Fast Active Set Methods for Online Deconvolution of Calcium Imaging Data

Johannes Friedrich¹,², Pengcheng Zhou³, Liam Paninski¹,⁴

¹Grossman Center for the Statistics of Mind and Department of Statistics, Columbia University, New York, NY
²Janelia Research Campus, Ashburn, VA
³Center for the Neural Basis of Cognition and Machine Learning Department, Carnegie Mellon University, Pittsburgh, PA
⁴Kavli Institute for Brain Science, and NeuroTechnology Center Columbia University, New York, NY, USA

j.friedrich@columbia.edu, pengchez@andrew.cmu.edu, liam@stat.columbia.edu

Abstract

Fluorescent calcium indicators are a popular means for observing the spiking activity of large neuronal populations, but extracting the activity of each neuron from raw fluorescence calcium imaging data is a nontrivial problem. We present a fast online active set method to solve this sparse non-negative deconvolution problem. Importantly, the algorithm progresses through each time series sequentially from beginning to end, thus enabling real-time online estimation of neural activity during the imaging session. Our algorithm is a generalization of the pool adjacent violators algorithm (PAVA) for isotonic regression and inherits its linear-time computational complexity. We gain remarkable increases in processing speed: more than one order of magnitude compared to currently employed state of the art convex solvers relying on interior point methods. Our method can exploit warm starts; therefore optimizing model hyperparameters only requires a handful of passes through the data. A minor modification can further improve the quality of activity inference by imposing a constraint on the minimum spike size. The algorithm enables real-time simultaneous deconvolution of $O(10^5)$ traces of whole-brain larval zebrafish imaging data on a laptop.

1 Introduction

Calcium imaging has become one of the most widely used techniques for recording activity from neural populations in vivo [1]. The basic principle of calcium imaging is that neural action potentials (or spikes), the point process signal of interest, each induce an optically measurable transient response in calcium dynamics. The nontrivial problem of extracting the activity of each neuron from a raw fluorescence trace has been addressed with several different approaches, including template matching [2] and linear deconvolution [3-4], which are outperformed by sparse non-negative deconvolution [5]. The latter can be interpreted as the maximum a posteriori (MAP) estimate under a simple generative model (linear convolution plus noise; Fig[1]), whereas fully Bayesian methods [6-8] can provide some further improvements, but are more computationally expensive. Supervised methods trained on simultaneously-recorded electrophysiological and imaging data [9-10] have also recently achieved state of the art results, but are more black-box in nature; Bayesian methods based on a well-defined generative model are somewhat easier to generalize to more complex multi-neuronal or multi-trial settings [11-13].

A shorter version of this article is in press for the 30th Conference on Neural Information Processing Systems (NIPS 2016), Barcelona, Spain.
The methods above are typically applied to imaging data offline, after the experiment is complete; however, there is a need for accurate and fast real-time processing to enable closed-loop experiments, a powerful strategy for causal investigation of neural circuitry [14]. In particular, observing and feeding back the effects of circuit interventions on physiologically relevant timescales will be valuable for directly testing whether inferred models of dynamics, connectivity, and causation are accurate in vivo, and recent experimental advances [15][16] are now enabling work in this direction. Brain-computer interfaces (BCIs) also rely on real-time estimates of neural activity. Whereas most BCI systems rely on electrical recordings, BCIs have been driven by optical signals too [17], providing new insight into how neurons change their activity during learning on a finer spatial scale than possible with intracortical electrodes. Finally, adaptive experimental design approaches [18][19][20] also rely on online estimates of neural activity.

Even in cases where we do not require the strict timing/latency constraints of real-time processing, we still need methods that scale to large data sets as for example in whole-brain imaging of larval zebrafish [21][22]. A further demand for scalability stems from the fact that the deconvolution problem is solved in the inner loop of constrained non-negative matrix factorization (CNMF) [13], the current state of the art for simultaneous denoising, deconvolution, and demixing of spatiotemporal calcium imaging data.

In this paper we address the pressing need for scalable online spike inference methods. Building on previous work, we frame this estimation problem as a sparse non-negative deconvolution. Current algorithms employ interior point methods to solve the ensuing optimization problem and are fast enough to process hundreds of neurons in about the same time as the recording [5], but can not handle larger data sets such as whole-brain zebrafish imaging in real time. Furthermore, these interior point methods scale linearly in the length of the recording, but they cannot be warm started [23], i.e., initialized with the solution from a previous iteration to gain speed-ups, and do not run online.

Here we note a close connection between the MAP problem and isotonic regression, which fits data by a monotone piecewise constant function. A classic algorithm for isotonic regression is the pool adjacent violators algorithm (PAVA) [24][25], which can be understood as an online active-set optimization method. We generalized PAVA to derive an Online Active Set method to Infer Spikes (OASIS); this new approach to solve the MAP problem yields speed-ups in processing time by at least one order of magnitude compared to interior point methods on both simulated and real data. Further, OASIS can be warm-started, which is useful in the inner loop of CNMF, and also when adjusting model hyperparameters, as we show below. Importantly, OASIS is not only much faster, but operates in an online fashion, progressing through the fluorescence time series sequentially from beginning to end. The advances in speed paired with the inherently online fashion of the algorithm enable true real-time online spike inference during the imaging session, with the potential to significantly impact experimental paradigms.

The sparse non-negative deconvolution recovers the neural activity well, but the inferred activity can contain many small false positives in low-SNR settings. These false positives are problematic in event detection. Therefore we also introduce a thresholding step in the deconvolution algorithm. The resulting problem is non-convex, and so we lose guarantees on finding global optima, but we can easily adapt OASIS to quickly find good solutions.

The rest of this paper is organized as follows: Section 2 introduces the autoregressive (AR(p)) model for calcium dynamics. In Section 3 we derive our active set method for the sparse non-negative deconvolution problem for the simple case of AR(1) dynamics. We further use the problem’s dual formulation to adjust the sparsity level in a principled way (following [13]), and describe methods for fitting model hyperparameters including the baseline and coefficients of the AR process. In Section 4 we generalize the active set method to AR(p) processes and arbitrary calcium response kernels. In Section 5 we show some results on simulated as well as real data. Finally, in Section 6 we discuss the relationship of our study to other models, as well as further extensions.

## 2 Model for calcium dynamics

We assume we observe the fluorescence signal for \( T \) timesteps, and denote by \( s_t \) the number of spikes that the neuron fired at the \( t \)-th timestep, \( t = 1, ..., T \), cf. Fig[1]. Following [5][13], we approximate the calcium concentration dynamics \( c \) using a stable autoregressive process of order \( p \) (AR(p)) where
Figure 1: Generative autoregressive model for calcium dynamics. Spike train s gets filtered to produce calcium trace c; here we used \( p = 2 \) as order of the AR process. Added noise yields the observed fluorescence \( y \).

\( p \) is a small positive integer, usually \( p = 1 \) or \( 2 \),

\[
c_t = \sum_{i=1}^{p} \gamma_i c_{t-i} + s_t. \quad (1)
\]

The observed fluorescence \( y \in \mathbb{R}^T \) is related to the calcium concentration as [5, 6, 7]:

\[
y_t = a c_t + b + \epsilon_t, \quad \epsilon_t \sim \mathcal{N}(0, \sigma^2) \quad (2)
\]

where \( a \) is a non-negative scalar, \( b \) is a scalar offset parameter, and the noise is assumed to be i.i.d. zero mean Gaussian with variance \( \sigma^2 \). For the remainder we assume units such that \( a = 1 \) without loss of generality. We begin by assuming \( b = 0 \) for simplicity, but we will relax this assumption later. The parameters \( \gamma_i \) and \( \sigma \) can be estimated from the autocovariance function and the power spectral density (PSD) of \( y \) respectively [13]. The autocovariance approach assumes that the spiking signal \( s \) comes from a homogeneous Poisson process and in practice often gives a crude estimate of \( \gamma_i \). We will improve on this below (Fig 4) by fitting the AR coefficients directly, which leads to better estimates, particularly when the spikes have some significant autocorrelation.

The goal of calcium deconvolution is to extract an estimate \( \hat{s} \) of the neural activity \( s \) from the vector of observations \( y \). As discussed in [5, 13], this leads to the following non-negative LASSO problem for estimating the calcium concentration:

\[
\min_{\hat{c}} \frac{1}{2} \| \hat{c} - y \|^2 + \lambda \| \hat{s} \|_1 \quad \text{subject to } \hat{s} = G\hat{c} \geq 0 \quad (3)
\]

where the \( \ell_1 \) penalty on \( \hat{s} \) enforces sparsity of the neural activity and the lower triangular matrix \( G \) is defined as:

\[
G = \begin{pmatrix}
1 & 0 & 0 & \cdots & 0 \\
-\gamma_1 & 1 & 0 & \cdots & 0 \\
-\gamma_2 & -\gamma_1 & 1 & \cdots & 0 \\
\vdots & \ddots & \ddots & \ddots & \vdots \\
0 & \cdots & -\gamma_2 & -\gamma_1 & 1
\end{pmatrix}. \quad (4)
\]

The matrix \( G \) is banded with bandwidth \( p \) for an AR(\( p \)) process. (Although our main focus is on the autoregressive model, we will discuss more general convolutional observation models below as well.) Following the approach in [5], note that the spike signal \( \hat{s} \) is relaxed from non-negative integers to arbitrary non-negative values.

3 Derivation of the active set algorithm

The optimization problem (3) could be solved using generic convex program solvers. Here we derive the much faster Online Active Set method to Infer Spikes (OASIS). The algorithm is inspired by the pool adjacent violators algorithm (PAVA) [24, 25], which we review first for readers not familiar with this classic algorithm before generalizing it to the non-negative LASSO problem.

3.0.1 Pool Adjacent Violators Algorithm (PAVA)

The pool adjacent violators algorithm (Alg 1) is a classic exact algorithm for isotonic regression, which fits data by a non-decreasing piecewise constant function. This algorithm is due to [24] and
We introduce temporary values

We first present the algorithm in a way that conveys its core ideas, then improve the algorithm’s efficiency by introducing “pools” of variables (adjacent \(x_t\) values) which are updated simultaneously. We introduce temporary values \(x’\) and initialize them to the unconstrained least squares solution, \(x’ = y\). Initially all constraints are in the “passive set” and possible violations are fixed by subsequently adding the respective constraints to the “active set”. Starting at \(t = 2\) the algorithm moves to the right until a violation of the constraint \(x’_t \geq x’_{t-1}\) at some time \(\tau\) is encountered. Now the monotonicity constraint is added to the active set and enforced by setting \(x’_t = x’_{\tau-1}\). (Supposing the opposite, i.e. \(x’_t > x’_{\tau-1}\), we could move \(x’_t\) and \(x’_{\tau-1}\) by some small \(\epsilon\) to decrease the objective without violating the constraints, yielding a proof by contradiction that the monotonicity constraint should be made “active” here - i.e., the constraint holds with strict equality.) We update the values \(x’_t = x’_{\tau-1}\) at the two time steps to the best possible fit with constraints. Minimizing their contribution to the objective yields an update that corresponds to averaging

\[
x’_t = x’_{t+1} = \ldots = x’_T = \frac{\sum_{t=t'}^T y_t}{\tau - t' + 1}
\]
with value $v_i$, weight $w_i$ and event time $t_i$ where $i$ indices the groups. Initially there is a pool $(v_s, w_s, t_s) = (y_s, 1, s)$ for each time point $s$. During the sweep through the data adjacent pools that violate the constraint $v_{t+1} \geq v_i$ are combined to a new pool $(\sum_i (w_i v_i + w_{i+1} v_{i+1}), w_i + w_{i+1}, t_i)$. Whenever pools $i$ and $i + 1$ are merged, former pool $i + 1$ is removed and the succeeding pool indices decreased by 1. It is easy to prove by induction that these updates guarantee that the value of a pool is indeed the average of the corresponding data points (see Supplementary Material) without having to explicitly calculate it using Eq 4. The latter would be expensive for long pools, whereas merging two pools has $O(1)$ complexity independent of the pool lengths. With pooling the considered worst case situation results in a single pool and only its value and weight are updated at every step forward, yielding $O(T)$ complexity. Constructing the optimal solution $x_t$ for all $t$ in a final effort after the optimal pool partition has been reached is also $O(T)$. At convergence all constraints have been enforced; further note that convergence occurs after a finite number of steps.

