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Benchmarking Spike Rate Inference in Population Calcium Imaging

Highlights

- We evaluate algorithms for spike inference from two-photon calcium recordings
- A new supervised algorithm performs best across neural tissues and indicators
- Its performance transfers to new datasets without a need for retraining
- Simulated data are not informative about performance on real data

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In Brief

Two-photon calcium imaging has become a widely used tool for studying neural populations. Theis et al. quantitatively evaluate a range of algorithms for spike inference on ground truth data and introduce a new algorithm, which consistently outperforms previous ones.
Benchmarking Spike Rate Inference in Population Calcium Imaging

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SUMMARY

A fundamental challenge in calcium imaging has been to infer spike rates of neurons from the measured noisy fluorescence traces. We systematically evaluate different spike inference algorithms on a large benchmark dataset (>100,000 spikes) recorded from varying neural tissue (V1 and retina) using different calcium indicators (OGB-1 and GCaMP6). In addition, we introduce a new algorithm based on supervised learning in flexible probabilistic models and find that it performs better than other published techniques. Importantly, it outperforms other algorithms even when applied to entirely new datasets for which no simultaneously recorded data is available. Future data acquired in new experimental conditions can be used to further improve the spike prediction accuracy and generalization performance of the model. Finally, we show that comparing algorithms on artificial data is not informative about performance on real data, suggesting that benchmarking different methods with real-world datasets may greatly facilitate future algorithmic developments in neuroscience.

INTRODUCTION

Over the past two decades, two-photon imaging has become one of the most widely used techniques for studying information processing in neural populations in vivo (Denk et al., 1990; Kerr and Denk, 2008). Typically, a calcium indicator such as the synthetic dye Oregon green BAPTA-1 (OGB-1) (Stosiek et al., 2003) or the genetically encoded GCaMP6 (Chen et al., 2013) is used to image a large fraction of cells in a neural tissue. Individual action potentials lead to a fast rise in fluorescence, followed by a slow decay with a time constant of hundreds of milliseconds (Chen et al., 2013; Kerr et al., 2005). Commonly, neural population activity from dozens or hundreds of cells is imaged using relatively slow scanning speeds (<15 Hz), but novel fast scanning methods (Cotton et al., 2013; Grewe et al., 2010; Valmianski et al., 2010) (up to several 100 Hz) have opened additional opportunities for studying neural population activity at increased temporal resolution.

A fundamental challenge has been to infer the time-varying spike rate of neurons from the measured noisy calcium fluorescence traces. To solve this problem of spike inference, several different approaches have been proposed, including template matching (Greenberg et al., 2008; Grewe et al., 2010; Öhav et al., 2013), deconvolution (Park et al., 2013; Yaksi and Friedrich, 2006) and approximate Bayesian inference (Pnevmatikakis et al., 2016, 2013; Vogelstein et al., 2010, 2009). These methods have in common that they assume a forward generative model of calcium signal generation that is then inverted to infer spike times. Forward models incorporate strong a priori assumptions about the shape of the calcium fluorescence signal induced by a single spike and the statistics of the noise. Alternatively, simple supervised learning techniques have been used to learn the relationship between calcium signals and spikes from data (Sasaki et al., 2008).

However, it is currently not known which approach is most successful at inferring spikes under typical experimental conditions used for population imaging, as a detailed quantitative comparison of different algorithms on large datasets has been lacking. Rather, most published algorithms have only been evaluated on relatively small experimental datasets often collected zooming in on individual cells. Also, performance measures differ between studies. In addition, the question of how well we can reconstruct the spikes of neurons given calcium measurements has been studied theoretically or using simulated datasets (Lütcke et al., 2013; Wilt et al., 2013). While such studies offer the advantage that many model parameters are under the control of the investigator, they still rely on model assumptions and thus do not answer the question of how well we can reconstruct spikes from actual measurements.
Here, we pursue two goals: (1) we systematically evaluate a range of spike inference algorithms on a large dataset including simultaneous measurements of spikes and calcium signals in primary visual cortex and the retina of mice using OGB-1 and GCaMP6 as calcium indicators collected ex vivo and in anesthetized and awake animals and (2) introduce a new data-driven approach based on supervised learning in flexible probabilistic models to infer spikes from calcium fluorescence traces. We show that our new method outperforms all previously published techniques even when tested on data collected under new experimental conditions not used for training.

RESULTS

A Flexible Probabilistic Model for Spike Inference

Here we introduce a new algorithm for spike inference from calcium data. We propose to model the probabilistic relationship between a segment of the fluorescence trace $x_t$ and the number of spikes $k_t$ in a small time bin, assuming they are Poisson distributed with rate $\lambda(x_t)$:

$$p(k_t|x_t) = \frac{\lambda(x_t)^k}{k!} e^{-\lambda(x_t)}.$$

Instead of relying on a specific forward model, we parameterize the firing rate $\lambda(x_t)$ using a recently introduced extension of generalized linear models, the factored spike-triggered mixture (STM) model (Theis et al., 2013) (Figure 1A; see Experimental Procedures):

$$\lambda_{STM}(x_t) = \sum_{k=1}^{K} \exp \left( \sum_{m=1}^{M} \theta_{km} (u_{km} x_t)^2 + w_{km} x_t + b_{km} \right).$$

We train this model on simultaneous recordings of spikes and calcium traces to learn a set of $K$ linear features $w_k$ and $M$ quadratic features $u_{km}$ (“supervised learning”), which are predictive of the occurrence of spikes in the fluorescence trace. Importantly, this model is sufficiently flexible to capture non-linear relationships between fluorescence traces and spikes but at the same time is sufficiently restricted to avoid overfitting when little data are available. Below we will evaluate whether this model is too simple or already more complex than necessary by comparing its performance to that of multi-layer neural networks and simple LNP-type models.

In contrast to many methods that result in a single most likely spike train (a “point estimate”), using a probabilistic model provides us with an estimate of the expected firing rate, $\lambda(x_t)$, and a distribution over spike counts, as fully Bayesian methods do (Pnevmatikakis et al., 2013; Vogelstein et al., 2009). An advantage of access to a distribution over spike trains is that it allows us, for example, to estimate the uncertainty in the predictions.
Example spike trains consistent with the calcium measurements can be easily generated from our model without spending considerable computational resources. While generating a single “most likely spike train” is also possible, its interpretation is less clear, as the result depends on the parametrization.

