Inferring the calcium current from fluorescent signals.

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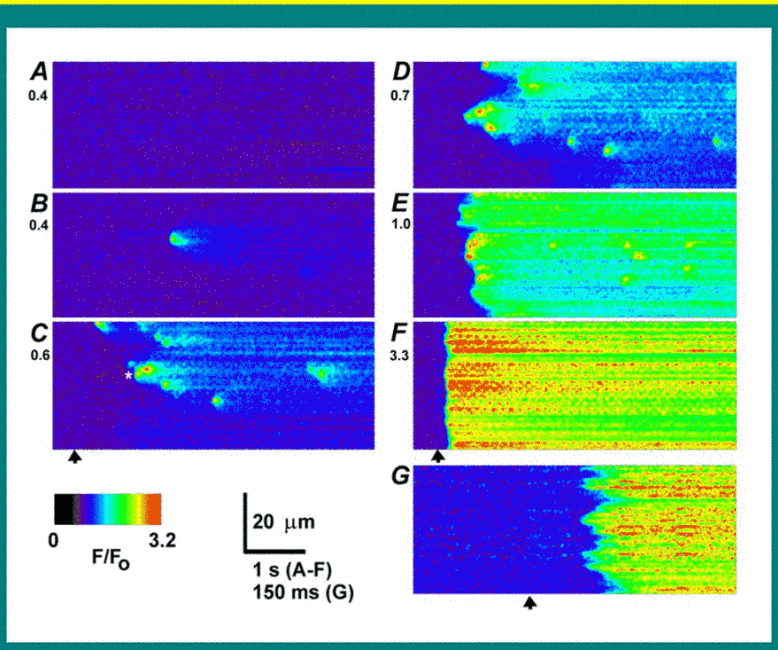
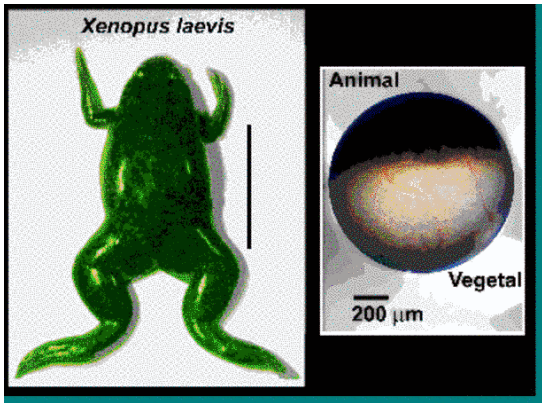
Optical methods provide a relatively non-invasive means by which calcium signals can be studied.

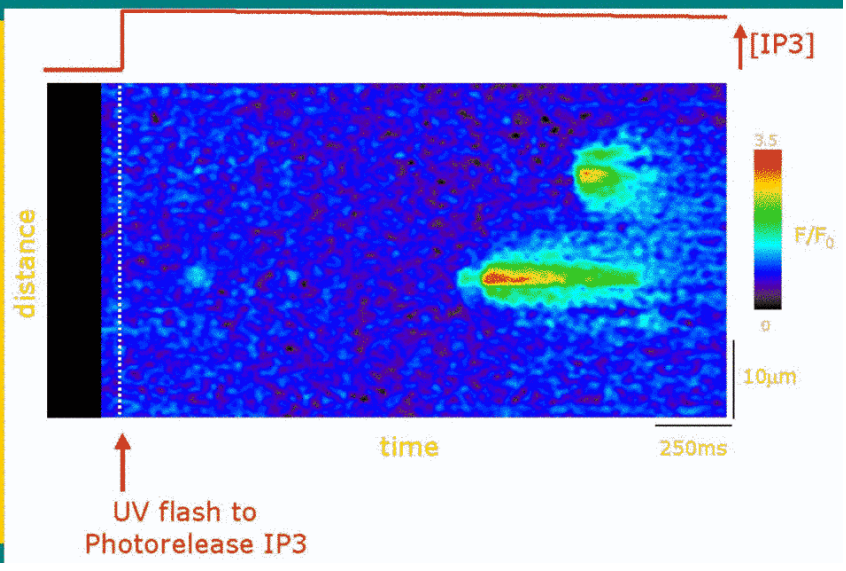


This is the approach followed by I. Parker (UC-Irvine) who studies calcium signals in the oocyte of *Xenopus Laevis*.



These experiments are done using the *Xenopus* laevis oocyte which the type-1 IP_3R.

Calcium signals are studied by photoreleasing caged IP_3, using different fluorescent indicators. (Experiments by I. Parker and co-workers)



One of the problems associated to optical techniques is the difficulty in being able to infer the value of the calcium current that underlies each signal.

Calcium does not diffuse freely in the cytosol. (A)

Calcium diffusion is affected by the presence of buffers.

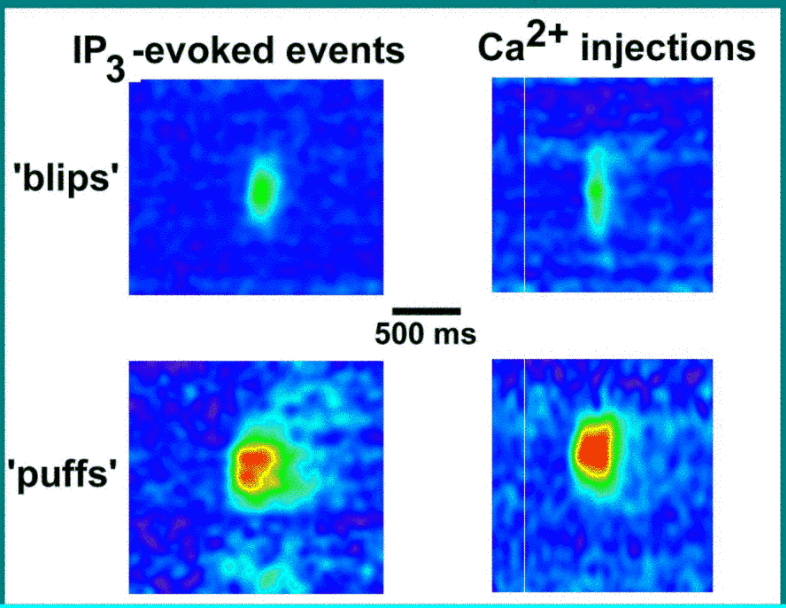
In particular, the fluorescent indicator is a calcium buffer.

