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Estimation of Templates and Timings of Spikes in Extracellular Voltage Signals Containing Overlaps of the Arbitrary Number of Spikes*

Tatsuya Haga, *Student Member, IEEE*, Yuzo Takayama, *Member, IEEE*, and Kunihiko Mabuchi, *Member, IEEE*

Abstract—**Development of methods to detect and classify neural spikes in extracellular voltage signals (e.g. commonly referred to as spike sorting) have been one of important subjects in neuroscience and neural engineering. Most of previous spike sorting methods suffer from unresolved overlaps of spike waveforms which make timings and shapes of spikes unclear. Some methods have got a handle on this problem, but they had restrictions about the type of electrodes or complexity of overlaps. In this paper, we attempted to develop a spike sorting method for the signal containing overlaps of the arbitrary number of spikes recorded with arbitrary electrodes. We estimated templates and timings of spikes by the inference based on hidden Markov model. In order to avoid the problem of too high computational cost and too much decomposition caused by assuming arbitrary overlaps, we imposed the weak probabilistic penalty on overlaps in the model and reduced computation of estimation by approximating low probabilities to zero. As the result of assessments using simulated signal and real extracellular recordings, we showed that proposed method could robustly detect and sort complexly overlapped spikes.**

I. Introduction

Multi-unit spike trains of neurons obtained by extracellular voltage recordings have been important bases for analyses of information processing in neural systems. However, in the extracellular recording, spikes of multiple neurons were often mixed in the signal recorded from an electrode. Therefore we have to not only estimate timings of spikes, but also discriminate spikes from different neurons in order to obtain spike trains of each neuron. The technology referred to as spike sorting has been developed to solve this problem [1][2].

It is common to discriminate spikes from different neurons based on the difference of spike waveforms [1]. In this approach, spike sorting can be interpreted as the estimation problem of the spike waveform corresponding to each neuron (spike templates) and spike timings of each neuron. In many previous spike sorting methods, spikes in the recorded signal were extracted by detecting voltage signals exceeding predefined threshold and extracted waveforms were classified by some clustering method to obtain spike templates and spike timings of multiple neurons [3-8]. These methods showed good performance when spikes were sparse.

However, when spikes were distributed densely and synchronously, unresolved overlaps of spikes worsened the performance of these methods. Dense and synchronous spiking has been the frequent phenomenon found in bursting over the neuronal network [9], responses evoked by electrical stimuli [10], and so on. Therefore, sorting of overlapped spikes has been one of the issues in spike sorting [1][2].

One of the solutions to the issue was the use of closely spaced multi-electrodes and decomposition of overlapped spikes by spatial information [11-14]. In spite of robustness to overlaps, the methods cannot be applied to sparsely-positioned electrode arrays e.g. Utah electrodes widely used in the Brain-Machine Interface [15][16] and the Multi-Electrode Array (MEA) for recording from cultured neurons [9][10]. On the other hand, methods to detect and sort overlapped spikes recorded without multi-electrodes have been also proposed [17-19]. However, these methods had limitation of the allowed number of spikes in an overlap to avoid high computational cost and over-decomposition of spikes. Previous methods have not been able to achieve the estimation of spike templates and timings in case of overlaps of the arbitrary number of spikes from the signal recorded without multi-electrodes. Though the recently developed method [20] can be a solution, it do not consider properties of neural spikes such as the refractory period and the variability of firing rates.

In this paper, we attempted to develop a spike sorting method for the signal containing overlapped spikes without these restrictions. Our method is based on the strategy by Herbst et al. [19]. Extracellular recordings and generation of spikes were modeled as the hidden Markov model (HMM) that can generate overlapped spikes and spike templates and spike timings were estimated using α-β algorithm and Expectation-Maximization (EM) algorithm. However, we imposed the probabilistic penalty on the number of overlaps in the model and reduced computation of estimation by approximating low probabilities to zero. They enable us to remove the restriction of the number of spikes in an overlap without the problem of high computational cost and over-decomposition of spikes.We applied our method to simulated neural signals and recordings from cortical neurons cultured on MEA and confirmed that our method could robustly estimate appropriate templates and timings of complexly overlapped spikes.

