CSHL 2019

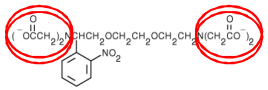
Organic calcium indicators

Jack Waters

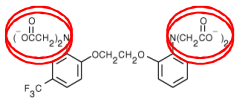
Loading

Carboxylic acid groups bind calcium ions

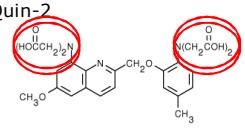
EGTA



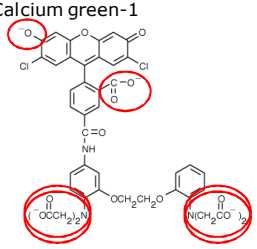
BAPTA



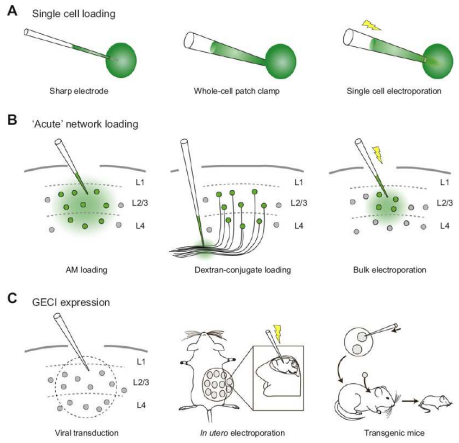
Quin-2



Calcium green-1



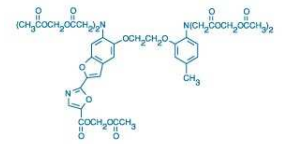
Loading / labeling



Grienberger & Konnerth, Neuron 2012

De-esterification

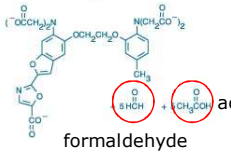
fura-2 AM



Cell membrane

Esterase

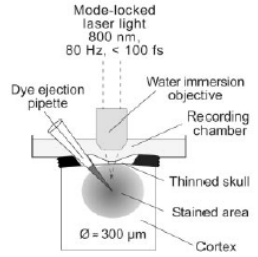
fura-2

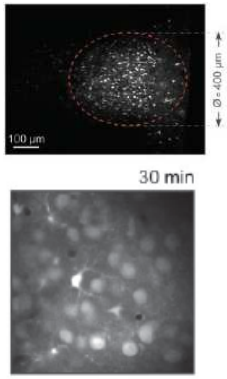


acetic acid

formaldehyde

AM ester loading in vivo



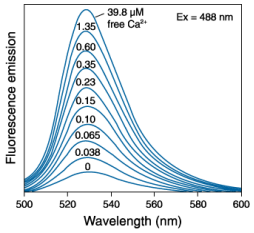


Stosiek, Garaschuk, Holthoff & Konnerth, 2003

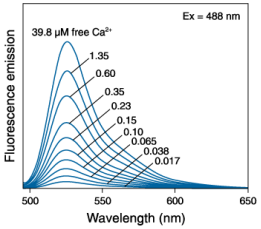
Quantification

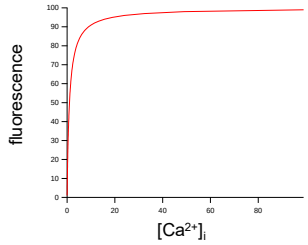
Emission spectra

calcium green -1



fluo-3



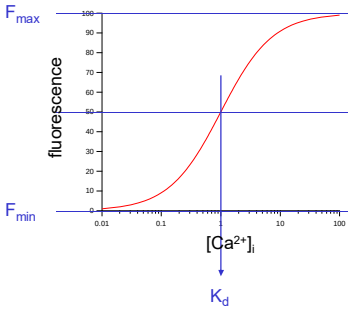


Saturable binding

-> rectangular hyperbola

$[Ca^{2+}]_i = K_d \cdot (F-F_{min}) / (F_{max}-F)$

$[Ca^{2+}]_i = K_d \cdot (F-F_{min}) / (F_{max}-F)$



$[Ca^{2+}]_i = K_d \cdot (F-F_{min}) / (F_{max}-F)$

F_{min} and F_{max} (and F) depend on the number of dye molecules excited

-> sensitive to dye concentration and volume of the compartment studied

- different indicator loading from cell to cell
- indicator concentration gradients, e.g. along dendrites
- volume differences, e.g. along dendrites

Therefore need to measure F_{min} and F_{max} in each experiment

Measure F_{min} and F_{max} at end of experiment

What if F_{min} and F_{max} change during experiment (e.g. photobleaching)?