### 3.1 Online Active Set method to Infer Spikes (OASIS)

Now we adapt the PAVA approach to problem (6). PAVA solves a regression problem subject to the constraint that the value at the current time bin must be greater than or equal to the last. The AR(1) model posits a more general but very similar constraint. The key insight is that problem (3) is a generalization of problem (5): if $p = 1$ in the AR model and we set $\gamma = 1$ (we skip the index of $\gamma$ for a single AR coefficient) and $\lambda = 0$ in Eq 6 we obtain Eq 8. Therefore we focus first on the $p = 1$ case and deal with $p > 1$ and arbitrary calcium response kernels in the next section.

We begin by inserting the definition of $\hat{s}$ (Eq 8). Using that $\hat{s}$ is constrained to be non-negative yields for the sparsity penalty

\[
\lambda \| \hat{s} \|_1 = \lambda \| \mu \|_\infty = \lambda \sum_{t=1}^{T} \sum_{i=1}^{T} G_{i,t} \hat{e}_t = \lambda \sum_{t=1}^{T} (1 - \gamma \delta_t) \hat{e}_t = \sum_{t=1}^{T} \mu_t \hat{e}_t = \mu^\top \hat{c} \tag{8}
\]

with $\mu_t := \lambda (1 - \gamma \delta_t)$ (with $\delta$ denoting Kronecker’s delta) by noting that the sum of the last column of $G$ is 1, whereas all other columns sum to $(1 - \gamma)$.

Now the problem

\[
\text{minimize} \quad \frac{1}{2} \sum_{t=1}^{T} (\hat{e}_t - y_t)^2 + \sum_{t=1}^{T} \mu_t \hat{e}_t \quad \text{subject to} \quad \hat{e}_{t+1} \geq \gamma \hat{e}_t \geq 0 \quad \forall t \tag{9}
\]

shares some similarity to isotonic regression with the constraint $\hat{e}_{t+1} \geq \hat{e}_t$ (Eq 5). However, our constraint $\hat{e}_{t+1} \geq \gamma \hat{e}_t$ bounds the rate of decay instead of enforcing monotonicity. Thus we need to generalize PAVA to handle the additional factor $\gamma$.

For clarity we mimic our approach from the last section: we first present the algorithm in a way that conveys its core ideas, and then improve the algorithm’s efficiency using pools. We introduce temporary values $c^t$ and initialize them to the unconstrained least squares solution, $c^t = y - \mu$. Starting at $t = 2$ one moves forward until a violation of the constraint $c^t \geq c^{t-1}$ at some time $\tau$ is detected (Fig 2A). Updating the two time steps by minimizing $\frac{1}{2} (y_{\tau-1} - c^{t-1})^2 + \frac{1}{2} (y_{\tau} - \gamma c^{t-1})^2 + \mu_{\tau-1} c^{t-1} + \mu_{\tau} \gamma c^{t-1}$ yields an updated value $c^{t-1}$. However, this updated value can violate the constraint $c^{t-1} \geq \gamma c^{t-2}$ and we need to update $c^{t-2}$ as well, etc., until we have backtracked some $\Delta t$ steps to time $t' = \tau - \Delta t$ where the constraint $c^{t'} \geq \gamma c^{t'-1}$ is already valid. At most one needs to backtrack to the most recent spike, because $c^{t'} > \gamma c^{t'-1}$ at spike times $t'$ (Eq 5). Solving

\[
\text{minimize} \quad \frac{1}{2} \sum_{t=0}^{\Delta t} (\gamma^t c^{t'} - y_{t'+v})^2 + \sum_{t=0}^{\Delta t} \mu_t \gamma^t c^{t'} \tag{10}
\]

by setting the derivative to zero yields

\[
c^{t'} = \frac{\sum_{t=0}^{\Delta t} (y_{t'+v} - \mu_t \gamma^t) c^{t'}}{\sum_{t=0}^{\Delta t} \gamma^t} \tag{11}
\]

and the next values are updated according to $c^{t'+1} = \gamma^t c^{t'}$ for $t = 1, \ldots, \Delta t$. Note the similarity of Eq 7 and 11, which differs by weighting the summands by powers of $\gamma$ due to the altered
Figure 2: Illustration of OASIS for an AR(1) process (see Supplementary Video). Red lines depict true spike times. The shaded background shows how the time points are gathered in pools. The pool currently under consideration is indicated by the blue crosses. A constraint violation is encountered for the second time step (A) leading to backtracking and merging (B). The algorithm proceeds moving forward (C-E) until the next violation occurs (E) and triggers backtracking and merging (F-G) as long as constraints are violated. When the most recent spike time has been reached (G) the algorithm proceeds forward again (H). The process continues until the end of the series has been reached (I). The solution is obtained and pools span the inter-spike-intervals.

constraints, and by subtracting \( \mu \) from the data \( y \) due to the sparsity penalty. (Along the way it is worth noting that, because a spike induces a calcium response described by kernel \( h \) with components \( h_{1:t} = \gamma^t, c'_t \) could be expressed in the more familiar regression form as \( \frac{h_{1:t+1}^\top (y - \mu_1:t)}{h_{1:t+1}^\top h_{1:t+1}} \), where we used the notation \( v_{i:j} \) to describe a vector formed by components \( i \) to \( j \) of \( v \).) Now one moves forward again (Fig 2C-E) until detection of the next violation (Fig 2E), backtracks again to the most recent spike (Fig 2G), etc. Once the end of the time series is reached (Fig 2I) we have found the optimal solution and set \( \hat{c} = c' \).

While this yields a valid algorithm, it frequently updates each value \( c'_t \) and recalculates the full sums in Eq (11) for each step of backtracking. A similar algorithm has been suggested by [31] for the problem without sparsity penalty. However, it passes through the time series in reverse direction, from its end to its beginning, and is thus not applicable to online processing. It considers directly the deconvolved activity \( s \) and efficiently does not update all time steps but only suspected spike times. However, their algorithm uses the inefficient updates of Eq 11, rendering it an \( O(T^2) \) algorithm.

In order to obtain a true \( O(T) \) algorithm, as in PAVA, we introduce pools which are now tuples of the form \((v_i, w_i, t_i, l_i)\) with value \( v_i \), weight \( w_i \), event time \( t_i \) and pool length \( l_i \). Here we explicitly track the pool length, which was identical to its weight for PAVA. Initially there is a pool \((y_1 = \mu_1, 1, 1)\) for each time step \( t \). During backtracking pools get combined and only the first value \( v_i = c'_t \) is explicitly considered, while the other values are merely defined implicitly via \( c'_{t+1} = \gamma c'_t \). The constraint \( c'_t + 1 \geq \gamma c'_t \) translates to \( v_{i+1} \geq \gamma v_i \) as the criterion determining whether pools need to be combined. The introduced weights allow efficient value updates whenever pools are merged by avoiding recalculating the sums in Eq (11). Values are updated according to

\[
v_i \leftarrow \frac{w_i v_i + \gamma v_i w_{i+1} v_{i+1}}{w_i + \gamma v_i w_{i+1}}
\]  

(12)

where the denominator is the new weight of the pool and the pool lengths are summed

\[
w_i \leftarrow w_i + \gamma v_i w_{i+1}
\]  

(13)

\[
l_i \leftarrow l_i + l_{i+1}
\]  

(14)

Whenever pools \( i \) and \( i+1 \) are merged, former pool \( i+1 \) is removed and the succeeding pool indices decreased by 1. It is easy to prove by induction that the updates according to Eqs (12-14) guarantee...
that Eq (11) holds for all values (see Supplementary Material). Analogous to PAVA, the updates solve Eq (9) not just greedily but optimally, finding the exact solution to the convex problem in \( O(T) \). The final algorithm is summarized in Algorithm 2 and illustrated in Fig 2 as well as in the Supplementary Video. Comparing Algorithm 1 with 2 clearly reveals the modifications made and shows that for \( \gamma = 1 \) and \( \lambda = 0 \) the algorithm reduces to PAVA.

**Algorithm 2** Fast online deconvolution algorithm for AR1 processes with positive jumps

**Require:** data \( y \), decay factor \( \gamma \), regularization parameter \( \lambda \)

1: initialize pools as \( P = \{ (v_i, w_i, t_i, l_i) \}^T_{i=1} \leftarrow \{ (y_t - \lambda(1 - \gamma + \gamma \delta_{i,T}), 1, t, 1) \}^T_{t=1} \)

2: \( i \leftarrow 1 \)

3: while \( i < |P| \) do ▶ iterate until end

4: \quad while \( i < |P| \) and \( v_{i+1} \geq \gamma^i v_i \) do \( i \leftarrow i + 1 \) ▶ move forward

5: \quad if \( i == |P| \) then break

6: \quad while \( i > 0 \) and \( v_{i+1} < \gamma^i v_i \) do ▶ track back

7: \quad \( P_i \leftarrow \left( \frac{v_{i+1}}{w_i + \gamma^{2i-1} w_{i+1}}, w_i + \gamma^{2i} w_{i+1}, t_i, l_i + l_{i+1} \right) \) ▶ Eqs (12-14)

8: \quad remove \( P_{i+1} \)

9: \quad \( i \leftarrow i - 1 \)

10: \( i \leftarrow i + 1 \)

11: for \( (v, w, t, l) \) in \( P \) do ▶ construct solution for all \( t \)

12: \quad for \( \tau = 0, \ldots, l - 1 \) do \( \hat{c}_{\tau+t} \leftarrow \gamma^\tau \max(0, v) \) ▶ enforce \( \hat{c}_t \geq 0 \) via max

13: return \( \hat{c} \)

### 3.2 Dual formulation with hard noise constraint

The formulation above contains a troublesome free sparsity parameter \( \lambda \) (implicit in \( \mu \)). A more robust deconvolution approach eliminates it by inclusion of the residual sum of squares (RSS) as a hard constraint and not as a penalty term in the objective function [13]. The expected RSS satisfies \( \| c - y \|^2 = \sigma^2 T \) and by the law of large numbers \( \| c - y \|^2 = \sigma^2 T \) with high probability, leading to the constrained problem

\[
\min_{\hat{c}} \| \hat{s} \|_1 \quad \text{subject to} \quad \hat{s} = G\hat{c} \geq 0 \quad \text{and} \quad \| \hat{c} - y \|^2 \leq \sigma^2 T. \tag{15}
\]

(As noted above, we estimate \( \sigma \) using the power spectral estimator described in [13]; see also [8] for a similar approach.)

