SCALPEL: EXTRACTING NEURONS FROM CALCIUM IMAGING DATA

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In the past few years, new technologies in the field of neuroscience have made it possible to simultaneously image activity in large populations of neurons at cellular resolution in behaving animals. In mid-2016, a huge repository of this so-called “calcium imaging” data was made publicly available. The availability of this large-scale data resource opens the door to a host of scientific questions, for which new statistical methods must be developed.

In this paper, we consider the first step in the analysis of calcium imaging data: namely, identifying the neurons in a calcium imaging video. We propose a dictionary learning approach for this task. First, we perform image segmentation to develop a dictionary containing a huge number of candidate neurons. Next, we refine the dictionary using clustering. Finally, we apply the dictionary to select neurons and estimate their corresponding activity over time, using a sparse group lasso optimization problem. We assess performance on simulated calcium imaging data and apply our proposal to three calcium imaging data sets.

Our proposed approach is implemented in the R package scalpel, which is available on CRAN.

1. Introduction. The field of neuroscience is undergoing a rapid transformation: new technologies are making it possible to image activity in large populations of neurons at cellular resolution in behaving animals (Ahrens et al., 2013; Dombeck et al., 2007; Huber et al., 2012; Prevedel et al., 2014). The resulting calcium imaging data sets promise to provide unprecedented insight into neural activity. However, they bring with them both statistical and computational challenges.

While calcium imaging data sets have been collected by individual labs for the past several years, up until quite recently large-scale calcium imaging data sets were not publicly available. Thus, attempts by statisticians to develop methods for the analysis of these data have been hampered by limited data access. However, in July 2016, the Allen Institute for Brain Science released the Allen Brain Observatory, which contains 30 terabytes of raw data cataloguing 25 mice over 360 different experimental sessions (Shen, 2016). This massive data repository is ripe for the development of statistical methods, which can be applied not only to the data from the Allen Institute, but also to calcium imaging data sets collected by individual labs world-wide.

We now briefly describe the science underlying calcium imaging data. When a neuron fires, voltage-gated calcium channels in the axon terminal open, and calcium floods the cell. Therefore, intracellular calcium concentration is a surrogate marker for the spiking activity of neurons (Grienberger and Konnerth, 2012). In recent years, genetically encoded calcium indicators have been developed (Chen et al., 2013; Looger and Griesbeck, 2012; Rochefort et al., 2008). These indicators bind to intracellular calcium molecules and fluoresce. Thus, the locations of neurons and the times at which they fire can be seen through a sequence of two-dimensional images taken over time, typically using two-photon microscopy (Helmchen and Denk, 2005; Svoboda and Yasuda, 2006).

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In (a), we display sample frames from the raw calcium imaging video described in the text in Section 4, and analyzed in greater detail in Section 6.2. We wish to construct a spatial map of the neurons, like that shown in (b). As a by-product, we will also obtain a crude estimate of the calcium trace for each neuron over time, as shown in (c).

A typical calcium imaging video consists of a 500 × 500 pixels frame over one hour, sampled at 15-30 Hz. A given pixel in a given frame is continuous-valued, with larger values representing higher fluorescent intensities due to greater calcium concentrations. An example frame from a calcium imaging video is shown in Figure 1(a). We have posted snippets of the three calcium imaging videos analyzed in this paper at www.ajpete.com/software.

On the basis of a calcium imaging video, two goals are typically of interest:

- **Neuron identification**: The goal is to assign pixels of the image frame to neurons. Due to the thickness of the brain slice captured by the imaging technology, neurons can overlap in the two-dimensional image. This means that a single pixel can be assigned to more than one neuron. This step is sometimes referred to as region of interest identification or cell sorting. An example of neurons identified from a calcium imaging video is shown in Figure 1(b).

- **Calcium quantification**: The goal is to estimate the intracellular calcium concentration for each neuron during each frame of the movie. An example of these estimated calcium traces is shown in Figure 1(c).

To a certain extent, these two goals can be accomplished by visual inspection. However, visual inspection suffers from several shortcomings:

- It is subjective and it is not reproducible. Two people who view the same video may identify a different set of neurons or different firing times.
- It does not yield numerical information regarding neuron firing times, which may be needed for downstream analyses.
- It may be inaccurate: for instance, a neuron that is very dim or that fires infrequently may not be identified by visual inspection.
- It is not feasible on videos with very large neuronal populations or very long durations. In fact, a typical calcium imaging video contains 250,000 pixels and more than 50,000 frames, making visual inspection essentially impossible.

In this paper, we propose a method that identifies the locations of neurons. Previous proposals to automatically accomplish this task have been proposed in the literature, and are reviewed in
Section 3. However, our method has several advantages over competing approaches. Unlike many existing approaches, it:

- Involves few tuning parameters, which are themselves interpretable to the user, can for the most part be set to default values, and can be varied independently;
- Yields results that are stable across a range of tuning parameters;
- Is computationally feasible even on very large data sets; and
- Uses spatial and temporal information to resolve individual neurons, from sets of overlapping neurons, without post-processing.

The methods proposed in this paper can be seen as a necessary step that precedes downstream modeling of calcium imaging data. For instance, there is substantial interest in modeling functional connectivity among populations of neurons, or using neural activity to decode stimuli (see, e.g., Ko et al. (2011); Mishchencko et al. (2011); Paninski et al. (2007)). However, before either of those tasks can be carried out, it is necessary to first identify the neurons; this is the task that we consider in this paper.

The remainder of this paper is organized as follows. We introduce notation in Section 2. In Section 3, we review related work. We present our proposal in Section 4, and discuss the selection of tuning parameters in Section 5. We apply our method to three calcium imaging videos in Section 6 and assess performance using simulated calcium imaging data in Section 7. We close with a discussion in Section 8. Proofs are in the Supplementary File.

2. Notation. Let $P$ denote the total number of pixels per image frame, and $T$ the number of frames of the video. We define $Y$ to be a $P \times T$ matrix for which the $(i,j)$th element, $y_{i,j}$, contains the fluorescence of the $i$th pixel in the $j$th frame. We let $\mathbf{y}_i = (y_{i,1} \ y_{i,2} \ \ldots \ y_{i,T})^\top$ represent the fluorescence of the $i$th pixel at each of the $T$ frames. We let $\mathbf{y}_{-,j} = (y_{1,j} \ y_{2,j} \ \ldots \ y_{P,j})^\top$ represent the fluorescence of all $P$ pixels during the $j$th frame. We use the same subscript conventions in order to reference the elements, rows, and columns of other matrices.

The primary goal of our work is to identify the locations of the neurons; as a by-product, we will also obtain a crude estimate of their calcium concentrations over time. We view these tasks in the framework of a matrix factorization problem: we decompose $Y$ into a matrix of spatial components, $A \in \mathbb{R}^{P \times K}$, and a matrix of temporal components, $Z \in \mathbb{R}^{K \times T}$, such that

$$Y \approx AZ,$$

where $K$ is the total number of estimated neurons. Note that $a_{i,k}$ specifies which of the $P$ pixels of the image frame are mapped to the $k$th neuron, and $z_k$ quantifies the calcium concentration for the $k$th neuron at each of the $T$ video frames. We note that the true number of neurons is unknown, and must be determined as part of the analysis. The vectorization of the image frames in $Y$ is simply for notational convenience: in estimating $A$, we will use spatial information from the two-dimensional structure of the image frames.

3. Related Work. There are two distinct lines of work in this area. The first focuses on simply identifying the regions of interest in the video, and then subsequently estimating the calcium traces. The second aims to simultaneously identify neurons and quantify their calcium concentrations.

Methods that focus solely on region of interest identification typically construct a summary image for the calcium imaging video and then segment this image using various approaches. For example, Pachitariu et al. (2013) calculate the mean image of the video and then apply convolutional sparse block coding to identify regions of interest. Alternatively, Smith and Häusser (2010) calculate a
local cross-correlation image, which is then thresholded using a locally adaptive filter to extract the regions of interest. Similar approaches have been used by others (Mellen and Tuong, 2009; Ozden et al., 2008). These region of interest approaches often do not fully exploit temporal information; furthermore, they typically are unable to resolve spatially-overlapping neurons.

We now focus on methods that simultaneously identify neurons and estimate calcium concentrations. One of the first methods in this area was proposed by Mukamel et al. (2009). This method first applies principal component analysis to reduce the dimensionality of the data, followed by spatio-temporal independent component analysis to produce spatial and temporal components that are statistically independent of one another. Though this method is widely used, it often requires heuristic post-processing of the spatial components, and typically fails to distinguish between spatially overlapping neurons (Pnevmatikakis et al., 2016).

To better handle overlapping neurons, Maruyama et al. (2014) proposed a non-negative matrix factorization approach, which estimates $A$ and $Z$ in (1) by solving

$$
\text{minimize} \quad \frac{1}{2} \|Y - AZ - a_bz_b^T\|_F^2.
$$

In (2), the term $a_bz_b^T$ is a rank-one correction for background noise: $z_b \in \mathbb{R}^T$ is a temporal representation of the background noise (known as the bleaching line, and estimated using a linear fit to average fluorescence over time of a background region), and $a_b \in \mathbb{R}^P$ is a spatial representation of the background noise. Element-wise positivity constraints are imposed on $A$, $Z$, and $a_b$ in (1). While (2) can handle overlapping neurons, there is no constraint on the sparsity of $Z$, and no effort to ensure that the non-zero elements of $A$ are sparse and spatially contiguous. Thus, the estimated temporal components are very noisy, and the estimated spatial components often require heuristic post-processing.

To overcome these shortcomings, Haeffele et al. (2014) modify (2) so that the temporal components $A$ are sparse and the spatial components $Z$ are sparse and have low total variation. Recently, Pnevmatikakis et al. (2016) further refine (2) by explicitly modeling the dynamics of the calcium when estimating the temporal components $Z$, and combining a sparsity constraint with intermediate image filtering when estimating the spatial components $A$. Zhou et al. (2016) extend the work of Pnevmatikakis et al. (2016) to better handle one-photon imaging data by (i) modeling the background in a more flexible way, and (ii) introducing a greedy initialization procedure for the neurons that is more robust to background noise. Related approaches are taken by Diego et al. (2013); Diego Andilla and Hamprecht (2014); Friedrich et al. (2015). Other recent approaches consider using convolutional networks trained on manual annotation (Apthorpe et al., 2016) and multi-level matrix factorization (Diego Andilla and Hamprecht, 2013).

While these existing approaches show substantial promise, and are a marked improvement over manual identification of neurons from the calcium imaging videos, they also suffer from some shortcomings:

- The optimization problems (see, e.g., (2)) are biconvex. Thus, algorithms typically get trapped in unattractive local optima. Furthermore, the results strongly depend on the choice of initialization.
- Each method involves several user-selected tuning parameters. There is no natural interpretation to these tuning parameters, which leads to challenges in selection. Furthermore, the tuning parameters are highly interdependent, so that changing one tuning parameter may necessitate updating all of them. Moreover, there is no natural nesting with respect to the tuning parameters: a slight increase or decrease in one tuning parameter can lead to a completely different set of identified neurons.
• The number of neurons $K$ must be specified in advance, and the estimates obtained for different values of $K$ will not be nested: two different values of $K$ can yield completely different answers.

• Post-processing of the identified neurons is often necessary.

• Implementation on very large data sets can be computationally burdensome.

To overcome these challenges, instead of simultaneously estimating $A$ and $Z$ in the model (1), we take a dictionary learning approach. We first leverage spatial information to build a preliminary dictionary of spatial components, which is then refined using a clustering approach to give an estimate of $A$. We then use our estimate of $A$ to obtain an accurate estimate of the temporal components $Z$, while simultaneously selecting the final set of neurons in $A$. This dictionary learning approach allows us to re-cast (1), a very challenging unsupervised learning problem, into a much easier supervised learning problem. Compared to existing approaches, our proposal is much faster to solve computationally, involves more interpretable tuning parameters, and yields substantially more accurate results.

