</sub>) tails in place of the saturated octadecyl tails (C<sub>18:0</sub>) of DiI and DiO increase rate of transport

## Sulfonate forms



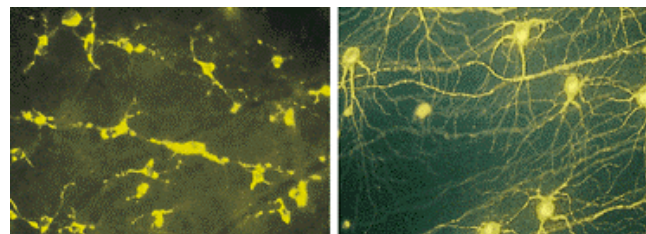
1,1'-dioctadecyl-6,6'-di(4-sulfophenyl)-3,3,3',3'-tetramethylindodicarbocyanine (SP-DiIC<sub>18</sub>(3))

## DiA



4-(4-(dihexadecylamino)styryl)-*N*-methylpyridinium iodide (DiA; 4-Di-16-ASP)

## DiA in mouse retina



3 days after optic nerve transection

10 days after optic nerve transection

## Dextrans

Substituted dextran polymers

Poly-( $\alpha$ -D-1,6-glucose) linkages, which render them resistant to cleavage by most endogenous cellular glycosidases

Wide molecular weight range (3000 – 300K)

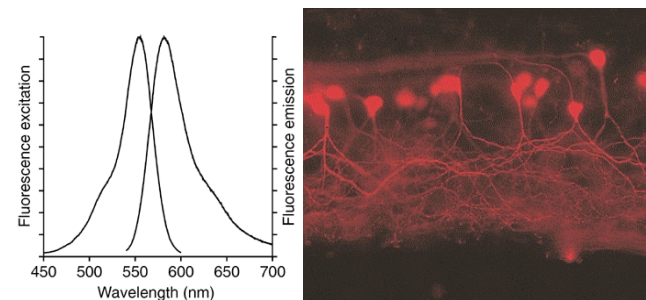
Multiple fluorophores

Biotin

Lysine

Fluoro-Ruby

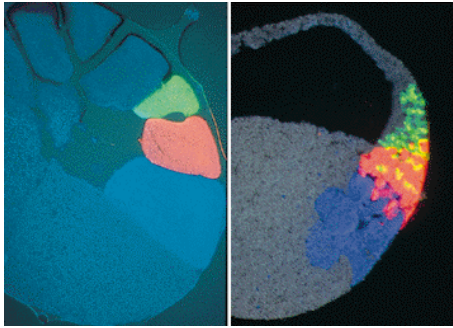
## Fluoro-Ruby



Fish spinal cord

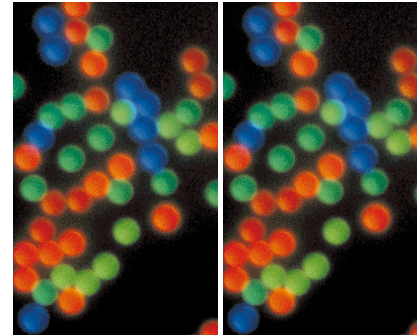


### Cell lineage with dextrans



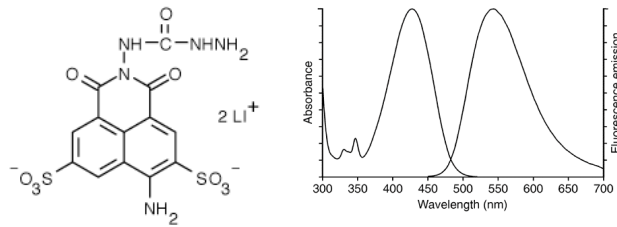
The image on the left shows a 13  $\mu\text{m}$ -thick section of a stage 6 (32-cell) *Xenopus* embryo fixed right after injection; this section exhibits significant autofluorescence due to the presence of residual yolk. The image on the right is a stage 10 (early gastrula) embryo

### Fluorescent Microspheres

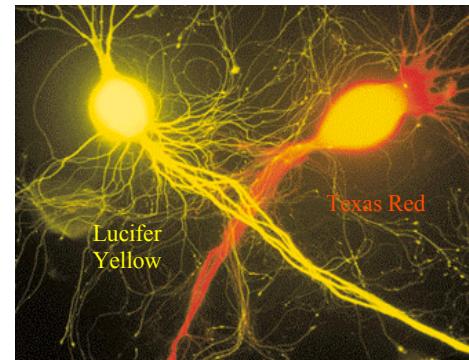


polystyrene microspheres 0.02 – 15  $\mu\text{m}$  diameter

### Lucifer Yellow



### Lucifer Yellow



## End of tract tracers

