

1 **MIN1PIPE: A Miniscope 1-photon-based Calcium Imaging Signal Extraction**
2 **Pipeline**

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19 **SUMMARY**

20 In vivo calcium imaging using 1-photon based miniscope and microendoscopic lens
21 enables studies of neural activities in freely behaving animals. However, the high and
22 fluctuating background, the inevitable movements and distortions of imaging field, and the
23 extensive spatial overlaps of fluorescent signals emitted from imaged neurons inherent in
24 this 1-photon imaging method present major challenges for extracting neuronal signals
25 reliably and automatically from the raw imaging data. Here we develop a unifying
26 algorithm called MINiscope 1-photon imaging PIPEline (MIN1PIPE) that contains several
27 standalone modules and can handle a wide range of imaging conditions and qualities with
28 minimal parameter tuning, and automatically and accurately isolate spatially localized
29 neural signals. We quantitatively compare MIN1PIPE with other existing partial methods
30 using both synthetic and real datasets obtained from different animal models, and show
31 that MIN1PIPE has a superior performance both in terms of efficiency and precision in
32 analyzing noisy miniscope calcium imaging data.

33 INTRODUCTION

34 In vivo calcium imaging of activities from large populations of neurons in awake and
35 behaving animals has become one of the staple technologies in neuroscience (Cai et al.,
36 2016; Flusberg et al., 2008; Ghosh et al., 2011). Recent advances in single-photon based
37 miniscope technology have further enabled imaging of neural ensemble activities in freely
38 moving animals (Cai et al., 2016; Flusberg et al., 2008; Ghosh et al., 2011), thereby
39 allowing circuits involved in a rich repertoire of animal behaviors to be examined. For
40 example, this technology has been successfully used in probing dynamics of neural circuits
41 involved in innate behaviors (Betley et al., 2015; Douglass et al., 2017; Jennings et al.,
42 2015), decision making (Pinto and Dan, 2015; Poyraz et al., 2016), motor control (Klaus
43 et al., 2017), learning and memory (Grewe et al., 2017; Kamigaki and Dan, 2017; Kitamura
44 et al., 2017; Roberts et al., 2017; Roy et al., 2017; Xu et al., 2016), social memory
45 (Okuyama et al., 2016), hippocampal place coding (Ziv et al., 2013), sleep (Cox et al., 2016;
46 Weber and Dan, 2016), bird song (Markowitz et al., 2015), and pathological processes
47 (Berdyeva et al., 2016).

48 The increasing popularity of the miniscope calcium imaging technology demands the
49 development of a fully automatic and robust signal processing method that can reliably
50 extract neuronal signals from the noisy single photon calcium imaging data. Ideally, the
51 processing method 1) should be able to handle a wide range of imaging conditions (*e.g.*
52 high fluctuating background) and results with minimal parameter tuning, and 2) should
53 have minimal assumptions about the quality of the data, such as free of movement or
54 distortion, or sufficiently good signal-to-noise ratio (SNR). Existing imaging processing
55 algorithms do not meet these two criteria.

56 For extraction of neuronal signals, many previous methods work well in situations with
57 high SNR and stable field of views, therefore they are best suited for processing two-photon
58 imaging data. These algorithms include linear unsupervised basis learning methods
59 (Mukamel et al., 2009; Reidl et al., 2007; Pachitariu et al., 2013), nonlinear unsupervised
60 methods (Maruyama et al., 2014; Pnevmatikakis et al., 2016), and the supervised learning
61 method (Apthorpe et al., 2016). The PCA/ICA (principal component analysis followed by
62 independent component analysis) method (Mukamel et al., 2009) was the first attempt to
63 automatize the signal extraction process from miniscope imaging data through manual
64 annotations of ROIs, but this method has difficulties in delineating localized ROIs and in
65 separating overlapping ROIs. Since single-photon based imaging collect lights from a large
66 depth of field, overlapping neurons in different depth is not uncommon. Another method
67 called CNMF (constraint matrix factorization framework) (Pnevmatikakis et al., 2016),
68 which combines nonlinearity in matrix factorization with simultaneous deconvolving spike
69 trains from calcium dynamics, returns more spatially localized maps of ROIs compared to
70 other methods and has better performance in identifying overlapping neurons. While
71 methods like CNMF achieve plausible results in processing two-photon imaging data, they
72 are ill-suited for processing the single-photon based miniscope imaging because: 1) the
73 data from miniscope imaging are dominated by noisy, uneven and fluctuating background,
74 2) such methods depend on sophisticated parameter tuning, especially requiring setting
75 parameters that are unknown *a priori* in practice such as “number of neurons”. Thus, a new
76 method that can effectively remove background yet preserve real neural signals is highly
77 desired.

78 Moreover, both PCA/ICA and CNMF rely on the stable imaging field and will fail if the
79 imaging field contains movement, but movements (including distortions) during imaging,
80 are often inevitable. Therefore, correcting movements and distortions is another major
81 problem needs solving before neural activity signals can be reliably extracted. Many
82 methods were developed independently in attempt to solve this problem. For example,
83 several approaches register frames through template matching based on the assumption
84 that the major form of movements is translational displacement (Dubbs et al., 2016;
85 Thévenaz et al., 1998). To eliminate such assumptions, methods with block-based
86 displacement field estimation were developed, with image feature matching algorithms
87 extended from Lucas-Kanade tracker (Greenberg and Kerr, 2009; Lucas and Kanade, 1981)
88 or Hidden Markov Models (Dombeck et al., 2007; Kaifosh et al., 2013). Some of these
89 movement correction methods have been included as a module in a larger toolbox, using
90 such frame-wise rigid registration approaches (Kaifosh et al., 2014; Pachitariu et al., 2017).
91 When applied to handle nonrigid movement registrations, existing methods make specific
92 assumptions about both the form and magnitude of the potential movements that result in
93 suboptimal performance when large deformation occurs during imaging. Furthermore,
94 errors in the movement correction can easily propagate since these methods all register
95 frames based on a single reference frame. Considering that extracting neural activity
96 signals is highly dependent on removing movement artifacts, an accurate and robust
97 movement correction module is imperative. A simple unimodal algorithm for either
98 translation/rigid or nonrigid registration is insufficient for this purpose.

99 Here we develop the MINiscope 1-photon imaging signal extraction PIPEline (MIN1PIPE)
100 that takes the very raw calcium videos as inputs, and automatically removes background

101 while preserving signals, corrects movements with no assumptions of the types of
102 movements, and delivers separated neuronal ROIs as well as deconvolved calcium traces
103 as outputs. The MIN1PIPE contains a neural enhancing module that minimizes the
104 influence of background unevenness and fluctuations, a hierarchical movement correction
105 module that can handle all kinds of deformation with minimal error propagation, and a
106 seeds-cleansed neural signal extraction module that identifies the set of real ROIs and their
107 corresponding calcium traces without setting unknown parameters *a priori* (Fig. 1).
108 Though our MIN1PIPE is primarily developed for single-photon based miniscope imaging,
109 individual modules can also be independently combined with other processing algorithms
110 to improve performance in analyzing two-photon imaging.

111 **RESULTS**

112 **Core modules in MIN1PIPE**

113 ***Neural Enhancing Module:*** The core of MIN1PIPE relies on turning the imaging data into
114 a stack of background free, baseline corrected frames as the first step. Due to the complex
115 spatiotemporal properties of background dynamics, our method applies a frame-wise
116 background estimator that is adaptive to the local properties. A natural idea can be
117 translated from mathematical morphology and computer vision, that the foreground neural
118 signals can be approximated by subtracting the estimated background in a denoised image.
119 Therefore, we first remove the grainy noise inherent to the single-photon system (see
120 example of such grainy noise in Supplementary Fig. S2) while preserving the boundary
121 between foreground and background, and this is achieved by applying an *anisotropic*
122 *diffusion* denoising operation (Perona and Malik, 1990) on the raw imaging frames. Next,
123 we use a simple straightforward *morphological opening* operation (Serra and Vincent,

124 1992) as the background estimator, with the size of the structure element similar to that of
125 the neurons in the imaging field. The opening operation removes structures smaller than
126 the desired structure element. Subsequently, the foreground that contains all the neuronal
127 signals with the minimal noise is computed as the difference between the denoised raw and
128 the morphological opened frames (Fig. 1a and Supplementary Fig. S1-S2).

129 ***Movement Correction Module***: After the neural signals are enhanced, we next correct for
130 movements in the imaging videos. The problem of movement correction can essentially be
131 broken down to image stack registration. However, without setting specific constraints on
132 the form or magnitude of the movements, even the most efficient registration algorithms
133 require a running time on the order of seconds to minutes per frame (Vercauteren et al.,
134 2009). Considering that the general imaging datasets contain tens of thousands of frames,
135 the time required for applying these sophisticated image registration methods to every
136 frame is inconceivable. Here we develop a hierarchical video registration framework for
137 the correction of all types of movement, without sacrificing the precision or the speed of
138 corrections. Our framework first decomposes the imaging video into two sections: the
139 *stable sections* whose movements can be approximated by small translational displacement,
140 and the *non-stable sections* that contain large general deformation. This step uses the KLT
141 tracker that estimates the displacement of potential corner-like features between two
142 neighboring frames (Shi and Tomasi, 1994). Next, we employ three levels of different
143 strategies to align images. At the first level, we correct the small translational displacement
144 within each stable section using the fast Lucas-Kanade tracker, which can be performed
145 efficiently in parallel on multiple sections (Lucas et al., 1981). We then incorporate a
146 diffeomorphic Log-Demons image registration method which can handle large

147 deformations while preserving the local geometrical properties (Vercauteren et al., 2009).
148 At the second level, we align all the stable sections. The overall information of each section
149 is extracted to form a sectional image. The current sectional image is then aligned to a
150 reference sectional image, which is generated as a linear summation of all previously
151 registered sectional images that is the closest to the current image. The summation weights
152 are determined by least square regression between the current and previous sectional
153 images. The estimated displacement field is then applied to each frame within the current
154 section. This will be iterated until all stable sections are aligned. At the third level, we use
155 a similar process to register the individual frames within each non-stable section, which
156 can be parallelized to boost performance efficiency (Fig. 1b). This hierarchical approach
157 significantly reduces the total registration time due to the balanced assignment of different
158 methods. Importantly, the common registration error does not propagate with this approach.

