The CalciOMatic package: a new tool for quantitative calcium imaging analysis

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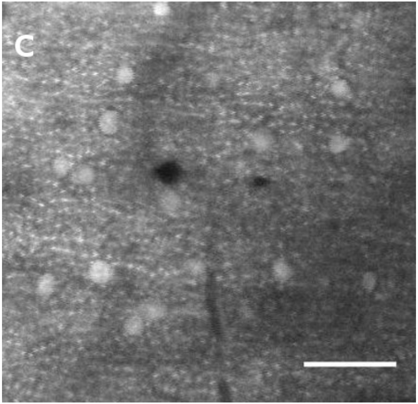
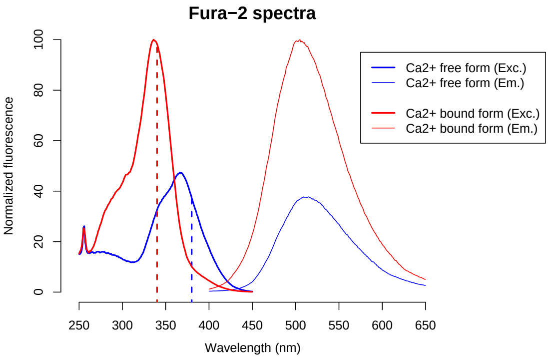
Calcium imaging: following neuronal activity



scale bar: 40 μm

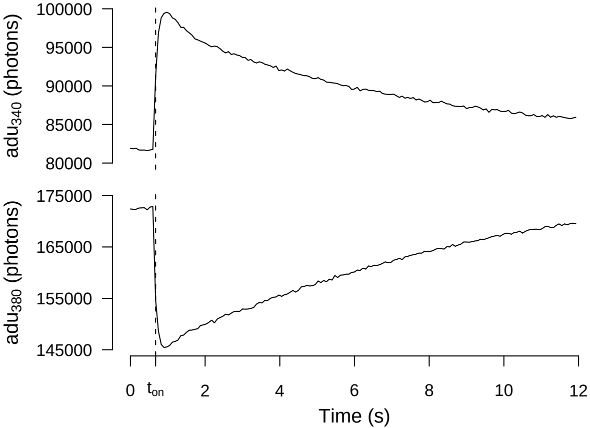
with courtesy of R. Franconville

Fura-2, a ratiometric calcium indicator



source: `www.invitrogen.com`

Experimental protocol



Fluorescence transient evoked in an olfactory neuron
of the cockroach *Periplaneta Americana*

Expression of the fluorescence intensity

$$
\begin{aligned}
\mathcal{F}_{340} &= \left(\frac{\phi \cdot [B_T]}{K_d + [Ca^{2+}]}(R_{min} \cdot K_{eff} + R_{max} \cdot [Ca^{2+}]) + s_{B,340}\right) \cdot T_{e,340} \cdot P, \\
\mathcal{F}_{380} &= \left(\frac{\phi \cdot [B_T]}{K_d + [Ca^{2+}]}(K_{eff} + [Ca^{2+}]) + s_{B,380}\right) \cdot T_{e,380} \cdot P.
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Calibration parameters

R_{min}, R_{max}, K_{eff} and K_d

are calibrated using a dedicated set of experiments

Getting the intracellular calcium concentration ?

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$$R = \frac{\mathcal{F}_{340} - \mathcal{F}_{B,340}}{\mathcal{F}_{380} - \mathcal{F}_{B,380}} \cdot \frac{T_{380}}{T_{340}}$$

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\gg [Ca^{2+}] = K_{eff} \cdot \frac{R - R_{min}}{R_{max} - R}
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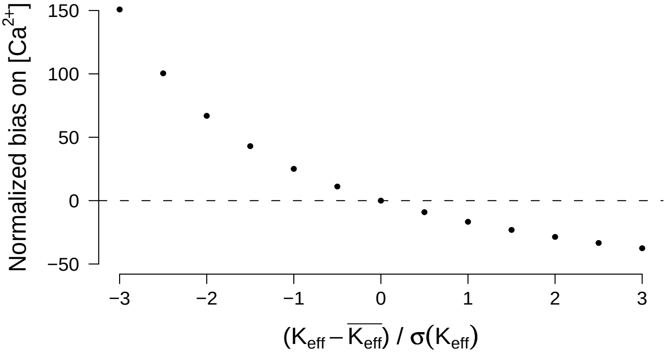
Bias on the absolute calcium concentration