4. Proposed Approach. Our proposed approach is based on dictionary learning. In Figure 2, we summarize our Segmentation, Clustering, and Lasso Penalties (SCALPEL) proposal, which consists of four steps:

Step 0. Data Pre-Processing: We apply standard pre-processing techniques to smooth the data both temporally and spatially, remove the bleaching effect, and calculate a standardized fluorescence. Details are provided in Section 9 of the Supplementary File. In what follows, $Y$ refers to the calcium imaging data after these three pre-processing steps have been performed.

Step 1. Construction of a Preliminary Spatial Component Dictionary: We apply a simple image segmentation procedure to each frame of the video to derive a spatial component dictionary, which is used to construct the matrix $A^0 \in \mathbb{R}^{P \times K^0}$ with $a^0_{j,k} = 1$ if the $j$th pixel is contained in the $k$th preliminary dictionary element, and $a^0_{j,k} = 0$ otherwise. This is discussed further in Section 4.1.

Step 2. Refinement of the Spatial Component Dictionary: To eliminate redundancy in the preliminary spatial component dictionary, we cluster together dictionary elements that co-localize in time and space. This results in a matrix $A \in \mathbb{R}^{P \times K}$ where $K < K^0$; $a_{j,k} = 1$ if the $j$th pixel is contained in the $k$th dictionary element, and $a_{j,k} = 0$ otherwise. More details are provided in Section 4.2.

Step 3. Application of the Spatial Component Dictionary: We remove dictionary elements corresponding to clusters with few members, resulting in a filtered dictionary $A^f \in \mathbb{R}^{P \times K^f}$, which contains a subset of the columns of $A$. We then estimate the temporal components $Z$ corresponding to the filtered elements of the dictionary by solving a sparse group lasso problem with a non-negativity constraint. The $k$th row of $\hat{Z}$ is the estimated calcium trace corresponding to the $k$th filtered dictionary element; if this is entirely equal to zero, then the $k$th dictionary element in $A^f$ has been eliminated. Thus, in this step, we finalize our estimates of the neurons’ locations, and as a by-product obtain a crude estimate of the temporal components associated with each estimated neuron. Additional details are in Section 4.3.

Step 1 is applied to each frame separately, and thus can be efficiently performed in parallel across the frames of the video. Similarly, parts of Step 0 can be parallelized across frames and across pixels.

Throughout this section, we illustrate SCALPEL on an example one-photon calcium imaging data set that has $205 \times 226$ pixels and 3000 frames, sampled at 10 Hz, collected in the lab of Ilana Witten at the Princeton Neuroscience Institute. Figures 1-8, as well as Figures S1, S2, and S4-S9
Fig 2. A summary of the SCALPEL procedure, along with the results of applying each step to an example data set with 205 × 226 pixels and 3000 frames, described in the text in Section 4, and analyzed in greater detail in Section 6.2.
Fig 3. In (a), we display a single frame of the example calcium imaging video after performing the pre-processing described in Section 9 of the Supplementary File. In (b), we show the binary image that results after thresholding using the negative of the 0.1% quantile of the video’s elements. In (c), we display the seven connected components from the image in (b) that contain at least 25 pixels.

in the Supplementary File, involve this data set. In Section 6, we present a more complete analysis of this data set, along with analyses of additional data sets.

4.1. Step 1: Construction of a Preliminary Spatial Component Dictionary. In this step, we identify a large set of preliminary dictionary elements, by applying a simple image segmentation procedure to each frame separately.

1. **Threshold Image:** We create a binary image by thresholding the image frame. Figure 3(b) displays the binary image that results from thresholding the frame shown in Figure 3(a).

2. **Identify Connected Components:** We identify the connected components of the thresholded image, using the notion of 4-connectivity: connected pixels are pairs of white pixels that are immediately to the left, right, above, or below one another (Sonka et al., 2014). Some of these connected components may represent neurons, whereas others are likely to be noise artifacts or snapshots of multiple nearby neurons.

3. **Filter Components:** To eliminate noise, we filter components based on their overall size, width, and height. In the examples in this paper, we discard connected components of fewer than 25 or more than 500 pixels, as well as those with a width or height larger than 30 pixels.

We now discuss the choice of threshold used above. After performing Step 0, we expect that the intensities of “noise pixels” (i.e., pixels that are not part of a firing neuron in that frame) will have a distribution that is approximately symmetric and approximately centered at zero. In contrast, non-noise pixels will have larger values. This implies that the noise pixels should have a value no larger than the negative of the minimum value of \( Y \). Therefore, we threshold each frame using the negative of the minimum value of \( Y \). We also repeat this procedure using a threshold equal to the negative of the 0.1% quantile of \( Y \), as well as with the average of these two threshold values. In Section 5.1, we discuss alternative approaches to choosing this threshold.

The \( K^0 \) connected components that arise from performing Step 1 on each frame, for each of the three threshold values, form a preliminary spatial component dictionary. We use them to construct the matrix \( A^0 \in \mathbb{R}^{P \times K^0} \): the \( k \)th column of \( A^0 \) is a vector of 1’s and 0’s, indicating whether each pixel is contained in the \( k \)th preliminary dictionary element.

4.2. Step 2: Refinement of the Spatial Component Dictionary. We will now refine the preliminary spatial component dictionary obtained in Step 1, by combining dictionary elements that are very similar to each other, as these likely represent multiple appearances of a single neuron. We proceed as follows:
1. **Calculate Dissimilarity Matrix**: We use a novel dissimilarity metric, which incorporates both spatial and temporal information, to calculate the dissimilarity between every pair of dictionary elements. More details are given in Section 4.2.1.

2. **Perform Prototype Clustering**: We use the aforementioned pair-wise dissimilarities to perform prototype clustering of dictionary elements (Bien and Tibshirani, 2011). We also identify a representative dictionary element for each cluster. More details are given in Section 4.2.2.

These elements of this refined dictionary make up the columns of the matrix \( A \), which will be used in Step 3, discussed in Section 4.3.

### 4.2.1. Choice of Dissimilarity Metric

Before performing clustering, we must decide how to quantify similarity between the \( K^0 \) elements of the preliminary dictionary obtained in Step 1. Dictionary elements that correspond to the same neuron are likely to have (i) similar spatial maps and (ii) similar average fluorescence over time. To this end, we construct a dissimilarity metric that leverages both spatial and temporal information.

We define

\[
p_{i,j} = \left( a^0_{i,j\cdot i} \right)^\top a^0_{j\cdot i},
\]

the number of pixels shared between the \( i \)th and \( j \)th dictionary elements. When \( i = j \), \( p_{i,i} \) is simply the number of pixels in the \( i \)th dictionary element. We then define the spatial dissimilarity between the \( i \)th and \( j \)th dictionary elements to be

\[
d^s_{i,j} = 1 - \frac{p_{i,j}}{\sqrt{p_{i,i}p_{j,j}}},
\]

Thus, \( d^s_{i,j} = 1 \) if and only if the \( i \)th and \( j \)th elements are non-overlapping in space, and \( d^s_{i,j} = 0 \) if and only if they are identical. Note that \( d^s_{i,j} \) is known as the cosine dissimilarity or Ochiai coefficient (Gower, 2006).

We now define the matrix \( Y^B \), a thresholded version of the pre-processed data matrix \( Y \) (obtained in Step 0), with elements of the form

\[
[Y^B]_{j,k} = \begin{cases} [Y]_{j,k} & \text{if } [Y]_{j,k} > \text{quantile}_{0.1\%}(Y) \\ 0 & \text{otherwise} \end{cases}
\]

Note that when a value other than the negative of the 0.1% quantile is used for image segmentation in Step 1, this value can also be used to threshold \( Y \) above. The temporal dissimilarity between the \( i \)th and \( j \)th dictionary elements is defined as

\[
d^t_{i,j} = 1 - \frac{\left( a^0_{i,j\cdot i} \right)^\top Y^B (Y^B)^\top a^0_{j\cdot i}}{\left\| (Y^B)^\top a^0_{i\cdot i} \right\|_2 \left\| (Y^B)^\top a^0_{j\cdot i} \right\|_2}.
\]

(Note that the elements of \( (Y^B)^\top a^0_{i\cdot j} \in \mathbb{R}^T \) represent the thresholded fluorescence of each time frame, summed over all pixels in the \( i \)th preliminary dictionary element.) We threshold \( Y \) before computing this dissimilarity, because (i) we are interested in the extent to which there is agreement between the peak fluorescences of the \( i \)th and \( j \)th preliminary dictionary elements; and (ii) the sparsity induced by thresholding is computationally advantageous.

Finally, the overall dissimilarity is

\[
d_{i,j} = \omega d^s_{i,j} + (1 - \omega) d^t_{i,j},
\]

where \( \omega \in [0,1] \) controls the relative weightings of the spatial and temporal dissimilarities. We use \( \omega = 0.2 \) to obtain the results shown throughout this paper. While we wish to incorporate
the spatial and temporal information equally, the magnitudes of the two dissimilarity measures translate to different degrees of similarity. That is, a pair of neurons will tend to have a larger spatial dissimilarity than temporal dissimilarity. Therefore, we weight the temporal information more heavily. A detailed justification for $\omega = 0.2$ is given in Section 10 of the Supplementary File; furthermore, a sensitivity analysis for the value of $\omega$ is presented in Section 7.3.

In Figure 4, we illustrate pairs of preliminary dictionary elements with various dissimilarities for $\omega = 0.2$.

4.2.2. Prototype Clustering. We now consider the task of clustering the elements of the preliminary dictionary. To avoid pre-specifying the number of clusters, and to obtain solutions that are nested as the number of clusters is varied, we opt to use hierarchical clustering (Hastie et al., 2009). In particular, we use prototype clustering, proposed in Bien and Tibshirani (2011), with the dissimilarity given in (5). Prototype clustering guarantees that at least one member of each cluster has a small dissimilarity with all other members of the cluster. To represent each cluster using a single dictionary element, we choose the member with the smallest median dissimilarity to all of the other members. Then we combine the representatives of the $K$ clusters to obtain a refined spatial component dictionary. We can represent this refined dictionary with the matrix $A \in \mathbb{R}^{P \times K}$, defined as follows: $a_{j,k} = 1$ if the $j$th pixel is contained in the $k$th cluster’s representative, and $a_{j,k} = 0$ otherwise.

We apply prototype clustering to the example calcium imaging data set, using the R package protoclust (Bien and Tibshirani, 2015). The resulting dendrogram is in Figure 5(a). In Section 5.2, we discuss choosing the cut-point, or height, at which to cut the dendrogram. Results for different cut-points are displayed in Figures 5(b)–(e). An additional example is provided in Section 11 of the Supplementary File, and a sensitivity analysis indicating that the results of SCALPEL are insensitive to the choice of cut-point is presented in Section 7.3.

4.3. Step 3: Application of the Spatial Component Dictionary. In this final step, we optionally filter the $K$ refined dictionary elements, and then estimate the temporal components associated with this filtered dictionary. We recommend performing the optional filtering of the dictionary elements based on the number of members in the cluster, as clusters with a larger number of

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**Fig 4.** Each column displays two pairs of preliminary dictionary elements with overall dissimilarities, as defined in (5), of 0.05, 0.1, 0.15, 0.2, and 0.25. For each preliminary dictionary element, the average thresholded fluorescence over time and the (zoomed-in) spatial map are shown. These results are based on the example calcium imaging video.
Fig 5. In (a), we display the dendrogram that results from applying prototype clustering to the example calcium imaging data set. Three different cut-points are indicated: 0.05 (---), 0.18 (--), and 0.4 (----). In (b), we display the number of clusters that result from these three cut-points. In (c)–(e), we show the refined dictionary elements that result from using these three cut-points. For simplicity, we only display dictionary elements corresponding to clusters with at least five members.
members are more likely to be true neurons. This filtering process is discussed more in Section 12 of the Supplementary File. After this filtering, we construct the filtered dictionary, $A^f \in \mathbb{R}^{P \times K_f}$, which contains the retained columns of $A$.