159 *Seeds-Cleansed Neural Signal Extraction Module*: Once movements are corrected and
160 images are aligned, the main task turns to the neural signal extraction. MINPIPE extracts
161 neural signals automatically in two main steps, 1) the seeds cleansing step to reliably detect
162 the set of real ROIs, and 2) an altered spatiotemporal CNMF to separate ROIs and
163 corresponding calcium traces (Pnevmatikakis et al., 2016). Previous methods contain either
164 no explicit seeds initialization step or only a coarse initialization that compromises between
165 precision and recall. In contrast, our seeds cleansing step forces the algorithm to find the
166 set of real ROIs. This is achieved by first generating an over-complete set of seeds
167 containing all potential centers of real ROIs at the cost of including false positives. This
168 over-complete set is then coarsely cleansed by applying a two-component Gaussian
169 Mixture Model (GMM) on the peak-valley difference of corresponding traces of the seeds,

170 where the traces of real neurons usually have larger fluctuations compared to the non-
171 neuron false positive seeds. The GMM removes most background false positives without
172 losing real neurons (Supplementary Fig. S3). To further remove the remaining false
173 positives, such as the ones with abnormal background fluctuations or hemodynamics, we
174 have trained an Recurrent Neural Networks (RNNs) with LSTM module offline as the
175 classifier for calcium spikes (Supplementary Fig. S4) (LeCun et al., 2015; Hochreiter and
176 Schmidhuber, 1997). Those seeds whose traces contain RNN-identified calcium spikes,
177 regardless of their temporal locations, are classified as true positives whereas the rest are
178 deemed as false positives. After such cleansing processes, there is still a low possibility of
179 identifying multiple seeds within a single ROI. Therefore, we merge potentially redundant
180 seeds by computing the temporal similarity of seeds within their neighborhoods, and
181 preserving the ones with maximum intensity. With the cleansed set of seeds as the initial
182 position of ROIs, we next perform the iterative spatial and temporal optimizations, as
183 proposed in CNMF, to update the spatial footprints of individual ROIs, and the temporal
184 traces with deconvolved spike trains. Notably, unlike previous CNMF, where the spatial
185 footprints are sequentially updated and subtracted from the preceding residuals, we extract
186 spatial footprints from the original data that does not depend on preceding iterations.
187 Therefore, the information loss/duplication is reduced and the optimization procedures can
188 be parallelized in our method.

189 We show example results obtained using the MIN1PIPE methodology including a raw
190 frame, the fully processed ROIs and the example traces from ROIs (Fig. 1c).

191 **Quantitative validation of the MIN1PIPE performance**

192 The neural signal extraction and movement correction are two independent problems that
193 can be tested separately. For the signal extraction, we test the performance on synthetic
194 datasets with various signal levels, while for the movement correction, we can directly test
195 it on real data. To measure the performance of the signal extraction, we use a scoring metric
196 that evaluates the spatial and temporal similarity between the ground truth and the
197 identified ROIs (see Online Methods), and calculate true positive, false positive and false
198 negative. To measure the performance of the movement correction, we use a metric based
199 on the average displacement of feature points between neighboring frames.

200 We synthesized 16 imaging videos with signal levels (SL, defined as the ratio of the
201 amplitude between the signals and the background) ranging from 0.05 to 0.8. Each video
202 contains 3000 frames with 100 neurons of various shapes and calcium dynamics, and
203 background fluctuations extracted from real datasets (details in Supplementary Notes S1-
204 S2). The properties of synthetic videos resemble those of real data, whose SL falls between
205 the range of 0.2 and 0.8. The condition at 0.05 SL is an extreme (Supplementary Fig. S5
206 and Video S1-S3). We compare MIN1PIPE with PCA/ICA and CNMF. We used
207 commercially available *Mosaic* software (Inscopix Inc.) which implements PCA/ICA
208 method (Mukamel et al., 2009), and processed the data following the standard workflow
209 in the software manual. In particular, we chose the number of principal components (PC)
210 and independent components (IC) based on the suggested rate (*e.g.* 20% more ICs and 50%
211 more PCs than the estimated number of ROIs). For CNMF, we used the default
212 initialization strategy in (Pnevmatikakis et al., 2016).

213 The results of ROI detection (Fig. 2a) and the calcium traces from one example ROI
214 obtained by different methods (Fig. 2b) at 0.2 and 0.8 SL are compared. The contours of

215 the identified ROIs using different methods are drawn and superimposed onto the max
216 projection of the ground truth. For both SLs, MIN1PIPE can identify the nearly complete
217 set of ROIs (93% and 100% for 0.2 and 0.8 SL respectively) with minimal false positives
218 (1% and 0% respectively), whereas the other two methods detect partial subsets of ROIs
219 (PCA/ICA: 0% at 0.2 SL and ~65% true positives at 0.8 SL; CNMF: ~32% at 0.2 SL and
220 ~95% true positives at 0.8 SL). In addition, the extracted spatial footprints are less realistic
221 with the PCA/ICA or previous CNMF methods. The example calcium traces indicate that
222 MIN1PIPE has a near-optimal performance in extracting individual ROIs even at low SLs
223 when compared to the ground truth traces (Fig. 2b). In contrast, PCA/ICA completely fails
224 to identify ROIs when SL is low, while CNMF fails to separate neurons from overlapping
225 ROIs. Fig. 2c summarizes the performance accuracy of these three methods at all SLs. The
226 plots of the true positive, false positive and false negative indicate that MIN1PIPE has a
227 significantly superior performance in all conditions, and outperforms the other two
228 methods in the extreme conditions. Fig. 2d summarizes the spatiotemporal similarities
229 between the extracted results and the ground truth. The clusters of point clouds confirm
230 again that MIN1PIPE outperforms the other two methods in best resembling both the
231 spatial and temporal properties of the ground truth signals. The results for all signal levels
232 are shown in Supplementary Fig. S6.

233 To validate the performance of the movement correction in MIN1PIPE, we applied the
234 module on video sections with large deformation movements of the imaging field.
235 Specifically, we chose a video obtained through two-photon imaging of the ferret's
236 posterior parietal cortex as an example due to its particularly frequent and large
237 deformations (Supplementary Video S4 as a demo section of the full video). Fig. 2e shows

238 an example of 3 consecutive frames (with each frame pseudo-colored as pink, green or
239 blue) with large nonlinear deformations between frames superimposed together. The two
240 rigid-transform-based methods (LK and KLT Affine) by themselves fail to fully remove
241 the large deformations, whereas MIN1PIPE (combining the Log-Demons transformation)
242 succeeds in correcting these movements. We further quantified the extent of correction by
243 calculating the average displacement of feature points between two neighboring frames
244 before and after the movement correction (Fig. 2f). Before correction, the score of average
245 displacement shows frequent burst of large deformation periods, whereas after correction,
246 the score of displacement shows a nearly flat line. Notably, the frames with large
247 deformations are all well aligned (Fig. 2f lower panels; Supplementary Video S4).

248 **Application of MIN1PIPE on real miniscope imaging datasets**

249 We next compare the performance of MIN1PIPE with PCA/ICA and CNMF methods on
250 real datasets. We first applied the three methods to the miniscope calcium imaging data
251 obtained using prism probe from layer 2 and 3 of the barrel cortex in freely moving mice.
252 GCaMP6f was expressed in layer 2/3 neurons using AAV, and signals were imaged
253 continuously over 5min when the mouse freely explored its environment. Following the
254 general pipeline of MIN1PIPE (Online Methods), our method removed the strong uneven
255 background structure, and automatically identified 210 putative ROI components without
256 the need for additional manual selection (Fig. 3a, Supplementary Video S5). In comparison,
257 PCA/ICA identified 79 ROIs whereas CNMF identified 71 ROIs. Note that with the same
258 computer configuration and dataset (~28 GB), the CNMF ran into memory issues and could
259 not process the full-scale video, thus we cropped a center patch as the input video to the
260 CNMF (Fig 3b-d middle panels). The contours of the ROIs identified by the different