We will solve this problem by increasing \( \lambda \) in the dual formulation until the noise constraint is tight. We start with some small \( \lambda \), e.g. \( \lambda = 0 \), to obtain a first partitioning into pools \( P \), cf. Fig 4A below. From Eqs (11-13) along with the definition of \( \mu \) (Eq 9) it follows that given the solution \( (v_i, w_i, t_i, l_i) \), where

\[
v_i = \sum_{t=0}^{l_i-1} \frac{(y_{i+t} - \mu_{i+t}) \gamma^t}{\sum_{i=0}^{l_i-1} \gamma^{2i}} = \sum_{t=0}^{l_i-1} \frac{(y_{i+t} - \lambda(1 - \gamma + \gamma \delta_{i+1,T}) \gamma^t}{w_i}
\]

for some \( \lambda \), the solution \( (v'_i, w'_i, t'_i, l'_i) \) for \( \lambda + \Delta \lambda \) is

\[
v'_i = v_i - \Delta \lambda \sum_{t=0}^{l_i-1} \frac{(1 - \gamma + \gamma \delta_{i+1,T}) \gamma^t}{w_i} = v_i - \Delta \lambda \frac{1 - \gamma^{l_i}(1 - \delta_{i+l})}{w_i}
\]

where \( z \) is the index of the last pool and because pools are updated independently we make the approximation that no changes in the pool structure occur. Inserting Eq (17) into the noise constraint (Eq 15) results in

\[
\sum_{i=1}^{z} \sum_{t=0}^{l_i-1} \left( \frac{v_i - \lambda \frac{1 - \gamma^{l_i}(1 - \delta_{i+l})}{w_i} \gamma^t - y_{i+t}}{w_i} \right)^2 = \sigma^2 T
\]

and solving the quadratic equation for \( \Delta \lambda \) yields

\[
\Delta \lambda = \frac{-\beta + \sqrt{\beta^2 - 4\alpha \epsilon}}{2\alpha}
\]

7
with $\alpha = \sum_{i,t} \xi_{it}^2$, $\beta = 2 \sum_{i,t} \chi_{it} \xi_{it}$ and $\epsilon = \sum_{i,t} \chi_{it}^2 - \hat{\sigma}^2 T$ where $\xi_{it} = \frac{1-\gamma^t (1-\delta_{iz})}{w_i} \gamma^t$ and $\chi_{it} = y_{i,t} - v_i \gamma^t$.

The solution $\Delta \lambda$ provides a good approximate proposal step for updating the pool values $v_i$ (using Eq.7). Since this update proposal is only approximate it can give rise to violated constraints (e.g., negative values of $v_i$). To satisfy all constraints Algorithm 2 is run to update the pool structure, cf. Fig 4C, but with a warm start: we initialize with the current set of merely $z$ pools $P^*$ instead of the $T$ pools for a cold start (Alg 2 line 1). This step returns a set of $v_i$ values that satisfy the constraints and may merge pools (i.e., delete spikes); then the procedure (update $\lambda$ then rerun the warm-started Algorithm 2) can be iterated until no further pools need to be merged, at which point the procedure has converged. In practice this leads to an increasing sequence of $\lambda$ values (corresponding to an increasingly sparse set of spikes), and no pool-split (i.e., add-spike) moves are necessary. (Note that it is possible to cheaply detect any violations of the KKT conditions in a candidate solution; if such a violation is detected, the corresponding pool could be split and the warm-started Algorithm 2 run locally near the detected violations. However, as we noted, due to the increasing $\lambda$ sequence we did not find this step to be necessary in the examples examined here.)

This warm-starting approach brings major speed benefits: after the residual is updated following a $\lambda$ update, the computational cost of the algorithm is linear in the number of pools $z$, hence warm starting drastically reduces computational costs from $k_1 T$ to $k_2 z$ with proportionality constants $k_1$ and $k_2$: if no pool boundary updates are needed then after warm starting the algorithm only needs to pass once through all pools to verify that no constraint is violated, whereas a cold start might involve a couple passes over the data to update pools, so $k_2$ is typically significantly smaller than $k_1$, and $z$ is typically much smaller than $T$ (especially in sparsely-spikeing regimes).

### 3.3 Additional baseline

For ease of exposition we thus far assumed no offsetting baseline. Adding a known baseline $b \neq 0$ the problem reads

$$\min_{\beta, \epsilon} \frac{1}{2} \|b1 + \hat{c} - y\|^2 + \lambda \|\hat{s}\|_1 \quad \text{subject to} \quad \hat{s} = G\hat{c} \geq 0. \quad \text{(20)}$$

For known baseline one merely needs to initialize OASIS by subtracting not only the sparsity parameter $\mu(\lambda)$ from the data $y$, cf. Eq (11) and Algorithm 2 but also the baseline $b$. The fluorescence $\hat{c}$ depends only on the sum $\phi = b1 + \mu$.

If the baseline is not known, we want to optimize it too by solving the noise constrained dual problem

$$\min_{b, \epsilon} \|\hat{s}\|_1 \quad \text{subject to} \quad \hat{s} = G\hat{c} \geq 0 \quad \text{and} \quad \|b1 + \hat{c} - y\|^2 \leq \hat{\sigma}^2 T. \quad \text{(21)}$$

We denote all except the differing last component of $\mu$ by $\mu = \lambda(1 - \gamma)$ (Eq 8) and of $\phi$ by $\phi = b + \lambda(1 - \gamma)$. $\phi$ is the total shift applied to the data (except for the last time step) due to the baseline and sparsity penalty before running OASIS. We increase $\phi$ until the noise constraint is tight. $\phi$ can be initialized by $\min y_t$ or better by a small percentile of $y$, e.g. 15%. Once OASIS has been run with some $\phi$ the baseline $b$ is obtained by minimizing the objective (20) with respect to it, yielding $\hat{b} = \langle y - \hat{c} \rangle = \frac{1}{T} \sum_{t=1}^{T} (y_t - \hat{c}_t)$, and the sparsity parameter is $\mu = \phi - \hat{b}$. We could hold the obtained $\hat{b}$ fixed and use Eq (18) with appropriately added $\hat{b}$

$$\sum_{i=1}^{z} \sum_{t=0}^{T-1} \left( v_i - \Delta \phi \frac{1 - \gamma^t (1 - \delta_{iz})}{(1 - \gamma)w_i} \right) \gamma^t - y_{i,t+1} + \hat{b} = \hat{\sigma}^2 T \tag{22}$$

To obtain $\Delta \phi$ and update all pools according to $v'_i = v_i - \Delta \phi \frac{1 - \gamma^t (1 - \delta_{iz})}{(1 - \gamma)w_i}$, cf. Eq (17). To satisfy all constraints Algorithm 2 is run, warm-started by initializing with the current set of pools. Without optimizing the baseline $\hat{b}$ the resulting RSS would be close to $\hat{\sigma}^2 T$ by construction, however optimizing it yields a reduction in the RSS, if the baseline has not already converged. While feasible, updating the coordinates $\phi$ and $\hat{b}$ sequentially takes small steps $\Delta \phi$ and requires more ($\approx$5-6 instead 2-3) iterations to converge. A better estimate of $\Delta \phi$ is obtained using a block coordinate update of $\phi$ and $\hat{b}$ by plugging the analytic expression $\hat{b} = \frac{1}{T} \sum_{t=1}^{T} (y_t - \hat{c}_t) = \frac{1}{T} \sum_{t=1}^{T} \left( w_i \right) \gamma^t x_{i,t}^{(t)}$ into Eq (18).
We have presented methods to estimate the hyperparameters $\lambda$, $b$, and $\gamma$, which require a handful of warm-started iterations of OASIS. To gain further speed-ups these parameters can be estimated on decimated data. When downsampling by a factor $k$, the average of $k$ subsequent frames is calculated, the noise $\sigma$ divided by a factor $\sqrt{k}$ and the initial estimate of the AR coefficient scaled to $\hat{\gamma}^k$. Alternatively, one could estimate $\sigma$ and $\gamma$ based on the decimated data. Once the hyperparameters have been obtained, the corresponding inverse transformations are performed: $\hat{\gamma} \rightarrow \hat{\gamma}^k$, $\hat{b} \rightarrow \hat{b}$ and $\lambda \rightarrow \lambda\frac{1-\hat{\gamma}}{1-\hat{\gamma}^k}$ such that the shrinkage $\mu = \lambda(1-\hat{\gamma})$ due to the penalty term stays invariant. The final run of OASIS on the full data is warm started using the solution obtained on the decimated data. Data points that are not in the proximity of a spike of the downsampled solution are already combined into large pools, instead of initializing each data point as its own separate pool. More precisely, if the deconvoluted decimated data has positive values at times $\{t_i\}$, for deconvolving the full data time steps $\bigcup \{k\mathcal{I}, k\mathcal{I}+1, ..., k\mathcal{I}+\mathcal{I}\}$ are initialized as individual pools, while the remaining time steps are pooled together into bigger pools, separated from each other by the individual ones, with values given by Eq (11) and weights by its denominator.

In particular the estimation of the AR coefficient $\gamma$ is computationally burdensome, because it involves expensive repeated evaluations of the RSS in order to minimize it as function of $\hat{\gamma}$ (and $\hat{b}$). The computing time depends linearly on the number of pools $z$ and we gain further speed-ups by restricting the attention to merely a subset of pools. In particular, because $\gamma$ can be well estimated based on large isolated calcium events, we restrict the calculation of the RSS to the pools with largest product of value and length. A large value indicates a large event and a long pool an isolated event. It is also worth noting that the hyperparameter estimation discussed above is performed in ‘batch’ mode, not online. However, once good hyperparameter values are obtained on a short initial batch we can switch into online mode (with the hyperparameters held fixed) and handle the remaining data in a stream.

### 3.4 Optimizing the AR coefficient

Thus far the parameter $\gamma$ has been known or been estimated based on the autocovariance function. We can improve upon this estimate by optimizing $\hat{\gamma}$ as well, which is illustrated in Fig 4. After updating $\lambda$ (and $\hat{b}$) followed by running Algorithm 2 we perform a coordinate descent step in $\hat{\gamma}$ that minimizes the RSS, cf. Fig 4. The RSS as a function of $\hat{\gamma}$ is a high order polynomial, cf. Eq (11), and we need to settle for numerical solutions. We used Brent’s method with bounds $0 < \hat{\gamma} < 1$. One iteration consists now of steps B-E in Fig 4 while for known $\gamma$ only B-C were necessary. If optimizing the baseline too, we obtained better results by minimizing the RSS jointly with respect to $\hat{\gamma}$ and $\hat{b}$ using L-BFGS-B instead of keeping the baseline $\hat{b}$ fixed.