We estimate the temporal components associated with the $K_f$ elements of the final dictionary by solving a sparse group lasso problem with a non-negativity constraint,

$$
\min_{Z \in \mathbb{R}^{K_f \times T}, Z \geq 0} \frac{1}{2} \| Y - \tilde{A}^f Z \|_F^2 + \lambda \alpha \sum_{k=1}^{K_f} \| z_{k,\cdot} \|_1 + \lambda (1 - \alpha) \sum_{k=1}^{K_f} \| z_{k,\cdot} \|_2^2,
$$

where $\alpha \in [0, 1]$ and $\lambda > 0$ are tuning parameters, and $\tilde{A}^f$ is defined as $\tilde{a}^f_{\cdot,k} = a^f_{\cdot,k} / \| a^f_{\cdot,k} \|_2^2$. This scaling of $A^f$ is justified in Section 13.3 of the Supplementary File.

The first term of the objective in (6) encourages the spatiotemporal factorization (1) to fit the data closely. The two penalty terms in (6) were specifically chosen to accomplish two goals:

- **Temporal components should be non-zero for a small number of frames:** The second term in (6) is a lasso penalty on each of the temporal components. A lasso penalty on a vector encourages a subset of the individual elements of the vector to be exactly zero (Tibshirani, 1996). In our application, this element-wise sparsity translates to each neuron being estimated to be active in a small number of frames, which fits with the scientific understanding of the activity of neurons.
- **Unneeded neurons should be removed:** The third term in (6) is a group lasso penalty on each of the temporal components. A group lasso penalty on a vector encourages the entire vector to equal zero (Yuan and Lin, 2006). This group-wise sparsity on the temporal components leads to selection of dictionary components: for $\lambda (1 - \alpha)$ sufficiently large, only a subset of the $K_f$ elements in the filtered dictionary will have a non-zero temporal component. This penalty is especially useful for removing any dictionary elements that are a combination of neighboring neurons; we elaborate on this point in Section 13.5 of the Supplementary File.

In Section 13 of the Supplementary File, we discuss the technical details related to solving (6). We summarize the practical implications of these results here:

- **Feasible computational time:** We show that solving (6) is decomposable into spatially overlapping groups of neurons. Furthermore, in many cases, a closed-form solution is available. These two results greatly reduce the computational time required in Step 3. See Sections 13.1 and 13.2 of the Supplementary File for details.
- **Scaling of the columns of $A^f$:** In Section 13.3 of the Supplementary File, we discuss how to scale the columns of $A^f$ so that the optimization problem (6) is invariant to the sizes of the dictionary elements.
- **Ease of tuning parameter selection:** Our results in Section 13.4 of the Supplementary File allow us to determine the largest value of $\lambda$ to consider when selecting $\lambda$ by cross-validation.
- **Zeroing out of double neurons:** Some of the preliminary dictionary elements in Step 1 may be double neurons, i.e., elements that are a combination of two neighboring neurons that happened to be active during the same frame. In Step 2, these double neurons are unlikely to cluster with elements representing either of the individual neurons they represent, and thus these double neurons may remain in the filtered set of dictionary elements, $A^f$, used in Step 3. Fortunately, as detailed in Section 13.5 of the Supplementary File, the group lasso penalty in (6) zeroes out these double neurons.

5. Tuning Parameter Selection. SCALPEL involves a number of tuning parameters:
• Step 1: Quantile thresholds for image segmentation
• Step 2: Cut-point for dendrogram and dissimilarity weight $\omega$
• Step 3: $\lambda$ and $\alpha$ for Equation 6

However, in marked contrast to competing methods (such as matrix factorization approaches) that involve a number of interdependent tuning parameter values, the tuning parameters in SCALPEL can be chosen independently at each step and are very interpretable to the user. We provide recommendations for fixed default values for all but the choice of $\lambda$ in Step 3. We recommend that users first run SCALPEL using the default values. Then, if needed to accommodate differences between labs, experimental conditions, brain regions, or calcium indicators, those default values can be modified. Furthermore, a sensitivity analysis showing that SCALPEL is robust to modest changes in the values of the tuning parameters will be presented in Section 7.3.

We note that in a typical statistical problem, tuning parameters must be carefully chosen using cross-validation or a validation set approach to avoid overfitting. By contrast, the goal of SCALPEL is to identify neurons in the video at hand, rather than in a future video. Furthermore, true positives and false positives can be assessed by visual inspection on a frame-by-frame basis. Thus, tuning parameter selection in SCALPEL is more straightforward than in a typical statistical problem.

5.1. Tuning Parameters for Step 1. The tuning parameters in Step 1 are the quantile thresholds used in image segmentation. In analyzing the example video considered thus far in this paper, we used three different threshold values to segment the video: the negative of the minimum value of $Y$, the negative of the 0.1% quantile of $Y$, and the average of these two values. In principle, these quantile thresholds may need to be adjusted; however, it is straightforward to select reasonable thresholds by visually examining a few frames and their corresponding binary images, as in Figures 3(a) and (b). Furthermore, the results of SCALPEL are insensitive to the exact values of these threshold values, as they serve only to generate a preliminary dictionary. We illustrate the robustness of the results to modest changes in the values of the quantile thresholds in Section 7.3 and Section 14 of the Supplementary File.

5.2. Tuning Parameters for Step 2. In Step 2, we must choose a height $h \in [0, 1]$ at which to cut the hierarchical clustering dendrogram, as shown in Figure 5(a). Fortunately, the cut-point has an intuitive interpretation, which can help guide our choice. If we cut the dendrogram at a height of $h$, then each cluster will contain an element of the preliminary dictionary that has dissimilarity no more than $h$ with each of the other members of that cluster. Figure 4 displays pairs of preliminary dictionary elements with a given dissimilarity. We have used a fixed cut-point of 0.18 to obtain all of the results shown in this paper. An investigator can either choose a cut-point by visual inspection of the resulting refined dictionary elements, or can simply use a fixed cut-point, such as 0.18. We recommend choosing a cut-point less than the dissimilarity weight $\omega$ in (5), as this guarantees that the dictionary elements within each cluster will have spatial overlap. We recommend using $\omega = 0.2$, as discussed in Section 10 of the Supplementary File. Furthermore, in Section 7.3 and Section 14 of the Supplementary File, we show that the results are robust to modest changes in the values of $\omega$ and the cut-point.

5.3. Tuning Parameters for Step 3. In Step 3, we must choose values of $\lambda$ and $\alpha$ in (6). We suggest using a fixed value of $\alpha = 0.9$, which is known to work well in settings like this with a high level of element-wise sparsity (Simon et al., 2013). To select the value of $\lambda$, we use cross-validation. We illustrate this approach in Section 6, and provide further details in Section 13.6 of the Supplementary File. Alternatively, both $\alpha$ and $\lambda$ can be chosen by cross-validation on a two-dimensional grid.
6. Results for Calcium Imaging Data. In this section, we compare SCALPEL’s performance to that of competitor methods on three calcium imaging data sets. In Section 6.1, we detail how we assess performance in this setting. We consider one-photon and two-photon calcium imaging data sets in Sections 6.2 and 6.3, respectively. In Section 6.4, we compare the running times for the various methods. Additional results for these three calcium imaging videos are available at www.ajpete.com/software.

6.1. Assessment of Performance. Given that we are in an unsupervised setting in which both $A$ and $Z$ are unknown, performance cannot be assessed in a traditional manner, such as cross-validation error. Instead, we will assess whether identified neurons are true positives or false positives by visually inspecting a small number of frames in which they are estimated to be active. In SCALPEL, these active frames can be chosen in one of two ways: (i) the frames in which the element was originally derived in Step 1 of SCALPEL or (ii) the frames for which the corresponding temporal component is estimated to be the largest in Step 3 of SCALPEL. We can then visually examine these frames to see if the fluorescence is consistent with the presence of a neuron.

We now briefly comment on the use of visual inspection to determine whether an estimated neuron is a true or false positive. We believe that if the goal were to identify neurons in a single calcium imaging frame, then visual inspection would be the gold standard. However, while it is feasible to identify neurons in a single frame by visual inspection, it would not be possible to identify neurons in an entire movie by visual inspection, because a movie consists of $O(10^5)$ frames, and because of difficulties in aligning neurons between frames (i.e., determining whether two neurons identified in different frames are in fact the same neuron). Thus, while we can confirm or disprove the presence of a given potential neuron by visual inspection, an automated procedure such as SCALPEL is needed to estimate neurons throughout the entire video.

6.2. Application to One-Photon Calcium Imaging Data. We now present the results from applying SCALPEL to the calcium imaging video used as an example in Section 4. This one-photon video, collected by the lab of Ilana Witten at the Princeton Neuroscience Institute, has 3000 frames of size $205 \times 226$ pixels sampled at 10 Hz. We used the default tuning parameters to analyze this video. Using the default quantile thresholds that corresponded to thresholds of 0.0544, 0.0743, and 0.0942, Step 1 of SCALPEL resulted in a preliminary dictionary with 2943 elements, which came from 997 different frames of the video. Using a cut-point of 0.18 in Step 2 resulted in a refined dictionary that contained 50 elements. In Step 3, we discarded the twenty-one components corresponding to clusters with fewer than five preliminary dictionary elements assigned to them, and then fit the sparse group lasso model with $\alpha = 0.9$ and $\lambda = 0.0416$, which was chosen using the validation set approach described in Section 13.6 of the Supplementary File. This resulted in 29 estimated neurons. The results are shown in Figures 6(a) and (b). In Figure 6(c), we compare the estimated neurons to a pixel-wise variance plot of the calcium imaging video. We expect pixels that are part of true neurons to have higher variance than pixels not associated with any neurons. Indeed, we see that many of the estimated neurons coincide with regions of high pixel-wise variance. However, some estimated neurons are in regions with low variance. Examining the frames from which the dictionary elements were derived can provide further evidence as to whether an estimated neuron is truly a neuron. For example, in Figure 7(a), we show that one of the estimated neurons in a low-variance region does indeed appear to be a true neuron, while Figure 7(b) shows evidence that one of the estimated neurons (element 22 in Figure 6) is not truly a neuron. Repeating this process for all of the estimated neurons, we see that element 22 in Figure 6 is the only estimated neuron that does not appear to be a true neuron.

We compare the performance of SCALPEL to that of CNMF-E (Zhou et al., 2016), a proposal
Fig 6. In (a), we plot the spatial maps for the 29 elements of the final dictionary for the calcium imaging video considered in Section 6.2. In (b), we plot their estimated intracellular calcium concentrations corresponding to \( \lambda \) chosen via a validation set approach. In (c), we compare the outlines of the 29 dictionary elements from (a) to a heat map of the pixel-wise variance of the calcium imaging video. That is, we plot the variance of each pixel over the 3000 frames, with whiter points indicating higher variance.