261 methods are drawn and superimposed onto the max projection of the neural enhanced data
262 (Fig. 3b). This reveals that MIN1PIPE can identify potentially all ROIs, whereas PCA/ICA
263 and CNMF miss a significant subset of ROIs. Meanwhile, both the PCA/ICA and CNMF
264 have some problems in separating overlapping ROIs and/or estimating the correct shape of
265 the ROIs, as revealed by the max projections of the extracted signals (Fig. 3c). The
266 projection of MIN1PIPE extracted signals closely resembles those of the neural enhanced
267 data (Fig. 3c, top). In comparison, the projection obtained using PCA/ICA has low SNR
268 with high background signals (Fig. 3c, bottom), whereas the projection derived from
269 CNMF shows unrealistic ROI shapes larger than the true shape of neurons, indicating that
270 the CNMF is sensitive to the contamination of the background dynamics (Fig. 3c, middle).
271 To further check the shape of individual ROIs, we choose to visualize one example ROI
272 embedded in the full imaging field using different methods (Fig. 3d). MIN1PIPE delineates
273 a well localized ROI footprint, whereas CNMF returns a less localized ROI difficult to
274 relate to the underlying neuron. PCA/ICA, on the other hand, fails to provide a localized
275 footprint as remnants of other ROI components can also be seen. To examine the temporal
276 traces extracted by the different methods, we selected 10 ROI components that were
277 identified by all three methods within the cropped image field and plotted their
278 corresponding calcium traces (Fig 3e, individual panels marked with 1-10). Again, the
279 spatial footprints of the 10 ROIs obtained through CNMF and PCA/ICA are less localized
280 as described above. In terms of calcium dynamics, both MIN1PIPE and CNMF give
281 denoised traces, whereas the traces obtained using PCA/ICA are noisy and show unrealistic
282 negative fluctuations. Furthermore, the traces extracted using CNMF include false positive
283 calcium events that likely reflect background noises (arrows in Fig. 3e). Supplementary

284 Video S6 shows the comparison of raw and processed results using three different methods
285 of the entire imaging video.

286 To further illustrate the general applicability of MIN1PIPE in processing miniscope
287 imaging data obtained over different brain areas and/or different animal models, we applied
288 it to process calcium imaging results from Area X in zebra finch and compared the results
289 with those obtained using the other two methods. Briefly, MIN1PIPE detected 55 ROI
290 components, whereas CNMF detected 15 and PCA/ICA detected 35 ROIs after manual
291 selection (Fig. 4a-b, Supplementary Video S7). Again, CNMF could only process a
292 cropped portion of the imaging video due to the computer memory issues. All of the ROIs
293 detected by CNMF and PCA/ICA are included in the set extracted by MIN1PIPE. In
294 addition to false negatives, CNMF also identifies a cluster of false positive ROIs that are
295 apparent upon visual inspections (Fig. 4b-c, e). The PCA/ICA again gives rise to
296 nonlocalized ROI footprints that contain other potential components (Fig. 4d). It was
297 known that Area X neurons show strong song selective activities when the bird is singing
298 (Goldberg and Fee, 2010; Kojima and Doupe, 2007; Woolley et al., 2014; Yazaki-
299 Sugiyama and Mooney, 2004), which can be used as partial ground truth to validate
300 MIN1PIPE. We plot the calcium traces of the ROIs identified by MIN1PIPE, and the
301 majority of the ROIs contain calcium events that are roughly phase-locked to the singing
302 onsets (Fig. 4f). Notably, a subset of the neurons shows precise singing-related activities
303 with minimal events unrelated to singing (Fig. 4g upper panel). Furthermore, we sorted
304 neurons according to their timing of peak calcium activities during each song production
305 event (Fig. 4g lower panel), and this analysis also reveals a subset of Area X neurons whose

306 activation patterns are closely related to the onset of the singing, consistent with previous
307 findings.

308 We also did studies (Supplementary Fig. S7-S8) showing the effectiveness of the two
309 modules (neural enhancing and seeds-cleansed signal extraction) when combined
310 separately with previous methods to improve the performance. Finally, we compare our
311 method with the CNMF-E method (Zhou et al., 2016) (Supplementary Fig. S9).

312 **DISCUSSION**

313 The key advances that set MIN1PIPE apart from the previous imaging processing methods
314 are the following. First, MIN1PIPE solves the full range of problems for signal extraction
315 in single-photon miniscope imaging with one pipeline. Specifically, we have developed
316 innovative and robust modules to solve different problems including: the neural enhancing
317 module that uses a novel algorithm to remove the ultra-high and fluctuating background
318 characteristic of single photon imaging, the novel hierarchical movement correction
319 module that is capable of efficiently registering any types of deformations of the imaging
320 field, and the seeds-cleansed neural signal extraction module that utilizes GMM and
321 pretrained RNN to enable automatic identification and extraction of ROIs and calcium
322 traces. Second, MIN1PIPE eliminates the need for heuristically setting many parameters
323 that are not only unknown *a priori*, but also influence the performance of the downstream
324 processing steps, such as setting the number of neurons, a central parameter required by all
325 previous neural signal extraction methods. The importance of this should not be neglected,
326 because pre-setting unknown parameters can become problematic in practice. For example,
327 overestimating the number of neurons may result in false positives in identified ROIs
328 before the calcium trace extraction step, and also result in the unnecessary consumption of

329 computing time. These false positives can only be removed with laborious manual selection
330 without a robust seeds-cleansing step. On the other hand, underestimating the number of
331 neurons will likely lead to false negatives that can never be identified by the downstream
332 steps. Therefore, tweaking this parameter is inevitable in practice using previous methods.
333 While we do not claim that MIN1PIPE completely eliminates the need for manually
334 pruning identified ROIs, our method does only involve minimal manual interference. Third,
335 MIN1PIPE contains a minimal set of parameters that are easy to interpret and error-tolerant
336 (Online Methods). These include a set of simplified and fixed parameters applicable to
337 various conditions of the popular miniscope platforms (*e.g.* Inscopix nVista, UCLA open-
338 source miniscope) in our brick algorithm. In addition, all modules use definitive criteria
339 independent of various imaging datasets, which ensures the robustness that the previous
340 combination of setting the number of neurons and the serial initialization procedure could
341 not provide.

342 In summary, MIN1PIPE provides a generally applicable high-performance toolbox with
343 the modular framework to handle and process miniscope imaging data. Interesting future
344 works may integrate more advanced methods to further improve the precision, such as
345 stricter choices of the kernel of anisotropic diffusion (Chen et al., 2011; Tsitsios and
346 Petrou, 2013), considering spectral invariants to handle very large deformations during
347 non-rigid registration (Lombaert et al., 2014), and using more biologically valid calcium
348 dynamics deconvolution methods (Speiser et al., 2017). Additionally, a more robust RNN
349 classifier for seeds cleansing can be trained with more available datasets.

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354 **AUTHOR CONTRIBUTIONS**

355 J.L. and F.W. conceived and designed the project; J.L. designed and implemented all
356 modules of MIN1PIPE; C.L. designed the RNN classifier; J.L. generated the synthetic data;
357 J.L., F.W., J.S.-A., R.M., Z.Z. and F.F. designed and/or conducted in vivo imaging
358 experiments; J.L. and C.L. analyzed the data; J.L., C.L., F.W. wrote the manuscript.

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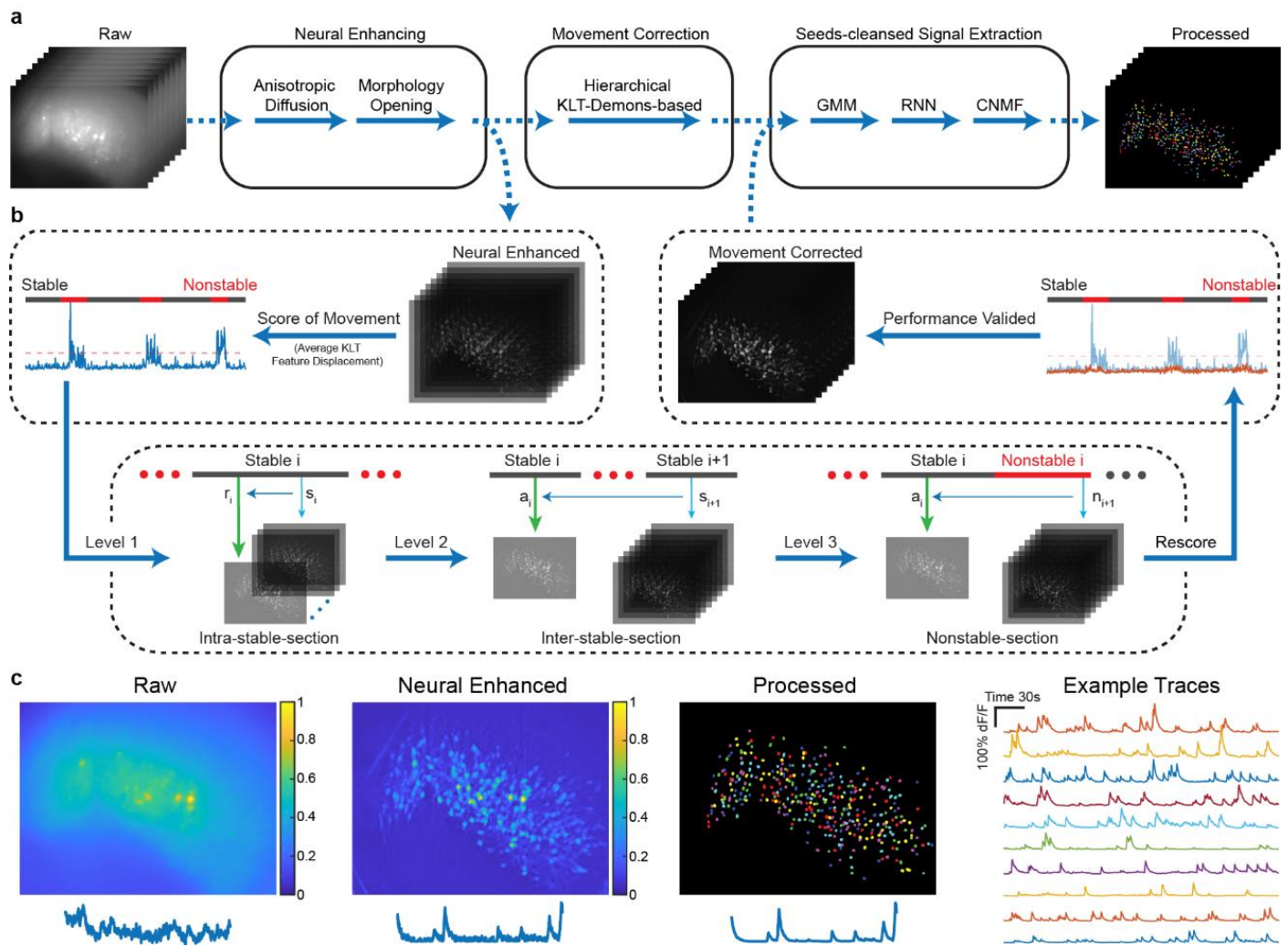
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506 Figure 1