**Benchmarking Spike Inference Algorithms on Experimental Data**

To quantitatively evaluate different spike inference approaches including our model, we acquired a large benchmark dataset with a total of 90 traces from 73 neurons, in which we simultaneously recorded calcium signals and spikes (Figure 1B; in total >100,000 spikes). These cells were recorded with different scanning methods, different calcium indicators, in different brain states and at different sampling rates (see Table 1 and Experimental Procedures). We used four datasets for our main analysis: dataset 1 consisted of 16 neurons recorded in vivo in V1 of anesthetized mice using fast 3D AOD-based imaging (Cotton et al., 2013) at ~320 Hz with OGB-1 as indicator. Dataset 2 consisted of 31 neurons recorded in vivo in anesthetized mouse V1 using raster scanning at ~12 Hz with OGB-1 as indicator. Dataset 3 consisted of 19 segments recorded from 11 neurons in vivo in anesthetized mouse V1 using the genetic calcium indicator GCaMP6s with a resonance scanner at ~59 Hz. Finally, dataset 4 consisted of 9 retinal ganglion cells recorded ex vivo at ~8 Hz using raster scanning with OGB-1 as indicator (Briggman and Euler, 2011). In addition, we collected a small dataset of 6 cells from V1 of awake mice using again the genetic calcium indicator GCaMP6s to demonstrate the performance during awake imaging (see below). We resampled the calcium traces from all datasets to a common resolution of 100 Hz. Importantly, all of our datasets were acquired at a zoom factor commonly used in population imaging such that the signal quality should match well that commonly encountered in these preparations (see Table 1).

We compared the performance of our algorithm (STM) and that of algorithms representative of the different approaches (see Table 2 and Experimental Procedures), including simple deconvolution (YF06; Yaksi and Friedrich, 2006), MAP (VP10, known as “fast-oopsi”; Vogelstein et al., 2010), and Bayesian inference (PP13 [Pnevmatikakis et al., 2013]; VP09 [Vogelstein et al., 2009]) in generative models, template matching by finite rate of innovation (OD13; Oñativia et al., 2013), and supervised learning using a support vector machine (SVM; Sasaki et al., 2008). To provide a baseline level of performance, we evaluated how closely the calcium trace followed the spike train without any further processing (raw).

We focus on two measures of spike reconstruction performance to provide a quantitative evaluation of the different techniques: (1) the correlation between the original and the reconstructed spike train and (2) the information gained about the spike train based on the calcium signal (see Experimental Procedures). For completeness, we computed (3) the area under the ROC curve (AUC), which has also been used in the literature. The AUC score is a less sensitive measure of spike reconstruction performance, as e.g., an algorithm could consistently overestimate high rates compared to low rates and yet yield the same AUC (for a more technical discussion, see Experimental Procedures).

To provide a fair comparison between the different algorithms, we evaluated their performance using leave-one-out cross-validation: we estimated the parameters of the algorithms on all but one cell from a dataset and tested them on the one remaining cell, repeating this procedure for each cell in the dataset (see Experimental Procedures). For the algorithms based on generative models, we selected the hyperparameters during cross-validation (VP10, VP09) or using a sampling-based approach (PP13; see Experimental Procedures).

**Supervised Learning Sets Benchmark**

We found that the spike rates predicted by our algorithm matched the true spike train closely, for cells from each dataset including both indicators OGB-1 and GCaMP6 (Figures 1C–1F). The other tested algorithms generally showed worse prediction performance: for example, YF06 typically resulted in very noisy estimates of the spike density function (Figures 1C–1F) and both VP10 and PP13 missed single spikes (Figures 1D–1F, marked by asterisk) and had difficulties modeling the dynamics of the GCaMP6 indicator (Figure 1E).

A quantitative comparison revealed that our STM method reconstructed the true spike trains better than its competitors, yielding a consistently higher correlation and information gain for all four datasets (Figures 2A and 2B; evaluated with bins of 40 ms; for statistics, see figure). The median improvement in correlation across all recordings achieved by the STM over its two closest competitors was 0.12 (0.07–0.14; median and bootstrapped 95% confidence interval, n = 75) for SI08—the other supervised learning approach based on SVMs—and 0.1
method is similar to the one used in Greenberg et al. (2008), but in as the relative rate fluctuations in the finer time bins matter. This correlation value for 10 ms bins requires much higher timing accuracy, finer variations are ignored. In contrast, achieving a similar correlation with average predicted firing rates in 50 ms bins, while at a timescale larger than 50 ms as it compares observed spike counts with average predicted firing rates in 50 ms bins, while finer variations are ignored. In contrast, achieving a similar correlation value for 10 ms bins requires much higher timing accuracy, as the relative rate fluctuations in the finer time bins matter. This method is similar to the one used in Greenberg et al. (2008), but in addition takes false positives/false negatives into account. Note that the binning affects the evaluation of the algorithm, not the spike inference. For all bin widths, the inference step was performed at the common sampling rate of 100 Hz (independent of scanning rate).

As a side remark, note that AUC is closely related to the cost function optimized by SI08, which is based on a support vector machine. Indeed, the features learned by the STM algorithm are more informative than those used in SI08. To show this, we trained a SVM using the STM features and find that this hybrid algorithm obtains higher AUC than SI08 on 3 out of the 4 datasets (Figure S1). To evaluate timing accuracy, we asked what correlation between the inferred and true rate was achieved when ignoring timing details finer than a certain bin width (between 10 and several hundreds of milliseconds; Figure 3): the correlation value reported for a bin width of 50 ms reflects only firing rate changes at a timescale larger than 50 ms as it compares observed spike counts with average predicted firing rates in 50 ms bins, while finer variations are ignored. In contrast, achieving a similar correlation value for 10 ms bins requires much higher timing accuracy, as the relative rate fluctuations in the finer time bins matter. This method is similar to the one used in Greenberg et al. (2008), but in addition takes false positives/false negatives into account. Note that the binning affects the evaluation of the algorithm, not the spike inference. For all bin widths, the inference step was performed at the common sampling rate of 100 Hz (independent of scanning rate).