Embedding a calcium model into the fluorescence model

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Calcium model

$$[Ca^{2+}](t) = Ca_0 + \Delta Ca \cdot exp\big(-(t - t_{on})/\tau\big)$$

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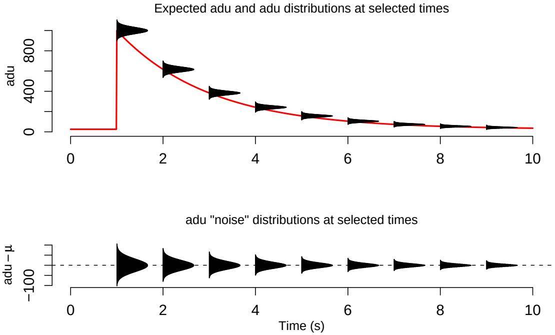
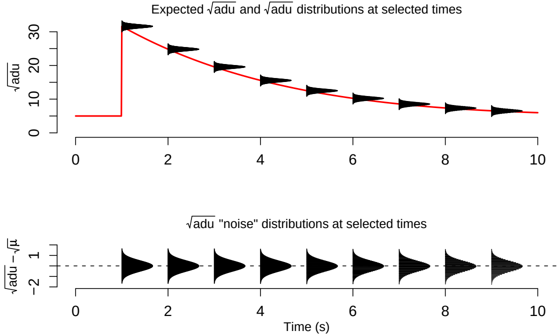
A CCD camera model

The fluorescence signals acquisition, with a CCD camera, induces a Poisson noise. At high photon counts, the Poisson distribution can be well approximated by a Gaussian with variance equal to the mean.



The square root transformation

Taking the square root of the fluorescence signals stabilizes the noise variance, which becomes equal to $1/4$ independently of the mean.



Fitting simultaneously both fluorescence transients

$$\texttt{nls}\Big(c\left(\sqrt{adu_{B,340}}, \sqrt{adu_{340}}, \sqrt{adu_{B,380}}, \sqrt{adu_{380}}\right) \\ \sim c\left(\sqrt{\mathcal{F}_{B,340}}, \sqrt{\mathcal{F}_{340}}, \sqrt{\mathcal{F}_{B,380}}, \sqrt{\mathcal{F}_{380}}\right)\Big)$$

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Estimated parameters

$$Ca_0, \Delta Ca, \tau, \phi, s_{B,340}, s_{B,380}$$

Fitting simultaneously both fluorescence transients and actual values of the calibration parameters

$$
\begin{aligned}
\texttt{nls}(c & \left(\sqrt{adu_{B,340}}, \sqrt{adu_{340}}, \sqrt{adu_{B,380}}, \sqrt{adu_{380}}, R_{min}, R_{max}, K_{eff}, K_d\right) \\
& \sim c\left(\sqrt{\mathcal{F}_{B,340}}, \sqrt{\mathcal{F}_{340}}, \sqrt{\mathcal{F}_{B,380}}, \sqrt{\mathcal{F}_{380}}, \overline{R_{min}}, \overline{R_{max}}, \overline{K_{eff}}, \overline{K_d}\right), \\
& weights = c\left(4, 4, 4, 4, \frac{1}{\sigma^2_{Rmin}}, \frac{1}{\sigma^2_{Rmax}}, \frac{1}{\sigma^2_{Keff}}, \frac{1}{\sigma^2_{Kd}}\right))
\end{aligned}
$$

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$$\sim c\left(\sqrt{\mathcal{F}_{B,340}}, \sqrt{\mathcal{F}_{340}}, \sqrt{\mathcal{F}_{B,380}}, \sqrt{\mathcal{F}_{380}}, \overline{R_{min}}, \overline{R_{max}}, \overline{K_{eff}}, \overline{K_d}\right),$$