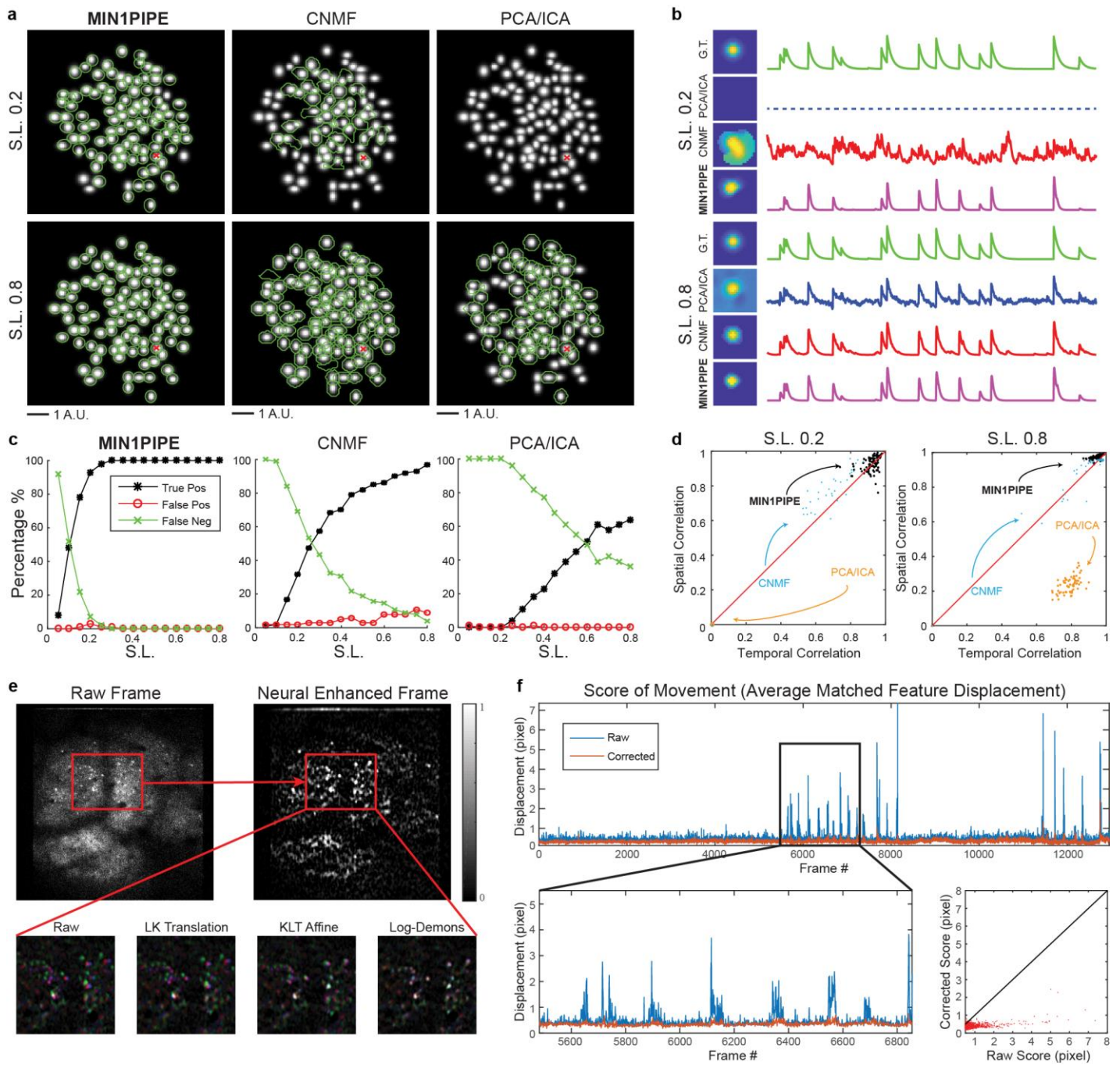


507

508 **Fig. 1.** The general pipeline and demonstrations of MIN1PIPE. **a.** The overall structure of
 509 MIN1PIPE. MIN1PIPE takes the very raw miniscope imaging data freshly collected from
 510 the imaging system as inputs, and returns fully processed ROI components with spatial
 511 footprints and temporal calcium traces as outputs. The data are processed in series by neural
 512 enhancing, hierarchical movement correction, and seeds-cleansed signal extraction
 513 modules. Each module is composed of specific brick functions. **b.** A zoom-in of the
 514 hierarchical movement correction module. The module is KLT-Log Demons based. It first
 515 scores all the neural enhanced frames and divides them into stable and nonstable sections,

516 then register frames at three different levels. The movement corrected frames are then fed
517 into the seeds-cleansed neural signal extraction module. **c.** The max projecting
518 demonstrations of MIN1PIPE. The raw imaging video contains large, dominant
519 background fluctuation, the neural enhanced video contains mainly neural signals, and the
520 fully processed video contains only extracted and denoised neural signals as independent
521 ROI components. Example traces are randomly selected from the processed video. See also
522 Fig. S1-S4.

523 Figure 2

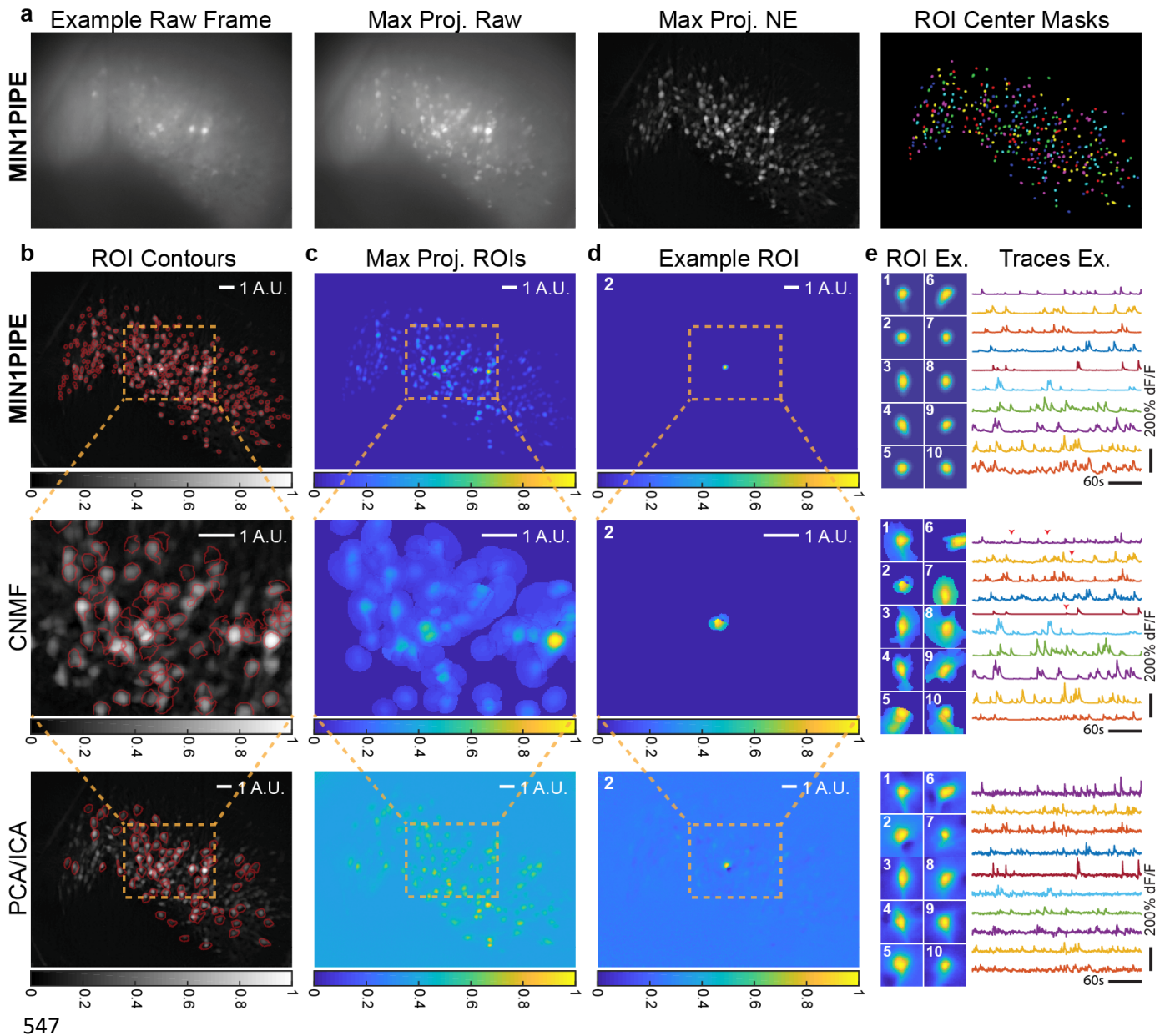


524

525 **Fig. 2.** Quantification of MIN1PIPE. **a-d.** The quantitative performance comparison
 526 between MIN1PIPE and the other methods on simulated datasets. **a.** The identified ROI
 527 contours of the three methods at two representative signal levels. S.L.: signal level (the

