Neurofunctional Techniques

Lesson 7

28 October 2026

Ca^{2+}-dependent fluorescence properties

Calendar

- W 30 Sept: Course introduction (14-16)
- F 2 Oct: Review neurophysiology + Functional imaging (14-18)
- F 23 Oct: Functional imaging + introduction to paper presentation (14-18)
- W 28 Oct: Functional imaging (14-16)
- F 30 Oct: Introduction to paper presentations (14-18)
- W 4 Nov: Modeling + paper assignment (14-16)
- F 6 Nov: Molecular tools + optogenetics (14-18)
- F 13 Nov: X-genetics + paper discussion (14-18)
- F 20 Nov: Laboratory (14-18)
- F 27 Nov: Paper presentation (14-18)
- F 4 Dec: Paper presentation (14-18)
- W 9 Dic: Prof. Agenor Limon (09-11)
- T 10 Dic: Prof. Agenor Limon (09-11)
- F 11 Dec: Paper presentation (14-18)
- Tue 15 Dec: Test (14-16)
- Fri 18 Dec: ----

Ca^{2+} imaging

- Review Ca^{2+} signaling
- Ca^{2+} indicators
- Ca^{2+} binding
- Ca^{2+} diffusion
- **Ca^{2+}-dependent fluorescence properties**
- **Simplified models of Ca^{2+} dynamics**
- **Imaging and network activity**

Objective

How fluorescence relates to [Ca^{2+}]

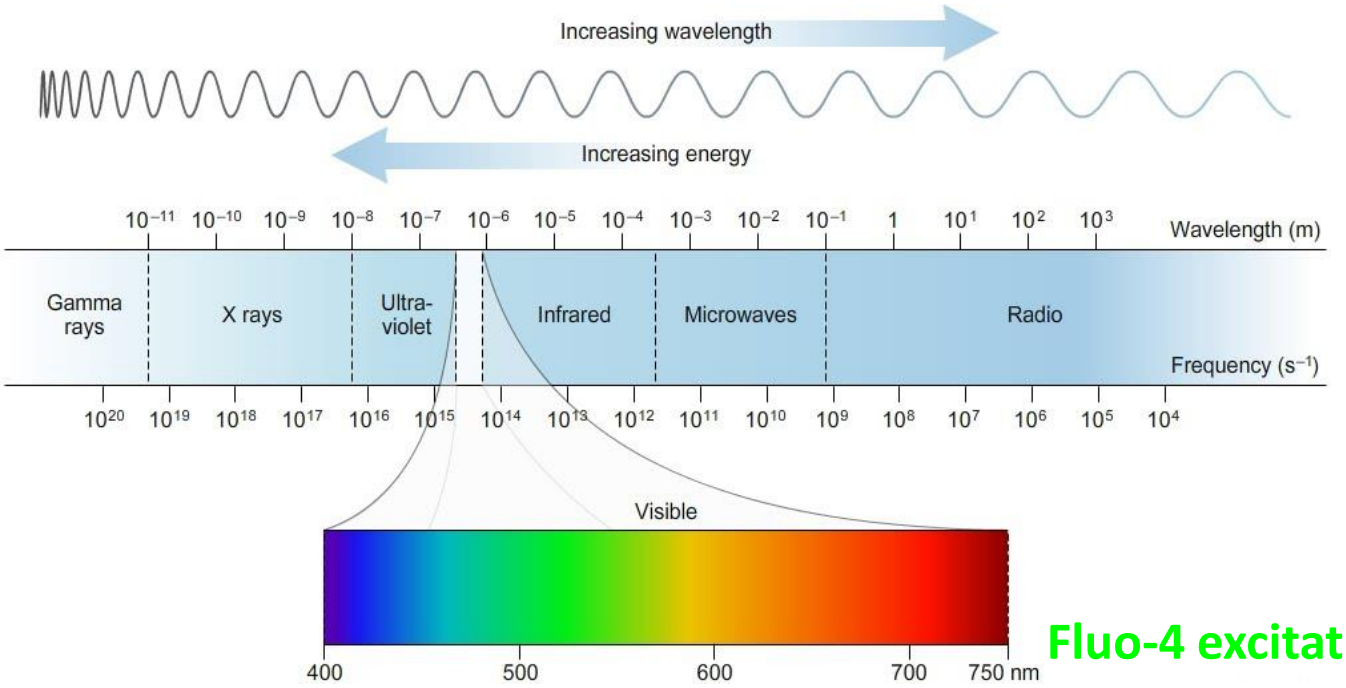
How do you make a fluorescent Ca^{2+} indicator?

It should have two moieties:

- **1 acting as Ca^{2+} buffer/chelator**
- **1 acting as fluorophore**
- **Ca^{2+} binding to the chelator moiety must affect some property of the fluorophore**

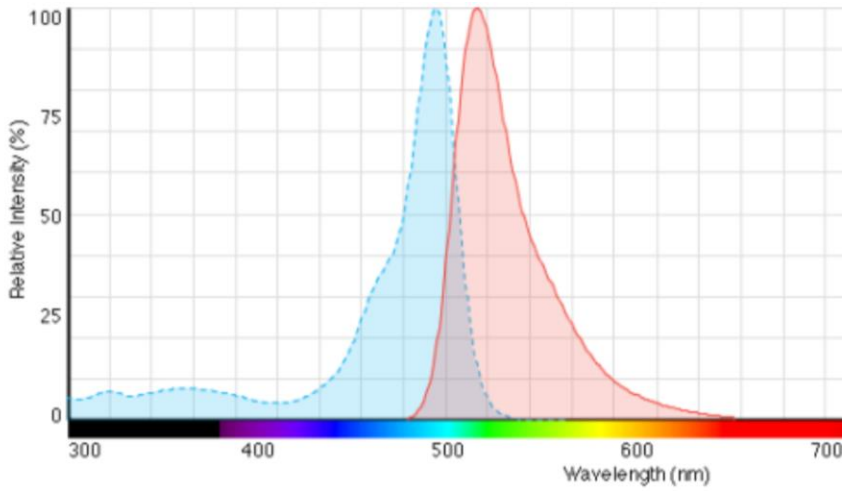
Fluorescence ⟺ Ca^{2+}

Electromagnetic spectrum



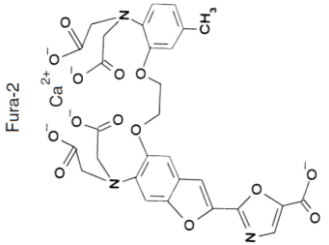
Example:

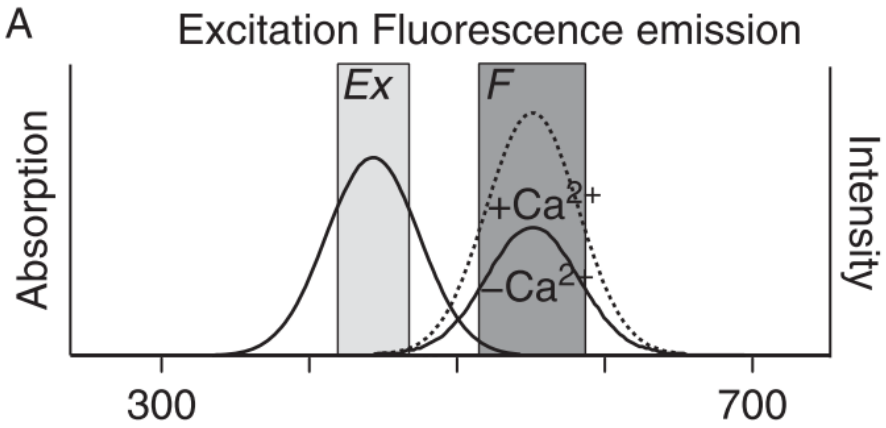
Fluo-4 excitation and emission spectra

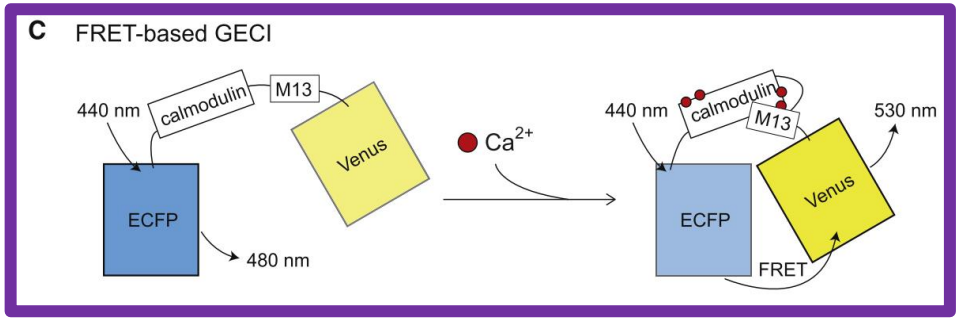


Ca^{2+}-dependent fluorescence changes

Intensity meusurements: Ca^{2+} binding increases (or decreases, not shown) fluorescence (**e.g. Fluo & GCaMP series**)



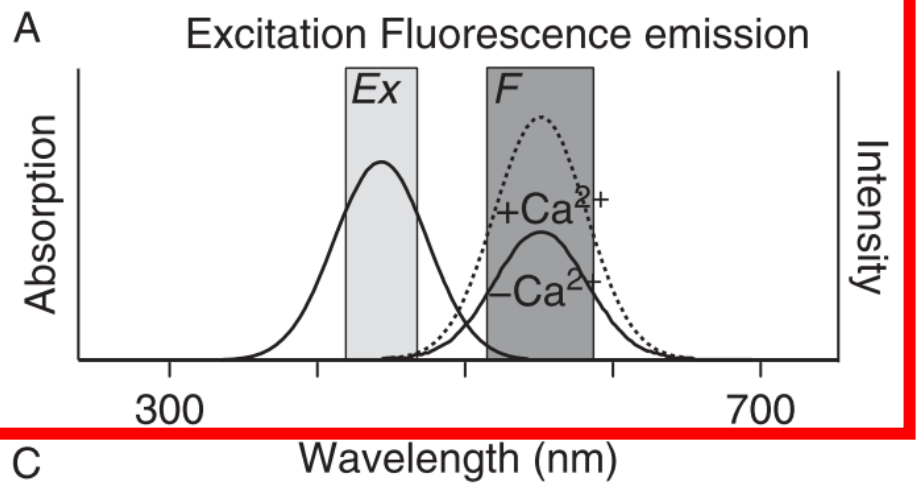


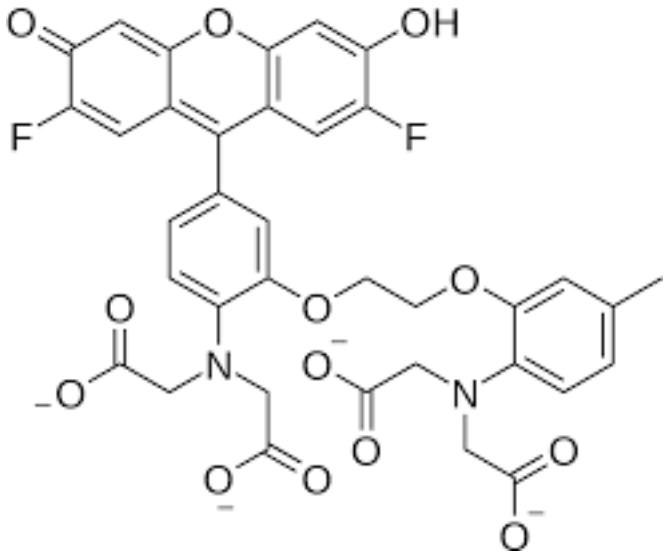


Fluorescence rationing: Ca^{2+} binding shifts either the emission (**e.g. Cameleon**)