There are other mechanisms by which Ca^{2+} is removed which also affect its spatio-temporal dynamics.

It is not easy to have quantitative information of all the processes that affect the Ca^{2+} spatio-temporal dynamics during a given experiment.

Thus, it is hard to determine the amplitude and temporal behavior of the Ca^{2+} current in each experiment and different methods have been tried to determine it.

How to infer the underlying calcium current experimentally (1):
"Simulated calcium puffs"

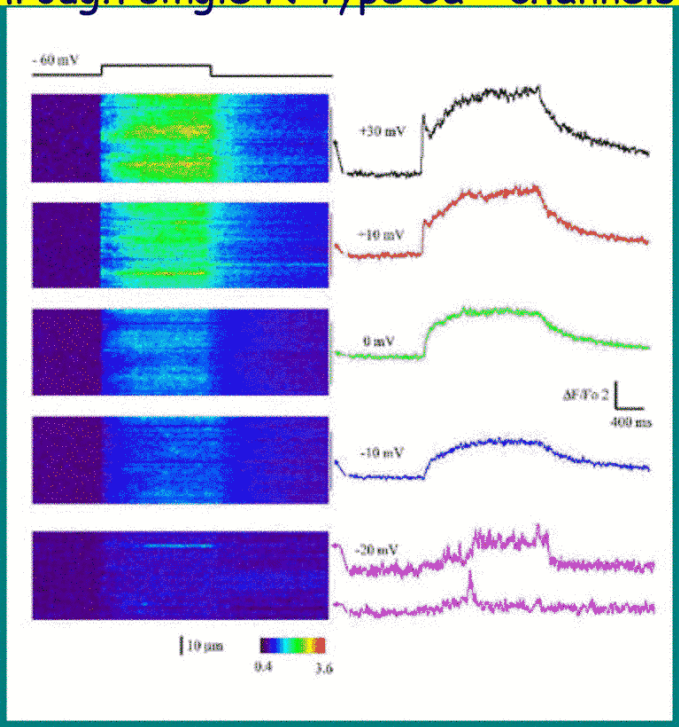


Experiments by I. Parker

Calcium is injected into the oocyte with a micropipette. However, the calcium current is still unknown.

How to infer the underlying calcium current experimentally (2): "Imaging calcium flux through single N-type Ca^{2+} channels"

Experiment by I. Parker using 10 mM extracellular Ca^{2+} and Fluo-4



These experiments can be used for calibration purposes, since the current through one of these channels is known.

Still, it is of interest to obtain a reliable method that gives the calcium current that underlies an image.

Some methods have been published to analyze signals from calcium sparks, *e.g.*:

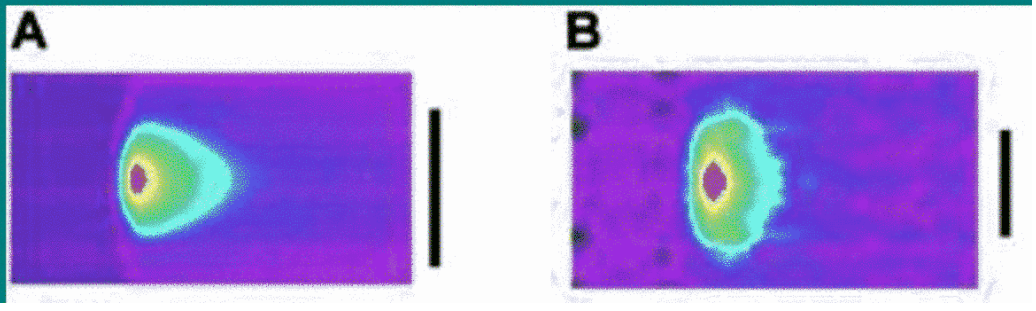
E. Ríos and G. Brum, Frontiers in Biosci. **7**, d1195 (2002)

C. Soeller and M.B. Cannell, Biophys. J. **82**, 3962 (2002)

Besides these methods, we can also cite a variety of studies in which spark models are simulated numerically and the images obtained in this way are compared to those obtained from real experiments, *e.g.*:

G.D. Smith et al, Biophys. J. **75**, 15 (1998)

More quantitative comparisons



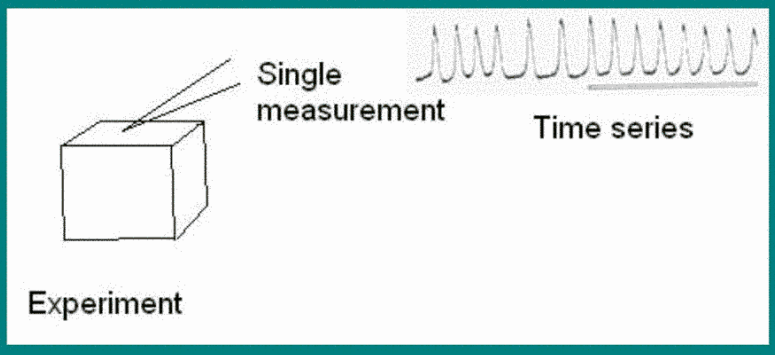
The methods of Soeller and Cannel and of Rios and Brum involve having a complete model of the calcium dynamics in the cytosol (calcium diffusion, interaction with buffers, pumps, etc).