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Estimated parameters

$$Ca_0, \Delta Ca, \tau, \phi, s_{B,340}, s_{B,380}, R_{min}, R_{max}, K_{eff}, K_d$$

Monte-Carlo simulations

Simulate data

1. Choose values for $[Ca^{2+}]$ and experiment-specific parameters

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2. Draw calibrated parameters from Gaussian distributions

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3. Create ideal background and transient fluorescence signals

Monte-Carlo simulations

Simulate data

1. Choose values for $[Ca^{2+}]$ and experiment-specific parameters
2. Draw calibrated parameters from Gaussian distributions
3. Create ideal background and transient fluorescence signals
4. Simulate noisy signals according to the Poisson distribution

Monte-Carlo simulations

Simulate data

Fit data

Ratiometric approach Compute an equivalent $[Ca^{2+}]$ transient and fit a monoexponential model

Monte-Carlo simulations

Simulate data

Fit data

Ratiometric approach Compute an equivalent $[Ca^{2+}]$ transient and fit a monoexponential model

Direct approach Fit the whole fluorescence model on the square-rooted signals and the calibrated parameters

Monte-Carlo simulations

Simulate data / Fit data

Test the reliability of the confidence intervals - Procedure

1. Test if the true value of each parameter is within the 95% confidence interval returned by `nls` (`TRUE` / `FALSE`)

Monte-Carlo simulations

Simulate data / Fit data

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Monte-Carlo simulations

Simulate data / Fit data

Test the reliability of the confidence intervals - Procedure

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2. Repeat the Simulation and Fitting steps 1000 times
3. Count the number of `TRUE`

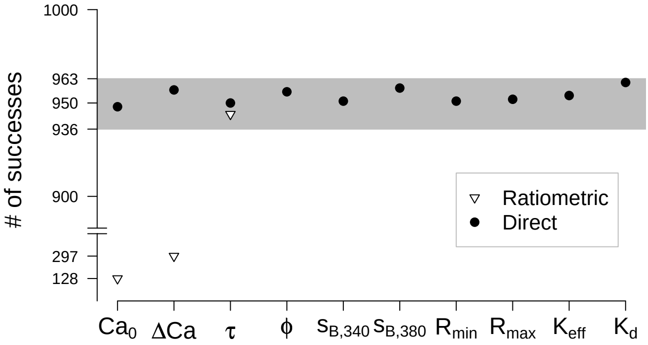
Monte-Carlo simulations

Simulate data / Fit data

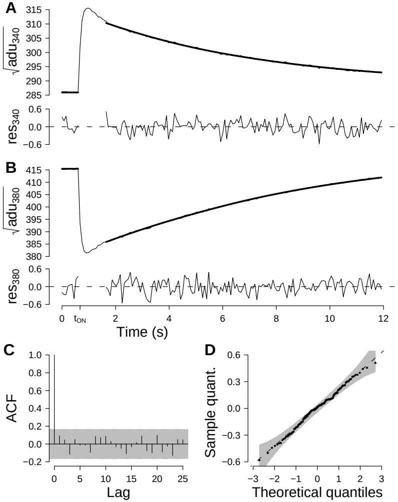
Test the reliability of the confidence intervals - Procedure

1. Test if the true value of each parameter is within the 95% confidence interval returned by `nls` (`TRUE` / `FALSE`)
2. Repeat the Simulation and Fitting steps 1000 times
3. Count the number of `TRUE`
4. Compare these values with the 2.5% and 97.5% quantiles of the Binomial distribution with probability $p = 0.95$.

Monte-Carlo simulations



Fitting cockroach's data



Summary

- Data generation model including a probabilistic model of the CCD camera
- The "ratiometric" transformation gives wrong confidence intervals
- The "direct" approach, combined with the square root transformation, gives meaningful confidence intervals
- We can take into account the uncertainty of calibration measurements
- The latter feature has been shown to improve the fits of physiological data

- The "direct" method is available from the `CRAN` website
  $\gg$ look for CalciOMatic