### 3.5 Faster optimization of hyperparameters

We have presented methods to estimate the hyperparameters $\lambda$, $b$ and $\gamma$, which require a handful of warm-started iterations of OASIS. To gain further speed-ups these parameters can be estimated on decimated data. When downsampling by a factor $k$, the average of $k$ subsequent frames is calculated, the noise $\sigma$ divided by a factor $\sqrt{k}$ and the initial estimate of the AR coefficient scaled to $\hat{\gamma}^k$. Alternatively, one could estimate $\sigma$ and $\gamma$ based on the decimated data. Once the hyperparameters have been obtained, the corresponding inverse transformations are performed: $\hat{\gamma} \rightarrow \hat{\gamma}^k$, $\hat{b} \rightarrow \hat{b}$ and $\lambda \rightarrow \lambda\frac{1-\hat{\gamma}}{1-\hat{\gamma}^k}$ such that the shrinkage $\mu = \lambda(1-\hat{\gamma})$ due to the penalty term stays invariant. The final run of OASIS on the full data is warm started using the solution obtained on the decimated data. Data points that are not in the proximity of a spike of the downsampled solution are already combined into large pools, instead of initializing each data point as its own separate pool. More precisely, if the deconvoluted decimated data has positive values at times $\{t_i\}$, for deconvolving the full data time steps $\bigcup \{k\mathcal{I}, k\mathcal{I}+1, ..., k\mathcal{I}+\mathcal{I}\}$ are initialized as individual pools, while the remaining time steps are pooled together into bigger pools, separated from each other by the individual ones, with values given by Eq (11) and weights by its denominator.

In particular the estimation of the AR coefficient $\gamma$ is computationally burdensome, because it involves expensive repeated evaluations of the RSS in order to minimize it as function of $\hat{\gamma}$ (and $\hat{b}$). The computing time depends linearly on the number of pools $z$ and we gain further speed-ups by restricting the attention to merely a subset of pools. In particular, because $\gamma$ can be well estimated based on large isolated calcium events, we restrict the calculation of the RSS to the pools with largest product of value and length. A large value indicates a large event and a long pool an isolated event. We present detailed results in the Results section, indicating that altogether we can save about an order of magnitude computation with the greatest savings obtained by reducing the optimization of $\hat{\gamma}$ from $O(z)$ to $O(1)$.

It is also worth noting that the hyperparameter estimation discussed above is performed in ‘batch’ mode, not online. However, once good hyperparameter values are obtained on a short initial batch we can switch into online mode (with the hyperparameters held fixed) and handle the remaining data in a stream.

### 3.6 Hard shrinkage and $\ell_0$ penalty

It is well-known that $\ell_1$ penalization results in “soft-thresholding”, in which small values are zeroed out and large values are shifted to lower values (where the size of this shift is proportional to the penalty $\lambda$). We can perform hard instead of soft thresholding (avoiding this shrinkage of...
While the updates resemble those of matching pursuit [35], in practice we found that adding spikes at
µ
An AR(1) process models the calcium response to a spike as an instantaneous increase followed by
s
As is the case with all optimized hyperparameters, once we have obtained a decent estimate of
not Markovian any more and the next value depends not only on the current but on possibly multiple
decay time constants) can also be modeled with higher values for the order
an exponential decay. This is a good description when the fluorescence rise time constant is small
compared to the length of a time-bin, e.g. when using GCaMP6f [36] with a slow imaging rate. For
fast imaging rates and slow indicators such as GCaMP6s it is more accurate to explicitly model

\[
\text{minimize } \frac{1}{2} \| \hat{c} - y \|^2 \quad \text{subject to } \hat{s} = G\hat{c} \text{ with } \hat{s}_i \geq s_{\text{min}} \text{ or } \hat{s}_i = 0 \quad (24)
\]

is non-convex and we are not guaranteed to find the global minimum. However, we obtain a good local
minimum by merely changing the condition to merge pools from \(v_{t+1} < \gamma v_t\) to \(v_{t+1} < \gamma v_t + s_{\text{min}}\),
modifying lines 3 and 5 in Algorithm 2.

Now we must choose a value for \(s_{\text{min}}\). In many cases we found that simply setting \(s_{\text{min}}\) as a small
multiple of the noise level led to good results. If the scaling factor \(\alpha\) (Eq 2) relating fluorescence to
action potentials was known, we could properly normalize the spike train such that \(\hat{s}_i = 1\) corresponds
to one spike and choose \(s_{\text{min}} = 0.5\), or a slightly higher value to avoid splitting one spike into two
of size 0.5. However, the factor is often unknown or difficult to estimate, rendering the choice of
\(s_{\text{min}}\) cumbersome. Analogous to the variation of \(\lambda\), we can start with \(s_{\text{min}} = 0\) and increase it until
the RSS crosses the \(\sigma^2 T\) threshold by sequentially removing the smallest ‘spike’ and merging the
pools it used to separate. By maximizing \(s_{\text{min}}\) under the noise constraint we minimize the number of
non-zero values of \(\hat{s}\). De facto, we try to find a parsimonious description of the data by minimizing
the number of non-zero values of \(\hat{s}\), thus solving a sparsity problem with \(\ell_0\) penalty:

\[
\text{minimize } ||\hat{s}||_1 \quad \text{subject to } \hat{s} = G\hat{c} \geq 0 \quad \text{and } ||\hat{c} - y||^2 \leq \sigma^2 T \quad (25)
\]

Instead of sequentially removing the smallest ‘spike’ we actually obtained the best performance by
sequentially adding spikes at the highest values of the \(\ell_1\)-solution \(\hat{s}\) until the RSS is smaller than \(\hat{\sigma}^2 T\).
While the updates resemble those of matching pursuit [55], in practice we found that adding spikes at
the positions suggested by the \(\ell_1\)-solution yields better results than matching pursuit (which adds
spikes at positions that greedily lead to the highest RSS reduction per step). Specifically, we found
that often matching pursuit cannot resolve spikes in close proximity, but instead results in erroneous
placement of one big spike as an explanation for all nearby spikes. Instead of merging pools we now
need to split pools. Denoting the time where to add a spike by \(t_s\), i.e. the time where the \(\ell_1\)-solution
has its highest value after ruling out times where spikes have already been added, one searches for the
pool \(i\) in which it falls, i.e. \(t_i < t_s < t_i + l_i\). Pool \(i\) gets updated as \(l'_i = t_s - t_i\), \(w'_i = \sum_{t=0}^{l'_i-1} \gamma^{2t}\), and
\(v'_i = \sum_{t=0}^{l'_i-1} y_{t+t_i} \gamma^t / w'_i\), which follows directly from Eq (11) with \(\mu_t = 0\). All pool indices greater
than \(i\) are increased by one and a new pool is inserted after pool \(i\) with \(l'_{i+1} = l_i - l'_i\), \(l'_{i+1} = t_s\),
\(w'_{i+1} = \sum_{t=0}^{l'_{i+1}-1} \gamma^{2t}\), and \(v'_{i+1} = \sum_{t=0}^{l'_{i+1}-1} y_{t+t_s} \gamma^t / w'_{i+1}\).

As is the case with all optimized hyperparameters, once we have obtained a decent estimate of \(s_{\text{min}}\)
on an initial subset of the data we can switch back into online mode. In online mode our algorithm is
typically faster than matching pursuit, since matching pursuit requires updating \(O(\Delta)\) points of the
residual with each update, where \(\Delta\) is the length of the calcium transient (in number of frames).

4 Generalization beyond the AR(1) case

4.1 A greedy solution for the AR(\(p > 1\)) processes

An AR(1) process models the calcium response to a spike as an instantaneous increase followed by
an exponential decay. This is a good description when the fluorescence rise time constant is small
compared to the length of a time-bin, e.g. when using GCaMP6f [36] with a slow imaging rate. For
fast imaging rates and slow indicators such as GCaMP6s it is more accurate to explicitly model
the finite rise time. Typically we choose an AR(2) process, though more structured responses (e.g.
multiple decay time constants) can also be modeled with higher values for the order \(p\).

For an AR(\(p\)) process the sparsity penalty \(\lambda ||\hat{s}||_1\) can again be expressed as \(\mu^\top \hat{c}\), because

\[
\lambda ||\hat{s}||_1 = \lambda \mathbf{1}^\top \hat{s} = \lambda \sum_{t=1}^{T} \sum_{k=1}^{T} G_k \hat{c}_t = \lambda \sum_{t=1}^{T} (1 - \sum_{i=1}^{\min(p,T-t)} \gamma_i) \hat{c}_t = \sum_{t=1}^{T} \mu_t \hat{c}_t = \mu^\top \hat{c},
\]

with \(\mu_t := \lambda (1 - \sum_{i=1}^{\min(p,T-t)} \gamma_i)\), by evaluating the column sums of \(G\). For \(p > 1\) the dynamics are
not Markovian any more and the next value depends not only on the current but on possibly multiple
previous time steps. Due to the non-Markovian dynamics, following along the lines of the previous section just leads to a greedy, approximate solution; we will present an exact algorithm later. We use matrix- and vector notation to describe the dynamics of \( c_t \). Let the transition matrix \( A \), multi time step calcium vectors \( \zeta_t \), and vector \( e \) be defined as

\[
A = \begin{pmatrix}
\gamma_1 & \gamma_2 & \cdots & \gamma_p \\
1 & 0 & \cdots & 0 \\
\vdots & \vdots & \ddots & \vdots \\
0 & \cdots & \cdots & 1
\end{pmatrix} \quad \zeta_t = \begin{pmatrix}
c_t \\
c_{t-1} \\
\vdots \\
c_{t-p+1}
\end{pmatrix} \quad e = \begin{pmatrix}
1 \\
0 \\
0 
\end{pmatrix}
\]

(27)

The calcium dynamics is given by \( \zeta_t = A\zeta_{t-1} + s_t e \). Analogously to the AR(1) case we derive an algorithm that moves through the time series until it finds a violation of the constraint \( c_t^2 \geq e^T A\zeta_{t-1}^2 \) for some time \( \tau \), updates \( c_t \) and \( c_{t-1} \), and backtracks further until the updates do not violate any constraints at previous time steps. Note that we also implicitly have constraints on \( \zeta_t \), enforcing the fact that \( \zeta_{t+1} \) is mostly a time-shifted version of \( \zeta_t \).

Assuming we need to backtrack by \( \Delta \tau \) steps and introducing again \( t' = \tau - \Delta \tau \), the objective is to minimize \( \sum_{t=t'}^{t'}(e^T A\zeta_t' - y_{t+t})^2 + \frac{\Delta \tau}{\mu} e^T A\zeta_t' \) with respect to \( c_{t'} \) under the active constraints \( \zeta_t = A\zeta_{t-1} \) for \( t = t' + 1, \ldots, \tau \). Plugging in the constraints on the dynamics the objective reads

\[
\text{minimize} \quad \frac{\Delta \tau}{2} \sum_{t=0}^{\Delta \tau} (e^T A\zeta_t' - y_{t+t})^2 + \sum_{t=0}^{\Delta \tau} \mu_t e^T A\zeta_t'
\]

(28)

Setting the derivative with respect to \( c_{t'} \) to zero and solving for \( c_{t'} \) yields

\[
c_{t'} = \frac{\sum_{t'=0}^{\Delta \tau} (y_{t+t'} - \mu_{t+t'} - \sum_{k=2}^p (A^t)_{1,k} c_{t',1-k}) (A^t)_{1,1}}{\sum_{t'=0}^{\Delta \tau} (A^t)_{1,1}^2}
\]

(29)

where \((A^t)_{1,1}^2\) denotes the square of the entry in the first row and column in the matrix obtained as \( t \)-th matrix power of \( A \). Again, note that these entries describe the calcium kernel \( h \) with components \( h_{1+t} = (A^t)_{1,1} \). Eq (29) reduces to Eq (11) for \( p = 1 \) where \( A \) is just a \( 1 \times 1 \)-matrix with entry \( \gamma \). The next values are updated according to \( c_{t'+1} = \sum_{k=1}^p \gamma_k c_{t'+1-k} \) for \( t = 1, \ldots, \Delta \tau \).