Not surprisingly, correlation decayed as a function of bin width for all algorithms, as the resolution of increasingly fine detail becomes an increasingly challenging problem. However, the STM model performed better than the other algorithms in particular for small bin widths, providing higher temporal resolution (Figure 3; also Figure S2). Consequently, if the desired average correlation between inferred and true spike rates deemed acceptable was 0.4, our method was able to achieve that using time bins of ~17 ms, whereas competing methods required ~29 and ~58 ms (PP13 and SI08, respectively; evaluated on dataset 1, Figure 3A). Interestingly, VP10 (“fast-oopsi”) performed similar to our method for low sampling rates, but its performance deteriorated consistently on all datasets to the performance level of VF06 with increasing sampling rates (Figure 3).

The performance of the STM model could not be further improved using a more flexible multilayer neural network for modeling the non-linear rate function $l_t$ (Figure 4 and Figure S3). To test this, we replaced the STM model by a neural network with two hidden layers but found that this change resulted in only marginal performance improvement (Figure 4). In addition, we tested whether a much simpler linear-nonlinear model would suffice to model $l_t$. We found that the STM model performed significantly better than the simple LNP model (Figure 4 and Figure S3). Therefore, the choice of the STM seems to provide a good compromise between flexibility of the model structure and generalization performance. In comparison to the neural network, the STM is derived from a fully interpretable probabilistic generative model (Theis et al., 2013).

Importantly, already a small training set about 5–10 cells or 10,000 spikes was sufficient to achieve good performance with
the STM model trained de novo (Figures 5A and 5B and Figures S4A–S4D). We tested the prediction performance of the STM model with training sets of various sizes and found that it saturated between 5 and 10 cells for all datasets, arguing that a few simultaneously recorded cells may suffice to directly adapt the algorithms to new datasets acquired in other laboratories or with new imaging methods. In addition, we analyzed the training performance as a function of the number of spikes used for training and found that beyond 10,000 spikes in the training set predictions do not improve much (Figure 5B and Figures S4C and S4D). Of course, these two factors are not independent: recording 10,000 spikes from a single neuron will likely not yield the same quality predictions as recording 1,000 spikes from 10 neurons each. Finally, the superior performance of the STM was largely independent of the firing rate of the neuron within the limited range of firing rate in our sample of cells (Figures 5C and 5D and Figure S4E).

Generalization of Performance to New Datasets
In addition, we tested how well our algorithm performs if no simultaneous spike-calcium recordings are available for a new preparation or if a researcher wants to apply our algorithm without collecting simultaneous spike-calcium recordings, such that de novo training of the model is impossible.

Remarkably, the STM model was able to generalize to new datasets that were recorded under different conditions than the data used for training. To test this, we trained the algorithms on three of the datasets and evaluated it on the remaining one (Figure 6A)—that is, we applied the algorithm to an entirely new set of cells not seen during training. The STM algorithm still showed better performance than the other algorithms (Figures 6B and 6C and Figure S5A), including superior performance on the GCamp6 dataset when trained solely on the three OGB datasets (Figures 6B and 6C).

Next, we tested whether the algorithm’s performance would also transfer to recordings in head-fixed awake animals running on a Styrofoam ball (Figure 7A) (Reimer et al., 2014). Brain movements and brain state fluctuations caused by the animal running on the ball may induce additional variability in the recordings, which renders spike inference under these conditions more difficult. Example neurons showed good spike inference performance for the STM model in periods without (Figure 7B) and with (Figure 7C) movement. Overall, the STM trained on all neurons recorded in anesthetized animals or ex vivo retina (n = 75 traces from 70 cells) performed better than or comparable to the other algorithms on the awake data recorded using GCamp6s (n = 15 traces from 6 cells; Figures 7D and 7E and Figure S5B), further underscoring its generalization abilities. In addition, when we split the data into parts with and without motion (410.1 s versus 2,056.9 s), we found that the STM model’s performance was not impaired during periods in which the mouse moved (Figure 7F, correlation 0.27 ± 0.03 versus 0.27 ± 0.02, mean ± SEM).

We finally tested the different algorithms on three datasets using different GCamp-indicators acquired focusing on individual cells (in contrast to our population imaging dataset; n = 29 cells; data publicly available from Svoboda lab, see Experimental Procedures). Similarly to above, our algorithm was trained on two of these datasets and tested on the third. In addition, we included all cells from datasets 1–4 in to the training set, as there are only comparably few spikes in the Svoboda lab datasets. Focusing on individual cells makes the data less noisy, resulting on overall much higher correlation and AUC values (Figure S6). The STM algorithm performed well and on a par with VP10 regarding all three measures used for evaluation (Figure S6).
We then applied the same experimental procedures; *p < 0.05, **p < 0.01). The evaluation was performed performing the same model comparison described in (A).

(B) Information gained about the true spike train by observing the calcium trace in bins of 40 ms.

Figure 4. Evaluating Model Complexity

(A) Correlation (mean ± 2 SEM for repeated-measure designs) between the true and inferred spike rate comparing the STM model (black) with a flexible multilayer neural network (dark gray) and a simple LNP model (light gray) evaluated on the four different datasets collected under anesthesia/ex vivo (with n = 16, 31, 19, and 9, respectively). Markers above bars show the result of an Wilcoxon signed-rank test between the STM model and the LNP model (see Experimental Procedures, *p < 0.05, **p < 0.01). The evaluation was performed in bins of 40 ms.

(B) Information gained about the true spike train by observing the calcium trace performing the same model comparison described in (A).

Taken together, our analysis indicates that good performance can be expected for our algorithm when it is directly applied on novel datasets without further training (see Discussion). A pretrained version of our algorithm is available for download (see Experimental Procedures).

Comparisons on Artificial Data

Finally, we evaluated the performance of the algorithms on simulated data and show that this was not predictive of the performance of the algorithms on the real datasets (Figure 7). To test this, we simulated data from a simple biophysical model of calcium fluorescence generation (Figure 7A, see Experimental Procedures; Vogelstein et al., 2009). We then applied the same cross-validation procedure as before to evaluate the performance of the algorithms (Figure 7B). Not surprisingly, we found that all algorithms based on this or a similar generative model (PP13, VP10, YF06) performed well. Interestingly, even the algorithms that performed least well for the real data (OD13, VP09) showed good performance on the artificial data. The STM model was among the top-performing algorithms, in contrast to the other supervised learning algorithm (SI08). A direct comparison of the performance on the simulated dataset and the experimental data clearly illustrates that the former is not a good predictor of the latter (Figure 7C).