All the information that is needed in order to develop such a model is not available in the case of the oocyte . On the other hand, we would like to have a method that could rely on as few assumptions as possible (a model independent method).

We then decided to follow a more "abstract" approach, based on ideas of dynamical systems theory.

This "more abstract" approach allowed us to develop a "model-independent" method to infer the underlying calcium current from a fluorescent image which relies on a minimal number of assumptions.

In the 80's the development of new tools for time series analyses started to increase (in conjunction with the increasing interest in chaotic systems, etc).



Given a scalar time series it was proved that a multi-dimensional phase space could be constructed where the dynamics was "equivalent" to the dynamics of the actual system that generated the time series.

Takens embedding theorem

Packard et al, PRL 45, 712 (1980)

Sauer et al, J. Stat. Phys. 65, 579 (1991)

At the beginning, most applications of these ideas focused on trying to determine statistical or topological properties of the reconstructed attractor. Regarding modeling, a model would be discarded if it was unable to reproduce these properties.

Later on, other types of strategies were developed:

- Reconstruction of vector fields (Gouesbet, PRA **46**, 1784 (1992) , Mindlin et al Europhys. Lett **42**, 31 (1998))
- Prediction (Farmer and co-workers)

The basic idea is then: if we have a long enough time series that is generated by an autonomous dynamical system then it is possible to "predict", at any given time, what is going to happen next. Moreover, it is possible to construct a dynamical system, one of whose variables will behave like the time series.

In particular, G. Mindlin and co-workers were able to reproduce various experimental time-series, u(t), using a dynamical system written in a standard form:

$$\frac{du}{dt} = v; \quad \frac{dv}{dt} = w; \quad \ldots$$

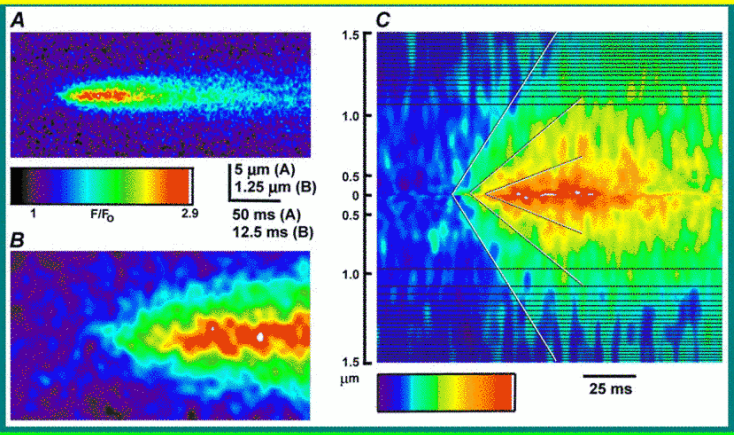
$$\frac{dw}{dt} = f(u, v, w)$$

where an expression for $f(u,v,w)$ can be obtained with a fitting procedure.

Using u(t) and its derivatives (equivalently, knowing u at the present time and at previous times) we can construct a dynamical system that reproduces the observed time series (that predicts what is going to happen next).

All the relevant information is contained in the time series!

What information do we have in the experiments that use fluorescent indicators?



The image occurs because calcium binds to the indicator.

$$Ca^{2+} + F \underset{k_+, k_-}{\longleftrightarrow} CaF$$

F: fluo4

The fluorescence, f, and [CaF] are related by:

$$[CaF](r,t) = \frac{f(r,t) - f_{\min}}{f_{\max} - f_{\min}} [F]_T$$

$[F]_T$ is known f_{min} and f_{max} can be measured.

Thus, the image gives information on the spatio-temporal distribution of [CaF].

It is a linescan. However, assuming spherical symmetry, we can obtain [CaF](r,t) from the image.

Defining the total concentration of indicator, $[F]_T=[F]+[CaF]$, and assuming that F and CaF diffuse at equal rates and that they are initially distributed more or less homogeneously in space, the evolution equation for [CaF] reads:

$$\frac{\partial [CaF]}{\partial t} = D_F \nabla^2 [CaF] + k_+ [Ca^{2+}]([F]_T - [CaF]) - k_- [CaF]$$

Since we know [CaF](r,t), we know its time and space derivatives. Therefore, we can determine $[Ca^{2+}](r,t)$ from the image using the evolution equation for [CaF].

We have a spatio-temporal series of the calcium concentration!

The spatio-temporal series of $[Ca^{2+}]$ is "generated" by the dynamical system that rules the dynamics of the calcium concentration in the oocyte.

Many factors affect this dynamics: buffers, pumps, etc, and the source of calcium ions (channels in "real" puffs, the current through the pipette in the simulated ones):

$$\frac{\partial[Ca^{2+}]}{\partial t} = D_{Ca}\nabla^2[Ca^{2+}] - k_+[Ca^{2+}]([F]_T - [CaF]) + k_-[CaF]$$
$$- k_{E+}[Ca^{2+}]([E]_T - [CaE]) + k_{E-}[CaE] + \ldots + source$$

here E stands for EGTA which is used in the experiments.