528 ratio of the amplitude between the signals and the background). A.U.: arbitrary unit. **b.** The
529 trace of an example ROI component identified by the three methods and the ground truth.
530 G.T.: ground truth. **c.** The identification precision and accuracy of the three methods. **d.**
531 The spatiotemporal identification accuracy with the three methods at two representative
532 signal levels. The similarity of the spatial footprints and temporal traces between the results
533 of the three methods and the ground truth of each ROI is plotted as a dot in the figure.
534 These quantifications confirm that MIN1PIPE not only outperforms the other two methods
535 in all aspects, but also verify that MIN1PIPE can provide satisfactory neural signal
536 extraction results of miniscope imaging data. **e-f.** The quantification of the movement
537 correction module. **e.** The demonstration of various image registration methods in a
538 consecutive three frames from two-photon imaging data. The deformation registration
539 algorithm we use in the module (Log-Demons) successfully corrects all the nonrigid
540 deformations within the frames that cannot be corrected by the methods that assume
541 translation or rigid transformation. The black and white color indicate overlapping of the
542 same structure between the frames, whereas other colors indicate nonaligned structures. **f.**
543 The score of movement before and after the correction. In general, the hierarchical
544 correction steps register large deformations while preserves the stable frames. See also Fig.
545 S5-S9.

546 Figure 3

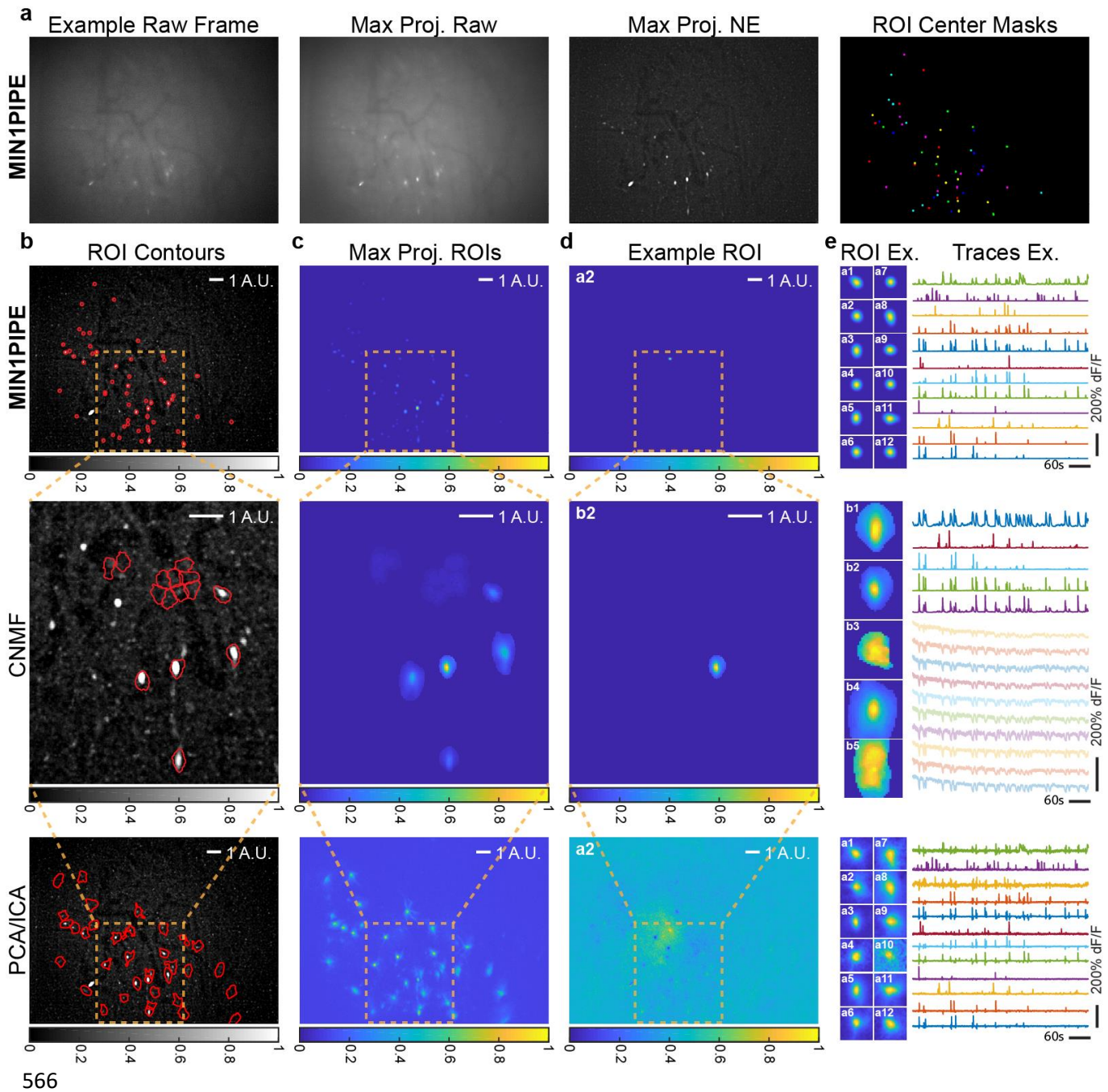


547

548 **Fig. 3.** Comparing different methods using miniscope imaging data from the mouse barrel
549 cortex. **a.** Demo of MIN1PIPE in processing imaging data from the barrel cortex. We show
550 an example raw frame, the max projection of raw video, the max projection of the neural
551 enhanced video, and the colored ROI masks. **b.** The identified ROI contours superimposed
552 on the max projection of neural enhanced video. MIN1PIPE returns a set of ROIs with

553 well-shaped, localized spatial footprints. CNMF and PCA/ICA identify only a small subset
554 of true positives (71 and 79 respectively), and either the footprints are not localized, or the
555 run into memory issues. The projection map is shown in grayscale colormap. **c.** The max
556 projections of all identified ROI footprints. This further demonstrates the general properties
557 of the extracted neural components. **d.** The spatial footprint of an example ROI. MIN1PIPE
558 extracted the most localized component that is close to the real shape shown in the neural
559 enhanced projection, whereas CNMF detected the noise-sensitive result and PCA/ICA
560 detected the unrealistic component as a mix of several other components. **e.** The temporal
561 traces of ten examples randomly selected from the ROIs that are detected by all three
562 methods. A similar conclusion can be drawn in spatial footprints, whereas MIN1PIPE
563 shows the best performance in temporal trace extraction (some potential false positive
564 events indicated by red arrows).