DISCUSSION

Here we provide a benchmark comparison of different algorithms for spike rate inference from calcium imaging recordings on ground truth data. We evaluate the algorithms for a wide range of recording conditions including OGB-1 and GCamp6 as calcium indicators, anesthetized and awake imaging, different scanning techniques, neural tissues, and with respect to different metrics. In addition, we introduced a new algorithm for inferring spike rates from calcium traces based on supervised training of a flexible probabilistic model and showed that this model performs currently better than all previously published algorithms for this problem under most conditions. Importantly, once trained, inferring spike rates using our algorithm is very fast, so even very large datasets can be processed rapidly. Interestingly, two of the three best algorithms rely on supervised learning to infer the relationship between calcium signal and spikes, suggesting that a data-driven approach offers distinct advantages over approaches based on forward models of the relationship between the two signals.

The superior performance of our algorithm carried over to new datasets not seen during training, promising good spike inference performance even when applied to a new dataset in which no simultaneous recordings are available. To use the algorithm “out of the box,” we provide it for download pre-trained with all experimental data used in this paper (see Experimental Procedures). In particular, its performance carried over to data recorded in awake animals, where brain movements or brain state fluctuations may render spike inference more difficult. In our recordings, motion in the z axis was small, on the order of 1–2 μm (Reimer et al., 2014, their Supplementary Information); if there was more brain movement in a given preparation and thus more neuropil contamination, generalization may be impaired. In addition, changing brain states during movement of the mouse compared to quiet restfulness (Niell and Stryker, 2010) may change the relationship between spikes and calcium signals. While we did not observe such effects in our data (Figure 7), it is certainly possible that they will become apparent with more data from awake animals with more frequent periods of running (here only ~20% of the data).

The fact that our algorithm can be used without extra training data is crucial, as this is often considered an important advantage of algorithms based on generative models. Note that for entirely new experimental conditions (e.g., a new calcium indicator), the performance of neither class of algorithms is guaranteed, however, and both need to be evaluated on a dataset with simultaneous recordings. For unsupervised methods, if such an evaluation reveals poor performance, e.g., because the assumed generative model does not match the structure of the dataset at hand (as seen, e.g., with the GCamp6 data; Figures 1E and 2), the only way to improve the algorithm would be to adapt the generative model and modify the inference procedures accordingly. In contrast, any simultaneous data collected...
in the future can be readily used to retrain our supervised algorithm and further improve its spike prediction and generalization performance. In fact, our choice of the spike triggered mixture model for estimating spike rates from calcium traces is motivated by its ability to automatically switch between different sub-models whenever the statistics of the data change (Theis et al., 2013). This property of the model might also allow the algorithm to accommodate different spike-calcium relationships in different brain states in awake animals, if they were to be found with more data from awake animals.

Interestingly, our evaluation shows that the correlation between inferred and real spike rates obtained at a temporal resolution of 40 ms is at best 0.4–0.6, depending on the dataset with substantial variability between cells (Figures 5C and 5D). This means that so far even the best spike inference algorithms make a substantial amount of errors, and one should be aware that for population imaging the inferred rates correspond to fairly coarse estimates of the true spike trains. It will be an interesting question whether new algorithmic ideas, new indicators (Chen et al., 2013; Inoue et al., 2015; St-Pierre et al., 2014; Thestrup et al., 2014), or scanning techniques will bring these values closer to 1, or whether these low correlations reflect a general limitation of population imaging approaches. Factors contributing to this limitation may include technical aspects of the imaging procedure such as neuropil contamination or activity-induced changes in blood vessel diameter and biophysical issues connected to the intracellular calcium dynamics. Our evaluation further shows that good spike inference performance on model data by no means guarantees good performance on real population imaging data (Figure 8).

We believe that theoretical model-based studies (Lütcke et al., 2013; Wilt et al., 2013) will remain useful to systematically explore how performance depends on model parameters, such as noise level or violations of the generative model, but will need to be followed up by systematic quantitative benchmark comparisons on datasets such as provided here.

Figure 5. Dependence on Training Set Size and Firing Rate

(A) Mean correlation for STM model on the four different datasets collected under anesthesia/ex vivo as a function of the number of neurons/segments in the training set.

(B) Mean correlation for STM model as a function of the number of neurons/segments in the training set as a function of the number of spikes in the training set. Large training sets (on the right) lead to less spikes in the test set, making the evaluation noisier.

(C) Correlation as a function of average firing rate of a cell. Dots mark correlation of STM model for individual traces. Solid lines indicate mean of a Gaussian process fit to correlation values for each of the indicated algorithms. Shaded areas are 95% CI.

(D) As in (C) for relative information gain.

Our proposed method is solely concerned with the problem of spike inference and does not infer the regions of interests (ROIs) from observed data or infers tuning properties of neurons simultaneously. Recently, several methods have been proposed to jointly infer ROIs and spikes (Diego and Hamprecht, 2014; Maruyama et al., 2014; Pnevmatikakis et al., 2016). These methods have the benefit that they exploit the full spatio-temporal structure of the problem of spike inference in calcium imaging and offer an unbiased approach for ROI placement. Since ROIs can also be placed using supervised learning (Valmianski et al., 2010), it should be feasible to develop supervised paradigms for simultaneous ROI placement and spike inference or combinations of unsupervised and supervised methods. Likewise, a recent study has combined spike rate inference with the estimation of response properties of neurons, such as tuning functions (Gannor et al., 2016), and it would be interesting to evaluate the use of supervised techniques for this problem as well.

We presented the first quantitative benchmarking approach to evaluating spike inference algorithms on a large dataset of population imaging data. We believe that such a benchmarking approach can also be an important catalyst for improvements on various computational problems in neuroscience, from systems identification to neuron reconstruction, as it is already used successfully in machine learning and related fields to drive new algorithmic developments. To catalyze the development of better spike inference algorithms for calcium imaging data, we will organize a competition, which will be announced separately.
Datasets and 5: Primary Visual Cortex (V1)—GCaMP6
We recorded calcium traces from neural populations expressing the genetic calcium indicator GCaMP6 in layer 2/3 of (1) isoloflurane-anesthetized and (2) awake wild-type mice (male C57CL/6J, age: 2–8 months; n = 2 and n = 1 mice for anesthetized and awake, respectively) using a resonant scanning microscope (ThorLabs) using methods described previously (Reimer et al., 2014). During awake experiments, the mouse was placed on a treadmill with its head restrained beneath the microscope objective (Reimer et al., 2014). Simultaneous loose-patch and two-photon calcium imaging recordings were conducted as described above. Data were split into segments involving movement or no movement by thresholding velocity traces. More details are provided in the Supplemental Experimental Procedures.