However, given the experience with time series, we expect to be able to construct a dynamical system based solely on $[Ca^{2+}]$ and its space and time derivatives. In fact, we assumed that:

$$\ldots = T([Ca^{2+}], \frac{\partial[Ca^{2+}]}{\partial t}, (\nabla[Ca^{2+}])^2, \nabla^2[Ca^{2+}])$$

Thus, we assume that the evolution equation for $[Ca^{2+}]$ can be written as:

$$\frac{\partial[Ca^{2+}]}{\partial t} = D_{Ca}\nabla^2[Ca^{2+}] - k_+[Ca^{2+}]([F]_T - [CaF]) + k_-[CaF]$$
$$- k_{E+}[Ca^{2+}]([E]_T - [CaE]) + k_{E-}[CaE]$$
$$+ T([Ca^{2+}], \frac{\partial[Ca^{2+}]}{\partial t}, (\nabla[Ca^{2+}])^2, \nabla^2[Ca^{2+}])$$
$$+ source$$

where there is the implicit assumption that T is the same function of $[Ca^{2+}]$ and its derivatives everywhere in space and for any given time.

Now, the source of calcium ions is only different from zero in a limited region of space: the region with open channels.

Thus, we can determine T by a fitting procedure applied to those points where there are no calcium sources.

Once we have T, then we can determine the source from:

$$source = \frac{\partial [Ca^{2+}]}{\partial t} - (D_{Ca}\nabla^2[Ca^{2+}] - k_+[Ca^{2+}]([F]_T - [CaF]) + k_-[CaF] - k_{E+}[Ca^{2+}]([E]_T - [CaE]) + k_{E-}[CaE] + T)$$

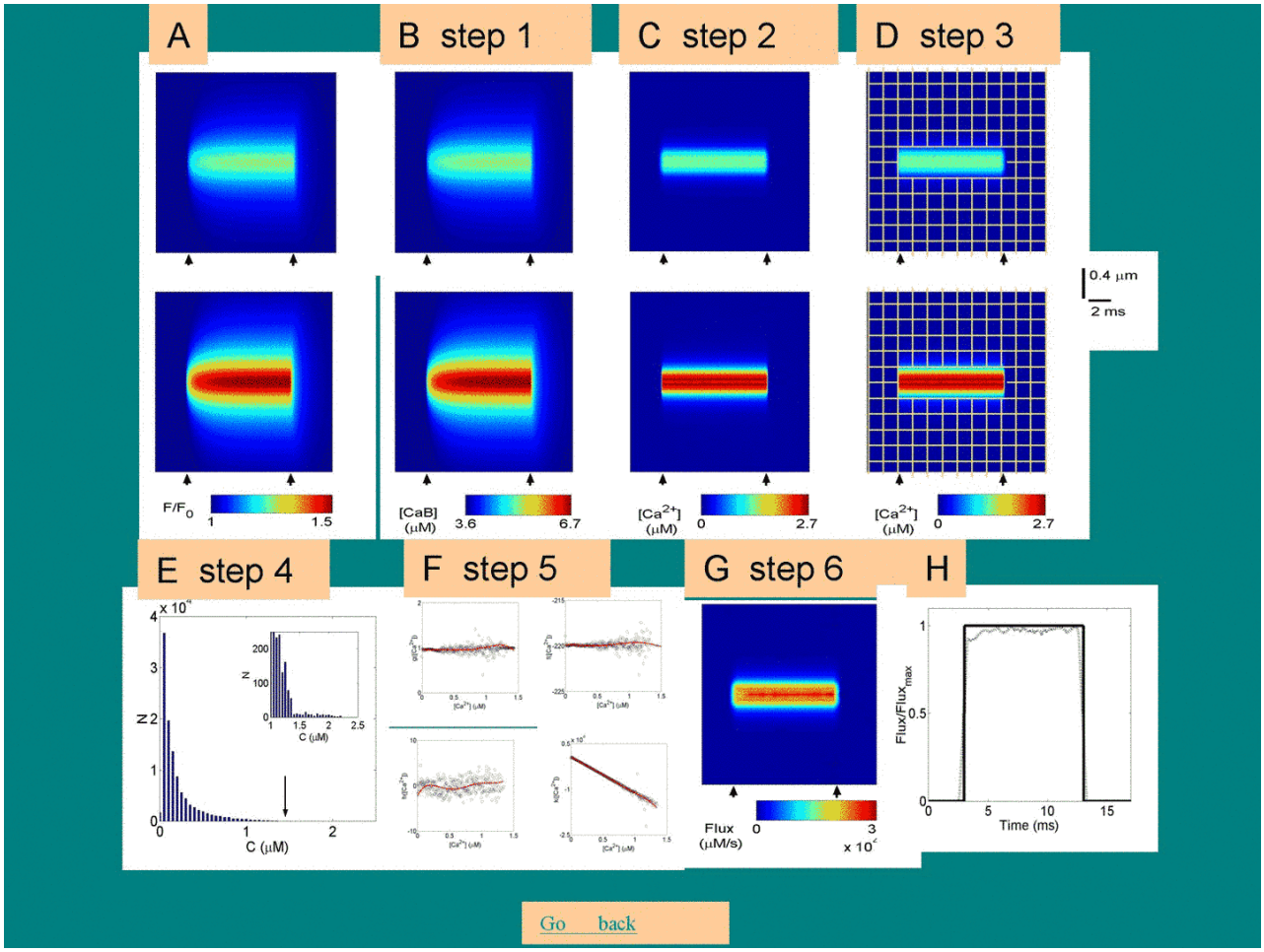
applying it to the region where there are sources (fitting).

We tried this procedure both with numerically simulated data (where we can check for sure whether it works or not) and with experimental data, and it seems to work pretty well.

Let us look at some results…

$$T = g([Ca^{2+}])\frac{\partial [Ca^{2+}]}{\partial t} + f([Ca^{2+}])(\nabla[Ca^{2+}])^2 + h([Ca^{2+}])\nabla^2[Ca^{2+}] + k([Ca^{2+}])$$

We fit the functions g, f, h and k in the region where there is no source. This requires the use of a set of experiments done on the same cell (or at least, cell type) so that we can obtain good estimates of these functions in regions with relatively large calcium concentrations (go back).