Fig 7. In (a), we see that one of the estimated neurons in a low-variance region in Figure 6(c) does correspond to a true neuron. In (b), we see a frame in which one of the estimated neurons was identified, though there does not appear to be a true neuron.
We display the estimated neurons that result from applying a competitor method, CNMF-E (Zhou et al., 2016), to the calcium imaging video considered in Section 6.2 for (a) the default parameters \( c_{\text{min}} = 0.85 \) and \( \alpha_{\text{min}} = 10 \), (b) the parameters \( c_{\text{min}} = 0.6 \) and \( \alpha_{\text{min}} = 7 \), and (c) the parameters \( c_{\text{min}} = 0.5 \) and \( \alpha_{\text{min}} = 3 \). The variation in darkness of the neurons estimated by CNMF-E is due to the fact that they take on continuous values, compared to the binary masks produced by SCALPEL. In each plot, the true neurons identified by SCALPEL are outlined in gray. Regardless of the tuning parameters used, CNMF-E yields a substantial number of false positives and false negatives.

for the analysis of one-photon data that takes a matrix factorization approach, as described in Section 3. The tuning parameters we consider are those noted in Algorithm 1 of Zhou et al. (2016): the average neuron size \( r \) and the width of the 2D Gaussian kernel \( \sigma \), which relate to the spatial filtering, and the minimum local correlation \( c_{\text{min}} \) and the minimum peak-to-noise ratio \( \alpha_{\text{min}} \), which relate to initializing neurons. We choose \( r = 11 \) in accordance with the average diameter of the neurons identified using SCALPEL. The default values suggested for the other tuning parameters are \( \sigma = 3 \), \( c_{\text{min}} = 0.85 \), and \( \alpha_{\text{min}} = 10 \). We present the results for these default values in Figure 8(a). Only 14 of the 29 neurons identified using SCALPEL were found by CNMF-E using these default parameters. To increase the number of neurons found, we consider lower values for \( c_{\text{min}} \) and \( \alpha_{\text{min}} \). We fit CNMF-E for all combinations of \( c_{\text{min}} = 0.5, 0.6, 0.7 \) and \( \alpha_{\text{min}} = 3, 5, 7 \). To assess the performance of these nine combinations of tuning parameters, we reviewed each estimated neuron for evidence of whether or not it appeared to be a true neuron, by visually inspecting the frames in which the neuron was estimated to be most active. In Figure 8(b), we present the results, chosen from the nine combinations of tuning parameter values considered, that has the smallest number of false positive neurons (i.e., estimated neurons that are noise or duplicates of other estimated neurons). These results consist of 24 estimated neurons: 21 elements correspond to neurons identified using SCALPEL, one element (element 23 in Figure 8(b)) corresponds to a neuron not identified using SCALPEL, and two elements (elements 22 and 24 in Figure 8(b)) appear to be duplicates of other estimated neurons. In Figure 8(c), we present the results, chosen from the nine combinations of tuning parameter values considered, with the highest number of true positive neurons (i.e., neurons that were identified by CNMF-E that appear to be real). These results consist of 41 estimated neurons: 25 elements correspond to neurons identified using SCALPEL, two elements (elements 27 and 34 in Figure 8(c)) correspond to neurons not identified using SCALPEL, 11 elements appear to be duplicates of other estimated neurons, and three elements (elements 39, 40, and 41 in Figure 8(c)) appear to be noise. So while this pair of tuning parameter values resulted in the identification of most of the neurons, it also resulted in a number of false positives. Some of the estimated neurons in Figure 8(c) are large and diffuse, making them difficult to interpret.
6.3. Application to Two-Photon Calcium Imaging Data. We now illustrate SCALPEL on two calcium imaging videos released by the Allen Institute as part of their Allen Brain Observatory. In addition to releasing the data, the Allen Institute also made available the spatial masks for the neurons they identified in each of the videos, using their own in-house software for this task. Thus we compare the estimated neurons from SCALPEL to those from the Allen Institute analysis. The two-photon videos we consider are those from experiments 496934409 and 502634578. The videos contain 105,698 and 105,710 frames, respectively, of size $512 \times 512$ pixels. In their analyses, the Allen Institute down-sampled the number of frames in each video by a factor of eight; we did the same in our analysis. For these videos, we found that using a value slightly smaller than the negative of the 0.1% quantile for the smallest threshold value in Step 0 was preferred based on visual inspection of the thresholding via the `plotThresholdedFrame` function in the SCALPEL R package. Otherwise, default values were used for all of the tuning parameters.

6.3.1. Allen Brain Observatory Experiment 496934409. Using thresholds of 0.250, 0.423, and 0.596, Step 1 of SCALPEL resulted in a preliminary dictionary with 68,630 elements, which came from 11,739 different frames of the video. After refining the dictionary via clustering in Step 2, we were left with 544 elements. In the analysis by the Allen Institute, neurons near the boundary of the field of view were eliminated from consideration. Thus we filtered out 259 elements that contained pixels outside of the region considered by the Allen Institute. Of the remaining 285 elements, 32 of these were determined to be dendrites, 131 were small elements not of primary interest, and 10 were duplicates of other neurons found. Thus in the end, we identified the same 87 neurons that the Allen Institute did, in addition to 25 potential neurons not identified by the Allen Institute. In Figure 9(a), we show the neurons identified by both SCALPEL and the Allen Institute. In Figure 9(b), we show the potential neurons uniquely identified by SCALPEL, along with evidence that they are, in fact, neurons in Figure 9(c).

6.3.2. Allen Brain Observatory Experiment 502634578. Using thresholds of 0.250, 0.481, and 0.712, Step 1 of SCALPEL resulted in a preliminary dictionary with 84,996 elements, which came from 12,272 different frames of the video. After refining the dictionary via clustering in Step 2, we...
We present the results for the calcium imaging video analyzed in Section 6.3.2. In (a), we plot the outlines of the neurons identified by the Allen Institute in blue, along with the outlines of the corresponding SCALPEL neurons in orange. Those shown in green are the Allen Institute neurons that appear to actually be a combination of two neurons. In (b), we plot the 94 potential neurons uniquely identified by SCALPEL in color, along with the SCALPEL neurons also identified by the Allen Institute in gray. In (c), we provide evidence for 4 of the 94 unique neurons. Similar plots for all of the potential neurons uniquely identified by SCALPEL are available at www.ajpete.com/software.

were left with 1297 elements. Once again, we filtered out the 390 elements that contained pixels outside of the region considered by the Allen Institute. Of the remaining 907 elements, 22 of these were determined to be dendrites, 382 were small elements not of primary interest, and 39 were duplicates of other neurons found. Thus in the end, we identified 370 of the 375 neurons that the Allen Institute did, in addition to 94 potential neurons not identified by the Allen Institute. Note that the five neurons identified by the Allen Institute, but not SCALPEL, each appear to be combinations of two neurons. SCALPEL did identify the 10 individual neurons of which these five Allen Institute neurons were a combination. In Figure 10(a), we show the neurons jointly identified by SCALPEL and the Allen Institute. In Figure 10(b), we show the potential neurons uniquely identified by SCALPEL, along with evidence that they are, in fact, real neurons in Figure 10(c).

6.4. Timing Results. All analyses were run on a Macbook Pro with a 2.0 GHz Intel Sandy Bridge Core i7 processor. Running the SCALPEL pipeline on the one-photon data presented in Section 6.2 took 6 minutes for Step 0 and 2 minutes for Steps 1-3. Running CNMF-E on the one-photon data presented in Section 6.2 took 5, 5, and 7 minutes for the analyses with a single set of tuning parameters presented in Figures 8(a), 8(b), and 8(c), respectively. Running the SCALPEL pipeline on the two-photon data presented in Section 6.3.1 took 12.85 hours for Step 0, 2.33 hours for Step 1, and 0.42 hours for Step 2. Running the SCALPEL pipeline on the two-photon data presented in Section 6.3.2 took 12.50 hours for Step 0, 2.55 hours for Step 1, and 0.43 hours for Step 2.

Further computational gains could be made by parallelizing the implementation of SCALPEL Steps 0 and 1. Also, recall that SCALPEL’s most time-intensive step, Step 0, is only ever run a single time for each data set, regardless of whether the user wishes to fit SCALPEL for different tuning parameters.

7. Results for Simulated Calcium Imaging Data. In this section, we apply SCALPEL and CNMF-E (Zhou et al., 2016) to simulated calcium imaging data to assess performance under a range of noise settings. In Section 7.1, we discuss the process of generating the data and as-
We illustrate the various noise scenarios that we consider for the simulated calcium imaging data described in Section 7.1. We vary the signal to spatially correlated noise (SSCN) ratio and the signal to independent noise (SIN) ratio. We show the simulated neurons truly active during a particular frame, along with the simulated data for that frame for each of the noise scenarios. The top row of frames has a variable strength of spatially correlated noise with a fixed strength of independent noise (SIN=1.5), and the bottom row of frames has a variable strength of independent noise with a fixed strength of spatially correlated noise (SSCN=1.5).

To construct the spatially correlated noise $E_{sc}$, we generate a spatially correlated two-dimensional image (i.e., a random field) whose intensity peaks and recedes over a span of 75 frames. There are 20 of these spatially correlated noise patterns in each video. We vary the strength of the noise patterns to explore different signal to noise ratios. We define the signal to noise ratio as the ratio of the peak intensity of the spiking neurons to the peak absolute intensity of the noise. We consider two noise scenarios. In the first, we keep the signal to independent noise ratio fixed at 1.5 and consider values of 1, 1.5, and 2 for the signal to spatially correlated noise ratio. In the second, we keep the signal to spatially correlated noise ratio fixed at 1.5 and consider values of 0.5, 1, 1.5, and 2 for the signal to independent noise ratio. In order to understand the difficulty of identifying neurons in this simulated data, example frames from each of these noise scenarios are shown in Figure 11.

Further details needed to replicate the generation of this simulated data are provided in the R code available at www.ajpete.com/software.

We measure performance in terms of sensitivity, defined as the percent of true neurons detected,
and precision, defined as the percent of neurons detected that are true neurons. We consider a detected neuron to be a match to a true neuron when (i) the pixels of the detected neuron contain at least 50% of the true neuron’s total intensity, and (ii) no more than 20% of the detected neuron’s intensity is contained in pixels not belonging to the true neuron. When more than one detected neuron matches these criteria for a true neuron, we match the detected neuron that captures the highest percentage of the true neuron’s intensity. We chose these fairly liberal matching criteria as to not put the competitor method, CNMF-E, at a disadvantage, since it tends to estimate more diffuse neuron masks than SCALPEL.

7.2. Comparison of Methods. We applied SCALPEL and CNMF-E (Zhou et al., 2016) to the simulated calcium imaging data. For all noise scenarios, SCALPEL was fit using the default tuning parameter values. We found that the default tuning parameter values performed poorly for the competitor method, CNMF-E. Thus we fit CNMF-E for all combinations of $c_{\text{min}} = 0.5, 0.6, 0.7, 0.85$ and $\alpha_{\text{min}} = 3, 5, 7, 10$ on five replicates of data for each noise scenario. For each noise scenario, we choose the tuning parameter combination that had the highest sum of the average sensitivity and average precision such that the average sensitivity was within 5% of the maximum average sensitivity. We then used this selected tuning parameter combination to analyze the remaining replicates of data for that noise scenario. Note that we used knowledge of the true neurons to select the tuning parameter values for CNMF-E so that we can compare SCALPEL’s performance to the best possible performance of CNMF-E in these settings. By contrast, no knowledge of the true neurons was used when applying SCALPEL, for which we just used the default tuning parameter values.

In Figure 12, we present the performance of SCALPEL and CNMF-E on the simulated calcium imaging data. In Figure 12(a), we see that sensitivity of SCALPEL improves as the strength of the spatially correlated noise is reduced, while the precision is fairly constant. Both the sensitivity and precision of CNMF-E improve as the strength of the spatially correlated noise is reduced. However, SCALPEL outperforms CNMF-E on both metrics under all noise scenarios. In Figure 12(b), we see that the sensitivity of SCALPEL is fairly constant as the strength of the independent noise is varied. However, the precision of SCALPEL drops as the strength of independent noise is reduced. While this might seem counterintuitive at first, the strong independent noise effectively counteracts the spatially correlated noise, since the former prevents spatial noise artifacts from being misconstrued as neurons. With the lowest strength of independent noise, CNMF-E has slightly higher precision, but still lower sensitivity than SCALPEL.

7.3. Sensitivity of Results to Tuning Parameter Selection. In Section 7.2, we presented the results for SCALPEL with all tuning parameters set to their default values. To determine how sensitive the results are to changes in the tuning parameters, we now consider the performance of SCALPEL for varied tuning parameter selections. In particular, we investigate the impact of modifying the quantile threshold in Step 1, the dissimilarity weight $\omega$ in Step 2, and the dendrogram cut-point in Step 2. The panels of Figure 13 plot the sensitivity and precision of SCALPEL when one of the tuning parameters is varied and the others are kept fixed at their default values. Note that the results presented are for a signal to spatially correlated noise ratio of 1.5 and a signal to independent noise ratio of 1.5. In Figure 13(a), we see that a high sensitivity is maintained regardless of the quantile threshold. Precision is slightly higher when a lower quantile threshold is used. In Figure 13(b), we see that the choice of $\omega$ does not have an impact on the precision, but choosing a large $\omega$ near 1 results in finding a lower percentage of the true neurons. Recall that when $\omega$ equals 1, only spatial information is used to cluster the preliminary dictionary elements. Without the benefit of temporal information, we are likely to erroneously cluster together spatially
overlapping neurons, resulting in reduced sensitivity. In Figure 13(c), we see that the performance is robust to modest variations in the dendrogram cut-point. These simulations illustrate that the performance of SCALPEL does not diminish with modest variations in the values of the tuning parameters. In Section 14 of the Supplementary File, we investigate the robustness of the results to modest changes in the tuning parameters for the one-photon data analyzed in Section 6.2.