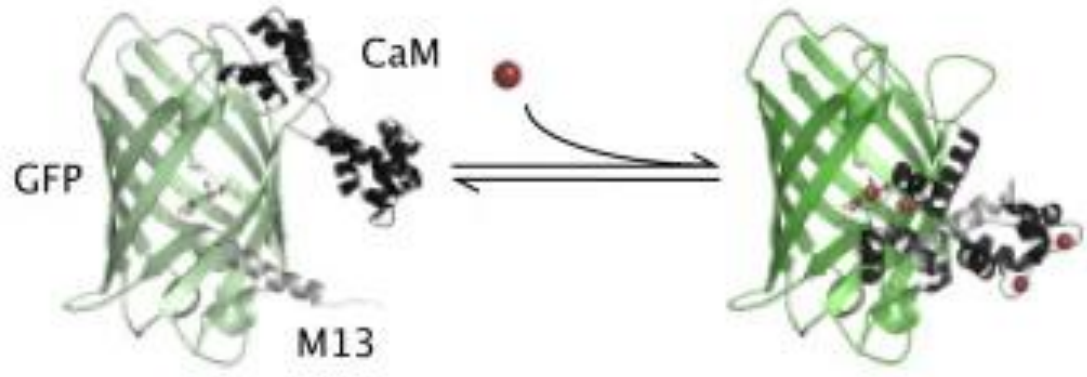
Ca^{2+}-dependent fluorescence changes

Intensity meusurements: Ca^{2+} **binding increases (or decreases, not shown) fluorescence (e.g. Fluo & GCaMP series)**





Fluos



GCaMPs

How does the fluorescence signal F relate to the concentration of the fluorofore X?

$$F = \quad = \quad = [X]$$

$I_{abs} = I_0 \ln(10) \varepsilon l [X]$ = absorbed light according to a linear approximation of the Beer-Lambert law (valid for [X] << 5 - 20 mM; ε = molar extinction coefficient of the fluorofore)

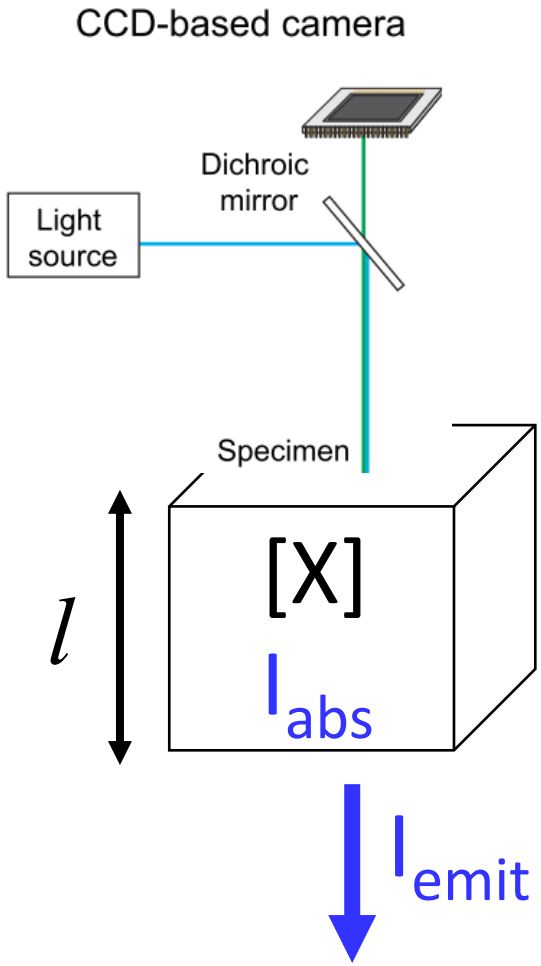
Q_F = quantum yield = $\frac{\text{photons emitted}}{\text{photons absorbed}}$