The alternative: $\Delta F/F$

Absolute calcium concentrations aren't always of interest

~~$[Ca^{2+}]_i = K_d \cdot (F-F_{min}) / (F_{max}-F)$~~

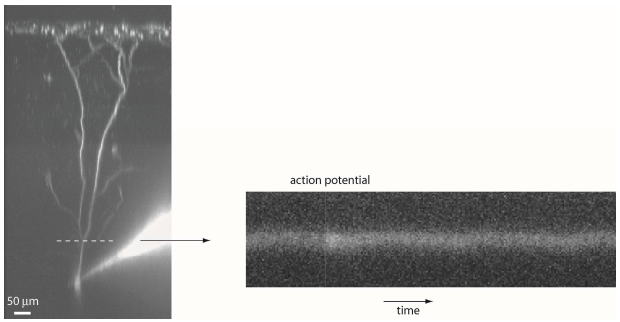
Quantify normalized changes in fluorescence

$$\Delta F/F = (F - F_{rest}) / F_{rest}$$

Unlike F_{min} and F_{max}, $\Delta F/F$ is insensitive to

- excitation intensity and detector efficiency
- cell to cell variability
- differences between regions of a cell
- slow changes in fluorescence during the experiment

$\Delta F/F = (F - F_{rest}) / F_{rest}$



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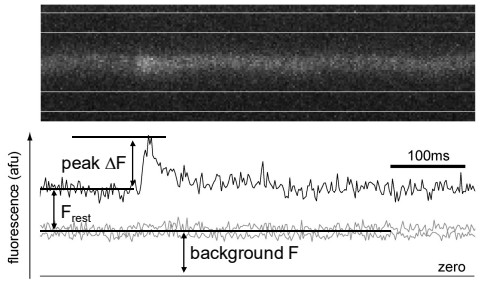

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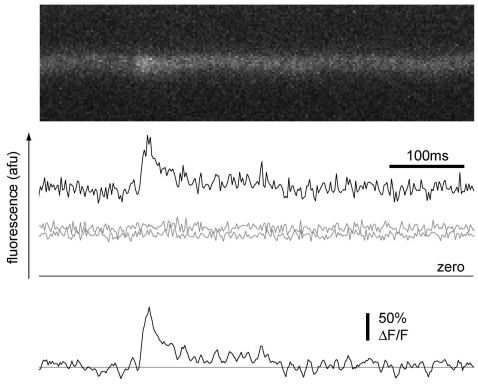
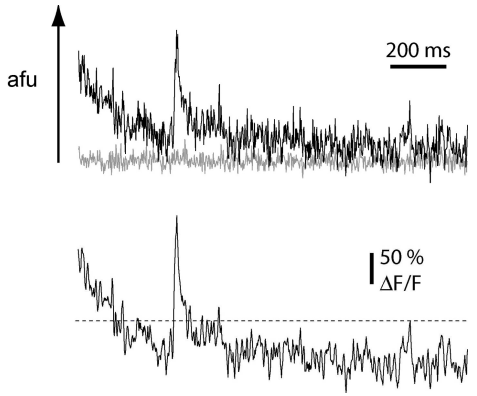
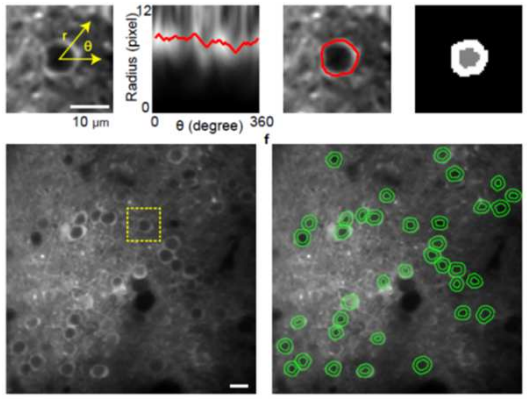


$\Delta F/F = (F - F_{rest}) / F_{rest}$



Neuropil subtraction



Chen *et al.* (2013) Nature

$\Delta F/F = (F - F_{rest}) / F_{rest}$

- don't forget to subtract background fluorescence
- beware changes in F_{rest} (e.g. from photobleaching)