II. Methods

A. The Model of Extracellular Voltage Recordings

We assumed that T time samples of the recorded signal $y_{1:T} = \{y_1, y_2, \dots, y_T\}$ contain spikes generated by N neurons

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T. Haga is with Graduate School of Information Science and Technology, University of Tokyo, Bunkyo-Ku 113-8656, Japan (corresponding author to provide phone: +81-3-5841-6880; fax: +81-3-5841-6882; e-mail: Tatsuya_Haga@ ipc.i.u-tokyo.ac.jp).

Y. Takayama is with Graduate School of Information Science and Technology, University of Tokyo, Bunkyo-Ku 113-8656, Japan and Research Fellow of the Japan Society for the Promotion of Science, Tokyo, Japan (e-mail: Yuzo_Takayama@ipc.i.u-tokyo.ac.jp).

K. Mabuchi is with Graduate School of Information Science and Technology, University of Tokyo, Bunkyo-Ku 113-8656, Japan (e-mail: Kunihiko_Mabuchi@ipc.i.u-tokyo.ac.jp).

with additive Gaussian noise with variance σ, and the vector $\boldsymbol{\mu}_n = (\mu_n[1], \mu_n[2], \ldots, \mu_n[M])$ was defined as the waveform of the spike generated by neuron n. Furthermore, $x_{1:T} = \{x_1, x_2, \ldots, x_T\}$ was defined as the number of the neuron which started to generate a spike at each time samples (if there is no spike, $x_t = 0$). Based on these assumptions, we had the recording model using the spike train vector $\mathbf{z}_t = (x[t], x[t-1], \ldots, x[t-M+1])$:

$$\mathrm{p}(y_t|\mathbf{z}_t, \boldsymbol{\mu}_{1:N}, \sigma) = \frac{1}{\sqrt{2\pi\sigma}} \exp\left(-\frac{\left(y_t - \sum_{\tau=1}^{M} \mu_{z_t[\tau]}[\tau]\right)^2}{\sigma}\right) \tag{1}$$

B. The Model of Spike Trains

We also derived the model of the spike train $\mathbf{z}_t$. We assumed x_t followed the multinomial distribution and the probability for a neuron to fire was determined with the parameters ω and $\boldsymbol{\pi} = (\pi_1, \ldots, \pi_N)$. We also took into account the refractory period, to be more precise, once a neuron fired, it cannot fire again in M time samples. Furthermore, in our model, the probability for a neuron to fire was reduced in M time samples after another neuron fired. This assumption made our method avoid decomposing the signal to too many overlapped spikes. After all, the model was $\mathrm{p}(\mathbf{z}_t|\mathbf{z}_{t-1}, \boldsymbol{\pi})$

$$= \begin{cases} \omega\pi_{z_t[1]}\mathrm{F}(\mathbf{z}_t, \mathbf{z}_t[1]) & , \text{if } z_t \in \mathrm{C_z} \wedge 1 \le z_t[1] \le N \\ \omega\left(1 + \sum_{k=1}^{N} \pi_k\left(1 - \mathrm{F}(\mathbf{z}_t, k)\right)\right) & , \text{if } z_t \in \mathrm{C_z} \wedge z_t[1] = 0 \\ 0 & , \text{if } \mathbf{z}_t \notin \mathrm{C_z} \end{cases} \tag{2}$$

$$\mathrm{C_z} = \{\mathbf{z}_t | 2 \le \forall\tau \le M, z_t[\tau] = z_{t-1}[\tau - 1]\} \tag{3}$$

$$\mathrm{F}(\mathbf{z}_t, n) = \begin{cases} 0, \text{if } 2 \le \exists\tau \le M, z_t[\tau] = n \\ \phi^{\sum_{\tau=2}^{M}\sum_{k=1}^{N}\delta_{k,z_t[\tau]}}, \text{else} \end{cases} \tag{4}$$

$$\omega\left(1 + \sum_{k=1}^{N} \pi_k\right) = 1 \tag{5}$$

$\delta_{0,z_{t-1}[\tau]}$ was Dirac's delta and ϕ was the weighting coefficient preventing over-decomposition.

C. Estimation of Hidden Variables and Parameters

Expectation-Maximization (EM) algorithm was used to estimate parameters in above-mentioned model. EM algorithm can estimate model parameters in the presence of hidden variables by repeating two steps, E-step and M-step.