We derive again an efficient formulation of the algorithm using pools. Considering the denominator in Eq (29) as a weight in analogy to the AR(1) case and calculating the weighted sum upon merging of pools is not valid for \( p > 1 \) because in general \((A^t)_{1,1}(A^u)_{1,1} \neq (A^{t+u})_{1,1}\). Introducing pools is still useful as it allows us to keep track of only a small number of \( p \) elements in each pool. While for the case of AR(1) we only kept track of each group’s first element, we now keep track of the first as well as the \( p - 1 \) last elements. In order to speed up the update in Eq (29), we can precompute the powers of \( A \) and store \((A^t)_{1,1}\) in memory. Note that only the powers up to the maximal inter-spike-interval are needed, which can be much smaller than \( T \); of course, for very large values of \( t \), \((A^t)_{1,1} \approx 0 \), by the stability of \( A \); thus for high powers the entries of \((A^t)_{1,1}\) can also be well approximated by a quickly computable exponential function or simply be truncated. Analogous to the case \( p = 1 \), we can also impose a constraint on the minimum spike size \( s_{\text{min}} \) at the expense of having to deal with a non-convex problem by merely changing the condition to merge pools from \( v_{i+1} < (A^t)_{1,1} v_i + (A^t)_{1,2} u_{i-1} \) to \( v_{i+1} < (A^t)_{1,1} v_i + (A^t)_{1,2} u_{i-1} + s_{\text{min}} \) where \( v_i \) and \( u_i \) denote the first and last value of pool \( i \).

According to Eq (29) the solution is a linear function of \( \mu \), and hence of \( \lambda \). Thus the hard noise constraint for the RSS \( \|e - y\|^2 = \sigma^2 T \) is a quadratic equation in \( \lambda \), that can be solved analytically, under the assumption of invariant pool structure analogous to above case of AR(1), but involving more lengthy expressions which we do not state explicitly. Updating all pools independently according to Eq (29) can give rise to violated constraints, requiring us to rerun the algorithm, warm-started by initializing with the current set of pools, as described above. After 2-3 iterations no pools need to be merged and the final solution has been found. We can again interleave an update step for optimizing the parameters \( \gamma_t \), as described above.

4.2 Online non-negative least squares (ONLNS)

We noted above that Eq (29) is non-Markovian: it includes a dependency on \( p - 1 \) previous time steps and hence in general the previous pool. In updating only the first value within a pool and using the current values of the \( p - 1 \) last values of the previous pool within the update Eq (29), we actually
We begin by reformulating the problem as was not necessary.) Further speedups can be obtained by restricting the set of possible spike times $s_t$ would be set smaller) and applying NNLS while holding the values of $A$ constant. A classic algorithm for solving a NNLS problem is the active set method of [38] and [37]. This algorithm alternates between normal equation matrix solves involving sub-matrices of $K^\top K$ and $K^\top$. The function NNLS implements a minor variation of the classic algorithm of [37] to solve $\min_{\hat{s} \in \mathbb{R}_+^n} \|y - K\hat{s}\|^2$:

$$K^\top K \text{ and } K^\top y \text{ are precomputed outside the function, to exploit that NNLS is called several times with the same } K. \text{ Further } \hat{s} \text{ is warm-started instead of initializing it as } 0.$$

performed greedy updates. These greedy updates can yield remarkably good results, in particular for long pools, such that the last value is already well constrained by a number of data points and hardly affected by the next pool. Nonetheless, in some cases these greedy updates lead to errors in the timing of inferred activity, in particular when the rise time of the calcium response is slow compared to the frame rate. The method described in this section can be used to correct these small errors.

We begin by reformulating the problem as

$$\min_\hat{s} \|K \hat{s} - y\|^2 + \lambda \|\hat{s}\|_1 \quad \text{subject to } \hat{s} \geq 0 \tag{30}$$

where $K = G^{-1}$ is the convolution matrix with entries $K_{t,u} = h_{t+u}$ if $t \geq u$ else zero; the kernel vector $h$ can be taken as an arbitrary response kernel for most of the development in this section. As noted earlier, $h_{t+u} = (A^\top)_{1,1}$ for the special case of an AR process. As we have seen previously, the effect of the sparsity penalty (together with the non-negative constraint) is to shift the data down by a vector $\mu = \lambda K^{-1} 1$, and the problem reduces to a non-negative least squares (NNLS) problem.

$$\min_\hat{s} \|K \hat{s} - (y - \lambda K^{-1} 1)\|^2 \quad \text{subject to } \hat{s} \geq 0. \tag{31}$$

(Note that the gradient of Eq (31) is the same as the gradient of Eq (30), $K^\top (K \hat{s} - (y - \lambda K^{-1} 1)) = K^\top (K \hat{s} - y) + \lambda 1$. In addition, $K$ is triangular with positive numbers on the main diagonal, hence $\det K > 0$ and $K$ is invertible.)

A classic algorithm for solving a NNLS problem is the active set method of [38] and [37]. This algorithm alternates between normal equation matrix solves involving sub-matrices of $K^\top K$ and updates of the active set. A naive application of this algorithm would scale cubically with the number of spikes. Instead, we exploit the locality of the problem (the fact that changing a spike height at time $t$ does not affect the solution at very distant times $s$) and apply the NNLS algorithm in the inner loop of a sequential coordinate block descent method. Specifically, we apply warm-started NNLS on blocks of size $\Delta$ (where $\Delta$ is the length of the calcium transient), stepping the block in steps of size $\Delta_m < \Delta$ (we found $\Delta_m = \frac{\Delta}{2}$ to be effective for offline applications; for online applications $\Delta_m$ would be set smaller) and applying NNLS while holding the values of $s$ outside the block fixed. We further exploit the Toeplitz structure of $K$ to precompute the necessary sub-matrices of $K^\top K$.

The resulting algorithm (Alg[3]) runs in $O(T)$ time. It involves solving a least squares problem for the time points within the considered window where $s_t > 0$; thus it scales cubically with the number of spikes per window and depends on the sparsity of $\hat{s}$. (In fact, for AR(p) models, the required matrix solves can be performed using linear-time (not cubic-time) Kalman filter-smoother methods, but the matrix sizes were sufficiently small in the examples examined here that the Kalman implementation was not necessary.) Further speedups can be obtained by restricting the set of possible spike times (for example, by running the $AR(1)$ version of OASIS on a temporally decimated version of the signal to crudely identify the set of spike times, then never updating $\hat{s}$ away from zero on the complement of this set).
5 Results

5.1 Benchmarking OASIS

We generated datasets of 20 fluorescence traces each for \( p = 1 \) and \( 2 \) with a duration of 100 s at a framerate of 30 Hz, such that \( T = 3,000 \) frames. The spiking signal came from a homogeneous Poisson process. We used \( \gamma = 0.95, \sigma = 0.3 \) for the AR(1) model and \( \gamma_1 = 1.7, \gamma_2 = -0.712, \sigma = 1 \) for the AR(2) model. Figures 3A-C are reassuring that our suggested (dual) active set method yields indeed the same results as other convex solvers for an AR(1) process and that spikes are extracted well. For an AR(2) process OASIS is greedy and yields good results that are similar to the one obtained with convex solvers (lower panels in Fig 3B and C), with virtually identical denoised fluorescence traces (upper panels).

Figures 3D,E report the computation time (±SEM) averaged over all 20 traces and ten runs per trace on a MacBook Pro with Intel Core i5 2.7 GHz CPU. We compared the run time of our algorithm to a variety of state of the art convex solvers that can all be conveniently called from the convex optimization toolbox CVXPY \([39]\): embedded conic solver (ECOS, \([40]\), MOSEK \([41]\), splitting

---

Figure 3: **OASIS produces the same high quality results as convex solvers at least an order of magnitude faster.** (A) Raw and inferred traces for simulated AR(1) data, (B) simulated AR(2) and (C) real data from \([36]\) modeled as AR(2) process. OASIS solves Eq (3) exactly for AR(1) and just approximately for AR(2) processes, nevertheless well extracting spikes. (D) Computation time for simulated AR(1) data with given \( \lambda \) (blue circles, Eq (3)) or inference with hard noise constraint (green x, Eq (15)). GUROBI failed on the noise constrained problem. (E) Computation time for simulated AR(2) data. (F) Normalized computation time of OASIS for simulated AR(1) data with given \( \lambda \) (blue circles, Eq (3)) and inference with hard noise constraint on full data (green x, Eq (15)) or small initial batch followed by processing in online mode (orange crosses).
We have shown that we can solve Eq (3) and Eq (15) faster than interior point methods. The AR
We also extracted each neuron’s fluorescence activity using CNMF from an unpublished whole-brain
We verified these results on real data as well. Running OASIS with the hard noise constraint and
We also ran the algorithms on longer traces up to a length of \( T = 300,000 \) frames (Fig 3F), confirming
that OASIS scales linearly with \( T \), where we obtained a proportionality constant of \( 1 \mu s/\text{frame} \). For
an unknown hyperparameter \( \lambda \) we obtained its value not only on the full data but on an initial small
batch (1,000 frames) and kept it fixed, which sped activity inference up by a factor of three once \( T 
\) is sufficiently large (Fig 3B, orange vs green) without compromising quality (correlation between
deconvolved activity and ground truth spike train \( 0.882 \pm 0.001 vs 0.881 \pm 0.002 \) for \( T = 300,000 \)).
Our active set method maintained its lead by 1-2 orders of magnitude in computing time. Further,
compared to our active set method the other algorithms required at least an order of magnitude more
RAM, confirming that OASIS is not only faster but much more memory efficient. Indeed, because
OASIS can run in online mode the memory footprint can be \( O(1) \), instead of \( O(T) \).
We verified these results on real data as well. Running OASIS with the hard noise constraint and
\( p = 2 \) on the GCaMP6s dataset of 14,400 frames collected at 60 Hz from [36, 44] took \( 0.101 \pm 0.005 \) s per trace, whereas the fastest other methods required \( 2.37 \pm 0.12 \) s. Fig 3C shows the real data together
with the inferred denoised and deconvolved traces as well as the true spike times, which were obtained
by simultaneous electrophysiological recordings [36].
We also extracted each neuron’s fluorescence activity using CNMF from an unpublished whole-brain
zebrafish imaging dataset from the M. Ahrens lab. Running OASIS with hard noise constraint and
\( p = 1 \) (chosen because the calcium onset was fast compared to the acquisition rate of 2 Hz) on 10,000
traces out of a total of 91,478 suspected neurons took 81.5 s whereas ECOS, the fastest competitor,
needed 2,818.1 s. For all neurons we would hence expect 745 s for OASIS, which is below the 1,500 s
recording duration (3,000 frames), and over 25,780 s for ECOS and other candidates.
OASIS solves the non-negative deconvolution problem exactly for an AR(1) process; however, as
discussed above, for \( p > 1 \) the solution is only a good (greedy) approximation. To obtain the exact
solution we ran the ONNLS algorithm on the simulated AR(2) traces using a window size of 200
frames, which was about ten times larger than the fluorescence decay time, and shifting the window
by 100 frames. We obtained higher accuracy results than all the state of the art convex solvers we
compared to, requiring merely \( 27.8 \pm 0.4 \) ms per trace for \( \lambda = 0 \) and \( 20.0 \pm 0.4 \) ms per trace for \( \lambda = 30 \).
the value that ensures that the hard noise constraint is tight. The choice of \( \lambda \) regulated the sparsity of
the solution, which affects the run time of ONNLS. The fastest state of the art convex solver (ECOS)
required \( 305 \pm 9 \) ms and was thus an order of magnitude slower. It took merely \( 8.56 \pm 0.04 \) ms to
obtain an approximate greedy solution using OASIS, independent of the choice of sparsity parameter
\( \lambda \). Though obtaining the exact solution requires more computing time, it is well within the same order
of magnitude. In contrast, running batch NNLS was significantly slower requiring \( 2,430 \pm 53 \) ms for
\( \lambda = 0 \) and \( 1,620 \pm 37 \) ms for \( \lambda = 30 \).