Dataset from Svoboda Lab
We used a publicly available dataset from the GENIE project, Svoboda lab, at Janelia farm on http://crcns.org (Akerboom et al., 2012; Chen et al., 2013; Svoboda, 2014). This dataset contains 9 cells recorded with GCaMP5, 11 cells recorded with GCaMP6f, and 9 cells recorded with GCaMP6s. The total number of spikes was 2,735, 4,536, and 2,123, respectively, and therefore much lower than for our datasets. Typically, these cells were recorded focusing on a single cell rather than recording from an entire population with lower zoom as in our dataset. For a detailed description of the data, see Akerboom et al. (2012) and Chen et al. (2013).

Preprocessing
We resampled all fluorescence traces and spike trains to 100 Hz (using scipy.signal.resample from the SciPy Python package). This allowed us to apply models across datasets independent of which dataset was used for training. We removed linear trends from the fluorescence traces by fitting a robust linear regression with Gaussian scale mixture residuals. That is, for each fluorescence trace $F_t$, we found parameters $a$, $b$, $n$, and $\sigma$ with maximal likelihood under the model

$$F_t = at + b + n \epsilon_t \sim \sum_{k=1}^{K} \pi_k N(-\epsilon, \sigma_k^2),$$

and computed $\hat{F}_t = F_t - at - b$. We used three different noise components ($K=3$). Afterward, we normalized the traces such that the 5th percentile of each trace’s fluorescence distribution is at zero, and the 80th percentile is at 1. Normalizing by percentiles instead of the minimum and maximum is more robust to outliers and less dependent on the firing rate of the neuron producing the fluorescence.

Supervised Learning in Flexible Probabilistic Models for Spike Inference
We predict the number of spikes $k_t$ falling in the $t$-th time bin of a neuron’s spike train based on 1,000 ms windows of the fluorescence trace centered around $t$ (preprocessed fluorescence snippets $s_t$). We reduced the dimensionality of the fluorescence windows via PCA, keeping at least 95% of the variance (resulting in 8 to 20 dimensions). Keeping 99% of the variance and slightly regularizing the model’s parameters gave similar results. Only for the Svoboda...
dataset we found it was necessary to keep 99% of the variance to achieve optimal results.

We assume that the spike counts $k_i$ given the preprocessed fluorescence snippets $x_i$ can be modeled using a Poisson distribution,

$$p(k_i|x_i) = \frac{(x_i^T \theta)^{k_i}}{k_i!} e^{-x_i^T \theta}.$$ 

We tested three models for the firing rate $\lambda(x_i)$ function:

1. A spike-triggered mixture (STM) model (Theis et al., 2013) with exponential nonlinearity,

$$\lambda_{\text{STM}}(x_i) = \sum_{k=1}^{K} \exp \left( \sum_{m=1}^{M} \beta_{km} (u_m x_i)^2 + w_k^T x_i + b_k \right),$$

where $w_k$ are linear filters, $u_m$ are quadratic filters weighted by $\beta_{km}$ for each of $K$ components, and $b_k$ is an offset for each component. We used three components and two quadratic features ($K=3$, $M=2$). The performance of the algorithm was not particularly sensitive to the choice of these parameters (we evaluated $K=1,\ldots,4$ and $M=1,\ldots,4$ in a grid search using one dataset).

2. As a simpler alternative, we use the linear-nonlinear-Poisson (LNP) neuron with exponential nonlinearity,

$$\lambda_{\text{LNP}}(x_i) = \exp(w^T x_i + b),$$

where $w$ is a linear filter and $b$ is an offset.

3. As a more flexible alternative, we used a multi-layer neural network (ML-NN) with two hidden layers,

$$\lambda_{\text{ML-NN}}(x_i) = \exp(w_2^T g(W_1 x_i + b_1) + b_2),$$

where $g(y) = \max(0,y)$ is a point-wise rectifying nonlinearity and $W_1$ and $W_2$ are matrices. We tested MLPs with 10 and 5 hidden units, and 5 and 3 hidden units for the first and second hidden layer, respectively. Again, the performance of the algorithm was not particularly sensitive to these parameters.

Parameters of all models were optimized by maximizing the average log-likelihood for a given training set,

$$\frac{1}{R} \sum_{k=1}^{R} \log p(k_i|x_i),$$

using limited-memory BFGS (Byrd et al., 1999), a standard quasi-Newton method. To increase robustness against potential local optima in the likelihood of the STM and the ML-NN, we trained four models with randomly initialized parameters and geometrically averaged their predictions. The geometric average of several Poisson distributions again yields a Poisson distribution whose rate parameter is the geometric average of the rate parameters of the individual Poisson distributions.

**Other Algorithms**

**S108**

This approach is based on applying a support-vector machine (SVM) on two PCA features of preprocessed segments of calcium traces. We re-implemented the features following closely the procedures described in (Sasaki et al., 2009). As the prediction signal, we used the distance of the input features to the SVM’s separating hyperplane, setting negative predictions to zero. We cross-validated the regularization parameter of the SVM but found that it had little impact on performance.

A Wilcoxon signed-rank test between the STM model and its closest competitor (see Experimental Procedures). *p < 0.05, **p < 0.01. The evaluation was performed in bins of 40 ms.

(D) Correlation (mean ± 2 SEM for repeated-measure designs) between the true spike rate and the inferred spike density function for a subset of the algorithms (see legend for color code) evaluated on awake data (n = 15 segments), trained on all anesthetized data. Markers above bars show the result of a Wilcoxon signed-rank test between the STM model and its closest competitor (see Experimental Procedures). *p < 0.05, **p < 0.01. The evaluation was performed in bins of 40 ms.

(E) As in (D) but for information gain.

(F) Evaluation of the effect of movement for the STM model. Recordings were separated into periods with and without motion (A, all; M, moving; S, stationary). Mouse movement left the performance unchanged.
Figure 8. Evaluating Algorithms on Artificial Data

(A) Example trace sampled from a generative model, true spikes, and binned rate as well as reconstructed spike rate from four different algorithms (conventions as in Figure 1). Numbers on the right denote correlations between true and inferred spike trains.

