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

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Measuring variations of intracellular free calcium concentration through the fluorescence changes of a calcium sensitive dye is an ubiquitous technique in Neuroscience. Despite its popularity confidence intervals on the estimated parameters of calcium dynamics models are never given. To address this issue, we have developed a model for ratiometric measurements obtained with a CCD camera.

We built a 2-stage model whose first element links the fluorescence intensity to the calcium dynamics and whose second element describes fluorescence measurements through a photon counting process. At each time sample and for both wavelengths, the photon count read out by the camera can be described as a realization of a Poisson random variable. In experimental situations encountered in practice, this distribution can be approximated by a Gaussian distribution with variance equal to the mean.

Using Monte-Carlo simulations, we first show that using the classical *ratiometric* transformation to fit calcium signals does not yield reliable confidence intervals on the fitted calcium dynamics parameters. This is due to the heteroscedasticity of the signal. We then introduce a *direct* approach, based on the *square-root* transformation of the original fluorescence signals. This transformation stabilizes the signal variance and leads us back to a standard nonlinear regression setting. Our *direct* approach has many advantages over the *ratiometric* approach:

1. The construction of confidence intervals is reliable, for both the calcium dynamics parameters and the experiment-specific ones (such as the background fluorescence at each wavelength).

2. Using approaches inspired by constrained linear regression, we can take into account the finite precision on calibrated parameters (such as the dye dissociation constant in the cell).

3. It is also possible to estimate the variations of the dye concentration during the experiment.

All these features will be illustrated on simulated data using the Monte-Carlo approach. Moreover, we show on experimental data that using the last two features leads to major improvements in the goodness of fit. These improvements are characterized with classical diagnostic plots of the `nls` function. Finally, the *direct* method allows us to formally decide between several nested models of the calcium decays.

The *direct* method was implemented in `R`, all pieces of codes being gathered in the `CalciOMatic` package, which will be submitted to `CRAN`. This package includes easy-to-use functions to simulate data, fit either simulated or experimental data, and plot results as well as diagnostic plots.

References

Joucla S, Pippow A, Kloppenburg P and Pouzat C. *Getting more out of ratiometric calcium measurements with an explicit data generation model.* Program No. 497.18. 2008 Neuroscience Meeting Planner. Washington, DC: Society for Neuroscience, 2008. Online.