8. Discussion. We have presented SCALPEL, a method for identifying neurons from calcium imaging data. SCALPEL takes a dictionary learning approach. We segment the frames of the calcium imaging video to construct a large preliminary dictionary of potential neurons, which is then refined through the use of clustering using a novel dissimilarity metric that leverages both spatial and temporal information. We then solve a sparse group lasso problem with a non-negativity constraint to obtain a final estimate of the neurons in the data, and to obtain a crude estimate of the calcium concentrations for these neurons.

Future work could consider alternative ways of deriving a preliminary dictionary in Step 1. Currently, we perform image segmentation via thresholding with multiple quantiles. This approach assumes that active neurons will have brightness, relative to their baseline fluorescence levels, that is within the range of our image segmentation threshold values. In practice, there is evidence that some neurons have comparatively lower fluorescence following spiking, which presents a challenge for optimal identification. Though SCALPEL performed well on the one-photon calcium imaging video we considered in Section 6.2, other one-photon videos may have more severe background effects. If this is the case, it may be desirable to incorporate more sophisticated modeling of the background noise, like that employed in Zhou et al. (2016). Additionally, in future work, we could modify Step 3 of SCALPEL to make use of a more refined model for neuron spiking, as in Friedrich et al. (2017); Jewell and Witten (2017); Vogelstein et al. (2010).

Our SCALPEL proposal is implemented in the R package scalpel, which is available on CRAN. A vignette illustrating how to use the package, and code to reproduce all results presented in this paper, are available at ajpete.com/software.
Fig 13. We present the sensitivity of SCALPEL’s performance to changes in the tuning parameters for the simulated calcium imaging data described in Section 7.1. In all panels, we plot the average sensitivity (—) and precision(—), along with 95% confidence intervals, as a function of the tuning parameter value. The dashed line indicates the default value of the tuning parameter. In (a), we consider the value of the quantile threshold used to construct the preliminary dictionary in Step 1. In (b), we consider the value of the dissimilarity weight $\omega$ in Step 2. In (c), we consider the value of the dendrogram cut-point in Step 2, as a proportion of $\omega = 0.2$.

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Supplemental Materials for SCALPEL: Extracting Neurons from Calcium Imaging Data

9. Data Pre-Processing. To begin, we perform three pre-processing steps on the raw data. These are briefly described in Step 0 of Section 4. First, we smooth the raw $P \times T$ data matrix spatially and temporally using a Gaussian kernel smoother with a bandwidth of one pixel (Hastie et al., 2009). Second, we adjust for any bleaching effect over time. Specifically, we fit a smoothing spline with 10 degrees of freedom to the median fluorescence for each frame over time, and subtract the frame-specific smoothed median from the corresponding frame. The smoothing spline fit for the example calcium imaging video is shown in Figure S1.

Finally, we apply a slight variation of the often-used $\Delta f/f$ transformation (Ahrens et al., 2013; Grewe et al., 2010; Grienberger and Konnerth, 2012). For the $i$th pixel in the $j$th frame, the standardized fluorescence is equal to

$$y_{i,j} = \frac{y_{i,j}^0 - \text{median}_{t=1,\ldots,T}(y_{i,t}^0)}{\text{median}_{t=1,\ldots,T}(y_{i,t}^0) + \text{quantile}_{10\%}(Y^0)},$$

where $y_{i,j}^0$ is the fluorescence (after smoothing and bleaching correction) of the $i$th pixel in the $j$th frame. This differs from the typical $\Delta f/f$ transformation in that (i) we standardize using the median across image frames instead of the mean; and (ii) we add a small number to the denominator. This adjustment in the denominator prevents small fluctuations in the amount of fluorescence at pixels with very little overall fluorescence from resulting in extremely high standardized fluorescences. In Figure S2, we show the resulting images after each stage of pre-processing for a sample frame.

10. Rationale for the Value of $\omega$ Used. In Section 4.2.1 of the main text, we suggested using a default value of $\omega = 0.2$ in Step 2 of SCALPEL. Here, we provide a justification for that choice of default value. To do this, we will derive the spatial and temporal dissimilarities for a pair of neurons. First, we make a couple of simplifying assumptions. We consider two neurons of the same size that share a fraction $k$ of their pixels. We assume that the neurons fire in distinct frames and
Fig S2. In (a), we show a sample frame from the raw example calcium imaging video. In (b), we show the same frame after spatial and temporal smoothing has been done and the bleaching effect has been removed. In (c), we show the frame after the $\Delta f/f$ transformation has been performed, which is the final result of the pre-processing in Step 0 of SCALPEL.

Fig S3. We compare the spatial and temporal dissimilarities, which are derived in Section 10, for a pair of neurons sharing a certain percent of pixels.

have the same overall activity (i.e., the $\ell_2$ norm of their calcium concentration over time is equal). Under these assumptions, the spatial dissimilarity will be $1 - k$ and the temporal dissimilarity will be $1 - \frac{2k}{1+k^2}$. These results follow directly from the definitions given in equations (3) and (4). In Figure S3, we plot the spatial and temporal dissimilarities over the range of possible $k$ values. We see that the spatial dissimilarity will always be larger than the temporal dissimilarity. Therefore, to put the spatial and temporal dissimilarities on equal footing, we should use $\omega < 0.5$. While the exact value of $\omega$ needed to balance the temporal and spatial information equally depends on the amount of overlap, we chose $\omega = 0.2$ as this corresponds to an intermediate amount of overlap (65%). Indeed, the exact choice of $\omega$ is not incredibly important: we show that values of $\omega$ between 0.1 and 0.4 perform similarly well for simulated data (Section 7.3) and real data (Section 14).

11. Example of a Cluster in Step 2. To see how the preliminary dictionary element in a cluster relates to the other preliminary dictionary elements assigned to that cluster, we give an example in Figure S4.

12. Filtering Dictionary Elements Prior to Fitting the Sparse Group Lasso. Optionally, at the beginning of Step 3, the elements in the refined dictionary from Step 2 may be filtered
prior to fitting the sparse group lasso in (6). One way to filter the dictionary elements is on the
basis of the number of members in the clusters. That is, we can discard any dictionary elements
representing clusters containing fewer than some minimum number of members. This number should
be chosen based on the goals of the analysis. If we retain all clusters, regardless of size, then we
may include some non-neuronal dictionary elements in the sparse group lasso problem. In contrast,
if we discard small clusters (e.g., those with fewer than five members), then we may erroneously
filter out some true neurons. In Figure S5, we illustrate the sensitivity of the results to the choice
of minimum cluster size, on the example video considered in Sections 4 and 6.2.

13. Further Discussion of Step 3. We now elaborate on issues related to solving the sparse
group lasso problem (6) in Step 3. This discussion is somewhat technical, and can be skipped by
readers interested in only the practical use of SCALPEL. We discuss the solution to (6) when
$K_f = 1$ in Section 13.1, our algorithm for solving (6) for any value of $K_f$ in Section 13.2, the
justification for the scaling of $A^f$ in Section 13.3, a result about the tuning parameters $\alpha$ and $\lambda$
that lead to a sparse solution in Section 13.4, and the ability of the group lasso penalty in (6) to
zero out unwanted dictionary elements in Section 13.5.

13.1. Single Component Problem. We first consider solving (6) in the setting with a single
spatial component ($K_f = 1$). While calcium imaging data will not have only a single neuron, this
setting provides intuition, and will prove useful when we later solve (6) for $K_f > 1$ in Section 13.2.

Lemma 13.1. The solution to

\[ \begin{align*}
\text{(S1)} & \quad \text{minimize } \quad \frac{1}{2} \left\| Y - \tilde{a}^f z^\top \right\|_F^2 + \lambda \alpha \| z \|_1 + \lambda (1 - \alpha) \| z \|_2 \\
\text{(S2)} & \quad \hat{z} = \left( 1 - \frac{\lambda (1 - \alpha)}{\| (Y^\top \tilde{a}^f - \lambda \alpha 1) \|_2} \right) \left( \frac{Y^\top \tilde{a}^f - \lambda \alpha 1}{(\tilde{a}^f)^\top \tilde{a}^f} \right)_+, 
\end{align*} \]

where $(a)_+ = \max(0, a)$ is applied element-wise.
Fig S5. On the example video considered in Sections 4 and 6.2, we manually inspected each dictionary element resulting from Step 2 of SCALPEL, by examining the frames from which each dictionary element was derived. Based on this manual inspection, we classified each of the 50 dictionary elements as a “neuron” or a “non-neuron”. Next, we considered whether simply filtering each dictionary element based on the number of elements in its cluster (as described at the beginning of Step 3 of SCALPEL) would accurately distinguish between “neurons” and “non-neurons”. In the figure, the y-axis shows the percentage of “neurons” that would remain after filtering (○), and the percentage of “non-neurons” that would be eliminated via filtering (●), as a function of the filtering threshold (shown on the x-axis). We find that in this video, a careful manual analysis of each dictionary element yields very similar results to simply filtering each dictionary element based on the number of elements in its cluster.

The proof of Lemma 13.1 is in Section 15.1. We can inspect the solution (S2) to gain intuition. Recall that $\tilde{a}^f_{i,k} \equiv a^f_{i,k}/\|a^f_{i,k}\|^2_2$, where $a^f_{i,k}$ has binary elements. Therefore, $Y^T \tilde{a}^f \in \mathbb{R}^T$ is the average fluorescence of pixels in the filtered dictionary element at each of the frames and $\frac{1}{\|a^f\|_1} a^T$ equals the number of pixels in the dictionary element. When $\lambda = 0$, $\hat{z} = \left( \frac{Y^T \tilde{a}^f}{\|a^f\|_1} \right) +$, which is simply the positive part of the total fluorescence at all pixels in the dictionary element over time. We now consider the impact of $\lambda$ for three different settings of $\alpha$:

- $\alpha = 1$: In this setting, $\hat{z}$ is the positive part of the soft-thresholded total fluorescence. This soft-thresholding encourages elements of $\hat{z}$ to be exactly zero for frames in which the dictionary element has low fluorescence.
- $\alpha = 0$: In this setting, $\hat{z}$ is found by scaling all elements of the total fluorescence by the same amount. Thus, individual elements of $\hat{z}$ are not encouraged to be 0, though $\hat{z} = 0$ if the dictionary element has a low amount of fluorescence across all frames (i.e., $\|Y^T \tilde{a}^f\|_2$ small) or $\lambda$ is very large.
- $\alpha \in (0, 1)$: Both soft-thresholding and soft-scaling are performed, which encourages sparsity of individual elements of $\hat{z}$ and the entire vector $\hat{z}$, respectively.

In Figure S6, we illustrate the values of $\hat{z}$ for the three scenarios described above.

13.2. Algorithm. We now consider how to solve (6) for $K_f > 1$. While generalized gradient descent (Beck and Teboulle, 2009) can be used to solve concurrently for $x_1, \ldots, x_{K_f}$ in (6), the problem is solved more efficiently by noting that (6) is decomposable into groups of overlapping spatial components.
Fig S6. For a single dictionary element in the example video, we plot the solution $\hat{z}$, as given in (S2), for a range of $\lambda$ when (a) only a lasso penalty is used ($\alpha = 1$), (b) a mixture of penalties is used ($\alpha = 0.5$), and (c) only a group lasso penalty is used ($\alpha = 0$).

Let $\mathcal{N}_1, \ldots, \mathcal{N}_S$ denote a partition of the $K_f$ elements of the filtered dictionary, such that $\mathcal{N}_s \cap \mathcal{N}_{s'} = \emptyset$ for $s \neq s'$, and $\bigcup_{s=1}^{S} \mathcal{N}_s = \{1, \ldots, K_f\}$. Define the mapping

(S3) $\mathcal{M}(\mathcal{N}_s) = \{ p \in (1, \ldots, P) : (\hat{A}_{p,\mathcal{N}_s})^\top 1 > 0 \}$.

