**Predicting calcium indicator fluorescence from spike trains using biophysical computational models**

Thomas J. Delaney1, Michael C. Ashby2, Cian O’Donnell1

1Department of Computer Science, 2School of Physiology, Pharmacology and Neuroscience, University of Bristol, Bristol, BS8 1UB, UK

**Abstract**

Although fluorescent calcium indicators such as GCaMP are widely used to monitor neuronal activity, in many cases the relationship between the fluorescence signal and action potential firing is poorly understood. This lack of knowledge makes it difficult for experimenters to decide between different indicator variants for a given application. We aimed to address this by building a basic biophysical model of calcium dynamics in neuronal soma. We fit the model parameters to data where GCaMP6s fluorescence and whole-cell electrophysiological recordings were made simultaneously in the same cells. We then systematically varied the model’s parameters to characterise the sensitivity of spike train inference algorithms to the calcium indicator’s main biophysical properties: binding rate, dissociation rate, and molecular concentration. This model should have two potential uses: experimental researchers may use it to help them select the optimal indicator for their desired experiment; and computational researchers may use it to generate simulated data to aid design of spike inference algorithms.

**Introduction**

Although fluorescent calcium indicators such as GCaMP are widely used to monitor neuronal activity, in many cases the relationship between the fluorescence signal and action potential firing is poorly understood. For example, the effects of the indicator characteristics on this fluorescence signal are unknown. For example, it is known that genetically encoded indicators accumulate within neurons over weeks and months. This makes comparison of activity levels at different time points difficult. As a result, whether or not spike train inference is always possible using fluorescent calcium indicators remains unknown.

The aim of this project was to model the fluorescence traces produced by a fluorescent calcium indicator in a neuron soma, given parameters such as binding rate, dissociation rate, and molecular concentration from a specific spike train. The ultimate goal of the model is to allow benchmarking of the various spike inference algorithms that have been developed, and to understand how indicator characteristics affect the quality of spike train inference.

The modelled cell contents consisted of free calcium, fluorescent indicator molecules, and mobile and immobile endogenous calcium buffers. The indicator molecules which were bound to a calcium molecule could be either excited, i.e. able to release a photon, or relaxed. In order to reproduce the noise in the system dynamics, we modelled the release of photons from the excited indicator bound calcium as a stochastic process.

The fluorescence traces produced by the simulation were calibrated to reproduce the signal-to-noise ratio observed in experimental data. Spike inference algorithms were used to infer spike trains from the experimental fluorescence traces and the modelled fluorescence traces. The parameters of the model were then varied in order to determine the effect on the system dynamics and the effects on spike inference.

**Results**

**1. A biophysical computational model can generate accurate fluorescence traces from spike trains**

To study the relationship between action potential firing and calcium fluorescence, we built a computational model of calcium dynamics in a neuronal soma. The model had one spatial compartment, and consisted of four dynamic variables: the concentration of free calcium, two types of endogenous buffer, and the calcium-sensitive fluorescent indicator. Each of the buffers and the indicator could independently bind and unbind with calcium. These reactions were modelled as

where X is the buffer concentration and Ca2+ the calcium concentration. Each molecule could therefore exist in two states: either bound or unbound with calcium. To model the imaging process, we also added a third, excited state to the indicator. When in the calcium-bound state, the indicator could be converted to an excited state, corresponding to the absorption of a photon. The rate of this excitation process could be interpreted as the intensity of the light illuminating the sample. Once excited, the species decayed back to the unexcited state at a fixed rate, corresponding to the spontaneous emission of photons. The total emitted fluorescence signal was interpreted as proportional to this de-excitation flux. To represent experimental noise in the photon capture process, we drew a random number of captured photons at each time step from a binomial distribution, parameterised by a number *p* that corresponds to the mean fraction of released photons that are captured.