5.2 Hyperparameter optimization
We have shown that we can solve Eq (3) and Eq (15) faster than interior point methods. The AR
coefficient \( \gamma \) was either known or estimated based on the autocorrelation in the above analyses. The
latter approach assumes that the spiking signal comes from a homogeneous Poisson process, which
does not generally hold for realistic data. Therefore we were interested in optimizing not only
the sparsity parameter \( \lambda \), but also the AR(1) coefficient \( \gamma \). To illustrate the optimization of both,
we generated a fluorescence trace with spiking signal from an inhomogeneous Poisson process
with sinusoidal instantaneous firing rate (Fig 3), thus mimicking realistic data. We conservatively
initialized \( \gamma \) to a small value of 0.9. The value obtained based on the autocorrelation was \( 0.9792 \)
and larger than the true value of 0.95. The left panels in Figures 4B and D illustrate the update of \( \lambda 
\) from the previous value \( \lambda^- \) to \( \lambda^+ \) by solving a quadratic equation analytically (Eq 15) and the update
of \( \gamma \) by numerical minimization of a high order polynomial respectively. Note that after merely
one iteration (Fig 4E) a good solution is obtained and after three iterations the solution is virtually
identical to the one obtained when the true value of \( \gamma \) has been provided (Fig 4F). This holds not only
visually, but also when judged by the correlation between deconvolved activity and ground truth spike
Figure 4: Optimizing sparsity parameter $\lambda$ and AR coefficient $\hat{\gamma}$. (A) Running the active set method, with conservatively small estimate $\hat{\gamma}$, yields an initial denoised estimate (blue) of the data (yellow) roughly capturing the truth (red). We also report the correlation between the deconvolved estimate and true spike train as a direct measure for the accuracy of spike train inference. (B) Updating sparsity parameter $\lambda$ according to Eq (18) such that $\text{RSS} = \sigma^2 T$ (left) shifts the current estimate downward (right, blue). (C) Running the active set method enforces the constraints again and is fast due to warm-starting. (D) Updating $\hat{\gamma}$ by minimizing the polynomial function $\text{RSS} (\hat{\gamma})$ and (E) running the warm-started active set method completes one iteration, which yields already a decent fit. (F) A few more iterations improve the solution further and the obtained estimate is hardly distinguishable from the one obtained with known true $\gamma$ (turquoise dashed on top of blue solid line). Note that determining $\hat{\gamma}$ based on the autocovariance (purple) yields a crude solution that even misses spikes (at 24.6 s and 46.5 s).

train, which was 0.869 compared to merely 0.773 if $\hat{\gamma}$ was obtained based on the autocorrelation. The optimization was robust to the initial value of $\hat{\gamma}$, as long as it was positive and not, or only marginally, greater than the true value. The value obtained based on the autocorrelation was considerably greater and partitioned the time series into pools in a way that missed entire spikes.

After illustrating the hyperparameter optimization we next quantify the computing time and quality of spike inference for various optimization scenarios. We generated 20 fluorescence traces with sinusoidal instantaneous firing rate as used in the illustration (Fig 4), again having a duration of 100 s at a framerate of 30 Hz, such that $T = 3,000$ frames, however we offset the data by an additional positive baseline $b$ that can be present in real data. This baseline can be optimized together with the sparsity parameter $\lambda$, as shown in Methods (subsection “Additional baseline”). The fastest deconvolution method is to merely estimate all parameters and run OASIS just once, cf. first row in Table 1 which shows the mean (±SEM) for computing time as well as correlation of the inferred spike train. As a baseline estimate we used the 15th percentile of the fluorescence trace. The sparsity penalty was set to $\lambda = 0$. A better choice of $\lambda$ is actually obtained by optimizing it, such that the hard noise constraint $\|b1 + \hat{c} - y\|^2 = \hat{\sigma}^2 T$ holds, cf. second row in Table 1. The next rows show that optimizing $b$ further improves the result, as does adding $\gamma$. However, the increased number of optimized parameters results in extra computational cost. The computation time can be reduced by estimating $\gamma$ not using the full data but only a limited number of pools, which does not affect the quality of the result, cf. row five and six in Table 1. Note that by restricting the optimization to a fixed number of pools, its computational load does not increase with the duration of the recording, hence the gain would be even more dramatic for longer time series. Further speed ups are obtained by estimating the parameters on a decimated version of the data, as the last rows in Table 1 illustrate. Here we decimated the fluorescence traces by a factor of ten, without harming the inference quality.
which improves the spike detection (correlation $\sigma$ wants to merely commit to one event within a time bin instead and get rid of remaining small values (Eq 15) (correlation $\sigma$).

The order in which the pools are decimated, use 5 pools $\lambda, b, \gamma$, yielding sparser solutions but rendering the problem non-convex. Although we are not guaranteed to find the global minimum, we obtained good results, cf. Fig [5]. To quantify directly the similarity between the inferred deconvolved trace and ground truth spike train we calculated the correlation between the two. The best results were obtained for $s_{\text{min}} = 0.5$ yielding correlation $0.899 \pm 0.009$ with the true spike train compared to $0.879 \pm 0.006$ for the solution of the problem with hard noise constraint (Eq [15]). However, in a practical application the scaling factor between calcium fluorescence and a single spike, which is 1 for our simulated data, is often unknown, rendering it impossible to simply set the threshold $s_{\text{min}}$ to the half of it. Instead, we can vary the threshold until the RSS crosses the threshold $\sigma^2 T$. The order in which the pools are merged or split matters for this non-convex case and sequentially adding spikes at the highest values of the $l_1$-solution yielded the best performance with correlation $0.888 \pm 0.007$.

Table 1: Cost and quality of spike inference with parameter optimization.

| optimize | accelerate | Time [ms] | Correlation |
|----------|------------|-----------|-------------|
| -        | -          | 3.25 ± 0.03 | 0.831 ± 0.006 |
| $\lambda$ | -          | 9.2 ± 0.1   | 0.849 ± 0.006 |
| $\lambda, b$ | -         | 8.4 ± 0.2   | 0.857 ± 0.007 |
| $\lambda, b, \gamma$ | -         | 48.4 ± 2.3  | 0.875 ± 0.006 |
| $\lambda, b, \gamma$ | use 10 pools | 16.0 ± 0.4  | 0.875 ± 0.006 |
| $\lambda, b, \gamma$ | use 5 pools | 14.2 ± 0.3   | 0.875 ± 0.006 |
| $\lambda, b, \gamma$ | decimate  | 29.4 ± 1.3   | 0.878 ± 0.006 |
| $\lambda, b, \gamma$ | decimate, use 10 pools | 12.1 ± 0.2    | 0.878 ± 0.006 |
| $\lambda, b, \gamma$ | decimate, use 5 pools | 10.6 ± 0.2  | 0.877 ± 0.006 |

The first column shows the quantities that have been optimized, the second methods used to accelerate the parameter optimization, the third the computing time per trace (±SEM) and the last shows the performance of spike train inference using the correlation between inferred activity and true spike train. We used 20 simulated fluorescence traces with a spiking signal coming from an inhomogeneous Poisson process and a duration of 100 s at a framerate of 30 Hz such that $T = 3,000$ frames.

### 5.3 Hard thresholding

OASIS solves a LASSO problem resulting in soft shrinkage. The deconvolved trace $\hat{s}$ typically has values smaller than 1 and often shows “partial spikes” in neighboring bins reflecting the uncertainty regarding the exact position of a spike, cf. Fig [3]. While this information can be useful, one sometimes wants to merely commit to one event within a time bin instead and get rid of remaining small values in $\hat{s}$. We ran a slightly modified version of the algorithm that replaces the sparsity penalty by a constraint on the minimal spike size $s_{\text{min}}$, yielding sparser solutions but rendering the problem non-convex. Although we are not guaranteed to find the global minimum, we obtained good results, cf. Fig [5]. To quantify directly the similarity between the inferred deconvolved trace and ground truth spike train we calculated the correlation between the two. The best results were obtained for $s_{\text{min}} = 0.5$ yielding correlation $0.899 \pm 0.009$ with the true spike train compared to $0.879 \pm 0.006$ for the solution of the problem with hard noise constraint (Eq [15]). However, in a practical application the scaling factor between calcium fluorescence and a single spike, which is 1 for our simulated data, is often unknown, rendering it impossible to simply set the threshold $s_{\text{min}}$ to the half of it. Instead, we can vary the threshold until the RSS crosses the threshold $\sigma^2 T$. The order in which the pools are merged or split matters for this non-convex case and sequentially adding spikes at the highest values of the $l_1$-solution yielded the best performance with correlation $0.888 \pm 0.007$.

Fig [5] also shows results with a constraint on the minimal spike size for an AR(2) process. Adding the constraint helps when pressed for a binary decision whether to assign a spike or not, yielding visually excellent results. However, with a finite rise time of the calcium response the onset detection is notoriously difficult, because for a low threshold there are a lot of false positives due to noise, whereas for a high threshold, closer to the peak of the calcium kernel, the onset has already occurred earlier. Indeed, the greedy method for an AR(2) process tends to register spikes too late, which is further exacerbated when a threshold on the spike size ($s_{\text{min}} = 0.5$) is introduced, leading to low values of spike similarity (correlation $0.419 \pm 0.016$) compared to the solution of basis pursuit denoising (Eq [15]) (correlation $0.497 \pm 0.013$). We can incorporate a correction step that whenever a new spike is added, slightly jitters the previous one and calculates the change in the optimization objective in order to determine the optimal placement of the spike. For simplicity and low computational burden, we restrict the consideration of the changing RSS to the pools prior and after the jittered spike, which improves the spike detection (correlation $0.462 \pm 0.015$) while only marginally increasing computational cost (from 8.65 ms to 11.65 ms). Further improvements can be obtained by following up with (O)NNLS. The solution obtained by OASIS with threshold on the minimal spike size and jittering can be used to restrict (O)NNLS to have non-zero values only in close proximity to the spikes of the greedily obtained solution. This processing step increased the performance of spike inference to correlation $0.530 \pm 0.010$, which is better than the already mentioned one obtained for exactly solving the convex problem (Eq [15]). Hence, though imposing a minimal spike size renders the problem non-convex, a tractable approximate solution to this problem can improve over the exact solution of the convex basis pursuit denoising problem.
Figure 5: **Thresholding can improve the accuracy of spike inference.** (A) Inferred trace using L1 penalty (L1, blue) and the thresholded OASIS (Thresh., green). The data (yellow) are simulated with AR(1) model. (B) Inferred spiking activity. (C) The detected events using thresholded OASIS depend on the selection of $s_{\text{min}}$. The ground truth is shown in red. (D,E,F), same as (A,B,C), but the data are simulated with AR(2).