(B) Correlation (mean ± 2 SEM for repeated-measure designs) and information gain computed on a simulated dataset with 20 traces. For algorithms see legend.

(C) Scatter plot comparing performance on simulated data with that on real data (averaged over cells from all datasets collected under anesthesia/ex vivo), suggesting little predictive value of performance on simulated data.

**PP13**

The algorithm performs Bayesian inference in a generative model, using maximum a posteriori (MAP) estimates for spike inference and MCMC on a portion of the calcium trace for estimating hyperparameters. We used a MATLAB implementation provided by the authors of Pnevmatikakis et al. (2013), which has contributed to the later published Pnevmatikakis et al. (2016). We also tried selecting the hyperparameters through cross-validation, which did not substantially change the overall results.

**VP10**

The fast-oopsi or non-negative deconvolution technique constrains the inferred spike rates to be positive (Vogelstein et al., 2009), performing approximate inference in a generative model. We used the implementation provided by the author (https://github.com/jovo/fast-oopsi). We adjusted the hyperparameters using cross-validation by performing a search over a grid of 54 parameter sets controlling the degree of assumed observation noise and the expected number of spikes (Figures 2A and 2B). In Figures 5B and 5C, the hyperparameters were instead directly inferred from the calcium traces by the algorithm.

**YF06**

The deconvolution algorithm (Yaksi and Friedrich, 2006) removes noise by local smoothing and the inverse filter resulting from the calcium transient. We used a MATLAB implementation provided by the authors. Using the cross-validation procedure outlined above, we automatically tuned the algorithm by testing 66 different parameter sets. The parameters controlled the cutoff frequency of a low-pass filter, a time constant of the filter used for deconvolution, and whether or not an iterative smoothing procedure was applied to the fluorescence traces.

**OD13**

This algorithm performs a template-matching-based approach by using the finite rate of innovation theory as described in Ofati et al. (2013). We used the implementation provided on the author’s homepage (http://www.commsp.ee.ic.ac.uk/%7Epld/software/ca_transient.zip). We adjusted the exponential time constant parameter using cross-validation.

**VP09**

This algorithm performs Bayesian inference in a generative model as described in Vogelstein et al. (2009). We used the implementation provided by the author (https://github.com/jovo/smc-oopsi). Since this algorithm is based on the same generative model as fast-oopsi but is much slower, we used the hyperparameters inferred by cross-validating fast-oopsi in Figures 2A and 2B and the hyperparameters automatically inferred by the algorithm in Figures 5B and 5C.

**Performance Evaluation**

Unless otherwise noted, we evaluated the performance of the algorithms on spike trains binned at 40 ms resolution. For Figure 3 and Figure S2, we changed the bin width between 10 ms and 500 ms. We used cross-validation to evaluate the performance of our framework, i.e., we estimated the parameters of our model on a training set, typically consisting of all but one cell for each dataset, and evaluated its performance on the remaining cell. This procedure was iterated such that each cell was held out as a test cell once. Results obtained using the different training and test sets were subsequently averaged.

**Correlation**

We computed the linear correlation coefficient between the true binned spike train and the inferred one. This is a widely used measure with a simple and intuitive interpretation, taking the overall shape of the spike density function into account. However, the correlation coefficient is invariant under affine transformations, which means that predictions optimized for this measure cannot be directly interpreted as spike counts or firing rates. In further contrast to information gain, it also does not take the uncertainty of the predictions into account. That is, a method that predicts the spike count to be 5 with absolute certainty will be treated the same as a method which predicts the spike count to be somewhere between 0 and 10 assigning equal probability to each possible outcome.

**Information Gain**

The information gain provides a model-based estimate of the amount of information about the spike train extracted from the calcium trace. Unlike AUC and correlation, it takes into account the uncertainty of the prediction. Assuming an average firing rate of \( \lambda \) and a predicted firing rate of \( \hat{\lambda} \) at time \( t \), the expected information gain (in bits per bin) can be estimated as

\[
I_B = \frac{1}{T} \sum_{t=1}^{T} \log_2 \frac{1}{\lambda} \sum_{t} \hat{\lambda} - \frac{1}{T} \sum_{t} \hat{\lambda}
\]

assuming Poisson statistics and independence of spike counts in different bins. The estimated information gain is bounded from above by the (unknown) amount of information about the spike train contained in the calcium trace, as well as by the marginal entropy of the spike train, which can be estimated using...
\[ H_m = \frac{1}{T} \sum_{k=1}^{n_H} \log(k_i) - \lambda \log(\lambda + \lambda). \]

We computed a relative information gain by dividing the information gain averaged over all cells by the average estimated entropy,

\[ \frac{\sum_{k=1}^{n_H} f_k}{\sum_{k=1}^{n_H} f_k + \lambda}, \]

where \( f_k \) is the information gain measured for the \( n \)-th cell in the dataset.

This can be interpreted as the fraction of entropy in the data explained away by the model (measured in percent points). Since our only method was optimized to yield Poisson firing rates, we allowed all methods a single monotonically increasing nonlinear function, which we optimized to maximize the average information gain over all cells. That is, we evaluated

\[ \frac{1}{T} \sum_{k=1}^{n_H} f_k, \]

where \( f \) is a piecewise linear monotonically increasing function optimized to maximize the information gain averaged over all cells (using an SLSQP implementation in SciPy).

**AUC**

The AUC score can be computed as the probability that a randomly picked prediction for a bin containing a spike is larger than a randomly picked prediction for a bin containing no spike (Fawcett, 2006). While this is a commonly used score for evaluating spike inference procedures (Vogelstein et al., 2010), it is not sensitive to changes in the relative height of different parts of the spike density function, as it is invariant under arbitrary strictly monotonically increasing transformations. For example, if predicted rates were squared, high rates would be over proportionally boosted compared to low rates, while yielding equivalent AUC scores.

**Statistical Analysis**

We used generalized Loftus and Masson SEM for repeated-measure designs (Frazn and Loftus, 2012) and report the mean ± 2 SEM. To assess statistical significance, we compare the performance of the STM model to the performance of its next best competitor, performing a one-sided Wilcoxon signed-rank test and report significance or the respective p value above a line spanning the respective columns. If the STM is not the best model, we perform the rank test and report significance or the respective p value above a line spanning the respective columns.