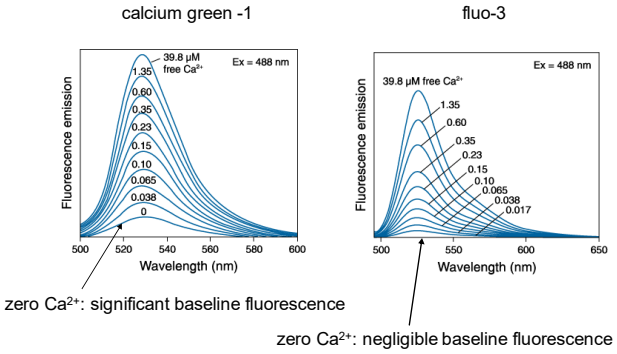
Main problem with $\Delta F/F$ calculation:

some dyes have very low resting fluorescence

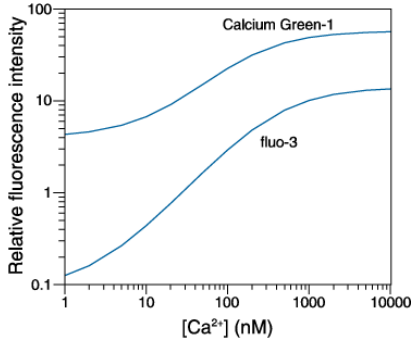
-> dividing by a small, noisy number

-> noisy $\Delta F/F$

Emission spectra



Basal fluorescence and dynamic range



calcium green:

moderate basal fluorescence

moderate dynamic range (F_{max}/F_{min} ~14)

fluo-3:

low basal fluorescence

large dynamic range (F_{max}/F_{min} > 100)

Solution to the low F_{rest} problem

F_{rest} is a measure of the indicator concentration

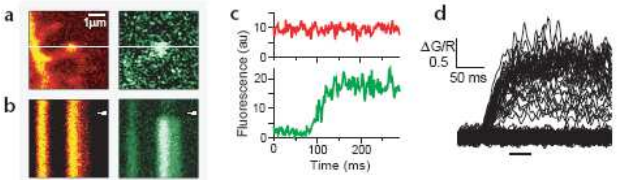
Co-load cells with two indicators

-> ratio fluorescence of calcium indicator to Ca^{2+}-insensitive indicator

$\Delta F/F = (F-F_{rest}) / F_{rest}$ $\Delta F/R = (F-F_{rest}) / R$

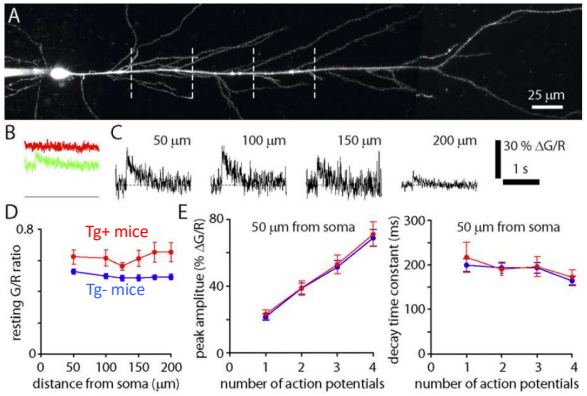
R is fluorescence of second indicator

Example of 2-indicator method



Oertner, Sabatini, Nimchinski & Svoboda, 2002

Example of 2-indicator method: change in resting calcium



Wykes et al., Neurobiol Aging 2012

Solution to the low F_{rest} problem

F_{rest} is a measure of the indicator concentration

Co-load cells with two indicators

-> ratio fluorescence of calcium indicator to Ca^{2+}-insensitive indicator

$\Delta F/F = (F-F_{rest}) / F_{rest}$ $\Delta F/R = (F-F_{rest}) / R$

Potential problem:
differential distribution
or photobleaching
of the two indicators

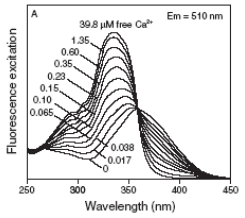
Quantification options for single-wavelength indicators