In the AR(2) case the exact solutions (ONNLS with $\lambda$ or ONNLS with support only in the proximity of the thresholded solution) consistently improved over the faster greedy methods, as measured by spike train correlation. The performance was hardly affected by whether the penalized or the thresholded version was chosen. Spike train correlation harshly penalizes spikes that are detected but at an incorrect time, no matter how close; therefore the activity plots and correlation values convey somewhat complementary information about the quality of the inference. We attribute the performance gap between greedy and exact solutions to greedy methods missing the exact time step more often. However, the optimally attainable time resolution is already limited by low SNR, in particular if the rise time of the calcium indicator is finite. Indeed, being more lenient regarding the exact spike timing we calculated the correlations after convolving the spike trains with a Gaussian with standard deviation of one bin width. The correlation values increased to $0.731 \pm 0.008$ for the greedy thresholded solution and to $0.800 \pm 0.007$ if followed up by ONNLS, but did not increase further for wider Gaussian kernels. This indicates that in the considered SNR regime single time bin resolution is out of reach, but spike times can be inferred with an uncertainty of about one time bin width.

### 5.4 Online spike inference with limited lag

For an exact solution of the non-negative deconvolution problem of an AR(1) process OASIS needs to backtrack to the most recent spike. (For an AR(2) process the solution is greedy and merely approximate. ONNLS yields an exact solution in this case but considers an even wider time window.) Such delays could be too long for some interesting closed loop experiments; therefore we were interested in how well the method performs if backtracking is limited to just a few frames. We varied the lag in the online estimator, i.e. the number of future samples observed before assigning a spike at time zero, for different signal-to-noise ratios (SNR). For each lag we chose the sparsity parameter $\lambda$ such that the noise constraint $\|\hat{c} - y\|^2 \leq \sigma^2T$ was tight. This yielded increasing values of $\lambda$ for smaller lags, compensating for the fact that limiting backtracking to fewer frames also imposes fewer constraints ($\hat{c}_t \geq \gamma \hat{c}_{t-1}$) on the dynamics. In the case of hard thresholding, better results were obtained with higher $s_{\text{min}}$ for smaller lags too, in order to avoid that one spike is split in two. We used a hand-chosen value of $s_{\text{min}} = 0.5 + 0.175 e^{-\tau}$ where $\tau$ is the lag, that asymptotically approaches the 0.5 for batch processing. The obtained results are depicted in Fig[6]. For realistic SNR (3-5, though [56] report even higher values, cf. Fig[3C]) and sample rates (30 Hz), lags of 2-5 yielded virtually the same results as offline estimation. The exact number depends on the noise; however, the
Figure 6: **Varied lag in the online estimator.** (A,B) Performance of spike inference as function of lag for various noise levels (i.e., inverse SNR) without (A) and with positive threshold $s_{\text{min}}$ (B). We used correlation of the inferred spike train as similarity measure and compared to ground truth as well as to the optimally recoverable activity when the lag is unlimited as in offline processing. (C) Inferred trace with positive threshold $s_{\text{min}}$ for increasing lag using the data depicted in Fig 3A with high noise level ($\sigma = 0.3$). The gray lines indicate the true spike times.

The main effect of noise was to reduce the optimal performance attainable even with batch processing, as the asymptotic values in Fig 6A and B reveal.

### 6 Conclusion

We presented an online active set method for spike inference from calcium imaging data. We assumed that the forward model to generate a fluorescence trace from a spike train is linear-Gaussian. Further development will extend the method to nonlinear models incorporating saturation effects and a noise variance that increases with the mean fluorescence to better resemble the Poissonian statistics of photon counts. In the Supplementary Material we already extend our mathematical formulation to include weights for each time point as a first step in this direction.

Our method considered spike inference as a sparse non-negative deconvolution problem. We focused on the formulation that imposes sparsity using an $\ell_1$ penalty that renders the problem convex. Using this problem formulation for spike inference has already long standing success within the neuroscientific community. We were able to speed it up by an order of magnitude compared to previously employed interior point methods and derived an algorithm that lends itself to online applications. However, recently several investigators have advocated sparser methods, e.g. by using an $\ell_q$-norm with $q < 1$ instead of $q = 1$[46] or by enforcing refractoriness[47] (see also [13] for some further discussion of sparsening beyond $\ell_1$ penalization). They report improved results, however in some cases at the expense of non-convexity, thus losing the guarantee of finding the global optimum. We leave it to future work to incorporate refractoriness into the methods developed here, but we did slightly modify the sparse non-negative deconvolution problem by adding the constraint that positive spikes need to be larger than some minimal value. A minor modification to our algorithm enabled it to find an (approximate) solution of this non-convex problem, which can be marginally better than the solution obtained with $\ell_1$ regularizer. The latter reflects the uncertainty regarding the exact position of a spike by distributing it as “partial spikes” over neighboring bins. The former lets go of this potentially useful information and instead commits to one event within the locally optimal time bin. We leave it up to the user which approach to choose.

### Code Availability

We provide Python and MATLAB implementations of our algorithm online ([https://github.com/j-friedrich/OASIS](https://github.com/j-friedrich/OASIS) and linked repositories therein). The code is readily usable on new data and includes example scripts that produce all figures and Table 1 of this article.
Acknowledgments

We would like to thank Misha Ahrens and Yu Mu for providing whole-brain imaging data of larval zebrafish. We thank John Cunningham and Eftychios Pnevmatikakis for helpful conversations as well as Scott Linderman and Daniel Soudry for valuable comments on the manuscript.

Funding for this research was provided by Swiss National Science Foundation Research Award P300P2_158428, Simons Foundation Global Brain Research Awards 325171 and 365002, ARO MURI W911NF-12-1-0594, NIH R01MH064537 and R09DA023426, DARPA N66001-15-C-4032 (SIMPLEX), and a Google Faculty Research award; in addition, this work was supported by the Intelligence Advanced Research Projects Activity (IARPA) via Department of Interior/Interior Business Center (DoI/IBC) contract number D16PC00003 and D16PC00007. The U.S. Government is authorized to reproduce and distribute reprints for Governmental purposes notwithstanding any copyright annotation thereon. Disclaimer: The views and conclusions contained herein are those of the authors and should not be interpreted as necessarily representing the official policies or endorsements, either expressed or implied, of IARPA, DoI/IBC, or the U.S. Government.

Supplementary Material

S.1 Weighted regression

For sake of generality we consider the case of weighted regression with weights $\theta$.

$$\text{minimize } \frac{1}{2} \sum_t \theta_t (\hat{c}_t - y_t)^2 + \lambda \sum_t \hat{s}_t \quad \text{subject to } \hat{s} = G\hat{c} \geq 0 \quad (S1)$$

These weights could be used to give lower weight to time points with higher variance for heteroscedastic data, for example for the Poissonian statistics of photon counts where the variance of the fluorescence increases with its mean. Further, instead of the linear relationship between fluorescence and calcium concentration (Eq 2) we could have a nonlinear observation model $y_t = f(c_t) + \epsilon_t$ (S2) where the nonlinear function $f$ can include saturation effects. This is often taken to be the Hill equation, i.e., $f(c) = \frac{a c^n}{c^n + k_d^n} + b$, with Hill coefficient $n$, dissociation constant $k_d$, scaling factor $a$ and baseline $b$ [45]. Applying Newton’s algorithm to optimize for $\hat{s}$ (or equivalently $\hat{c}$) results for each Newton step in a weighted constrained regression problem as in Eq (S1).

For an AR(1) process introducing weights changes Eq (10) to

$$\text{minimize } \frac{1}{2} \sum_{t=0}^{\Delta t} \theta_{t+\tau} (\gamma^\tau c'_t - y_{t+\tau})^2 + \sum_{t=0}^{\Delta t} \mu_{t+\tau} \gamma^\tau c'_t \quad (S3)$$

and its solution is a modification of Eq (11) by adding the weights

$$c'_t = \frac{\sum_{t=0}^{\Delta t} (\theta_{t+\tau} y_{t+\tau} - \mu_{t+\tau} \gamma^\tau)}{\sum_{t=0}^{\Delta t} \theta_{t+\tau} \gamma^2} \quad (S4)$$

We merely need to initialize each pool as $(v_t, w_t, t_t, l_t) = (y_t - \frac{\mu_{t+1}}{\gamma}, \theta_t, t, 1)$ for each time step $t$ and the updates according to Eqs (12-14) guarantee that Eq (S4) holds for all values $v_t = c'_t$ as we prove in the next section.

For an AR(p) process introducing weights changes Eq (29) to

$$c'_t = \frac{\sum_{t=0}^{\Delta t} (\theta_{t+\tau} (y_{t+\tau} - \sum_{k=2}^{p} (A^t)_{1,k} c'_{t+1-k}) - \mu_{t+\tau} \gamma^t) (A^t)_{1,1}}{\sum_{t=0}^{\Delta t} \theta_{t+\tau} (A^t)_{1,1}^2} \quad (S5)$$

and the same modified initialization holds.

S.2 Validity of updates according to equations (12-14)

Theorem 1. The updates according to Eqs (12-14) guarantee that Eqs (11), (S4) hold for all values $v_t = c'_t$.

Proof. We proceed by induction.
**Assumption:** Let for the denominator and numerator of Eq (S4) hold

\[
w_i = \sum_{t=0}^{l_i-1} \theta_{t+t_i} \gamma^{2t}
\]  

(S6)

and

\[
w_iv_i = \sum_{t=0}^{l_i-1} (\theta_{t+t_i} y_{t+t_i} - \mu_{t+t_i}) \gamma^t
\]  

(S7)

**Base case:** Pools are initialized as \((v_t, w_t, t_t, l_t) = (y_t - \frac{\mu_t}{\theta_t}, \theta_t, t, 1)\) for each time step \(t\) such that Eqs (S6, S7) hold.