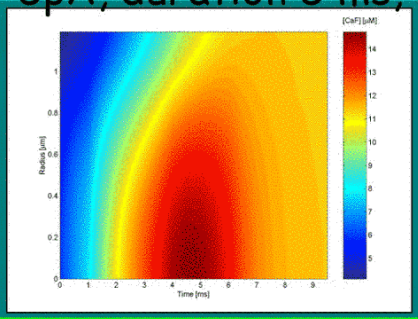


Numerical Simulations.

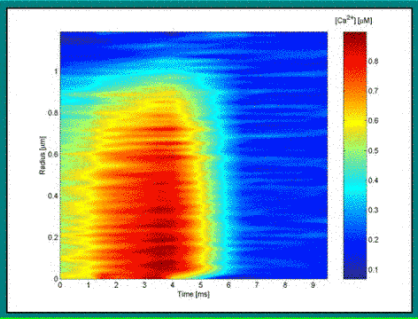
Starting from uniform initial conditions with $[Ca^{2+}]$= 50nM. A 5% white noise was added to the results.

Example 1: Radial constant source in the presence of EGTA. Current 5pA, duration 5 ms, radius 1 μm.

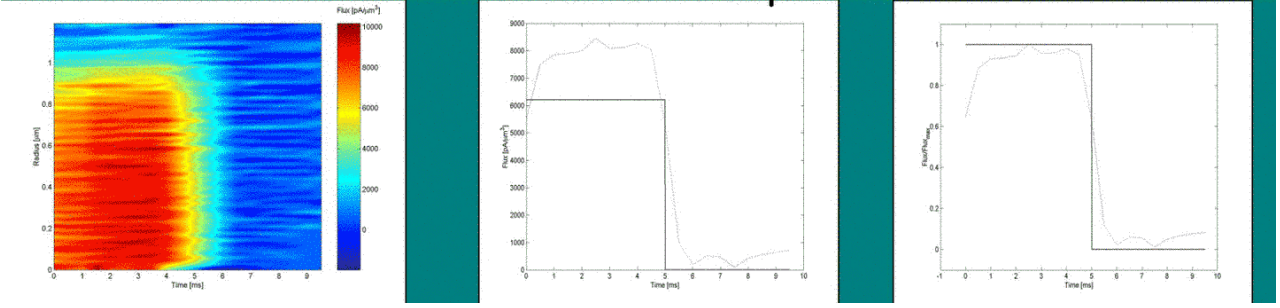
[CaF]



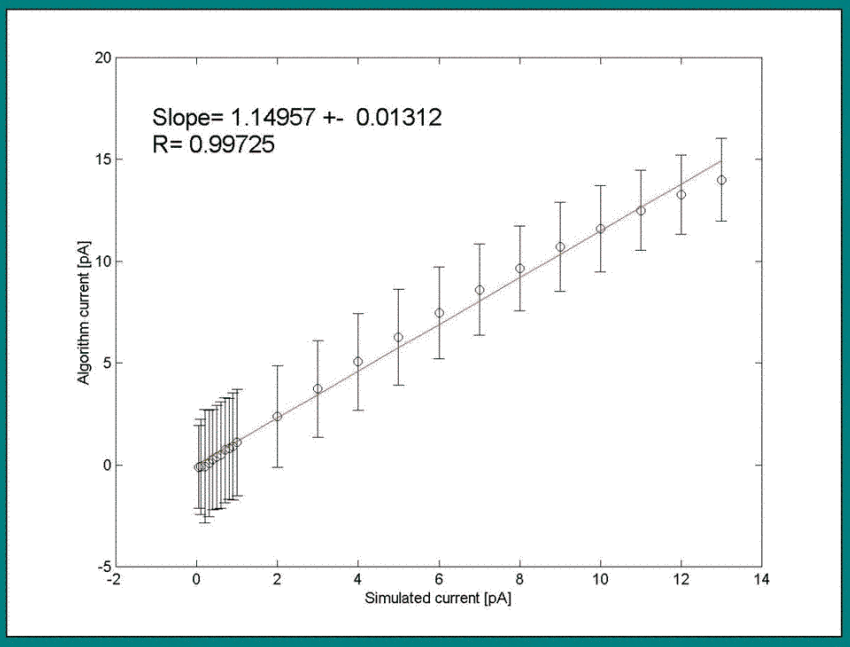
$[Ca^{2+}]$



The reconstructed source and its comparison with the real one



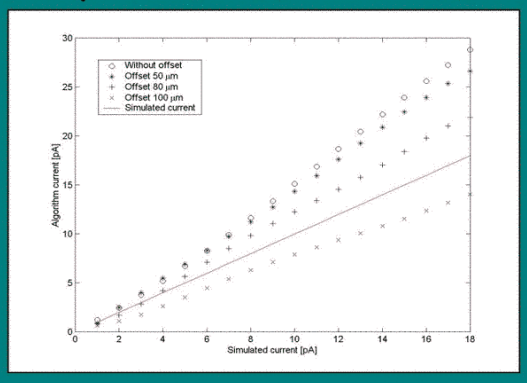
Results of various simulations of the same type with different calcium currents.