One indicator	Two indicators
$\Delta F/F = (F-F_{rest}) / F_{rest}$	$\Delta F/R = (F-F_{rest}) / R$
$[Ca^{2+}] = K_d \cdot (F-F_{min}) / (F_{max}-F)$	$[Ca^{2+}] = K_d \cdot \frac{(F/R) - (F/R)_{min}}{(F/R)_{max} - (F/R)}$

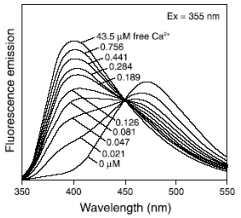
but see Maravall *et al*. (2000) BiophysJ

Ca induces spectral changes in ratiometric dyes

Excitation spectrum of fura



Emission spectrum of indo

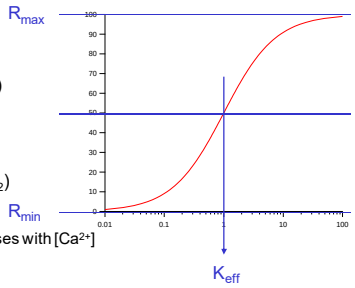


$[Ca^{2+}]_i = K_{eff} \cdot (R-R_{min}) / (R_{max}-R)$

where $R = F_{\lambda 1}/F_{\lambda 2}$

$K_{eff} = K_d * (F_{max,\lambda 2}/F_{min,\lambda 2})$

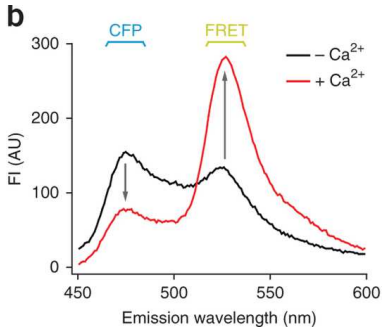
λ_2 is wavelength at which F decreases with $[Ca^{2+}]$



$[Ca^{2+}]_i = K_{eff} \cdot (R-R_{min}) / (R_{max}-R)$

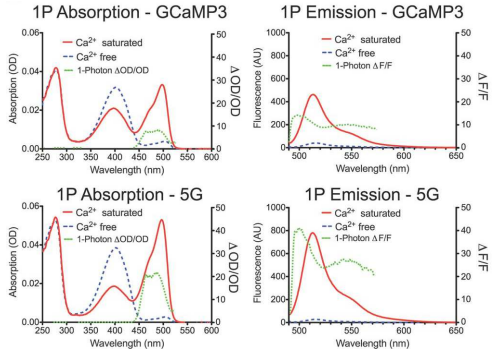
- need to measure R_{min}, R_{max}, $F_{max,\lambda 2}$ and $F_{min,\lambda 2}$
- unaffected by photobleaching, etc.
- disadvantages: UV-excitable dyes, poor signal-to-noise ratio

TN-XXL



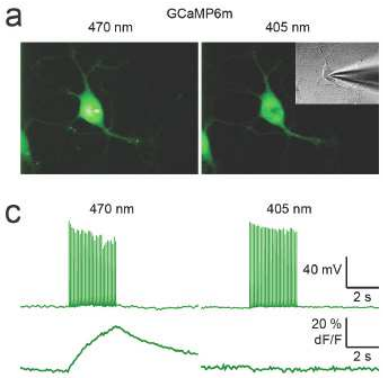
Nature Medicine 19, 778–783 (2013)

GCaMP



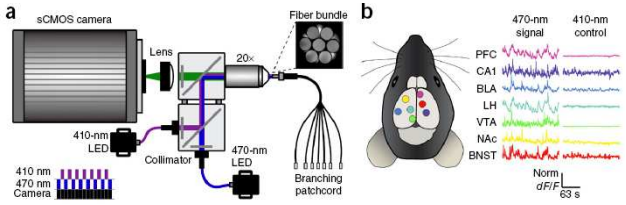
Ackerboom et al., J Neurosci 2012

GCaMP6



Kim et al., Nat Meth 2016

GCaMP6



Kim et al., Nat Meth 2016

Quantification -summary

One indicator	Two indicators
$\Delta F/F = (F-F_{rest}) / F_{rest}$	$\Delta F/R = (F-F_{rest}) / R$
$[Ca^{2+}] = K_d . (F-F_{min}) / (F_{max}-F)$	$[Ca^{2+}] = K_d . \frac{(F/R) - (F/R)_{min}}{(F/R)_{max} - (F/R)}$

Ratiometric

$$[Ca^{2+}]_i = K_{eff} . (R-R_{min}) / (R_{max}-R)$$

Quantification - pitfalls

Main pitfalls

1. Other ions
   - From Molecular Probes:

   K_d measured *in vitro* at 22°C in 100 mM KCl, 10 mM MOPS, pH 7.2, unless otherwise noted.