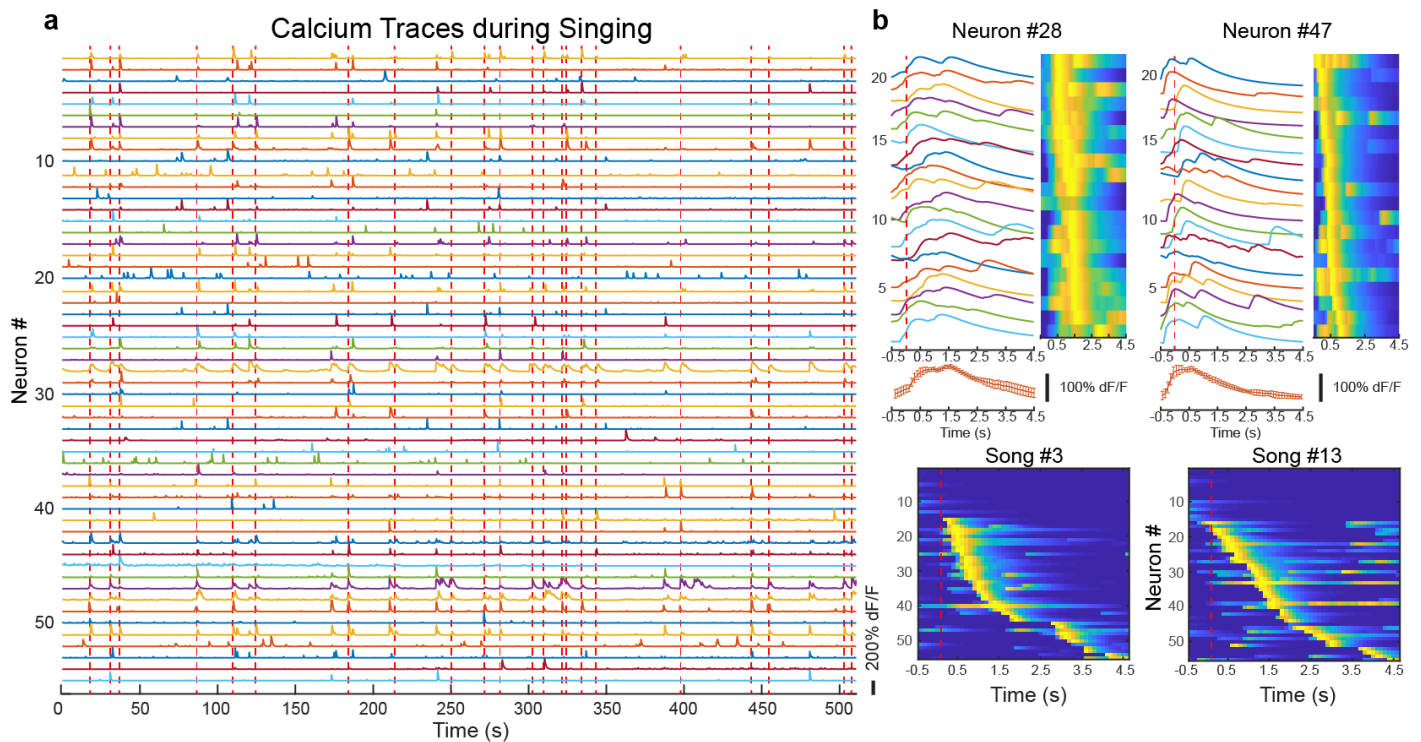
565 Figure 4



567 **Fig. 4.** Comparing different methods using miniscope imaging data from the Area X in
568 zebra finch. **a.** Demo of MIN1PIPE in processing data from Area X in the zebra finch brain.
569 We show an example of the raw frame, the max projection of raw video, the max projection
570 of the neural enhanced video, and the colored ROI masks. **b.** The identified ROI contours

571 superimposed on the max projection of neural enhanced video. MIN1PIPE returns a set of
572 ROIs with well-shaped, localized spatial footprints. CNMF and PCA/ICA identify only a
573 small subset of true positives (15 and 35 respectively), and either the footprints are not
574 localized, or the processes run into memory issues. The projection map is shown in
575 grayscale colormap. **c.** The max projections of all the identified ROI footprints. **d.** The
576 spatial footprint of an example ROI. MIN1PIPE extracted the most localized component
577 that is close to the real shape shown in the neural enhanced projection, whereas PCA/ICA
578 extracted the unrealistic component as a mix of several other components. CNMF on the
579 other hand, did not detect this ROI. **e.** The temporal traces of twelve examples selected
580 from the ROIs detected by both MIN1PIPE and PCA/ICA. A similar conclusion can be
581 drawn in spatial footprints, whereas MIN1PIPE shows the best performance of temporal
582 trace extraction. Faded traces in CNMF indicate the traces of the false positives.

583 Figure 5



584

585 **Fig. 5.** Analysis of neural correlation in Area X of zebra finch to singing behavior. **a.** The
586 traces of all automatically detected ROIs with MIN1PIPE superimposed with complete
587 singing onsets in red dashed lines. Note that incomplete song events are not labeled and
588 taken into analysis. **b.** Upper panel: two example neurons with precise song selectivity.
589 The traces represent the neuronal activity of the first 20 complete song singing events with
590 a window of 0.5 second before and 4.5 seconds after the song onset. The heatmap is another
591 way of visualizing the analysis. The error bar graphs at the bottom show the trial average
592 traces of the two neurons. Lower panel: population activity pattern of two example song
593 singing events. The neurons are sorted to the latency of the peak. Red dashed lines indicate
594 the onset of songs.

595 ONLINE METHODS

596 **NEURAL ENHANCING** The neural enhancing module contains two steps of framewise
597 operations. We denote $\mathbf{X} \in \mathbb{R}^{P \times T}$ (P is number of pixels per frame, and T is number of
598 frames) as the raw video after converting each two dimensional frame into a vector, and
599 $\mathbf{I} \in \mathbb{R}^{M \times J}$ (M and J are the height and width of a frame respectively) as a frame before
600 vectorization.

601 **Denoising** To first remove the spatial noise embedded in each frame introduced by the
602 photoelectric process of the sensor, we apply denoising operation on each frame \mathbf{I} using
603 anisotropic diffusion [36]. For a given diffusion time τ , the evolution follows the equation
604 $\frac{\partial \mathbf{I}}{\partial \tau} = \text{div}(\mathbf{C} \nabla \mathbf{I}) \triangleq \nabla \mathbf{C} \cdot \nabla \mathbf{I} + \mathbf{C} \Delta \mathbf{I}$ where \mathbf{C} is the diffusive coefficient matrix depending
605 on pixels and τ . By choosing concrete form of \mathbf{C} and τ , we can control the smoothing
606 level along or perpendicular to the boundaries between neurons and the background. Due
607 to the simple structures in the cleaned imaging field, we choose the classical Perona-Malik
608 filter $\mathbf{C} = \exp\left(-\frac{\|\nabla \mathbf{I}\|^2}{\kappa^2}\right)$, where κ controls the threshold of high-contrast. The diffusivity is
609 selected to preferentially smooth high-contrast regions, and κ and τ are chosen to allow high
610 tolerance. We use $\kappa = 0.5$ and $\tau = 0.5$ with a step size $\delta t = 0.05$ as the default parameters
611 in anisotropic diffusion. The output image \mathbf{I}^s contains reduced spatial noise while preserves
612 the boundary information of ROIs.

613 **Background Removal** Based on the observation that neuronal ROIs are small in size
614 compared to background structures, the gray-scale morphological opening operator with a
615 binary structuring element matrix Φ can well estimate the background \mathbf{B} from the frame
616 \mathbf{I} adaptively. The opening operator is the combination of erosion \ominus and dilation \oplus : $\mathbf{B} =$
617 $(\mathbf{I} \ominus \Phi) \oplus \Phi$ (Van Den Boomgaard and Van Balen, 1992), where the morphological erosion
618 returns minimum value $(\mathbf{I} \ominus \Phi)(x, y) = \min_{(s,t) \in \Phi} \{\mathbf{I}(x + s, y + t)\}$ and dilation returns

619 maximum value $(\mathbf{I} \oplus \Phi)(x, y) = \max_{(s,t) \in \Phi} \{\mathbf{I}(x + s, y + t)\}$ within the same structuring
620 window at each point. In practice, the choice of the structuring element should be comparable
621 to the overall size of the neurons in the imaging field. We choose the structuring element
622 to be of disk shape with a default size of 9 (pixels). The foreground is then computed as:
623 $\mathbf{I}^f = \mathbf{I} - \mathbf{B}$. After the dynamic background removal, \mathbf{I}^f contains only neuronal information
624 with minimal background noise corruption.

625 **MOVEMENT CORRECTION** The movement correction module performs a hierarchi-
626 cal registration framework over the frames of the imaging video. The neural enhanced video
627 is first scored with a measuring metric on the relative displacement between two neighboring
628 frames. The video is then segmented into stable or nonstable sections based on the score,
629 followed by three levels of registration: the intra-stable-section, inter-stable-section and
630 nonstable-section registration.

631 **Scoring Metric** We track features containing corner information of every two neighboring
632 frames using the KLT tracker, and then calculate the average displacement of these features.
633 The KLT tracker first selects good features to track, where the feature points contains enough
634 information on both directions within the neighborhood p . The problem can be formulated
635 as $\nabla p = 0$ over the neighborhood. A good feature point is selected if the smaller eigenvalue
636 $\sigma_{min} \geq \sigma_0$ and the *condition number* $\kappa = \frac{\sigma_{max}}{\sigma_{min}} \leq \kappa_{max}$. Then the algorithm tracks the
637 feature points based on Newton-Raphson minimization on the normal equation:

$$\mathbf{A} \mathbf{s} = \mathbf{b}$$

638 where

$$\mathbf{A} = \sum_{\mathbf{x}} \nabla \mathbf{J}_t^f(\mathbf{x}) \nabla \mathbf{J}_t^f(\mathbf{x})^\top \omega(\mathbf{x} - \mathbf{x}_{\mathbf{I}_t^f}) \quad \text{and} \quad \mathbf{b} = \sum_{\mathbf{x}} \nabla \mathbf{J}_t^f(\mathbf{x}) [\mathbf{I}_t^f(\mathbf{x}) - \mathbf{J}_t^f(\mathbf{x})]^\top \omega(\mathbf{x} - \mathbf{x}_{\mathbf{I}_t^f})$$

639 J_t^f and I_t^f are the moving and fixed frame of the t th registration pair after neural enhancing
640 respectively (in this case, $J_t^f = I_{t+1}^f$), and $\omega(\mathbf{x})$ is the window of the neighborhood. For the
641 scoring, we choose 31 pixels as the default window size of the neighborhood. To segment
642 the frames into stable/nonstable sections, we compute the minimum value between the 3
643 times of median absolute deviation (MAD) above median and upper limit threshold (0.5
644 pixel as default) to be the threshold of segmentation. Any frame whose score is above
645 the threshold is considered as one belonging to some stable section. The frames that are
646 connected together are labeled as stable sections while the remaining connected components
647 are nonstable ones.