The model had X total parameters describing the molecules’ concentrations and binding and unbinding rates. We set Y of these parameters to values from the literature. The remaining Z parameter values we fit to publicly-available data, briefly explained as follows (see Methods for full details). Single neurons from acute rat cortical slices expressing GCaMP6f were imaged with two-photon microscopy while the membrane potential of the soma of the same neurons were simultaneously recorded via whole-cell patch clamp electrophysiology. In this dataset, the electrical recording gave us unambiguous information about neuron’s spike times. To do the parameter fitting, we can inject these spike trains as inputs to the computational model, which after running returns a simulated fluorescence trace. We would like to find the model parameter values that give the best match between this simulated fluorescence trace and the real fluorescence time series recorded in the corresponding neuron. To do this we used a suite of optimisation procedures to jointly fit both the real neuron’s fluorescence time series and power spectrum, which capture complementary information about the spikes-to-fluorescence mapping (Methods). We performed the fitting procedure independently for each of the X neurons in the spikefinder dataset (http://spikefinder.org). After fitting the model produced realistic-looking fluorescence time series (Figure 1).

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| Figure 1. Comparison of model and real neuron fluorescence time series in response to the same identical spike trains.  **A:** Schematic diagram explaining goal of model.  **B:** Real vs modelled fluorescence time series from three example recordings from the spikefinder.org dataset [REF].  **C:** Comparison of data power spectra. |

**2. Spike inference algorithms perform similarly on real data compared with time series simulated from the model.**

Commonly researchers do not perform statistical analyses on fluorescence time series themselves, but first pass the time series through a spike inference tool. These algorithms take the fluorescence trace as input and attempt to estimate the neuronal spike train that triggered them. Part of our motivation for building this model was to allow us to ask the question: how do the properties of the cell and the calcium indicator affect the quality of spike inference? In order to trust the conclusions from our model, we should first be confident that spike inference from our simulated fluorescence traces is similar to that from the real data. To test this we passed each of the simulated fluorescence traces through three previously published spike inference algorithms, quantified their performance against the ground-truth electrophysiology data, repeated the procedure for the real calcium fluorescence time series, and compared the accuracy of the inference processes in all cases. The mean quality of spike inference varied across the three inference algorithms we tried (p=XXX, statistical test [ANOVA?]), Paninski correctly detected ~45%, OASIS detected ~35%, and ML spike detected ~15%. These low and heterogenous results show that spike inference is not an easy task and that the accuracy depends on the choice of inference algorithm. However the quality of inference on the simulated fluorescence time series was not statistically different than that on the real data for, any particular algorithm (p=XXX, statistical test [ANOVA?]). Notably the quality of inference was also fairly consistent for individual spike trains, not just the group means (p=XXX, statistical test). In contrast, when we feed the original spike trains into the spike inference algorithms, decoding accuracy was very high (NEED TO DO THIS). This demonstrates that the models were generating fluorescence time series that were similarly difficult to decode as the real data, in ways that were not specific to any one inference algorithm. This is evidence that the models captured real aspects of the spikes-to-fluorescence transform.

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| **Figure 2.** Accuracy (true positive rate) of three different spike inference algorithms for real data vs matched simulated fluorescence spike trains. |

**3. Relative effects of various buffers to the fluorescence signal.** One of the benefits of computational models over laboratory experiments is that we can observe all the variables in the simulation to gain insight into the system’s dynamics. We plotted the concentrations of the various species over time for a version of the model fit to one data set, in response to the same train of spikes used for fitting (Figure 1). Figure 1A shows the absolute values of the species concentrations summed. Consistent with experimental estimates [REF], only a small fraction ~X% of calcium is free and unbound to any buffer. Of the bound calcium, the vast majority, ~X% is bound to the GCaMP indicator. The two types of endogenous buffer account for the remaining ~X%. An influx of calcium from a single spike adds very little to the total calcium, in relative terms.