Fluorofore

Φ_D = fraction of emitted photons collected by the photodetection system

Q_D = detector's quantum efficiency

Setup

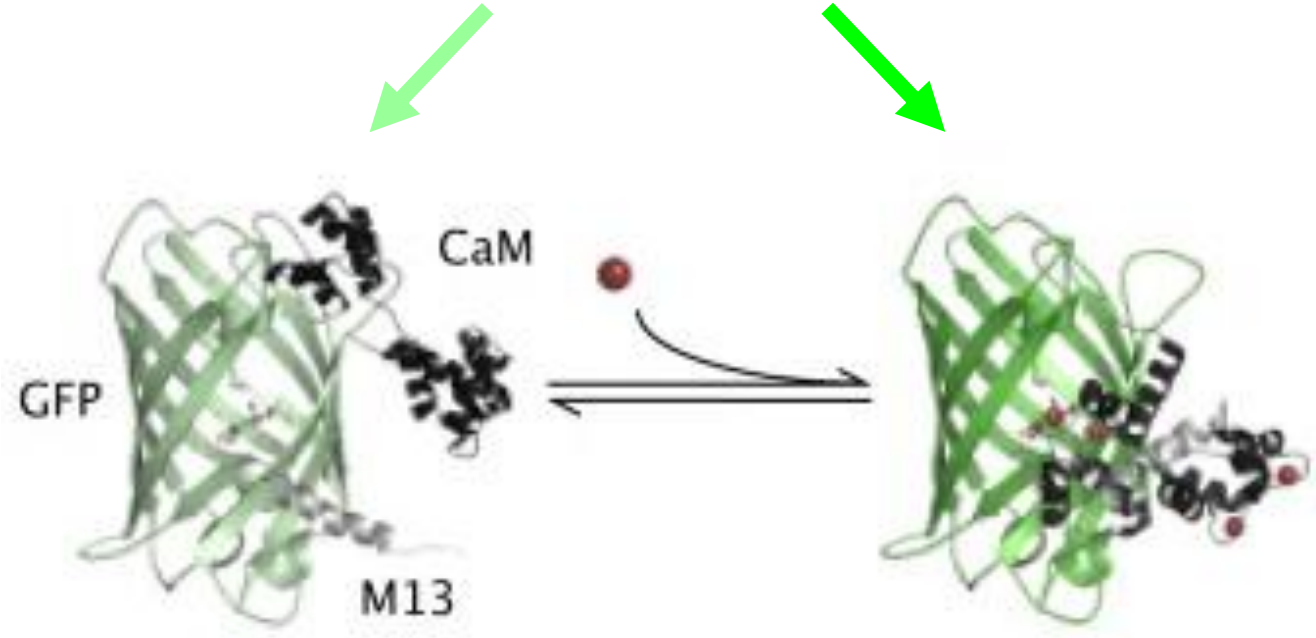


S = all fluorofore- and setup-specific factors merged in a single proportionality constant

How does the fluorescence signal F relate to [Ca^{2+}]?

For Ca^{2+} indicators we need to consider separately B and CaB because they have their own Q_F and I_{abs} and therefore they contribute to F with different factors S_f and S_b

$$F = S_f\ [\mathrm{B}] + S_b\ [\mathrm{CaB}]$$



How does the fluorescence signal F relate to [Ca^{2+}]?

? ? ? ?

$$F = S_f\,[\mathrm{B}] + S_b\,[\mathrm{CaB}]$$

$$= F_{min} + (S_b - S_f)\,[\mathrm{CaB}]$$

$$= F_{max} - (S_b - S_f)\,[\mathrm{B}]$$

$$[\mathrm{B}]_T = [\mathrm{B}] + [\mathrm{CaB}]$$

$$F_{min} = S_f[\mathrm{B}]_T$$

$$F_{max} = S_b[\mathrm{B}]_T$$

We need to calibrate the system

$$K_d = \frac{[\mathrm{Ca}]\,[\mathrm{B}]}{[\mathrm{CaB}]} \rightarrow [\mathrm{Ca}] = K_d \frac{[\mathrm{CaB}]}{[\mathrm{B}]}$$

Free!

- We do not know [B] at equilibrium but we can experimentally determine F_{min} at [Ca] = 0
- We do not know [CaB] at equilibrium but we can experimentally determine F_{max} at the end of the experiment with a maximal stimulation protocol (F_{max} assumes an increase in F upon Ca^{2+} binding)
- R_f = Dynamic range = $\frac{F_{max}}{F_{min}}$
- [Ca^{2+}] = free Ca^{2+} at equilibrium

Fluorescence ⟺ Free [Ca^{2+}]

How does the fluorescence signal F relate to $[Ca^{2+}]$?

$$[\mathrm{Ca}] = K_d \frac{F - F_{min}}{F_{max} - F}$$

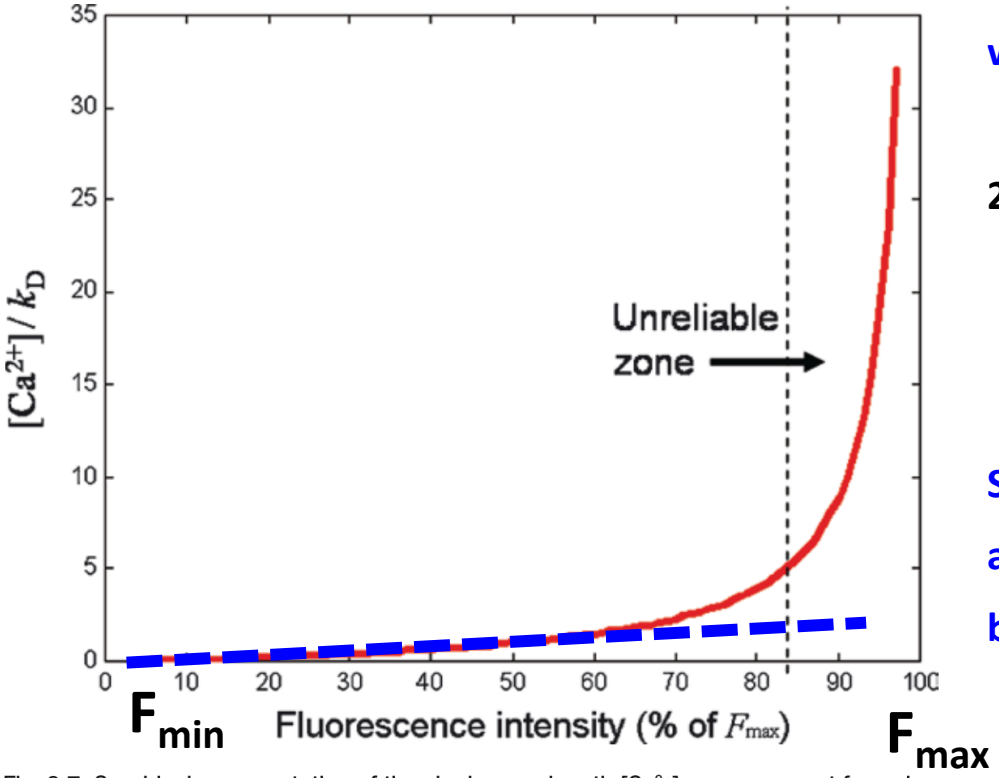


Fig. 3.7. Graphical representation of the single wavelength $[Ca^{2+}]$-measurement formula.