648 **Intra-stable-section Registration** Displacements with small magnitude or consistent
649 directions can be approximated as translational displacement. Therefore, after the segmen-
650 tation, movements within the stable sections can be considered translational. Within each
651 stable section, an efficient displacement matching method with subpixel resolution is used
652 to register the neighboring frames. Here we use a similar Lucas-Kanade tracker to track
653 the center of the moving frames with a sufficiently large window (80% of the frame size)
654 between two neighboring frames. However, when frame number within a section grows, the
655 registration error may cumulate for later frames. Therefore, in practice we update the fixed
656 frame as reference every second and all moving frames within that period are aligned to the
657 fixed frame. This is acceptable due to the slow calcium dynamics so that the imaging field
658 remains essentially similar within a second.

659 **Inter-stable-section Registration** The imaging field is stable within each stable section
660 after the intra-section registration. However, the imaging fields of different stable sections
661 are not necessarily similar. Therefore, we need to align different stable sections without
662 specific assumptions of the movement type. We first extract the overall information of each
663 stable section by averaging the frames with significant neuronal activation, we then sort

664 these sectional images $\mathbf{I}_i^{\text{sec}}$ according to their similarity. To register the i th sectional image
665 based on the sorted similarity, a reference image is generated by calculating the weights \hat{x}
666 using the least square regression between the registered frames and the current sectional
667 image:

$$\mathbf{A}^{\text{ref}}x = \mathbf{b}^{\text{cur}}$$

668 where

$$\mathbf{A}^{\text{ref}} = (\text{vec}(\mathbf{I}_1^{\text{sec}}), \dots, \text{vec}(\mathbf{I}_{i-1}^{\text{sec}})) \quad \text{and} \quad \mathbf{b}^{\text{cur}} = \text{vec}(\mathbf{I}_i^{\text{sec}})$$

669 The reference image is then reshaped into two dimensional matrix $\mathbf{R}_i^{\text{sec}}$ from $\mathbf{b}^{\text{ref}} = \mathbf{A}^{\text{ref}}\hat{x}$.
670 Once we have the moving and fixed sectional images, we apply rigid preregistration to the
671 moving image using similar KLT tracker. To finally correct for the nonrigid deformation, we
672 apply the diffeomorphic Log-Demons to the preregistered image $\hat{\mathbf{I}}_i^{\text{sec}}$ based on the reference
673 $\mathbf{R}_i^{\text{sec}}$. In general, the diffeomorphic Log-Demons is a non-parametric registration method,
674 which aims at finding the displacement of all pixels by minimizing the global energy:

$$E(c, s) = \frac{1}{\sigma_i^2} \text{Sim}(\mathbf{F}, \mathbf{M} \circ c) + \frac{1}{\sigma_x^2} \text{dist}(s, c)^2 + \frac{1}{\sigma_T^2} \text{Reg}(s)$$

675 It consists of the similarity, correspondence and regularization items, where \mathbf{F} and \mathbf{M} are
676 fixed and moving image respectively, and σ_i , σ_x and σ_T account for similarity, spatial
677 uncertainty on the correspondence and regularization level. In specific, Log-Demons
678 represents everything in log domain. It spells out two regularizing terms, fluid and diffusion,
679 and uses a Lie group structure to impose diffeomorphism. In our implementation, we choose
680 $\sigma_i = \sigma_x = 1$, and $\sigma_{\text{fluid}} = \sigma_{\text{diffusion}} = 3$. Because all the frames within the same stable
681 section share the same imaging field, the deformation field of the current stable section is
682 then applied to warp all the frames. After registering all the stable sections iteratively, all
683 the stable frames are efficiently aligned.

684 **Nonstable-section Registration** At the third level, only nonstable sections remain to be
685 aligned. We use a similar approach as in the inter-section registration, where we first
686 preregister the current frame to the reference image, and then apply Log-Demons to finely
687 align the frame. Similarly, the reference image is computed based on some of the registered
688 frames. For the i th nonstable section, this set of frames includes the last frame of the i th
689 stable section, the first frame of the $i + 1$ th stable section, and the frames that have already
690 been registered in the i th nonstable section.

691 **SEEDS-CLEANSSED SIGNAL EXTRACTION** We generate and cleanse the potential
692 seeds of ROIs in this section, and create the initial spatial regions and the time series of
693 ROIs for later refinement of the spatiotemporal signals.

694 **Over-complete Seeds Initialization** To generate the initial set containing centers of all
695 potential ROIs, we construct a *randomized max pooling* process to create an over-complete
696 set of seeds. This process first randomly selects a portion of frames $\mathbf{I}_\alpha^s = \{\mathbf{I}_t^s\}_{t \in \alpha}$, $\alpha \subseteq$
697 $\{1, \dots, T\}$, where α is a randomized subset of frame number. We then compute the max-
698 projection map across selected frames $\mathbf{I}^{\max} = \max(\mathbf{I}_\alpha^s)$, and further detect all the local max
699 points on this map as \mathcal{S} . We repeat the above procedures multiple times, and collect the
700 union of each map \mathcal{S} as the final over-complete set of neural seeds $\mathcal{S}_{\text{seeds}}$. Our randomized
701 max pooling can improve the true positives compared with max-pooling over the entire video,
702 because a real seed can be buried in the uneven florescence of an ROI over a long period but
703 can most likely be discovered in a small temporal vicinity. To exclude false positive seeds
704 from the set, we propose the following two-stage algorithm for seeds refinement.

705 **Seeds Refinement with GMM** The temporal properties of ROIs and non-ROIs can be
706 very different. In one aspect, the ROIs often have prominent peak-valley difference d , while
707 non-ROIs tend to be less spiky. We assume $\{d_s | s \in \mathcal{S}_{\text{seeds}}\}$ are generated from a mixture

708 of two Gaussians $d_s \sim \sum_{i=1}^2 \omega_i \mathcal{N}(d|\mu_i, \sigma_i^2)$, where $\mu_i, \sigma_i^2, \omega_i$ indicate the mean, variance,
709 mixture proportion of the i th Gaussian component. Therefore, we can cluster the seeds
710 based on their probabilities of belonging to each component, and only consider those having
711 higher probabilities to the Gaussian with a larger mean as positive seeds.

712 **Seeds Refinement with LSTM** To select true ROI seeds from the over-complete set,
713 we need to classify the seeds based on their patterns of neuronal calcium dynamics. We
714 propose to employ Recurrent Neural Networks (RNNs) (LeCun et al., 2015) for calcium
715 signal sequence classification. We offline trained the RNNs with a separate training dataset,
716 composed of both positive and negative sequence chunks of length $T_0 = 100$, obtained
717 with 10Hz frame rate. The training data for the RNN module were selected from a separate
718 real dataset. The labels were manually selected by experienced neuroscientists with a
719 conservative standard: the positive trials were the ones with most obvious calcium dynamics
720 that were aligned to the peak of the spike, whereas the negative trials were randomly selected
721 from the rest of the data. The training dataset contained 1000 positive and 1000 negative
722 labels, and both the validation and the test set contained 600 balanced trials. Specifically,
723 consider training data $\mathcal{D} = \{\mathbf{D}_1, \dots, \mathbf{D}_N\}$, where $\mathbf{D}_n \triangleq (\mathbf{y}_n, l_n)$, with input sequence \mathbf{y}_n
724 and output label $l_n \in \{0, 1\}$. Our goal is to learn model parameters θ to best characterize
725 the mapping from \mathbf{y}_n to l_n with likelihood $p(\mathcal{D}|\theta) = \prod_{n=1}^N p(\mathbf{D}_n|\theta)$. In our setting for
726 sequence classification, the input is a sequence, $\mathbf{y} = \{y_1, \dots, y_{T_0}\}$, where y_t is the input
727 data at time t . There is a corresponding hidden state vector $\mathbf{h}_t \in \mathbb{R}^K$ at each time t , obtained
728 by recursively applying the *transition function* $\mathbf{h}_t = g(\mathbf{h}_{t-1}, \mathbf{y}_t; \mathbf{W}, \mathbf{U})$. \mathbf{W} is *encoding*
729 *weights*, and \mathbf{U} is *recurrent weights*. The output c for our classification is defined as the
730 corresponding *decoding function* $p(c|\mathbf{h}_{T_0}; \mathbf{V}) = \sigma(\mathbf{V}\mathbf{h}_{T_0})$, where $\sigma(\cdot)$ denotes the *logistic*
731 *sigmoid function*, and \mathbf{V} is *decoding weights*.