   K_d values depend on temperature, ionic strength and pH

   - most Ca indicators bind other divalents, which may enhance or quench fluoresence

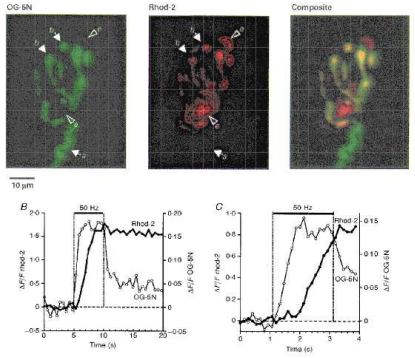
   e.g. Zn^{2+} binds readily to fluo-3 and produces bright fluorescence

   e.g. Mn^{2+} binds & quenches many dyes, including fura-2

Main pitfalls

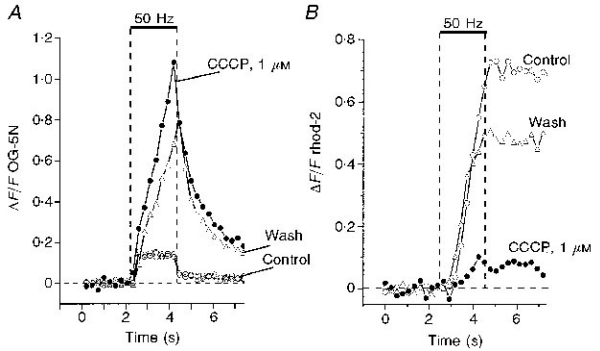
1. Other ions
2. Compartmentalization
   - dyes can be taken up into intracellular compartments

Example of compartmentalization



David, Barrett & Barrett, 1998

Example of compartmentalization



David, Barrett & Barrett, 1998

Main pitfalls

1. Other ions
2. Compartmentalization
   - dyes can be taken up into intracellular compartments

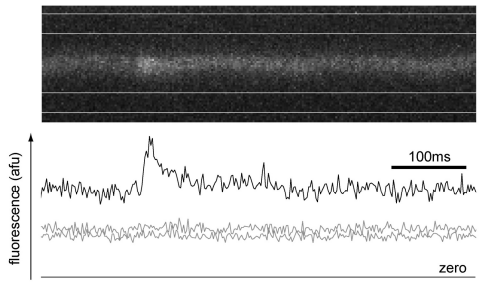
Main pitfalls

1. Other ions
2. Compartmentalization
3. Background problems

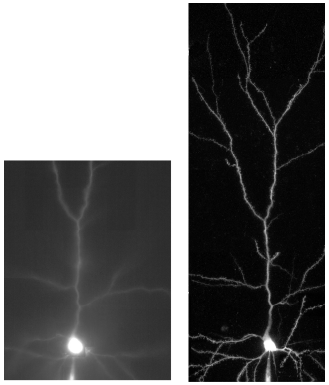
$$\Delta F/F = (F - F_{rest}) / F_{rest}$$

F_{rest} is fluorescence <u>from intracellular indicator</u>
-> need to subtract background fluorescence

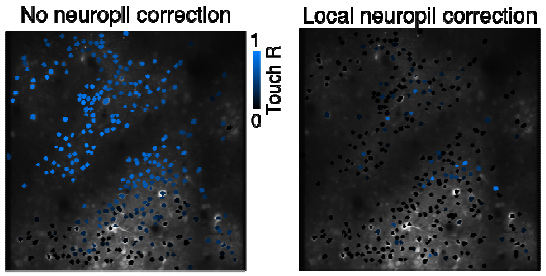
So what's the problem?