1) **Unreliable zone:** Even small fluctuations in the estimation of F may cause large fluctuations in the estimation of $[Ca^{2+}]$

Simple solution: we work far from saturation (and we use a linearization of the eq.)

2) The quantitative approach requires **optical path length (l)**, **dye concentration ($[B]_T$)** and **illumination intensity (I_0)** being uniform, which is the case for a cuvette <u>but not for a cell</u>

Solutions:

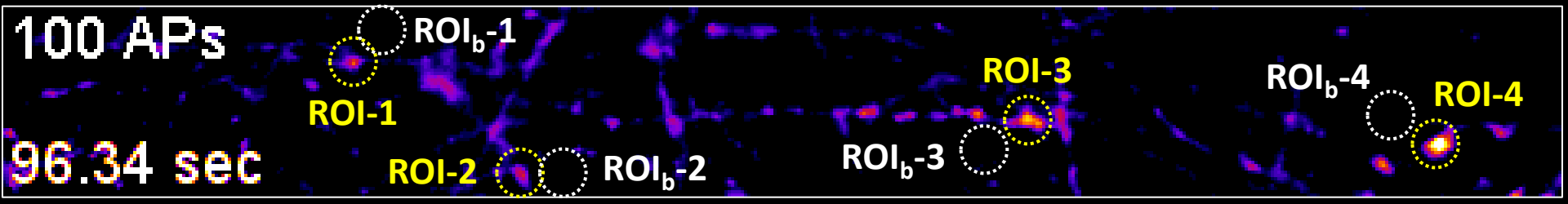
a. (We determine F_{min}, F_{max} and F for each pixel)

b. We limit ourselves to quantifying $\Delta[Ca^{2+}]$, rather than $[Ca^{2+}]$

Relative fluorescence change



Relative fluorescence change



For each ROI:

$F_{(i)} = F_{ROI(i)} - F_{ROIb(i)}$ (i) = frame number

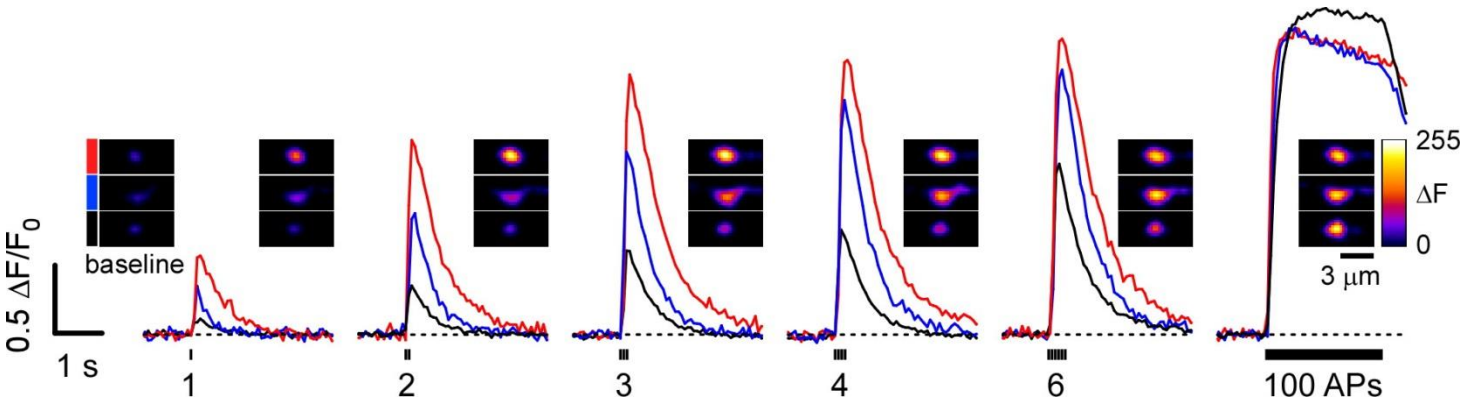
$$\Delta F/F_0 = \frac{F_i - F_0}{F_0}$$

(to correct for inhomogeneous illumination + rundown)

F_0 = F_i averaged over several frames before each stimulation

(to correct for rundown)