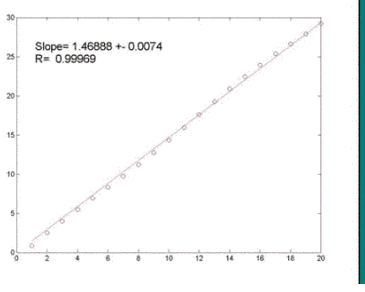
The method has a tendency to overestimate the calcium current

The estimate may improve if we take offsets into account

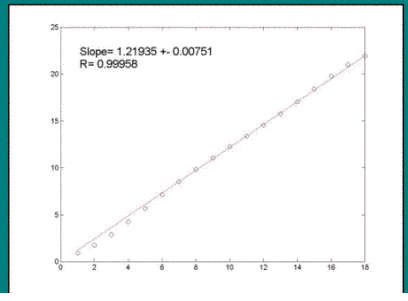
Radial source, EGTA + uptake, different offsets.



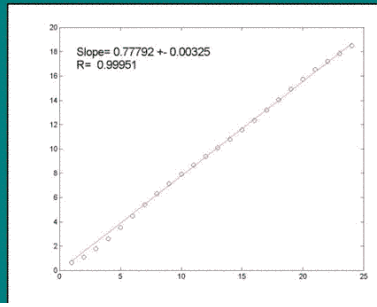
Offset 50



Offset 80



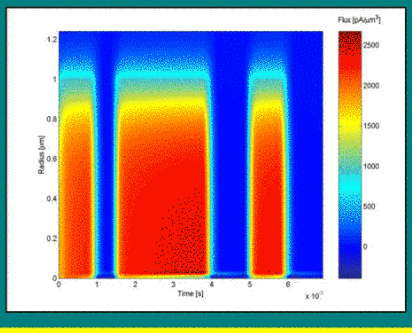
Offset 100

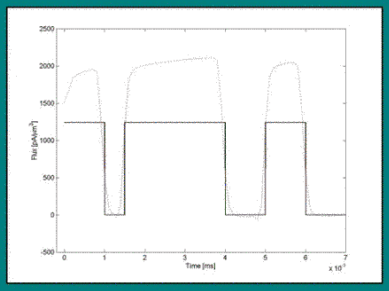


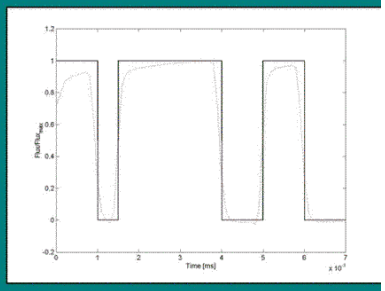
Example 2: Radial source, three openings in the presence of EGTA and uptake.

The reconstructed source and its comparison with the real one

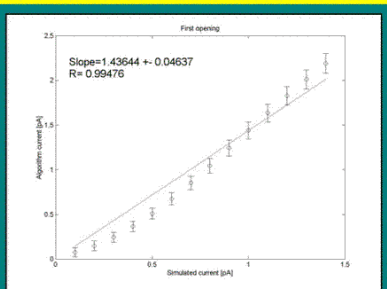
Case shown: Current 10pA, durations 1, 2.5 and 1 ms, radius 1 μm.

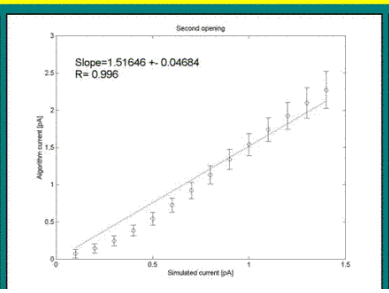


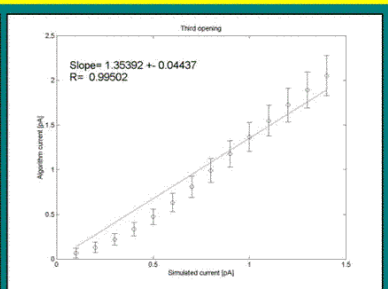




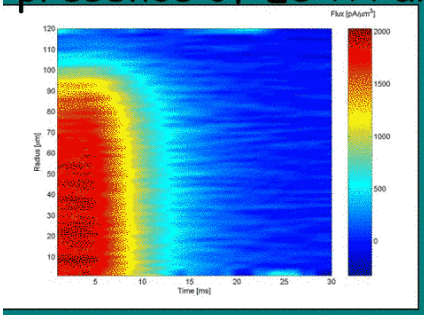
Results of various simulations of the same type with different calcium currents.



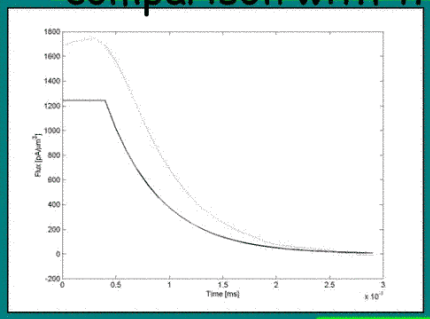


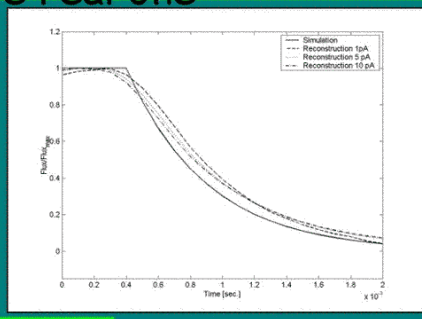


Example 3: Radial source with exponential decay in the presence of EGTA and uptake.