Pyramidal neuron in slice - camera vs 2P scope



Neuropil subtraction



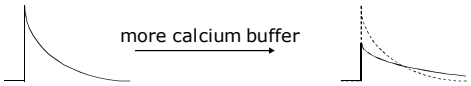
Peron et al., Neuron 2015

Main pitfalls

1. Other ions
2. Compartmentalization
3. Background problems
   - choice of background can strongly affect $\Delta F/F$
     - contamination with signal
     - non-uniform background
     - non-linear gradient

Main pitfalls

1. Other ions
2. Compartmentalization
3. Background problems
4. Buffering
   - indicators bind (buffer) calcium
   - added indicators alter calcium dynamics



The calcium binding ratio

κ: 'buffer capacity' or 'binding ratio'

$\kappa = [Ca^{2+}]_{bound} / [Ca^{2+}]_i$

If κ = 100 then there are 100 bound Ca^{2+} ions for every free Ca^{2+} ion

The calcium binding ratio

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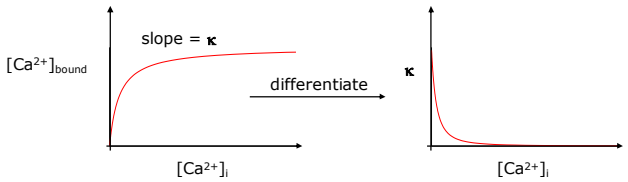
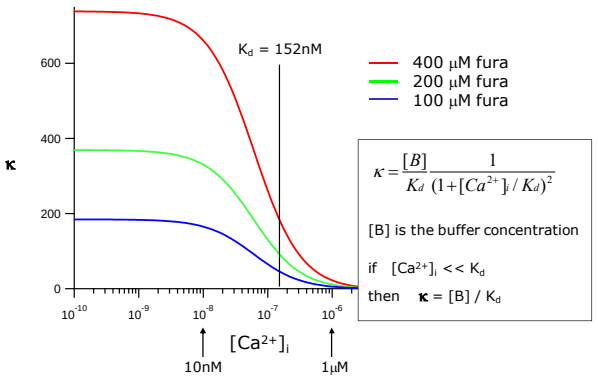
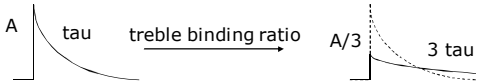


Calcium binding ratio for fura-2



Calcium buffers affect calcium dynamics



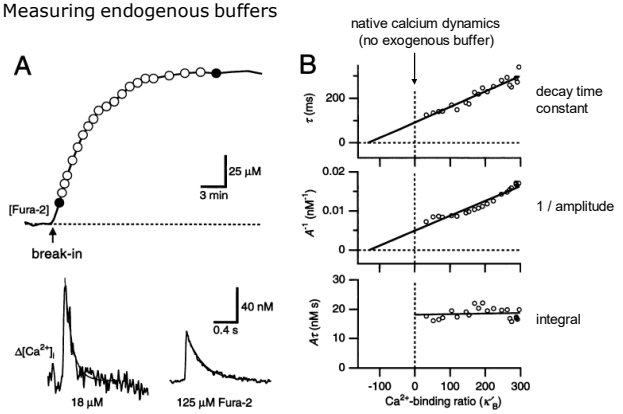
(integral is unchanged)

$$A \propto \frac{1}{1+\kappa} \qquad \text{tau} \propto 1+\kappa$$

For a mixture of endogenous and exogenous buffers:

$$A \propto \frac{1}{1+\kappa_S+\kappa_B} \qquad \text{tau} \propto 1+\kappa_S+\kappa_B$$

Measuring endogenous buffers



Helmchen, Imoto & Sakmann, BiophysJ 1996

Measuring endogenous buffers

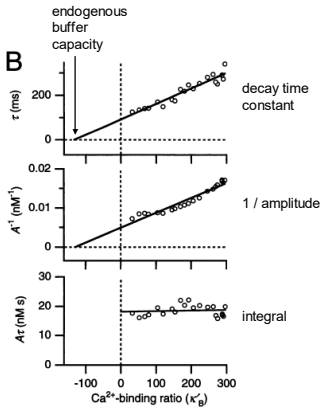
$$\text{tau} \propto 1+\kappa_S+\kappa_B$$