Relative fluorescence change



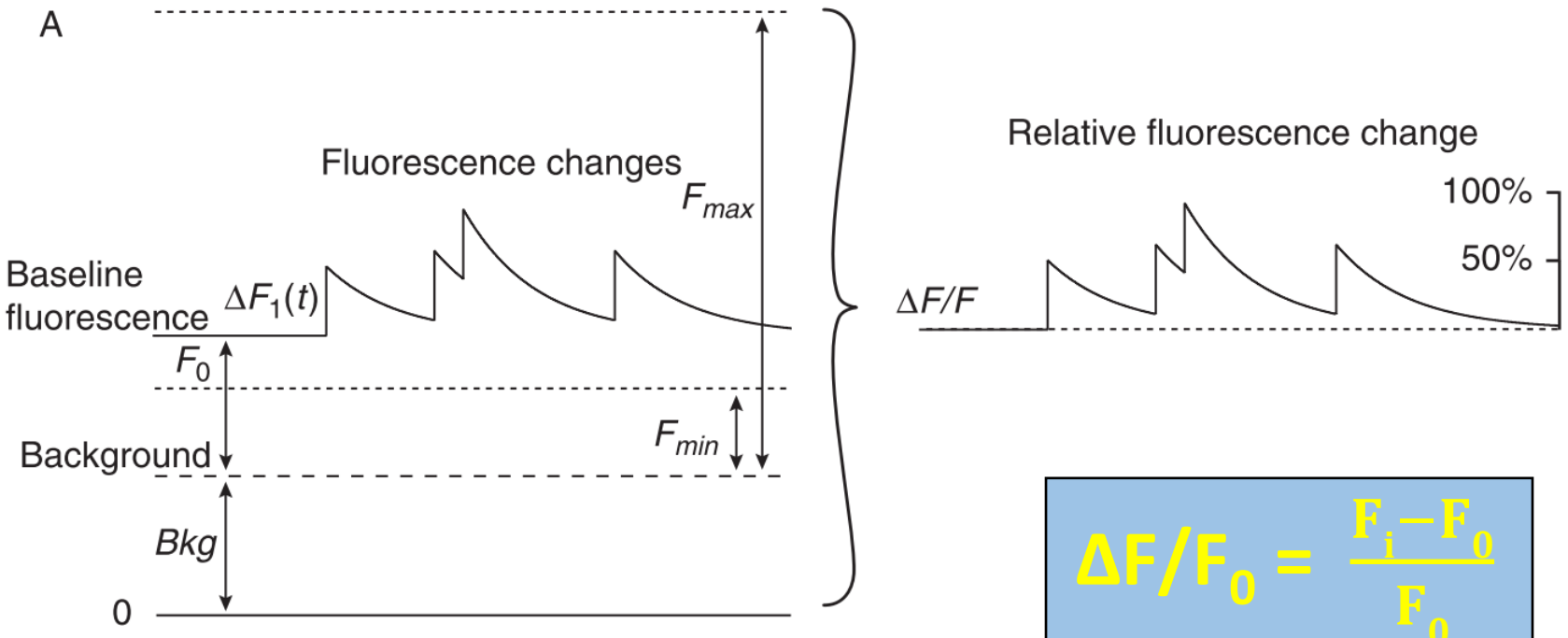
$Ca_V2.1$[EFa]

$Ca_V2.1$[EFb]

Control

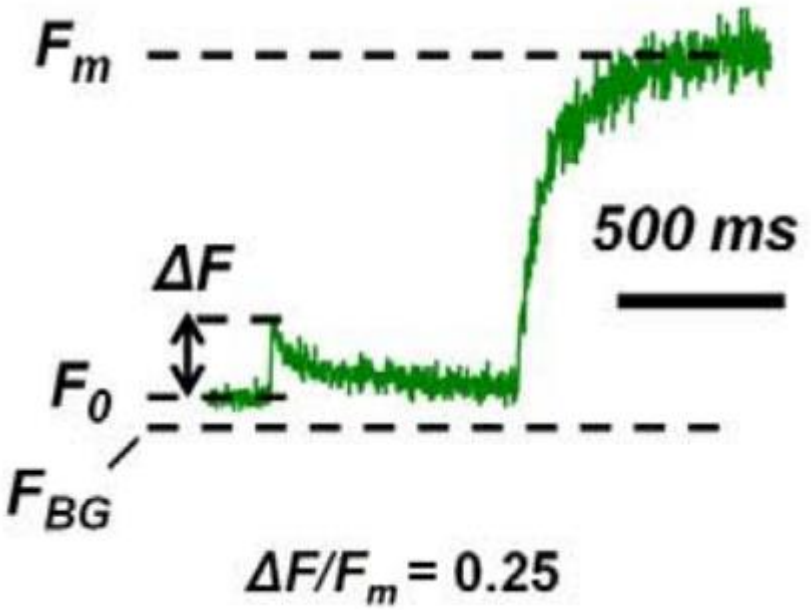
$$\Delta F/F_0 = \frac{F_i - F_0}{F_0}$$

Relative fluorescence change



$$\Delta F/F_0 = \frac{F_i - F_0}{F_0}$$

Relative fluorescence change



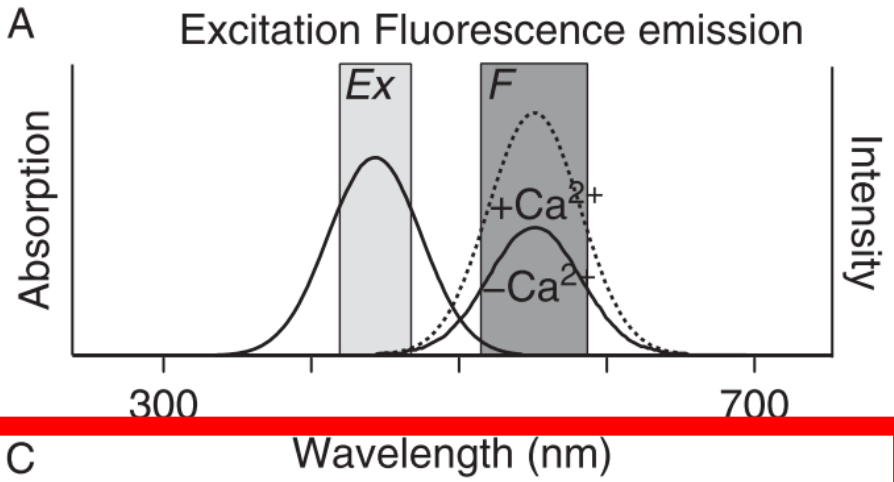
$$\Delta\,[\mathrm{Ca}] = \frac{K_d}{(\Delta F/F)_{max}}(\Delta F/F) \quad (\Delta F/F \ll (\Delta F/F)_{max})$$

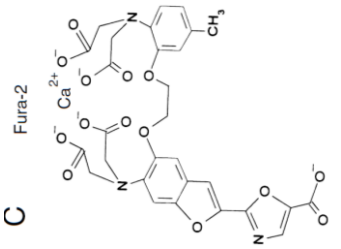
This equation provides a quantitative estimate of the change in [Ca^{2+}] but not of its absolute value