**Generation of Artificial Data**

We simulated data by sampling from the generative model used by Vogelstein et al. (2010). That is, we first generated spike counts by independently sampling each bin of a spike train from a Poisson distribution, then convolving the spike train with an exponential kernel to arrive at an artificial calcium concentration, and finally adding Poisson noise to generate a Fluorescence signal \( x_i \).

\[ k_i \sim \text{Poisson}(\lambda), \]

\[ C_t = \gamma C_{t-1} + k_i, \]

\[ x_i \sim \text{Poisson}(a C_t + b). \]

The firing rate \( \lambda \) for each cell was randomly chosen to be between 0 and 400 spikes/s. The parameters \( \gamma, a \), and \( b \) were fixed to 0.98, 100, and 1, respectively, and data were generated at a sampling rate of 100 Hz.

**Code Availability**

We provide a Python implementation of our algorithm online (https://github.com/lucastheis/c2s). The package includes a pre-trained version of our algorithm, which is readily usable even without simultaneous recordings and has been trained on our entire dataset. The pre-trained algorithm has been trained on all five datasets presented in this paper as well as the publicly available data from the Svoboda lab. To accommodate the wider range of data, we made the model slightly more flexible allowing 6 linear and 4 quadratic components as well as accounting for 99% of the variance in the dimensionality reduction step.

**SUPPLEMENTAL INFORMATION**

Supplemental Information includes Supplemental Experimental Procedures and six figures and can be found with this article online at http://dx.doi.org/10.1016/j.neuron.2016.04.014.

**AUTHOR CONTRIBUTIONS**

P.B., M.B., and L.T. designed the project. L.T. analyzed the data with input from P.B.; E.F., J.R., and A.S.T. acquired V1 data. M.R.R., T.B., and T.E. acquired retinal data. P.B. wrote the paper with input from all authors. P.B. and M.B. supervised the project.

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Supplemental Information

Benchmarking Spike Rate Inference
in Population Calcium Imaging

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Supplementary Data

**Figure S1** (related to Fig. 2)

AUC (mean± 2 SEM for repeated measure designs) between the true spike rate and the inferred spike density function for different algorithms (see legend for color code) evaluated on the four different datasets collected under anesthesia/ex-vivo (with n=16, 31, 19 and 9, respectively). In addition to the models shown in Fig. 2, we investigate the performance of STM+, which uses the STM features as input to an SVM. This yields better AUC values than the STM, as the AUC is closely related to the cost function of the SVM, and shows that the STM features are better than those used by SI08. Above bars show the result of a Wilcoxon signed rank test between the STM model and its closest competitor (see Methods, * denotes P<0.05, ** denotes P<0.01) disregarding STM+. 

![Graph showing AUC values for different datasets and algorithms](image-url)
Figure S2 (related to Fig. 3)

a) AUC (mean ± 2 SEM for repeated measure designs) between the true and inferred spike rate as a function of frequency for all four datasets (a-d) with n=16, 31, 19 and 9, respectively. See legend for color code.

b) Information gain, as above.
Figure S3 (related to Fig. 4)

AUC (mean ± 2 SEM for repeated measure designs) between the true and inferred spike rate comparing the STM model (black) with a more flexible multilayer neural network (dark grey) and a simple LNP model (light grey) evaluated on the four different datasets (with n=16, 31, 19 and 9, respectively).
Figure S4: Training set size and firing rate (related to Fig. 5)

a) Rel. information gain as a function training set size in terms of number of neurons for the four different datasets (see legend). Error bars are omitted for clarity.

b) AUC as a function training set size in terms of number of neurons (as in a.).

c) Rel. information gain as a function training set size in terms of number of spikes for the four different datasets (see legend). Error bars are omitted for clarity.

d) AUC as a function training set size in terms of number of spikes (as in c.).

e) AUC as a function for firing rate. Dots represent AUC of STM model for individual traces. Solid lines indicate mean of a Gaussian process fit to AUC values for each of the indicated algorithms. Shaded areas are 95%-CI.
a) AUC (mean ± 2 SEM for repeated measure designs) between the true spike rate and the inferred spike density function for a subset of the algorithms (see legend for color code) evaluated on each of the four different datasets (with n=16, 31, 19 and 9, respectively), trained on the remaining three. Markers above bars show the result of a Wilcoxon sign rank test between the STM model and its closest competitor (see Methods, n.s., not significant; ** denotes P<0.01).

b) AUC as in a) for awake dataset recorded in V1 using GCamp6s (n = 15). The STM was trained on datasets 1-4.
Figure S6: Svoboda lab data (related to Fig. 6/7)

a-c) Correlation, Information gain and AUC (mean± 2 SEM for repeated measure designs) between the true spike rate and the inferred spike density function for a subset of the algorithms (see legend for color code) for the three datasets provided by the Svoboda lab. In these datasets, data was typically recorded at a higher zoom factor, focusing on individual cells and yielding higher signal to noise ratio. The STM model was trained on two of the three datasets and datasets 1-4 and tested on the remaining dataset. Markers above bars show the result of a Wilcoxon sign rank test between the STM model and its closest competitor (see Methods, * denotes P<0.05).
Supplementary Experimental Procedures