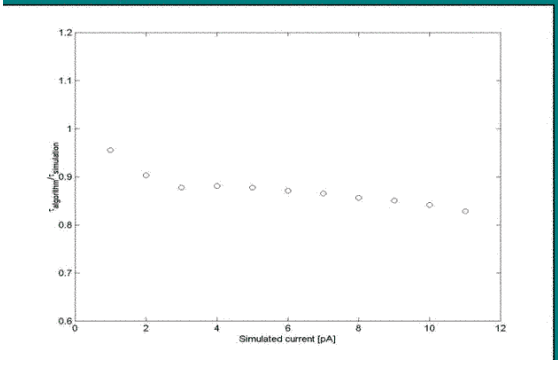


The reconstructed source and its comparison with the real one

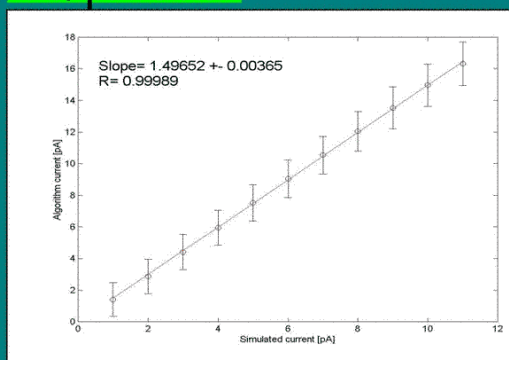




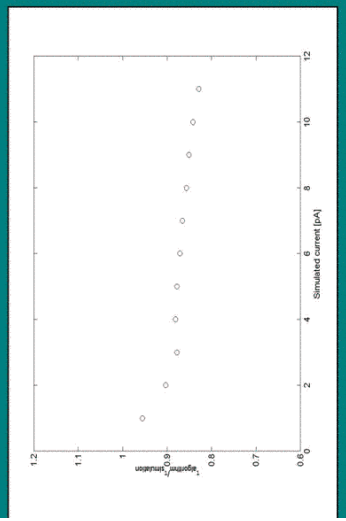
Decay time



Amplitude



Comparison of the algorithm's performance.



Our algorithm

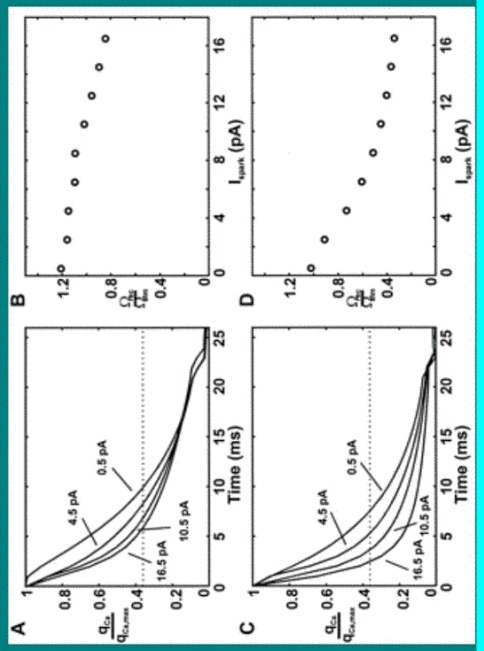
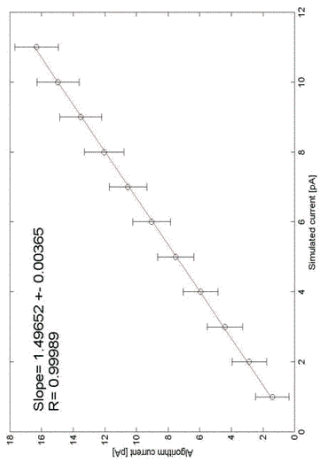
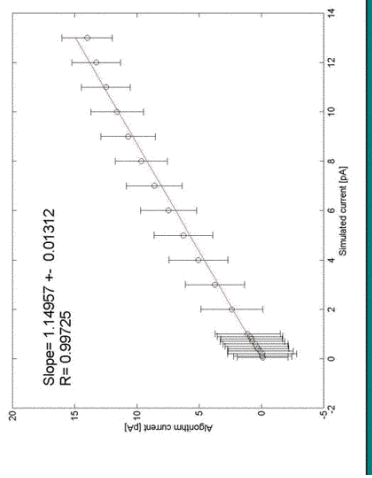


FIGURE 4 -- Soeller and Cannell, Biophysical Journal **82** (5) 2396. Amplitude dependence of reconstructed release flux time course.



Our algorithm



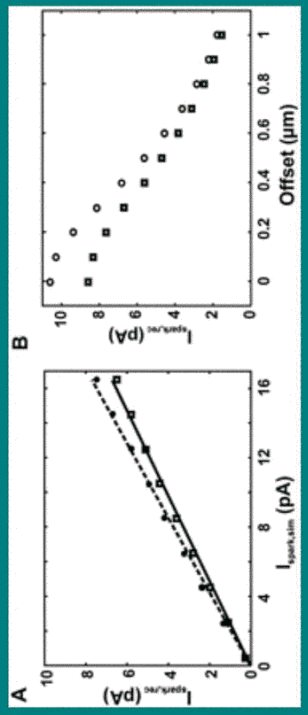
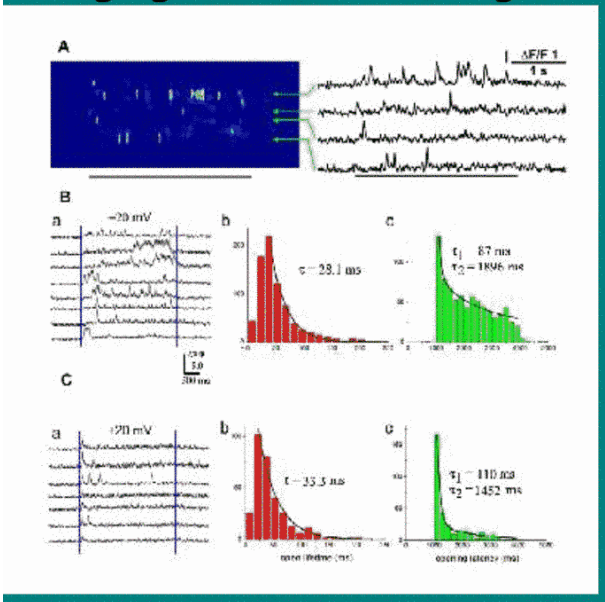


FIGURE 5 -- Soeller and Cannell, Biophysical Journal **82** (5) 2396. Summary graphs of algorithm performance.