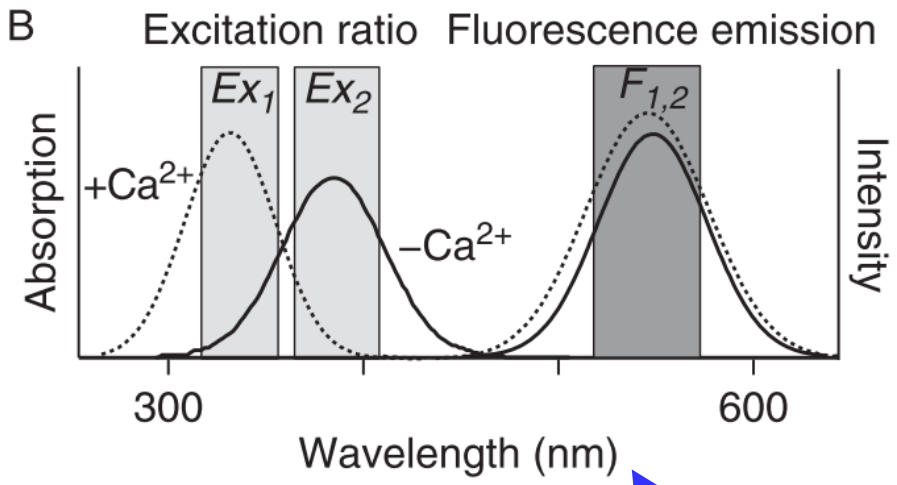
In most papers investigating Ca^{2+} in neurons (where transients are fast), you will find this approach, either Δ[Ca^{2+}] (e.g. with Fluos, which present independent binding) or simply as $\Delta F/F_0$ (e.g. with GCaMPs, which present cooperative binding)

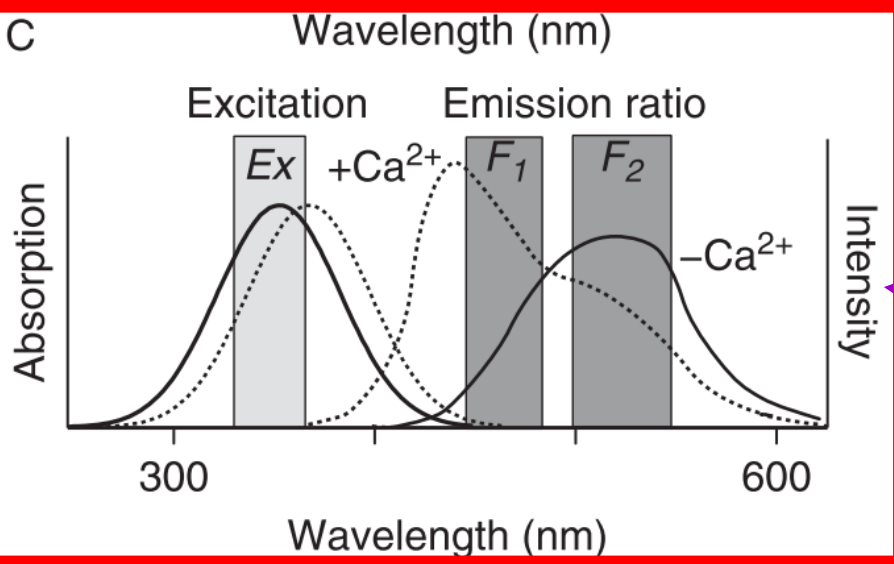
Ca^{2+}-dependent fluorescence changes

Intensity meusurements: Ca^{2+} binding increases (or decreases, not shown) fluorescence (**e.g. Fluo & GCaMP series**)



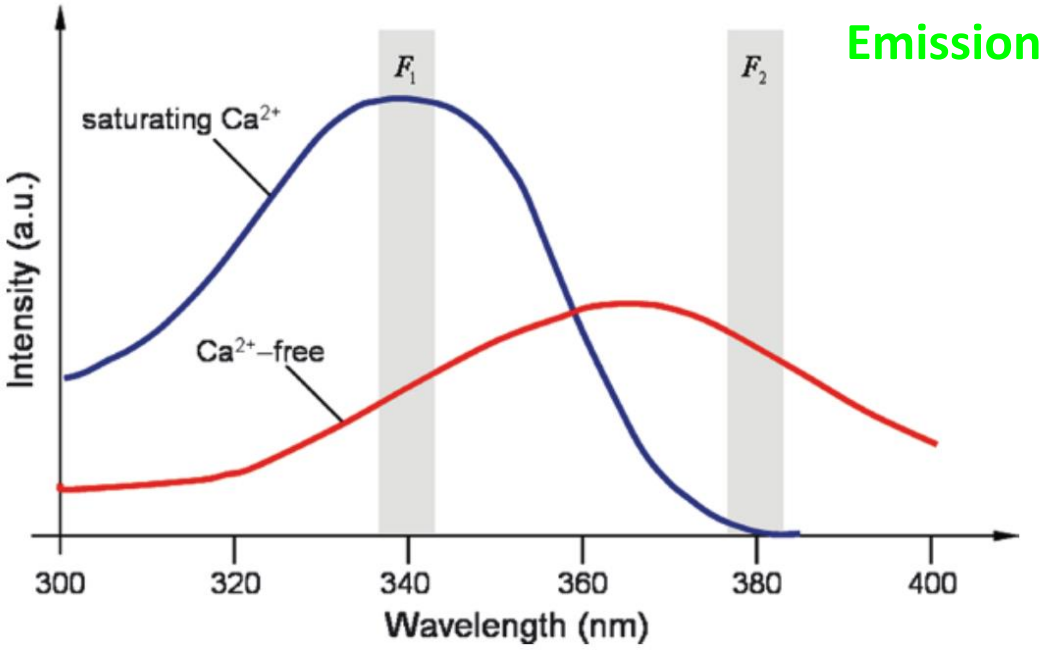


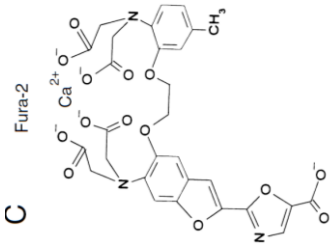




Fluorescence rationing: Ca^{2+} binding shifts either the emission (**e.g. Cameleon**) or absorption spectrum (**e.g. Fura-2**) or the