That is, $\mathcal{M}(\mathcal{N}_s)$ indexes the set of pixels that are active in that subset of neurons.

**Lemma 13.2.** Suppose that $\mathcal{M}(\mathcal{N}_s) \cap \mathcal{M}(\mathcal{N}_{s'}) = \emptyset$ for all $s \neq s'$, so that there is no spatial overlap between the sets of filtered dictionary elements $\mathcal{N}_1, \ldots, \mathcal{N}_S$. Then solving (6) gives the same solution as solving

(S4) $\min_{Z_{\mathcal{N}_s} \in \mathbb{R}^{\left|\mathcal{N}_s\right| \times T}} \frac{1}{2} \left\| Y_{\mathcal{M}(\mathcal{N}_s)} - \tilde{A}_{\mathcal{M}(\mathcal{N}_s),\mathcal{N}_s} Z_{\mathcal{N}_s} \right\|^2_F + \lambda \alpha \sum_{n \in \mathcal{N}_s} \| z_n \|_1 + \lambda (1 - \alpha) \sum_{n \in \mathcal{N}_s} \| z_n \|_2$,

for $s = 1, 2, \ldots, S$.

The proof of Lemma 13.2 is in Section 15.2.

Our approach to solving (S4) depends on the size of $\mathcal{N}_s$. For $|\mathcal{N}_s| = 1$, we can simply use the closed-form solution for $z_{\mathcal{N}_s}$, given by Lemma 13.1. This is advantageous as the calcium imaging data sets that we have analyzed often have some dictionary elements that do not overlap with any others. For $|\mathcal{N}_s| > 1$, we use generalized gradient descent to solve for the global optimum of (S4) (Beck and Teboulle, 2009).

In light of Lemma 13.2, in order to solve (6), we first partition the filtered dictionary elements into $S$ sets, $\mathcal{N}_1, \ldots, \mathcal{N}_S$, such that there is no overlap between the pixels in the $S$ sets, and so that no set can be partitioned further. This can be done quickly, as outlined in Step 1 of Algorithm 1. Then, we solve (S4) for $s = 1, \ldots, S$. Details are provided in Algorithm 1.

We typically solve (6) for a sequence of exponentially decreasing $\lambda$ values. To improve computational performance, Step 2(b) of Algorithm 1 can be implemented using warm starts, in which $Z_{\mathcal{N}_s}^{(0)}$ is initialized as the solution for $Z_{\mathcal{N}_s}$, for the previous value of $\lambda$. Additional details regarding the derivation of the generalized gradient descent algorithm used in Step 2(b) of Algorithm 1 are given in Section 15.3.
Algorithm 1 — Algorithm for Solving Equation (6)

1. Construct the adjacency matrix $N \in \mathbb{R}^{K \times K}$ with $n_{i,j} = \begin{cases} 1 & \text{if } (a_f^{(i)})^T a_f^{(j)} > 0 \\ 0 & \text{if } (a_f^{(i)})^T a_f^{(j)} = 0 \end{cases}$. Let $N_1, N_2, \ldots, N_S$ denote the connected components of the graph corresponding to $N$. That is, $N_s$ indexes the filtered dictionary elements in the $s$th connected component. Define the mapping $\mathcal{M}(N_s) = \{p \in (1, \ldots, P) : (\tilde{A}_{p,N_s}^f)^T 1 > 0\}$.

2. For $s = 1, 2, \ldots, S$, solve

$$(S5) \quad \text{minimize } \frac{1}{2} \| Y_{\mathcal{M}(N_s)} - \tilde{A}_{\mathcal{M}(N_s),N_s} Z_{N_s} \|^2_F + \lambda\alpha \sum_{n \in N_s} \| z_n \|_1 + \lambda(1-\alpha) \sum_{n \in N_s} \| z_n \|_2,$$

using one of the two following approaches:

(a) By Lemma 13.1, if $|N_s| = 1$, the closed-form solution for $z_{N_s, n}$ in (S5) is

$$(S6) \quad \hat{z}_{N_s, n} = \left(1 - \frac{\lambda(1-\alpha)}{\| (Y_{\mathcal{M}(N_s),n})^T \tilde{a}_{\mathcal{M}(N_s),N_s} - \lambda\alpha 1 \|_2} \right) \left( (Y_{\mathcal{M}(N_s),n})^T \tilde{a}_{\mathcal{M}(N_s),N_s} - \lambda\alpha 1 \right) + \left( (Y_{\mathcal{M}(N_s),n})^T \tilde{a}_{\mathcal{M}(N_s),N_s} - \lambda\alpha 1 \right) + .$$

(b) If $|N_s| > 1$, use generalized gradient descent to solve (S5) for $Z_{N_s, n}$:

i. Let $f(Z_{N_s, n}) = \frac{1}{2} \| Y_{\mathcal{M}(N_s),n} - \tilde{A}_{\mathcal{M}(N_s),N_s} Z_{N_s, n} \|^2_F + \lambda\alpha 1^T Z_{N_s, n}$. Initialize $Z_{N_s, n}^{(0)} := 0$ and let $t := (\max_{n \in N_s} \sum_{j \in N_s} (\tilde{a}_{\mathcal{M}(N_s),n})^T \tilde{a}_{\mathcal{M}(N_s),n})^{-1}$.

ii. For $b = 1, 2, \ldots$, until convergence, iterate:

$$\nabla f(Z_{N_s, n}^{(b-1)}) := -(\tilde{A}_{\mathcal{M}(N_s),N_s})^T \left( Y_{\mathcal{M}(N_s),n} - \tilde{A}_{\mathcal{M}(N_s),N_s} Z_{N_s, n}^{(b-1)} \right) + \lambda\alpha 11^T,$$

$$Y_{N_s, n} := Z_{N_s, n}^{(b-1)} - t \nabla f(Z_{N_s, n}^{(b-1)}),$$

and

$$z_{n, n}^{(b)} := \left(1 - \frac{\lambda(1-\alpha)}{\| (y_{n, n})^T \|_2} \right) \left( \tilde{y}_{n, n} \right),$$

for $n \in N_s$. 

13.3. Scaling of $\mathbf{A}^f$. In (6), the $k$th column of the matrix $\tilde{\mathbf{A}}^f$ encodes the spatial mapping of the $k$th filtered dictionary element, after scaling. To obtain $\tilde{\mathbf{A}}^f$, we divide the $k$th column of $\mathbf{A}^f$ by $\|\mathbf{a}^f_{k}\|_2$, the number of pixels in the $k$th filtered dictionary element. This scaling is performed so that the sizes of the dictionary elements do not impact when the components enter the model. That is, we would like $\|\mathbf{a}^f_{k}\|_2$ to be independent of the largest value of $\lambda$ for which $\hat{z}_k, \neq 0$. The following lemma supports this particular scaling of the columns of $\mathbf{A}^f$.

**Lemma 13.3.** Suppose $\mathbf{Y} = \mathbf{A}^f \mathbf{Z}^*$ where the following conditions hold:

(i) $\mathbf{A}^f \in \mathbb{R}^{P \times K_f}$ with $(\mathbf{a}^f_{1,1})^\top \mathbf{a}^f_{1,2} = 0$, $(\mathbf{a}^f_{1,1})^\top \mathbf{a}^f_{2,k} = 0$ for $k = 3, \ldots, K_f$, and $(\mathbf{a}^f_{2,2})^\top \mathbf{a}^f_{2,k} = 0$ for $k = 3, \ldots, K_f$ and

(ii) $\mathbf{Z}^* \in \mathbb{R}^{K_f \times T}$ with $z^*_1 = \mathbf{P} z^*_2$, for some $T \times T$ permutation matrix $\mathbf{P}$.

If we solve (6) for $\mathbf{Z}$ with $\tilde{\mathbf{A}}^f$ such that $\tilde{\mathbf{a}}^f_{k} = \mathbf{a}^f_{k}/\|\mathbf{a}^f_{k}\|_2^2$, then $\hat{z}_1, = \mathbf{0}$ if and only if $\hat{z}_2, = \mathbf{0}$.

The proof of Lemma 13.3 is in Section 15.4. Lemma 13.3 indicates that two non-overlapping spatial components, possibly of different sizes, whose temporal components are identical up to a permutation, will enter the model at the same value of $\lambda$. In Figure S7, we provide empirical evidence for the chosen scaling of $\mathbf{A}^f$.

13.4. Sparsity of the Solution. We now consider the range of $\lambda$ for which the solution to (6) is completely sparse (i.e., $\hat{\mathbf{Z}} = \mathbf{0}$) for a fixed value of $\alpha$.

**Lemma 13.4.** For any $\alpha \in [0, 1]$, the solution to (6) is completely sparse if and only if

(S7) \[ \lambda(1 - \alpha) \geq \left\| \left( (\tilde{\mathbf{A}}^f)^\top \mathbf{Y} \right)_{k,} - \lambda \mathbf{a}^f_1 \right\|_2 \]

for $k = 1, \ldots, K_f$. 

---

**Fig S7.** We solve (6) for different scalings of $\tilde{\mathbf{A}}^f$. For each spatial component, we note the value of $\lambda$ at which the spatial component enters the model (i.e., the largest $\lambda$ for which $\hat{z}_k, \neq 0$). We plot the size of each spatial component versus the value of $\lambda$ at which the spatial component enters for (a) $\tilde{\mathbf{a}}^f_{k} = \mathbf{a}^f_{k}$, (b) $\tilde{\mathbf{a}}^f_{k} = \mathbf{a}^f_{k}/\|\mathbf{a}^f_{k}\|_2$, and (c) $\tilde{\mathbf{a}}^f_{k} = \mathbf{a}^f_{k}/\|\mathbf{a}^f_{k}\|_2^2$. There is a high correlation in the scatterplots in panels (a) and (b), but little correlation in (c). This lack of correlation motivates us to use the scaling $\tilde{\mathbf{a}}^f_{k} = \mathbf{a}^f_{k}/\|\mathbf{a}^f_{k}\|_2^2$ in Step 3, so that dictionary elements receive a fair shot of selection by the sparse group lasso (6), regardless of their size.
Fig S8. We plot the value of the objective of (6) at $\hat{Z}(\lambda)$, the minimizer of (6) at $\lambda$, for a replicate of data as $\lambda$ varies. We compare two ways of finding a $\lambda$ large enough such that $\hat{Z}(\lambda) = 0$, which results in the objective shown as $\cdots$. We take $\lambda$ that satisfies Lemma 13.4 ($\longrightarrow$) or $\lambda$ as defined in Corollary 13.5 ($\cdots$). The former ($\longrightarrow$) gives the smallest $\lambda$ such that $\hat{Z}(\lambda) = 0$. We can see this from the fact that the line ($\longrightarrow$) is on the boundary of the shaded box, which indicates the range of $\lambda$ for which the objective value at $\hat{Z}(\lambda)$ equals the objective value at 0, i.e., the range of $\lambda$ for which $\hat{Z}(\lambda) = 0$.

Unfortunately, when $\alpha \in (0,1)$, $\lambda$ is on both sides of the inequality in (S7). Though we can solve for $\lambda$ in (S7) using a root finder when $\alpha \in (0,1)$, the following corollary provides a simple alternative.

**Corollary 13.5.** For any $\alpha \in (0,1)$, if

$$\lambda \geq \max_{k=1,\ldots,K_f} \left[ \min \left( \max_{l=1,\ldots,T} \left( \frac{\left( (\tilde{A}^f)^\top Y \right)_{k,l}}{\alpha} + \left\| \left( (\tilde{A}^f)^\top Y \right)_{k,l} \right\|_2 \right) \right), \frac{1}{1-\alpha} \right],$$

then the solution to (6) is completely sparse.

The condition in Corollary 13.5 is sufficient, but not necessary. Proofs of Lemma 13.4 and Corollary 13.5 can be found in Sections 15.5 and 15.6, respectively, of the Supplementary File. An illustration of Lemma 13.4 and Corollary 13.5 is provided in Figure S8.