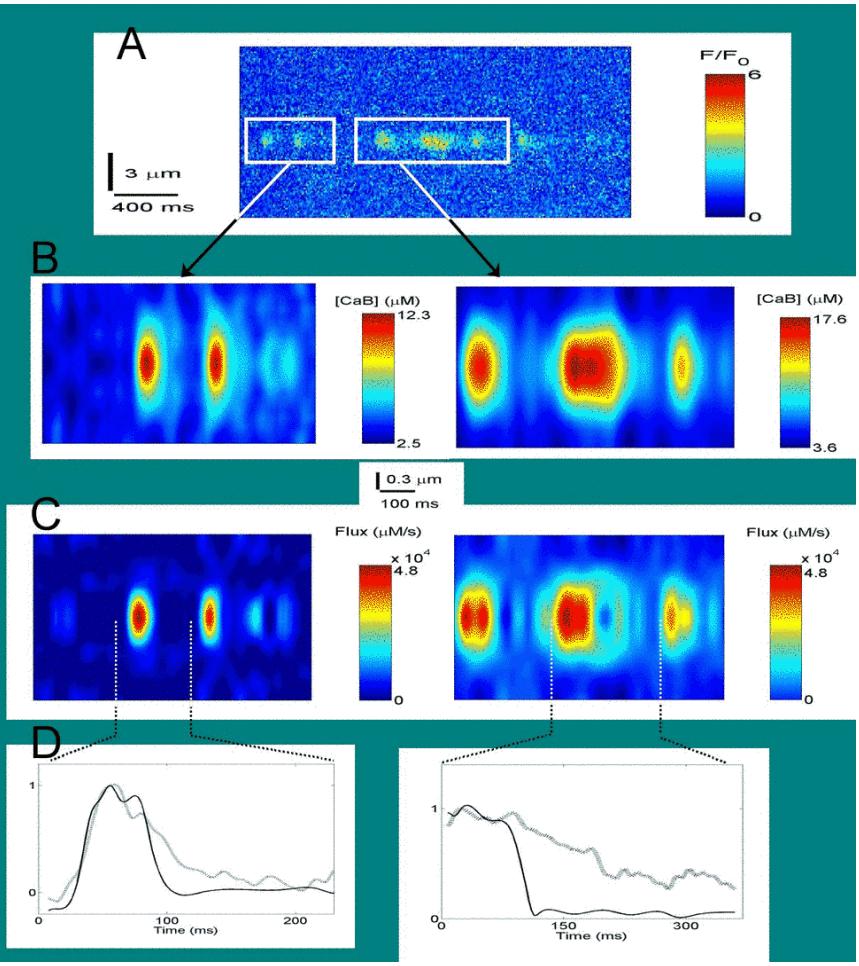
A.Demuro and I. Parker, "Optical single-channel recording: Imaging Ca2+ flux through individual voltage-gated channels".

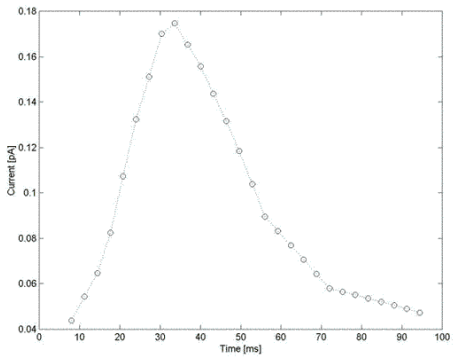


Optical recording of single channels.
B. Depolarization from –60mV to –20mV.
C. Depolarization to +20mV.

Fluorescence signals track the gaing of N-type Ca2+ channels with a kinetic resolution of about 10ms. The method fails to resolve signals that arise from very brief openings (although the dyes track [Ca2+] with sub-millisecond resolution).

Applying our algorithm to sparklets obtained from voltage gated channels.



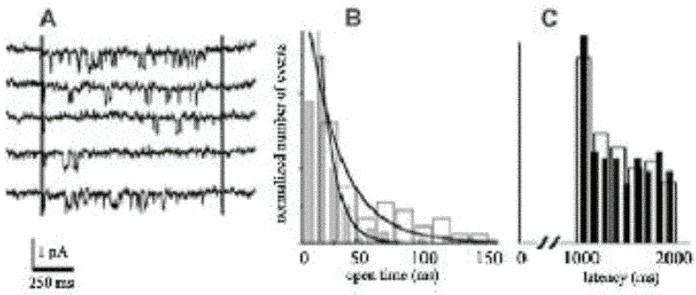


The calcium current is expected to be < .2 pA given the experimental conditions.

Results of our algorithm: Average over 7 individual events

Patch clamp data (using Ba2+ as carrier).

Patch clamp data follow a single exponential with a mean of 11.5 ms at -20mV. If brief openings are excluded, the mean open time is 27 ms.



Summary

We have developed a model-independent method that allows us to obtain the calcium current that underlies fluorescent images.

The method is based on very few assumptions. It does not involve the development of any model of how calcium behaves in the cell that is being imaged.

We applied the method to numerically generated images under various conditions. We determined that it may overestimate the calcium current, but the error is below 20%. This overestimate may be reduced.

The method gives the timescale of time-dependent calcium currents with an error that is less than 20%. It can also resolve several succesive openings and closings.

Now, we will apply it to different types of experiments. It will help us develop more accurate models of intracellular calcium dynamics. (to IP3R)