When calcium entered the model neuron it was rapidly buffered [REFS]. However the relative fractions of which buffer molecules bound to the influxed calcium was dynamic, and changed over time (NEW Figure 3X). [More commentary on this once figure is added]. Figure 3B-F shows the time course of the various species over time in response to a calcium influx event from a single action potential. Crucially, the indicator *BCa* competed with the endogenous buffers *ImCa* and *ECa* – all three bind calcium on similar timescales. This implies that the timecourse and amplitude of the *BCa* variable will also depend on the binding rates and availabilities of the endogenous buffers. For example if we decreased the concentration of an endogenous buffer, we might expect both a faster rise time and greater peak amplitude of the BCa signal in response to a calcium influx event. The slowest component of the decay had a similar time constant for BCa, ImCa and ECa, which in turn matched the Ca extrusion time constant in our model [ADD APPROXIMATE NUMBER]. This implies that the buffers and the indicator had reached an equilibrium and were simply tracking the free calcium concentration as calcium was slowly extruded from the cell.

Interestingly the excited bound calcium species (BCa\*) showed a qualitatively different timecourse in response to a calcium influx event [more comment].

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| **Figure 3.** Competition between various buffers for calcium.  (a) The proportions of bound and free calcium concentrations within a cell, with the associated spike train. (b)-(f) The dynamics of the concentration of (b) excited indicator bound calcium, (c) indicator bound calcium, (d) immobile endogeneous buffer bound calcium, (e) mobile endogenous buffer bound calcium, and (f) free calcium in response to an action potential at 23.17s. |

**4. Spike inference accuracy is sensitive to indicator properties, and likely varies within and between cells.**

The above results imply that the fluorescence signal depends on the relative properties of both GCaMP and the endogenous buffers. We next used the model to ask how sensitive spike inference was these properties. We focused on three key parameters that likely vary from cell to cell and experiment to experiment: GCaMP binding kinetics, GCaMP concentration, and endogenous buffer concentration.

Several variants of GCaMP itself have been made that differ in calcium binding kinetics, baseline fluorescence, fluorescence efficiency, and other factors. For example, GCaMP6f has a decay time constant of X, while GCaMP6s has a decay time constant of X. Here we asked how these differences in binding kinetics affect spike inference. We jointly varied the calcium binding and unbinding rates of the indicator by the same factor over a range from 100-fold slower to 100-fold faster from the fitted values, and simulated the fluorescence response for each of the parameter settings in response to the same spike trains as before. We computed two output measures from the simulated fluorescence traces: the signal-to-noise ratio for a single spike, and the accuracy of spike inference for each of the spike trains.

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[ARE FIGURES SWAPPED FOR INDICATOR CONCENTRATION VS KINETICS IN THOMAS’S DOCUMENT?]

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| **Figure 4.** Effect of calcium indicator kinetics on spike inference.  (a) Example fluorescence traces from |

Second, the overall concentrations of GCaMP often varies from cell to cell. For example different cells, even of the same type in the same tissue, can express different levels of GCaMP [REFS]. Also, GCaMP is often used for longitudinal experiments where the same cells are re-imaged across multiple days or weeks. However since GCaMP expression can ramp up across time [REF], the accuracy of spike inference may differ across multiple longitudinal recordings in the same cell. We addressed this by varying the concentration of calcium indicator in the model, simulating spike trains and measuring signal-to-noise ratio and spike inference accuracy on the resulting fluorescence traces. Surprisingly, indicator concentration had little effect on either the signal-to-noise ratio or the spike inference accuracy over a wide range of values (Figure 4b-c). Only when concentrations were 100-fold lower than their fitted values did we see an appreciable drop in signal-to-noise ratio and spike inference accuracy. In contrast, increasing the indicator concentration from the fitted values had very modest effects on spike inference.