Fluorescence ratio: Fura-2 (independent binding)





Emission constant at 505 nm

$$K_d = \frac{k_{off}}{k_{on}} = \frac{[\mathrm{Ca}]\,[\mathrm{B}]}{[\mathrm{CaB}]}$$

$$[\mathrm{Ca}] = K_{eff}\,\frac{R - R_{min}}{R_{max} - R}$$

$$F = S_f\,[\mathrm{B}] + S_b\,[\mathrm{CaB}]$$

$$F_1 = S_{f1}\,[\mathrm{B}] + S_{b1}\,[\mathrm{CaB}]$$

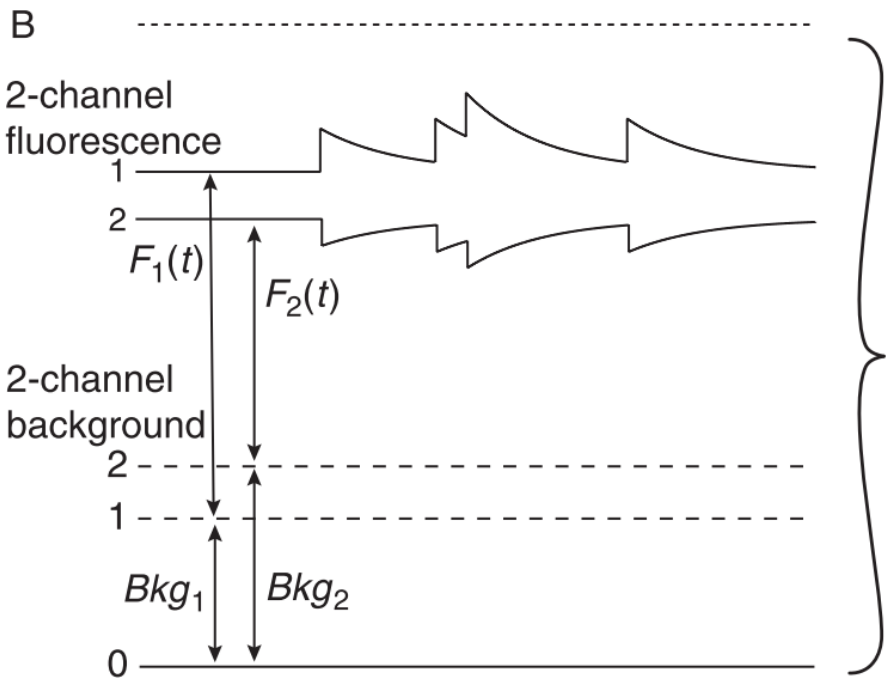
$$F_2 = S_{f2}\,[\mathrm{B}] + S_{b2}\,[\mathrm{CaB}]$$

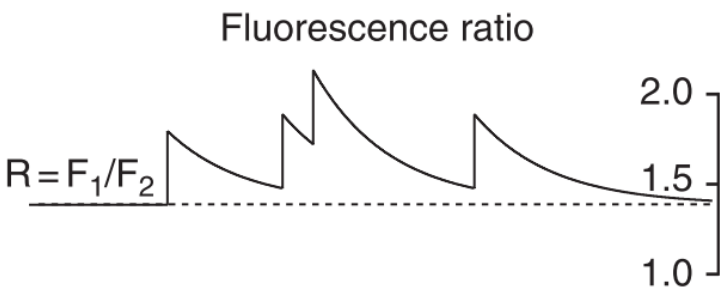
$R = \frac{F_1}{F_2}$ $R_{min} = \frac{S_{f1}}{S_{f2}}$ $R_{max} = \frac{S_{b1}}{S_{b2}}$ $K_{eff} = K_d \frac{S_{f2}}{S_{b2}}$

R_{max} and R_{min} are ratios under conditions of saturating Ca^{2+} levels and in the absence of Ca^{2+}, respectively

Calibration is specific to the experimental setup

Fluorescence ratio: Fura-2





$$[\mathrm{Ca}] = K_{eff}\frac{R - R_{min}}{R_{max} - R}$$

Advantage: A ratiometric approach normalizes for inhomogeneities in dye concentration, optical path length and illumination intensities → it can be used to estimate absolute [Ca^{2+}]

Drawbacks: The excitation at two wavelength makes it slow

In most papers looking at Ca^{2+} in astrocytes (where transients are slow), you will find this approach with Fura-2

Conclusions

1. **Theoretically, we can always estimate $[Ca^{2+}]_{free}$ from F**

2. **Practically/generally:**
   a. **For fast Ca^{2+} signals, we use:**
      - **intensity measurements with Fluo dyes (independent binding) to estimate $\Delta[Ca^{2+}]_{free}$ from $\Delta F/F_0$**
      - **intensity measurements with GCaMP dyes (cooperative binding) to estimate $\Delta F/F_0$**

   b. **For slow Ca^{2+} signals, we can use ratiometric measurements with Fura-2 (independent binding) to estimate $[Ca^{2+}]_{free}$**