13.5. Zeroing Out of Double Neurons. Some of the elements in the preliminary dictionary obtained in Step 1 may be double neurons, i.e., elements that are a combination of two separate neurons. This occurs when two neighboring neurons are active during the same frame. In Step 2 of SCALPEL, these double neurons are unlikely to cluster with elements representing either of the individual neurons they combine, and thus there may be double neurons that remain in the filtered set of dictionary elements, $A^f$, used in Step 3 of SCALPEL. Fortunately, as detailed in the following lemma, the group lasso penalty in (6) filters out these double neurons by estimating their temporal components to be the zero vector.

**Lemma 13.6.** Suppose that the following conditions hold on $A^f \in \mathbb{R}^{P \times K_f}$:

(i) $a^f_{i,3} = a^f_{i,1} + a^f_{i,2}$,
(ii) $(a^f_{i,1})^\top a^f_{i,2} = 0,$
Then, define \( \tilde{a}_{j,k} = \frac{a_{j,k}}{\|a_{j,k}\|_2} \), and consider solving (6) for \( Z \) with \( \alpha < 1 \). Then, \( \hat{z}_{3,\cdot} = 0 \).

The proof of Lemma 13.6 is in Section 15.7. Note that Lemma 13.6 assumes that the individual elements for the neighboring neurons, \( a_{j,1} \) and \( a_{j,2} \), do not overlap at all. The group lasso penalty can also be effective at zeroing out double neurons resulting from overlapping neurons, though this depends on the amount of overlap, among other factors.

13.6. Selecting \( \lambda \) in (6) in Step 3. To choose \( \lambda \) for (6) via a validation set approach, we perform the following steps:

1. Obtain \( \tilde{A} \in \mathbb{R}^{P \times K_f} \) by dividing the \( k \)th column of \( A \) by \( \|a_{j,k}\|_2^2 \), which ensures that the sizes of the dictionary elements do not impact when the components enter the model.
2. Construct a training set \( T \) by sampling 60% of the pixels in each overlapping group of neurons. That is, we sample 60% of the elements in \( \mathcal{M}(\mathcal{N}_1), \mathcal{M}(\mathcal{N}_2), \ldots, \mathcal{M}(\mathcal{N}_S) \), which were defined in (S3). Assign the remaining pixels to the validation set, \( V = \{ v \in \{1, \ldots, P\} : v \notin T \} \).
3. Using Algorithm 1, solve (6) on the training set of pixels for a decreasing sequence of 20 \( \lambda \) values, \( \lambda_1, \ldots, \lambda_{20} \):

   \[
   \hat{Z}(\lambda_i) = \arg\min_{Z \in \mathbb{R}^{K_f \times P}, Z \geq 0} \frac{1}{2} \|Y_{T,\cdot} - \tilde{A} \hat{f}, \hat{Z}\|_F^2 + \lambda_i \alpha \sum_{k=1}^{K_f} \|z_{k,\cdot}\|_1 + \lambda_i (1 - \alpha) \sum_{k=1}^{K_f} \|z_{k,\cdot}\|_2.
   \]

4. For each \( \lambda_i \), calculate the validation error,

   \[ err_{V}(\lambda_i) = \frac{1}{|V|} \|Y_{V,\cdot}^B - \tilde{A} \hat{f}, \hat{Z}(\lambda_i)\|_F^2, \]

   where \([Y_{V,\cdot}^B]_{j,k} = \begin{cases} [Y_{V,\cdot}]_{j,k} & \text{if } [Y_{V,\cdot}]_{j,k} > -\text{quantile}_{0.1\%}(Y) \\ 0 & \text{otherwise} \end{cases} \). We use a thresholded version of \( Y \) when calculating the validation error, as we only care about the reconstruction error on the brightest parts of the video. Select the optimal value of \( \lambda \) as

   \[ \lambda^* = \arg\max_{\lambda_i} \left\{ \lambda_i : \frac{err_{V}(\lambda_i) - \min_{\lambda_j} err_{V}(\lambda_j)}{\min_{\lambda_j} err_{V}(\lambda_j)} \leq 0.05 \right\} ; \]

   this is the largest value of \( \lambda \) that results in a validation error within 5% of the minimum validation error achieved by any value of \( \lambda \) considered.

5. Solve (6) on all pixels:

   \[
   \min_{Z \in \mathbb{R}^{K_f \times P}, Z \geq 0} \frac{1}{2} \|Y - \tilde{A} \hat{f}, Z\|_F^2 + \frac{\lambda^*}{|T|/P} \alpha \sum_{k=1}^{K_f} \|z_{k,\cdot}\|_1 + \frac{\lambda^*}{|T|/P} (1 - \alpha) \sum_{k=1}^{K_f} \|z_{k,\cdot}\|_2,
   \]

   where we have scaled the tuning parameter by the percent of pixels in the training set, to account for the fact that the sum of squared errors in the loss function is not scaled by the number of pixels.

   This process can be done separately for each group of overlapping neurons \( \mathcal{N}_1, \ldots, \mathcal{N}_S \) to select a different value of \( \lambda \) for each group, or for all groups at once to select a single value of \( \lambda \). By following steps similar to those described above, \( \lambda \) can alternatively be selected via cross-validation.
Fig S9. We present the results of analyzing the one-photon data from Section 6.2 using non-default values of the tuning parameters. In (a), we plot the number of potential neurons identified as a function of the quantile threshold in Step 1. The dashed line indicates the default value for the quantile threshold. In (b), we plot the outlines of the neurons identified using the default value (—) and the non-default values (—) for the quantile threshold. In (c), we plot the number of potential neurons identified as a function of the dendrogram cut-point and $\omega$ in Step 2. The dashed line indicates the default value for $\omega$. In (d), we plot the outlines of the neurons identified using the default values (—) and the non-default values (—) for the dendrogram cut-point and $\omega$ values that produced the same number of neurons as the default values.

14. Sensitivity of Results to Changes in the Tuning Parameters. In analyzing the one-photon data in Section 6.2, we utilized default values for all of the tuning parameters. We now consider what impact varying these default values has on the results of our analysis. In particular, we consider the effect of modifying the quantile threshold in Step 1, the dissimilarity weight $\omega$ in Step 2, and the dendrogram cut-point in Step 2. In Figure S9(a), we see that varying the quantile threshold used for producing the preliminary dictionary in Step 1 results in a small variation in the final number of neurons identified, producing between 28 and 33 neurons, compared to the 29 neurons identified using the default value. Additionally, the shapes of the neurons identified using different quantile thresholds are quite similar (Figure S9(b)). In Figure S9(c), we see that a large range of values of $\omega$ and the dendrogram cut-point produce the exact same number of neurons as the default values of these parameters. Indeed, in Figure S9(d), we see that there is very little change in the neurons identified. These results illustrate the performance of SCALPEL does not diminish with modest variations in the values of the tuning parameters.

Similar analyses for the simulated calcium imaging data are provided in Section 7.3.

15. Technical Proofs Related to Section 13.

15.1. Proof of Lemma 13.1. We first prove a result that we will use later.

**Lemma 15.1.** The following holds: $\arg\min_{\beta \geq 0} \frac{1}{2} \| y - \beta \|_2^2 + \lambda \| \beta \|_2 = \left( 1 - \frac{\lambda}{\| y \|_2} \right) + (y)$. 

**Proof.** Let $\hat{\beta} = \arg\min_{\beta \geq 0} \frac{1}{2} \| y - \beta \|_2^2 + \lambda \| \beta \|_2$ and $C = \{ i : y_i \geq 0 \}$. First, we show $\hat{\beta}_C = 0$. In anticipation of contradiction, assume there exists $j$ such that $j \notin C$ and $\hat{\beta}_j > 0$. Define $\tilde{\beta}$ as $\tilde{\beta}_i = \begin{cases} \hat{\beta}_i & \text{if } i \neq j \\ 0 & \text{if } i = j \end{cases}$. Then

$$\frac{1}{2} \| y - \tilde{\beta} \|_2^2 + \lambda \| \tilde{\beta} \|_2 < \frac{1}{2} \| y - \hat{\beta} \|_2^2 + \lambda \| \hat{\beta} \|_2.$$
This is a contradiction, so we conclude that \( \hat{\beta}_i = 0 \) for all \( i \notin \mathcal{C} \). It remains to solve

\[
\begin{aligned}
\text{(S8)} \quad \min_{\beta_c \geq 0} \quad & \frac{1}{2} \| \mathbf{y} \mathbf{c} - \beta_c \|^2_F + \lambda \| \beta_c \|_2.
\end{aligned}
\]

By a result in Section 3.1 of Simon et al. (2013), the solution to (S8) without the non-negativity constraint on \( \beta_c \) is \( (1 - \frac{\lambda}{\| \mathbf{y} \mathbf{c} \|_2}) \mathbf{y} \mathbf{c} \), which has all non-negative elements. Therefore, it is also the solution to (S8).

We now proceed to prove Lemma 13.1.

**Proof.** Our goal is to solve

\[
\begin{aligned}
\text{(S9)} \quad \min_{z \in \mathbb{R}^T, z \geq 0} \quad & \frac{1}{2} \| \mathbf{Y} - \tilde{\mathbf{a}}^f z^T \|_F^2 + \lambda \alpha \mathbf{1}^T z + \lambda (1 - \alpha) \| z \|_2.
\end{aligned}
\]

Note that solving (S9) is equivalent to solving (S1), as \( \| z \|_1 = \mathbf{1}^T z \) when \( z \geq 0 \). By algebraic manipulation, we can show that

\[
\| \mathbf{Y} - \tilde{\mathbf{a}}^f z^T \|_F^2 + \lambda \alpha \mathbf{1}^T z = \left\| \mathbf{Y}^T \tilde{\mathbf{a}}^f - \lambda \alpha \mathbf{1} \sqrt{\langle \tilde{\mathbf{a}}^f \rangle \mathbf{1}} - \sqrt{\langle \tilde{\mathbf{a}}^f \rangle \mathbf{1}} \tilde{\mathbf{a}}^f z \right\|_2^2 + C,
\]

where \( C \) is a constant that does not depend on \( z \). Therefore, the solution to (S9) is the same as the solution to

\[
\begin{aligned}
\text{(S10)} \quad \min_{z \geq 0} \quad & \frac{1}{2} \left\| \mathbf{Y}^T \tilde{\mathbf{a}}^f - \lambda \alpha \mathbf{1} \right\|_F^2 + \lambda (1 - \alpha) \| z \|_2.
\end{aligned}
\]

We solve (S10) by applying Lemma 15.1.

15.2. *Proof of Lemma 13.2.*

**Proof.** Recall the definition \( \mathcal{M}(\mathcal{N}_s) \) of in (S3). Then, the result follows simply from observing that

\[
\| \mathbf{Y} - \tilde{\mathbf{A}}^f \mathbf{Z} \|_F^2 = \sum_{s=1}^S \left\| \mathbf{Y}_{\mathcal{M}(\mathcal{N}_s), \cdot} - \tilde{\mathbf{A}}^f_{\mathcal{M}(\mathcal{N}_s), \cdot} \mathbf{Z} \right\|_F^2
\]

\[
= \sum_{s=1}^S \left\| \mathbf{Y}_{\mathcal{M}(\mathcal{N}_s), \cdot} - \sum_{s'=1}^S \tilde{\mathbf{A}}^f_{\mathcal{M}(\mathcal{N}_s), \mathcal{N}_{s'}, \cdot} \mathbf{Z}_{\mathcal{N}_{s'}, \cdot} \right\|_F^2
\]

\[
= \sum_{s=1}^S \left\| \mathbf{Y}_{\mathcal{M}(\mathcal{N}_s), \cdot} - \tilde{\mathbf{A}}^f_{\mathcal{M}(\mathcal{N}_s), \mathcal{N}_s, \cdot} \mathbf{Z}_{\mathcal{N}_s, \cdot} \right\|_F^2.
\]

The last equality follows from the condition of the lemma, which guarantees that \( \tilde{\mathbf{A}}^f_{\mathcal{M}(\mathcal{N}_s), \mathcal{N}_{s'}} = 0 \) for all \( s \neq s' \).
15.3. Details of Step 2(b) of Algorithm 1. Note that minimizing the objective in (S5) subject to 
\( Z_{\mathcal{N}_s} \geq 0 \) is equivalent to minimizing

\[
\frac{1}{2} \left\| Y_{M(\mathcal{N}_s)} - \tilde{A}_{M(\mathcal{N}_s),\mathcal{N}_s} Z_{\mathcal{N}_s} \right\|_F^2 + \lambda \alpha^t Z_{\mathcal{N}_s} \cdot 1 + \lambda (1 - \alpha) \sum_{n \in \mathcal{N}_s} \| z_n \|_2
\]

subject to \( Z_{\mathcal{N}_s} \geq 0 \), since \( 1^t Z_{\mathcal{N}_s} \cdot 1 = \sum_{n \in \mathcal{N}_s} \| z_n \|_1 \) when \( Z_{\mathcal{N}_s} \geq 0 \).