$$A \propto \frac{1}{1+\kappa_S+\kappa_B}$$

$\tau = 0$ and $A^{-1} = 0$ when

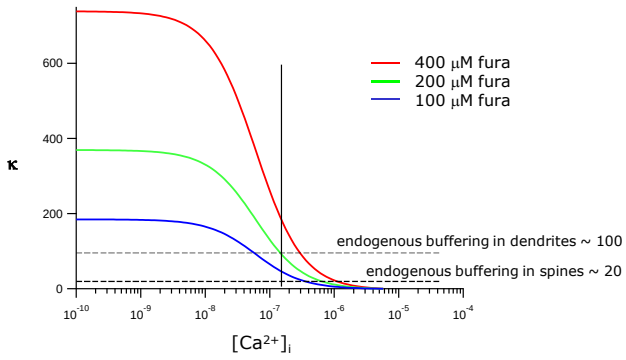
$1 + \kappa_s + \kappa_B = 0$

i.e. when $1+\kappa_s = -\kappa_B$



Helmchen, Imoto & Sakmann, BiophysJ 1996

Calcium binding ratio for fura-2



Main pitfalls

1. Other ions
2. Compartmentalization
3. Background problems
4. Buffering
   - added indicators (strongly) alter calcium dynamics
   - added indicators alter diffusion of calcium

Buffers compete for calcium binding (with each other, with pumps, etc.)

- immobile buffers 'hold calcium in place'
- mobile buffers facilitate diffusion

Endogenous buffers are typically immobile.

Indicators are mobile buffers.

-> Indicators increase spatial spread of calcium transients.

Simple diffusion: Fick's law

$$J = D \cdot dC/dx$$

J	diffusive flux (the rate of diffusion)
D	diffusion coefficient
dC/dx	concentration gradient

Buffered diffusion (one mobile and one immobile buffer)

$$D_{eff} = \frac{D_{Ca} + \kappa_B . D_B}{1 + \kappa_B + \kappa_S}$$

D_{eff}	effective diffusion coefficient
D_{Ca}	diffusion coefficient of calcium
D_B	diffusion coefficient of exogenous (mobile) buffer
κ_B & κ_S	binding ratios of exogenous (mobile) and endogenous (immobile) buffers

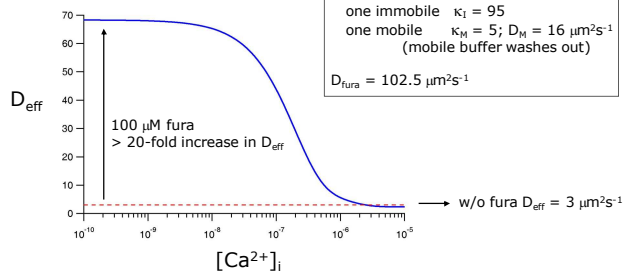
Effect of fura on D_{eff}

$$D_{eff} = \frac{D_{Ca} + \kappa_B . D_B + \kappa_M . D_M}{1 + \kappa_B + \kappa_M + \kappa_I}$$

D_{Ca} = 223 $\mu m^2 s^{-1}$

2 endogenous buffers
one immobile κ_I = 95
one mobile κ_M = 5; D_M = 16 $\mu m^2 s^{-1}$
(mobile buffer washes out)

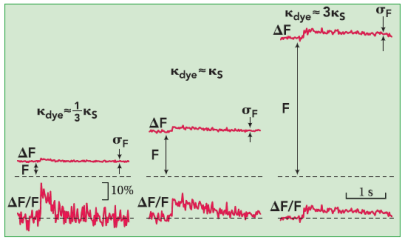
D_{fura} = 102.5 $\mu m^2 s^{-1}$



Main pitfalls

1. Other ions
2. Compartmentalization
3. Background problems
4. Buffering
   - added indicators (strongly) alter calcium dynamics
   - added indicators alter diffusion of calcium

Optimal signal-to-noise ratio



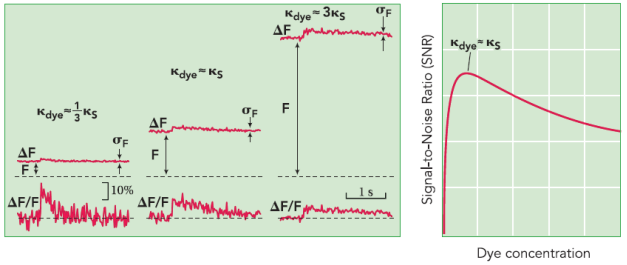
More dye
• more signal
• more shot noise
• smaller ΔF/F

→ΔF/F amplitude and noise both decrease with increasing amplitude

→ optimal signal-to-noise ratio is related to buffer capacities of dye and endogenous buffers

from Goebel & Helmchen, Physiology 2007

Optimal signal-to-noise ratio



from Goebel & Helmchen, Physiology 2007