732 The transition function $g(\cdot)$ can be implemented with a *gated* activation function, such as
733 LSTM (Hochreiter and Schmidhuber, 1997) or a Gated Recurrent Unit (GRU) (Cho et al.,

734 2014). Both LSTM and GRU have been proposed to address the issue of learning long-term
 735 sequential dependencies. Each LSTM unit has a cell containing a state \mathbf{c}_t at time t . This cell
 736 can be viewed as a memory unit. Reading or writing the memory unit is controlled through
 737 sigmoid gates: input gate \mathbf{i}_t , forget gate \mathbf{f}_t , and output gate \mathbf{o}_t . The hidden units \mathbf{h}_t are
 738 updated as follows:

$$\begin{aligned} \mathbf{i}_t &= \sigma(\mathbf{W}_i \mathbf{y}_t + \mathbf{U}_i \mathbf{h}_{t-1} + \mathbf{b}_i), & \tilde{\mathbf{c}}_t &= \tanh(\mathbf{W}_c \mathbf{y}_t + \mathbf{U}_c \mathbf{h}_{t-1} + \mathbf{b}_c), \\ \mathbf{f}_t &= \sigma(\mathbf{W}_f \mathbf{y}_t + \mathbf{U}_f \mathbf{h}_{t-1} + \mathbf{b}_f), & \mathbf{c}_t &= \mathbf{f}_t \odot \mathbf{c}_{t-1} + \mathbf{i}_t \odot \tilde{\mathbf{c}}_t, \\ \mathbf{o}_t &= \sigma(\mathbf{W}_o \mathbf{y}_t + \mathbf{U}_o \mathbf{h}_{t-1} + \mathbf{b}_o), & \mathbf{h}_t &= \mathbf{o}_t \odot \tanh(\mathbf{c}_t), \end{aligned} \quad (1)$$

739 where \odot represents the element-wise matrix multiplication operator. Note that the training of
 740 RNNs is completed off-line, only the efficient testing stage is performed for seeds refinement.
 741 In the testing stage, given an input $\tilde{\mathbf{y}}$ (with missing label \tilde{l}), the estimate for the output is
 742 $p(\tilde{l}|\tilde{\mathbf{y}}, \hat{\boldsymbol{\theta}})$, where $\hat{\boldsymbol{\theta}} = \arg \max \log p(\mathcal{D}|\boldsymbol{\theta})$. In our practical application, the testing sequence
 743 $\tilde{\mathbf{y}} \in \mathbb{R}^T$ is often of length $T > T_0$. We first convert it into a bag of subsequences $\{\tilde{\mathbf{y}}_i\}_{i=1}^{T-T_0+1}$,
 744 with a sliding window of width T_0 and moving step size 1. The well trained LSTMs are then
 745 used to label the subsequences. We consider $\tilde{\mathbf{y}}$ as positive if at least one subsequence in its
 746 bag is classified as "calcium spike" ($l = 1$), otherwise negative. Intuitively, this means that
 747 we only care about the patterns of neural spikes, regardless of their temporal positions.

748 **Seeds Merging** Once the set of seeds is cleansed, there is still a low possibility of identi-
 749 fying multiple seeds within a single ROI. Therefore, we merge all these redundant seeds
 750 by computing the temporal similarity of seeds within their neighborhood, and preserving
 751 the one with maximum intensity. Specifically, we compute the similarity based on phase-
 752 locking information (Hahn et al., 2006). For a sequence, we extract the instantaneous phase
 753 dynamics using Hilbert transform, and only consider the subsequences containing prominent
 754 peaks, because all the pixels within the same ROI are highly correlated only during the
 755 calcium spiking periods, but not necessarily during baseline period. After seeds merging,

756 we obtain K seeds, as the number of ROIs in our MINPIPE.

757 **Spatial and Temporal Initialization** The time series of the k th seed is used as initial
 758 guess of temporal signal $\hat{\mathbf{s}}_k$. The spatial map of the corresponding ROI, $\hat{\mathbf{r}}_k$, is estimated by
 759 pooling neighbor pixels with temporal similarity above a threshold. Because of our seeds
 760 generating and cleansing approach, the spatiotemporal initialization in our method can be
 761 readily parallelized. Then we fine-tune the corresponding spatial and temporal guess, by
 762 applying semi-NMF:

$$\min \|\mathbf{z}_k - \hat{\mathbf{r}}_k \hat{\mathbf{s}}_k^\top\|$$

763 where $\mathbf{z}_k \subset \mathbf{I}^s$ is a region containing the k th ROI. The spatial map and the temporal signal
 764 are updated respectively:

$$\mathbf{r}_k \leftarrow \mathbf{z}_k \mathbf{s}_k (\mathbf{s}_k^\top \mathbf{s}_k)^{-1}, \quad \mathbf{s}_k \leftarrow \mathbf{s}_k \sqrt{\frac{(\mathbf{r}_k \mathbf{z}_k)^+ + \mathbf{s}_k (\mathbf{r}_k^\top \mathbf{r}_k)^-}{(\mathbf{r}_k \mathbf{z}_k)^- + \mathbf{s}_k (\mathbf{r}_k^\top \mathbf{r}_k)^+}}$$

765 where $\mathbf{M}^+ \triangleq \frac{|\mathbf{M}| + \mathbf{M}}{2}$ and $\mathbf{M}^- \triangleq \frac{|\mathbf{M}| - \mathbf{M}}{2}$ for any matrix \mathbf{M} (Ding et al., 2010). In total, we
 766 now have K ROIs $\mathbf{S}^0 = [\mathbf{s}_1, \dots, \mathbf{s}_K]$, and temporal signals $\mathbf{R}^0 = [\mathbf{r}_1, \dots, \mathbf{r}_K]$. Similarly,
 767 the background parameters $\mathbf{b}^0, \mathbf{f}^0$ are also estimated by the same semi-NMF procedure,
 768 using $(\mathbf{I}^s - \sum_{k=1}^K \mathbf{r}_k \mathbf{s}_k^\top)$. $\mathbf{S}^0, \mathbf{R}^0, \mathbf{b}^0, \mathbf{f}^0$ are then used as initializations of the spatiotemporal
 769 signal refinement.

770 **Spatiotemporal Signal Refinement** We perform the iterative spatial and temporal op-
 771 timizations to update the spatial footprints of individual ROIs, and the temporal traces
 772 with deconvolved spike trains, as proposed in CNMF. Specifically, for the neural enhanced
 773 video \mathbf{X}^f , the method decomposes \mathbf{X}^f into a spatial dictionary $\mathbf{R}^f \in \mathbb{R}^{P \times K}$ representing
 774 individual ROIs, and corresponding temporal dynamics matrix $\mathbf{S}^f \in \mathbb{R}^{T \times K}$, in addition to

775 the background \mathbf{B}^f and the background dynamics \mathbf{E} :

$$\mathbf{X}^f = \mathbf{R}^f \mathbf{S}^{f\top} + \mathbf{B}^f + \mathbf{E}$$

776 Similarly, with the rank-1 assumption on \mathbf{B}^f , CNMF decomposes the background $\mathbf{B}^f =$
 777 $\mathbf{b}^f \mathbf{f}^{f\top}$, where $\mathbf{b}^f \in \mathbb{R}^P$ and $\mathbf{f}^f \in \mathbb{R}^T$. Meanwhile, \mathbf{S}^f is also correlated with underlying
 778 action potential events: $\mathbf{A}^f = \mathbf{S}^{f\top} \mathbf{G}^f$, where $\mathbf{A}^f \in \mathbb{R}^{K \times T}$, and $\mathbf{G}^f \in \mathbb{R}^{T \times T}$ is the coefficient
 779 matrix of the low-order autoregression. The variables $\mathbf{R}^f, \mathbf{S}^f, \mathbf{b}^f, \mathbf{f}^f$ are therefore estimated
 780 via iteratively alternating between the following two steps [26].

781 **Estimating Spatial Variables** Given the estimates of temporal variables $\mathbf{S}^{f(\ell-1)}$ and
 782 $\mathbf{f}^{f(\ell-1)}$ from the last iteration, the spatial parameters can be updated by solving the problem:

$$\begin{aligned} & \min_{\mathbf{R}^f, \mathbf{b}^f} \|\mathbf{R}^f\|_1, \text{ s.t. } \mathbf{R}^f, \mathbf{b}^f \geq 0 \\ & \|\mathbf{X}^f(i, :) - \mathbf{R}^f(i, :) \mathbf{S}^{f(\ell-1)\top} - \mathbf{b}^f(i) \mathbf{f}^{f(\ell-1)\top}\| \leq \sigma_i \sqrt{T} \end{aligned}$$

783 where $\mathbf{X}^f(i, :)$ is the i th row of the matrix \mathbf{X}^f , $\mathbf{b}^f(i, :)$ is the i th element of the vector. $\epsilon_i \sqrt{T}$
 784 is the empirically selected noise residual constraint of the corresponding pixel. This is
 785 essentially a basis pursuit denoising problem, and it is solved by the least angular regression
 786 (Efron et al., 2004) in implementation.

787 **Estimating Temporal Variables** Given the estimates of spatial variables $\mathbf{R}^f, \mathbf{b}^f$ and
 788 temporal parameters $\mathbf{S}^{f(\ell-1)}, \mathbf{f}^{f(\ell-1)}$ from the last iteration, the temporal parameters can be
 789 updated by solving the problem:

$$\begin{aligned} & \min_{\mathbf{S}^f, \mathbf{f}^f} \sum_{k=1}^K \mathbf{1}^\top \mathbf{G}^f \mathbf{S}^f, \text{ s.t. } \mathbf{G}^f \mathbf{s}_k^f \geq 0, k = 1, \dots, K \\ & \|\mathbf{X}^f(i, :) - \mathbf{R}^f(i, :) \mathbf{S}^{f(\ell-1)} - \mathbf{b}^f(i) \mathbf{f}^{f(\ell-1)\top}\| \leq \sigma_i \sqrt{T} \end{aligned}$$

790 Unlike in CNMF, where the spatial footprints are serially updated and subtracted from
791 the preceding residuals, we extract spatial footprints from the original data that does not
792 depend on preceding iterations. Therefore, the information loss/duplication is reduced and
793 the optimization procedures can be parallelized in our method.

794 **Data availability** The data that support the findings of this study are available from the
795 corresponding authors upon request.

796 **Code availability** The codes will be freely available upon publication, and the beta version
797 is available for reviewers upon request.