Let \( f(Z_{\mathcal{N}_s}) = \frac{1}{2} \left\| Y_{M(\mathcal{N}_s)} - \tilde{A}_{M(\mathcal{N}_s),\mathcal{N}_s} Z_{\mathcal{N}_s} \right\|_F^2 + \lambda \alpha^t Z_{\mathcal{N}_s} \cdot 1 \), which is the differentiable part of (S11), and let \( g(Z_{\mathcal{N}_s}) = (1 - \alpha) \sum_{n \in \mathcal{N}_s} \| z_n \|_2 \), the non-differentiable part.

Generalized gradient descent (Beck and Teboulle, 2009; Parikh and Boyd, 2014) is a majorization-minimization scheme. First, we find a quadratic approximation to \( f(Z_{\mathcal{N}_s}) \) centered at our previous estimate for \( Z_{\mathcal{N}_s} \), \( Z_{\mathcal{N}_s}^0 \), that majorizes \( f(Z_{\mathcal{N}_s}) \). That is,

\[
f(Z_{\mathcal{N}_s}) \leq f(Z_{\mathcal{N}_s}^0) + \text{Tr} \left[ (Z_{\mathcal{N}_s} - Z_{\mathcal{N}_s}^0)^t \nabla f(Z_{\mathcal{N}_s}^0) \right] + \frac{1}{2t} \left\| Z_{\mathcal{N}_s} - Z_{\mathcal{N}_s}^0 \right\|_F^2,
\]

where \( t \) is the step size such that \( \nabla^2 f(\cdot) \preceq \frac{1}{t} I \). After completing the square, we can see that minimizing the quadratic approximation to \( f(Z_{\mathcal{N}_s}) \) gives the same solution as solving

\[
\text{minimize}_{Z_{\mathcal{N}_s}} \quad \frac{1}{2t} \left\| Z_{\mathcal{N}_s} - (Z_{\mathcal{N}_s}^0 - t \nabla f(Z_{\mathcal{N}_s}^0)) \right\|_F^2.
\]

Thus we perform this minimization with \( g(Z_{\mathcal{N}_s}) \) added to the objective function, which gives the proximal problem

\[
\text{minimize}_{Z_{\mathcal{N}_s} \geq 0} \quad \frac{1}{2} \left\| Z_{\mathcal{N}_s} - \bar{Y}_{\mathcal{N}_s} \right\|_F^2 + \lambda (1 - \alpha) t \sum_{n \in \mathcal{N}_s} \| z_n \|_2,
\]

where \( \bar{Y}_{\mathcal{N}_s} = Z_{\mathcal{N}_s}^0 - t \left( -\tilde{A}_{M(\mathcal{N}_s),\mathcal{N}_s}^t \left( Y_{M(\mathcal{N}_s)} - \tilde{A}_{M(\mathcal{N}_s),\mathcal{N}_s} Z_{\mathcal{N}_s}^0 \right) + \lambda \alpha 1^t \right) \). The minimization in (S12) is separable in \( z_{\mathcal{N}_s} \), so for \( n \in \mathcal{N}_s \), we solve

\[
\text{minimize}_{z_n \geq 0} \quad \frac{1}{2} \left\| z_n - \bar{y}_n \right\|_2^2 + \lambda (1 - \alpha) t \| z_n \|_2.
\]

By Lemma 15.1 in Section 15.1, the solution to (S13) is

\[
\hat{z}_n = \left( 1 - \frac{t (1 - \alpha) \| \bar{y}_n \|_2}{\| \bar{y}_n \|_2} \right)^+ \bar{y}_n.
\]

It only remains to derive a suitable step size \( t \) so that \( \nabla^2 f(\cdot) = (\tilde{A}_{M(\mathcal{N}_s),\mathcal{N}_s}^t \tilde{A}_{M(\mathcal{N}_s),\mathcal{N}_s}) \preceq \frac{1}{t} I \). A sufficient condition for \( \frac{1}{t} I - (\tilde{A}_{M(\mathcal{N}_s),\mathcal{N}_s})^t \tilde{A}_{M(\mathcal{N}_s),\mathcal{N}_s} \) to be positive semi-definite is that \( \frac{1}{t} I - (\tilde{A}_{M(\mathcal{N}_s),\mathcal{N}_s})^t \tilde{A}_{M(\mathcal{N}_s),\mathcal{N}_s} \) be diagonally dominant. That is,

\[
\frac{1}{t} - (\tilde{a}_{M(\mathcal{N}_s),n})^t \tilde{a}_{M(\mathcal{N}_s),n} \geq \sum_{j \in \mathcal{N}_s, j \neq n} (\tilde{a}_{M(\mathcal{N}_s),j})^t \tilde{a}_{M(\mathcal{N}_s),n}
\]

for all \( n \in \mathcal{N}_s \). Thus we choose \( t = (\max_{n \in \mathcal{N}_s} \sum_{j \in \mathcal{N}_s} (\tilde{a}_{M(\mathcal{N}_s),j})^t \tilde{a}_{M(\mathcal{N}_s),n})^{-1} \).
15.4. Proof of Lemma 13.3.

Proof. Since \( a_{f_1}^f \) does not overlap any other spatial components (i.e., \((a_{f_1}^f)^\top a_{f_k}^f = 0\) for \(k = 2, \ldots, K_f\)), we know by the results in Lemmas 13.1 and 13.2 that
\[
\hat{z}_{1,s} = \left(1 - \frac{\lambda(1 - \alpha)}{\|Y^\top \tilde{a}_{f_1}^f - \lambda \alpha 1\|_2}\right) + \left(\frac{Y^\top \tilde{a}_{f_1}^f - \lambda \alpha 1}{(\tilde{a}_{f_1}^f)^\top \tilde{a}_{f_1}^f}\right).
\]
Note that \( Y^\top \tilde{a}_{f_1}^f = (Z^*)^\top (A^f)^\top a_{f_1}^f/\|a_{f_1}^f\|_2^2 \), so \( Y^\top \tilde{a}_{f_1}^f = z_{1,s}^* \). Since \((a_{f_1}^f)^\top a_{f_k}^f = 0\) for \(k = 2, \ldots, K_f\), Thus \( \hat{z}_{1,s} = 0 \) if and only if \( \lambda(1 - \alpha) \geq \|z_{1,s}^* - \lambda \alpha 1\|_2 \). Similarly, \( \hat{z}_{2,s} = 0 \) if and only if \( \lambda(1 - \alpha) \geq \|z_{2,s}^* - \lambda \alpha 1\|_2 \).

15.5. Proof of Lemma 13.4.

Proof. Recall that solving (6) gives the same solution as solving (S4). Thus we focus on deriving a condition on \( \lambda \) that guarantees that \( \hat{Z}_{N_{s'}} \), the solution to (S4), equals zero for \( s = 1, \ldots, S \). If \( |N_s| = 1 \), we see from (S6) that \( \hat{z}_{N_{s'}} = 0 \) if and only if
\[
\lambda(1 - \alpha) \geq \left\| \left((Y_{M(N_s)})^\top \tilde{a}_{f_{M(N_s)}}^f - \lambda \alpha 1\right)_{+} \right\|_2.
\]
Recall that if \( |N_s| > 1 \), we iteratively solve for \( \hat{Z}_{N_{s'}} \) using Step 2(b) of Algorithm 1. We initialize at the sparse solution \( Z_{N_{s'}}^{(0)} = 0 \) and thus for \( n \in N_s \)
\[
z_{n,s}^{(1)} = \left(1 - \frac{\lambda(1 - \alpha)t}{\|\tilde{y}_{n,s}^*\|_2}\right)_{+} (\tilde{y}_{n,s}^*),
\]
where \( \tilde{y}_{N_{s'}} = t(\tilde{A}_{M(N_{s'})}^f)^\top Y_{M(N_{s'})}^\top - t\lambda 11^\top \). We will have \( \hat{Z}_{N_{s'}} = 0 \) if \( z_{n,s}^{(1)} = 0 \) for all \( n \in N_s \).

Note that \( z_{n,s}^{(1)} = 0 \) if
\[
\lambda(1 - \alpha)t \geq \left\| \left((\tilde{A}_{M(N_{s'})}^f)^\top Y_{M(N_{s'})}^\top \right)_{+} - t\lambda 1\right\|_2.
\]
By algebraic manipulation, the sparsity conditions given in (S14) and (S15) can be shown to be equivalent to the condition given in Lemma 13.4. Alternatively, this lemma’s result also follows from inspection of the optimality condition for (6).

15.6. Proof of Corollary 13.5.

Proof. The sufficient condition given in Corollary 13.5 follows from noting that (S15) is satisfied if \( \lambda(1 - \alpha) \geq \left\| (\tilde{A}_l^f)^\top Y_{k,l} \right\|_2 \) or if \( \lambda 1 \geq \left\| (\tilde{A}_l^f)^\top Y_{k,l} \right\|_2 \) for \( l = 1, \ldots, T \). Thus, when at least one of these two conditions is satisfied for all \( k = 1, \ldots, K_f \), then the solution to (6) will be sparse.
15.7. **Proof of Lemma 13.6.**

**Proof.** Let $\hat{Z}$ be the solution to (6). In anticipation of contradiction, assume there exists $j \in \{1, \ldots, T\}$ such that $\hat{z}_{3,j} > 0$. Define $\tilde{Z}$ as $\tilde{z}_1 = \hat{z}_1 + \left( \frac{1^T a_{f,j}}{1^T (a_{f,1}^j + a_{f,2}^j)} \right) \hat{z}_3$, $\tilde{z}_2 = \hat{z}_2 + \left( \frac{1^T a_{f,2}^j}{1^T (a_{f,1}^j + a_{f,2}^j)} \right) \hat{z}_3$, $\tilde{z}_3 = 0$, and $\tilde{z}_k = \hat{z}_k$ for $k = 4, \ldots, K_f$. Let $\text{obj}(Z)$ be the value of the objective function of (6) at $Z$ for some fixed $\lambda$ and $\alpha$. We have

$$
\text{obj}(\tilde{Z}) - \text{obj}(\hat{Z}) = \lambda(1 - \alpha) \sum_{k=1}^{3} (\|\tilde{z}_k\|_2 - \|\hat{z}_k\|_2)
$$

$$
= \lambda(1 - \alpha)[\|\tilde{z}_1\|_2 + (1^T a_{f,1}^j)/(1^T (a_{f,1}^j + a_{f,2}^j))\|\tilde{z}_3\|_2]
$$

$$
+ \|\tilde{z}_2\|_2 + (1^T a_{f,2}^j)/(1^T (a_{f,1}^j + a_{f,2}^j))\|\tilde{z}_3\|_2 - (\|\tilde{z}_1\|_2 + \|\tilde{z}_2\|_2 + \|\tilde{z}_3\|_2)
$$

$$
< \lambda(1 - \alpha)[\|\tilde{z}_1\|_2 + (1^T a_{f,1}^j)/(1^T (a_{f,1}^j + a_{f,2}^j))\|\tilde{z}_3\|_2 + \|\tilde{z}_2\|_2
$$

$$
+ (1^T a_{f,2}^j)/(1^T (a_{f,1}^j + a_{f,2}^j))\|\tilde{z}_3\|_2 - (\|\tilde{z}_1\|_2 + \|\tilde{z}_2\|_2 + \|\tilde{z}_3\|_2)]
$$

$$
= 0.
$$

This is a contradiction, so we conclude $\hat{z}_{3,j} = 0$. \qed