**Primary visual cortex (V1) – OGB-1**

We recorded calcium traces from neural populations in layer 2/3 of anesthetized wild type mice (male C57CL/6J, age: p40–p60) using a custom-built two-photon microscope using previously described methods (Cotton et al., 2013; Froudarakis et al., 2014). Briefly, the temperature of the mouse was maintained between 36.5 °C and 37.5 °C throughout the experiment using a homeothermic blanket system (Harvard Instruments). While recording we either provided no visual stimulation, moving gratings, or natural and phase scrambled movies as previously described (Froudarakis et al., 2014). A ~1 mm craniotomy was performed over the primary visual cortex of the mouse. The details of surgical techniques and anesthesia protocol have been described elsewhere (Cotton et al., 2013). We then used bolus-loaded Oregon green BAPTA-1 (OGB-1, Invitrogen) as calcium indicator and the injections were performed by using a continuous-pulse low pressure protocol with a glass micropipette to inject ~300 μm below the surface of the cortex. The cortical window was sealed using a glass coverslip. After allowing 1h for the dye uptake we recorded calcium traces using a custom-built two-photon microscope equipped with a Chameleon Ti-sapphire laser (Coherent) tuned at 800 nm and a 20×, 1.0 NA Olympus objective. Scanning was controlled by either a set of galvanometric mirrors (Galvo) or a custom-built acousto-optic deflector system (AODs) (Cotton et al., 2013). The average power output of the objective was kept < 50 mW for galvanometric scanning and 120 mW for AODs. Calcium activity was typically sampled at ~12 Hz with the galvanometric mirrors and at ~320 Hz with the AODs. The field of view was typically 200x200x100μm and 250x250μm for AODs and galvanometric imaging, respectively, imaging dozens to hundreds of neurons simultaneously (Cotton et al., 2013). To perform simultaneous loose-patch and two-photon calcium imaging recordings, we used glass pipettes with 5–7 MΩ resistance filled with Alexa Fluor 594 (Invitrogen) for targeted two-photon-guided loose cell patching of single cells. Spike times were extracted by thresholding. All procedures performed on mice were conducted in accordance with the ethical guidelines of the National Institutes of Health and were approved by the Baylor College of Medicine IACUC.

**Primary visual cortex (V1) – GCaMP6**

We recorded calcium traces from neural populations in layer 2/3 of (1) isoflurane-anesthetized and (2) awake wild type mice (male C57CL/6J, age: 2-8 months; N=2 and N=1 mice for anesthetized and awake, respectively) using a resonant scanning microscope (ThorLabs). Surgical procedures were similar to those described in Reimer et al (Reimer et al., 2014). Briefly, mice were initially injected with approximately 1 μL of AAV1.Syn.GCamp6s.WPRE.SV40 (University of Pennsylvania Vector Core) through a burr hole. The injection was performed with the pipette at a steep (~60 deg) angle, in order to infect cells in the cortex lateral to the injection site under an untouched region of the skull. The mice were allowed to recover and were returned to their cages. Typically three to five weeks later (4 months for the awake experiment), a 3 mm circular craniotomy was performed above the injection site and the craniotomy was sealed with a circular 3 mm coverslip with a ~0.5 μm hole to allow pipette access to infected cells. For anesthetized experiments, the temperature of the mouse was maintained between 36.5 °C and 37.5 °C throughout the experiment using a homeothermic blanket system (Harvard Instruments). During awake experiments, the mouse was placed on a treadmill with its head restrained beneath the microscope objective (Reimer et al., 2014). Recordings were of spontaneous activity without visual stimulation, and injected current was manually adjusted to maintain a moderate level of firing. Calcium traces were recorded using a Chameleon Ti-sapphire laser (Coherent) tuned at 920 nm and a 16×, .85 NA Nikon objective. The average power output of the objective was kept < 40 mW. To perform simultaneous loose-patch and two-photon calcium imaging recordings, we used glass pipettes with 7–10 MΩ resistance filled with ACSF and Alexa Fluor 594 (Invitrogen) as described above. For awake data, imaging data was motion corrected in the X-Y plane with post-hoc raster
correction and sub-pixel motion correction prior to extracting calcium traces. Motion along the Z-axis could not be corrected, but could be measured via correlation with a surrounding stack and in good preparations was typically small (running: mean 1.2 µm, s.d. 0.6 µm; quiet: mean 0.88 µm, s.d. 0.46 µm; data from (Reimer et al., 2014)). Calcium traces were extracted after manually segmenting patched cells and spike times were extracted by thresholding after excluding any periods where the patch was deemed unstable or of low quality. Data was split into segments involving movement or no movement by thresholding velocity traces. If the ball’s velocity reached the threshold (0.5 cm/s), at least 5 seconds of the trace before and after the detected movement were classified as moving. All procedures performed on mice were conducted in accordance with the ethical guidelines of the National Institutes of Health and were approved by the Baylor College of Medicine IACUC.

Retina
Imaging experiments were performed as described previously (Briggman and Euler, 2011). In short, the retina was enucleated and dissected from dark-adapted wild-type mice (both genders, C57BL/6J, p21-42), flattened, mounted onto an Anodisc (13, 0.1 mm pores, Whatman) with ganglion cells facing up, and electroporated with Oregon green BAPTA-1 (OGB-1, Invitrogen). The tissue was placed under the microscope, where it was constantly perfused with tempered (36°C) carboxygenated (95% O₂, 5% CO₂) artificial cerebral spinal fluid (ACSF). Cells were left to recover for at least 1 hour before recordings were performed. We used a MOM-type two-photon microscope equipped with a mode-locked Ti:Sapphire laser (MaiTai-HP DeepSee, Newport Spectra-Physics) tuned to 927 nm (Euler et al., 2009). OGB-1 Fluorescence was detected at 520 BP 30 nm (AHF) under a 20x objective (W Plan-Apochromat, 1.0 NA, Zeiss). Data were acquired with custom software (ScanM by M. Müller and T. Euler running under IgorPro 6.3, Wavemetrics), taking 64 x 64 pixel images at 7.8 Hz. Light stimuli were presented through the objective from a DLP projector (K11, Acer), fitted with band-pass-filtered LEDs (amber, z 578 BP 10; and blue/UV, HC 405 BP 10, AHF/Croma), synchronized with the microscope’s scanner. Stimulator intensity (as photoisomerization rate, 10⁴ R*/s/cone) was calibrated as described to range from 0.1 (LEDs off) to ~1.3 (Euler et al., 2009). Mostly due to two-photon excitation of photopigments, an additional, steady illumination component of ~10⁴ R*/s/cone was present during the recordings. The field of view was 100x100µm, imaging 50-100 cells in the ganglion cell layer simultaneously (Briggman and Euler, 2011). For juxtacellular spike recordings, OGB-1 labeled somata were targeted with a 5 MΩ glass-pipette under dim IR illumination to establish a loose (<1GΩ) seal. Signals were amplified using an Axopatch 200A amplifier (Molecular Devices) in I=0 mode and digitized at 10 kHz on a Digidata 1440A (Molecular Devices). Imaging and spike data were aligned offline using a trigger signal recorded in both acquisition systems, and spike times were extracted by thresholding. All procedures were performed in accordance with the law on animal protection (Tierschutzgesetz) issued by the German Federal Government and were approved by the institutional animal welfare committee of the University of Tübingen.