**Induction step:** Consider two pools \((v_{i}, w_{i}, t_{i}, l_{i})\) and \((v_{i+1}, w_{i+1}, t_{i+1}, l_{i+1})\) that satisfy Eqs (S6, S7) and are merged to pool \((v_i', w_i', t_i', l_i')\) according to Eqs (12-14). \(\gamma\theta\)

\[
w_i' = w_i + \gamma^{2l_i} w_{i+1} = \sum_{t=0}^{l_i-1} \theta_{t+t_i} \gamma^{2t} + \sum_{t=0}^{l_{i+1}-1} \theta_{t+t_{i+1}} \gamma^{2t_i+2t}
\]

\[
= \sum_{t=0}^{l_i+l_{i+1}-1} \theta_{t+t_i} \gamma^{2t_i+2t}
\]

where we used the contingency of the pools, \(t_{i+1} = t_i + l_i\). Thus after the update Eq (S6) holds for the merged pool too. It remains to show this also for the values:

\[
w_i'v_i' = w_iv_i + \gamma^{t_i} w_{i+1}v_{i+1}
\]

\[
= \sum_{t=0}^{l_i-1} (\theta_{t+t_i} y_{t+t_i} - \mu_{t+t_i}) \gamma^t + \sum_{t=0}^{l_{i+1}-1} (\theta_{t+t_{i+1}} y_{t+t_{i+1}} - \mu_{t+t_{i+1}}) \gamma^{t_i+2t}
\]

\[
= \sum_{t=0}^{l_i+l_{i+1}-1} (\theta_{t+t_i} y_{t+t_i} - \mu_{t+t_i}) \gamma^t
\]

\[
= \sum_{t=0}^{l_i'+1} (\theta_{t+t_i'} y_{t+t_i'} - \mu_{t+t_i'}) \gamma^t
\]

\[\square\]

**S.3 Supplementary video**

The supplementary video illustrates OASIS for an AR(1) process. As in Figure 2 red lines depict true spike times and the shaded background shows how the time points are gathered in pools. The pool currently under consideration is indicated by the blue crosses. The upper panel shows how the calcium fluorescence trace \(c'\) develops while the algorithm runs, cf. Figure 2. The video additionally shows the deconvolved trace \(s' = Ge'\) (Eq. 3) in the lower panel. The algorithm sweeps through the time series and enforces the constraint \(s' \geq 0\).

**References**

[1] C Grienberger and C Konnerth. Imaging calcium in neurons. *Neuron*, 73(5):862–885, 2012.

[2] B F Grewe, D Langer, H Kasper, B M Kampa, and F Helmchen. High-speed in vivo calcium imaging reveals neuronal network activity with near-millisecond precision. *Nat Methods*, 7(5):399–405, 2010.

[3] E Yaksi and R W Friedrich. Reconstruction of firing rate changes across neuronal populations by temporally deconvolved Ca\(^{2+}\) imaging. *Nat Methods*, 3(5):377–383, 2006.

[4] T F Holekamp, D Turaga, and T E Holy. Fast three-dimensional fluorescence imaging of activity in neural populations by objective-coupled planar illumination microscopy. *Neuron*, 57(5):661–672, 2008.

[5] J T Vogelstein, A M Packer, T A Machado, T Sippy, B Babadi, R Yuste, and L Paninski. Fast nonnegative deconvolution for spike train inference from population calcium imaging. *J Neurophysiol*, 104(6):3691–3704, 2010.

20
[6] J T Vogelstein, B O Watson, A M Packer, R Yuste, B Jedynak, and L Paninski. Spike inference from calcium imaging using sequential monte carlo methods. *Biophys J*, 97(2):636–655, 2009.

[7] E A Pnevmatikakis, J Merel, A Pakman, and L Paninski. Bayesian spike inference from calcium imaging data. *Asilomar Conference on Signals, Systems and Computers*, 2013.

[8] T Deneux, A Kaszas, G Szalay, G Katona, T Lakner, A Grinvald, B Rózsa, and I Vanzetta. Accurate spike estimation from noisy calcium signals for ultrafast three-dimensional imaging of large neuronal populations in vivo. *Nat Commun*, 7, 2016.

[9] T Sasaki, N Takahashi, N Matsuki, and Y Ikegaya. Fast and accurate detection of action potentials from somatic calcium fluctuations. *J Neurophysiol*, 100(3):1668–1676, 2008.

[10] L Theis, P Berens, E Froudarakis, J Reimer, M R Rosón, T Baden, T Euler, A S Tolias, and M Bethge. Benchmarking spike rate inference in population calcium imaging. *Neuron*, 90(3):471–482, 2016.

[11] Y Mishchencko, J T Vogelstein, and L Paninski. A bayesian approach for inferring neuronal connectivity from calcium fluorescent imaging data. *Ann Appl Stat*, pages 1229–1261, 2011.

[12] M A Picardo, J Merel, K A Katlowitz, D Vallentin, D E Okobi, S E Benezza, R C Clary, E A Pnevmatikakis, L Paninski, and M A Long. Population-level representation of a temporal sequence underlying song production in the zebra finch. *Neuron*, 90(4):866–876, 2016.

[13] E A Pnevmatikakis, D Soudry, Y Gao, T A Machado, J Merel, D Pfau, T Reardon, Y Mu, C Lacefield, W Yang, M Ahrens, R Bruno, T M Jessell, D S Peterka, R Yuste, and L Paninski. Simultaneous denoising, deconvolution, and demixing of calcium imaging data. *Neuron*, 89(2):285–299, 2016.

[14] L Grosenick, J H Marshel, and K Deisseroth. Closed-loop and activity-guided optogenetic control. *Neuron*, 86(1):106–139, 2015.

[15] J P Rickgauer, K Deisseroth, and D W Tank. Simultaneous cellular-resolution optical perturbation and imaging of place cell firing fields. *Nat Neurosci*, 17(12):1816–1824, 2014.

[16] A M Packer, L E Russell, H WP Dalgleish, and M Häusser. Simultaneous all-optical manipulation and recording of neural circuit activity with cellular resolution in vivo. *Nat Methods*, 12(2):140–146, 2015.

[17] K B Clancy, A C Koralek, R M Costa, D E Feldman, and J M Carmena. Volitional modulation of optically recorded calcium signals during neuroprosthetic learning. *Nat Neurosci*, 17(6):807–809, 2014.

[18] J Lewi, R Butera, and L Paninski. Sequential optimal design of neurophysiology experiments. *Neural Comput*, 21(3):619–687, 2009.

[19] M Park and J W Pillow. Bayesian active learning with localized priors for fast receptive field characterization. In *Adv Neural Inf Process Syst*, pages 2348–2356, 2012.

[20] B Shababo, B Paige, A Pakman, and L Paninski. Bayesian inference and online experimental design for mapping neural microcircuits. In *Adv Neural Inf Process Syst*, pages 1304–1312, 2013.

[21] M B Ahrens, M B Orger, D N Robson, J M Li, and P J Keller. Whole-brain functional imaging at cellular resolution using light-sheet microscopy. *Nat Methods*, 10(5):413–420, 2013.

[22] N Vladimirov, Y Mu, T Kawashima, D V Bennett, C-T Yang, L L Looger, P J Keller, J Freeman, and M B Ahrens. Light-sheet functional imaging in fictively behaving zebrafish. *Nat Methods*, 2014.

[23] F A Potra and S J Wright. Interior-point methods. *J Comput Appl Math*, 124(1):281–302, 2000.

[24] M Ayer, H D Brunk, G M Ewing, W T Reid, and E Silverman. An empirical distribution function for sampling with incomplete information. *Ann Math Stat*, 26(4):641–647, 1955.

[25] R E Barlow, D J Bartholomew, J M Bremner, and H D Brunk. *Statistical inference under order restrictions: The theory and application of isotonic regression*. Wiley New York, 1972.

[26] C van Eeden. *Testing and Estimating Ordered Parameters of Probability Distributions*. PhD thesis, University of Amsterdam, 1958.

[27] R E Miles. The complete amalgamation into blocks, by weighted means, of a finite set of real numbers. *Biometrika*, 46(3/4):317–327, 1959.
[28] P Mair, K Hornik, and J de Leeuw. Isotone optimization in R: pool-adjacent-violators algorithm (PAVA) and active set methods. J Stat Softw, 32(5):1–24, 2009.

[29] M J Best and N Chakravarti. Active set algorithms for isotonic regression; a unifying framework. Math Prog, 47(1-3):425–439, 1990.

[30] S J Grotzinger and C Witzgall. Projections onto order simplexes. Appl Math Optim, 12(1):247–270, 1984.

[31] K Podgorski and K Haas. Fast non-negative temporal deconvolution for laser scanning microscopy. J Biophotonics, 6(2):153–162, 2013.

[32] R P Brent. Algorithms for Minimization Without Derivatives. Courier Corporation, 1973.

[33] J Nocedal and S Wright. Numerical optimization. Springer Science & Business Media, 2006.

[34] D L Donoho. De-noising by soft-thresholding. IEEE Trans Inf Theory, 41(3):613–627, 1995.

[35] S. G. Mallat and Z. Zhang. Matching pursuits with time-frequency dictionaries. IEEE Trans Signal Processing, 41(12):3397–3415, 1993.

[36] T-W Chen, Tvl Wardill, Y Sun, S R Pulver, S L Renninger, A Baohan, E R Schreiter, R A Kerr, M B Orger, V Jayaraman, and L L Looger. Ultrasensitive fluorescent proteins for imaging neuronal activity. Nature, 499(7458):295–300, 2013.

[37] R Bro and S De Jong. A fast non-negativity-constrained least squares algorithm. J Chemometrics, 11(5):393–401, 1997.

[38] C L Lawson and R J Hanson. Solving least squares problems, volume 15. SIAM, 1995.

[39] S Diamond and S Boyd. CVXPY: A Python-embedded modeling language for convex optimization. J Mach Learn Res, 17(83):1–5, 2016.

[40] A Domahidi, E Chu, and S Boyd. ECOS: An SOCP solver for embedded systems. In European Control Conference (ECC), pages 3071–3076, 2013.

[41] E D Andersen and K D Andersen. The MOSEK interior point optimizer for linear programming: an implementation of the homogeneous algorithm. In High performance optimization, pages 197–232. Springer, 2000.

[42] B O’Donoghue, E Chu, N Parikh, and S Boyd. Conic optimization via operator splitting and homogeneous self-dual embedding. J Optim Theory Appl, pages 1–27, 2016.

[43] Gurobi Optimization Inc. Gurobi optimizer reference manual, 2015.

[44] GENIE project, Janelia Research Campus, HHMI; Karel Svoboda (contact). Simultaneous imaging and loose-seal cell-attached electrical recordings from neurons expressing a variety of genetically encoded calcium indicators. CRCNS.org, http://dx.doi.org/10.6080/K02R3PMN, 2015.

[45] T A Pologruto, R Yasuda, and K Svoboda. Monitoring neural activity and [Ca^{2+}] with genetically encoded Ca^{2+} indicators. J Neurosci, 24(43):9572–9579, 2004.

[46] T Quan, X Lv, X Liu, and S Zeng. Reconstruction of burst activity from calcium imaging of neuronal population via Lq minimization and interval screening. Biomed Opt Express, 7(6):2103–2117, 2016.

[47] E L Dyer, C Studer, J T Robinson, and R G Baraniuk. A robust and efficient method to recover neural events from noisy and corrupted data. In 6th Int IEEE/EMBS Conf Neural Eng (NER), pages 593–596. IEEE, 2013.

[48] M Pachitariu, C Stringer, S Schröder, M Dipoppa, L F Rossi, M Carandini, and K D Harris. Suite2p: beyond 10,000 neurons with standard two-photon microscopy. bioRxiv, 2016.