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| **Figure 5.** Effect of calcium indicator concentration on spike inference.  (a) |

Third, the concentration and types of endogenous calcium buffers also vary from neuron to neuron, both within and between cell types [REF calbindin hippocampal paper, MORE]. Since the calcium buffer capacity of neurons is high, around X-Y [REF numbers for pyramidal and purkinje cells], these endogenous buffers may compete with GCaMP for binding to calcium, and variations in endogenous buffer concentration may affect GCaMP signal and therefore spike inference. To address this we varied the concentration of the endogenous buffer in the model neuron over five orders of magnitude from XuM to YuM [put in values], simulated calcium fluorescence traces in response to the same set of spike trains, and performed spike inference on the resulting fluorescence time series. Increasing the endogenous buffer concentration had a substantial effect on the GCaMP fluorescence signal, both decreasing its amplitude and slowing its kinetics (Figure 6a). This corresponded with a decrease in both single-spike signal-to-noise ratio (Figure 6b) and spike inference accuracy (Figure 6c). In contrast, decreasing endogenous buffer capacity from the fitted value had little effect on either the GCaMP signal or spike inference (Figure 6a-c).

**5. Single spike inference accuracy drops for high firing rates, but firing rate itself can be estimated from mean fluorescence amplitude.**

The fluorescence signal recorded from neurons using calcium indicators is typically much slower than changes in membrane potential for two reasons: first, because the calcium and the indicator have slow binding and unbinding kinetics, the signal is a low-pass filtered version of the membrane potential. Second, neuronal two-photon imaging experiments are often performed in scanning mode, which limits their frame rate to ~10Hz or slower. This implies that multiple spike events that occur close in time might be difficult to resolve from a calcium indicator time series. Many cells, especially several types of inhibitory interneurons, fire tonically at rates higher than 10Hz. We used the model to test whether spike inference accuracy depended on the neuron’s firing frequency by driving the cell with spike trains sampled from Poisson processes of varying frequency. Surprisingly, spike inference accuracy increased with increasing firing rate, for up to 10 Hz Poisson spike trains. We also plotted the average Delta\_F as a function of stimulation firing rate. [We found that it increased monotonically as a function of firing rate.]

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| **Figure 6.** Effect of endogenous buffer concentration on spike inference.  (a) |

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| **Figure 7.** Effect of cell firing rate on spike inference   1. Simulated fluorescence time series from a cell spiking at 1Hz, 5Hz or 10Hz. 2. Accuracy of spike inference as a function of spike rate. 3. Average fluorescence signal over time for three neural firing rates. |

**Discussion**

[Para recapping what we did and what we found]

[Para commenting on possible explanations for the effects from varying model parameters]

[Para commenting on possibility of developing firing rate inference algorithms for high-firing rate cells where individual spike inference is not feasible. The challenge is how to calibrate them?]

Although the model produced visually similar time series to the real data, there were a few aspects it did not capture. First, the real data featured some low-frequency components that did not appear related to the spike events. These were not captured by the models we used in this study, but could be added in future by adding a suitable low-frequency term to the resulting time series. Second, the real data seemed to have some nonlinearities not captured in the model, for example the response to two nearby spikes was greater than expected from the linear sum of two single spikes. This may be due to the co-operative binding of Calmodulin to calcium, which gives calmodulin a supra-linear sensitivity to calcium concentration. The model, in contrast behaved much more linearly, but could be extended in future to include such nonlinearities. Third, in the real data the fluorescence peak amplitude seemed to vary from spike to spike, even well-isolated spike events. However in our model we assumed each spike lead to the same fixed-amplitude injection of calcium to the cell, leading to much greater regularity in fluorescence peak amplitudes. This variability could be added in future version of the model by making the injected calcium peak a random variable. Fourth, we modelled the soma as a single compartment, but in reality there is likely a non-uniform spatial profile of calcium concentration. This may matter because some endogenous buffers might access calcium right as it influxes from the extracellular space, whereas the fluorescence signal is more likely majority coming from the bulk of the cytoplasm. Future models could attempt to model these spatial dependencies to assess whether they affect the overall spike inference procedure.

[Para commenting on recent Jason kerr group paper, Pillow and Tank paper]

[Para exploring the idea of the model as a tool, with suggestions for how it could be used as part of an experimental project.]

[Para exploring how model could be used for future development of spike inference algorithms and genetically encoded calcium indicators]

**Methods**