In E-step, probability distributions of hidden variables $\gamma(\mathbf{z}_t) = p(\mathbf{z}_t|y_{1:T}, \Theta)$, $\xi(\mathbf{z}_t, \mathbf{z}_{t-1}) = \mathrm{p}(\mathbf{z}_t, \mathbf{z}_{t-1}|y_{1:T}, \Theta)$ were estimated under fixed model parameters using α-β algorithm. In this algorithm, alpha-messages $\hat{\alpha}(\mathbf{z}_t) = p(\mathbf{z}_t|y_{1:t}, \Theta)$ were recursively estimated as

$$\hat{\alpha}(\mathbf{z}_1) = \frac{p(y_1|\mathbf{z}_1, \Theta)p(\mathbf{z}_1)}{\sum_{z_1} p(y_1|\mathbf{z}_1, \Theta)p(\mathbf{z}_1)} \tag{6}$$

$$\alpha(\mathbf{z}_t) = p(y_t|\mathbf{z}_t, \Theta)\sum_{\mathbf{z}_{t-1}} p(\mathbf{z}_t|\mathbf{z}_{t-1}, \Theta)\hat{\alpha}(\mathbf{z}_{t-1}) \tag{7}$$

$$c_t = p(y_t|y_{1:t-1}, \Theta) = \sum_{\mathbf{z}_t} \alpha(\mathbf{z}_t) \tag{8}$$

$$\hat{\alpha}(\mathbf{z}_t) = \frac{\alpha(\mathbf{z}_t)}{c_t} \tag{9}$$

After that, beta-messages $\hat{\beta}(\mathbf{z}_t) = \frac{p(y_{t+1:T}|\mathbf{z}_t, \Theta)}{p(y_{t+1:T}|y_{1:t}, \Theta)}$ were recursively estimated as

$$\hat{\beta}(\mathbf{z}_T) = 1 \tag{10}$$

$$\hat{\beta}(\mathbf{z}_t) = \frac{1}{c_{t+1}}\sum_{\mathbf{z}_{t+1}} \hat{\beta}(\mathbf{z}_{t+1})p(y_{t+1}|\mathbf{z}_{t+1}, \Theta)p(\mathbf{z}_{t+1}|\mathbf{z}_t, \Theta) \tag{11}$$

$\gamma(\mathbf{z}_t)$ and $\xi(\mathbf{z}_t, \mathbf{z}_{t-1})$ were calculated from alpha-messages and beta-messages.

$$\gamma(\mathbf{z}_t) = \hat{\alpha}(\mathbf{z}_t)\hat{\beta}(\mathbf{z}_t) \tag{12}$$

$$\xi(\mathbf{z}_t, \mathbf{z}_{t-1}) = (c_n)^{-1}\hat{\alpha}(\mathbf{z}_{t-1})\mathrm{p}(y_t|\mathbf{z}_t)\mathrm{p}(\mathbf{z}_t|\mathbf{z}_{t-1})\hat{\beta}(\mathbf{z}_t) \tag{13}$$

In M-step, model parameters were updated to maximize the expected value of the complete data log-likelihood (Q-function). ω and $\boldsymbol{\pi}$ were updated as

$$\omega = \frac{1}{(1 + \sum_{k=1}^{N} \pi_k)} \tag{14}$$

$$\pi_n = \frac{\sum_{t=1}^{T}\sum_{\mathbf{z}_{t-1}}\sum_{\mathbf{z}_t\, s.t.\, z_t[1]=n} \xi(\mathbf{z}_t, \mathbf{z}_{t-1})}{\sum_{t=1}^{T}\sum_{\mathbf{z}_{t-1}}\left(\sum_{\mathbf{z}_t} \xi(\mathbf{z}_t, \mathbf{z}_{t-1}) - \sum_{\mathbf{z}_t\, s.t.\, z_t[1]=0} \xi(\mathbf{z}_t, \mathbf{z}_{t-1})\left(1 - \mathrm{F}(\mathbf{z}_t, n)\right)\right)} \tag{15}$$

$\boldsymbol{\mu}_n$ was updated by solving following equation:

$$\mathrm{A}\boldsymbol{\mu}_{\mathrm{all}} = \mathbf{b} \tag{16}$$

$$\boldsymbol{\mu}_{\mathrm{all}} = (\mu_1[1], \mu_1[2], \ldots, \mu_1[M], \mu_2[1], \mu_2[2], \ldots, \mu_N[M])^T \tag{17}$$

$$\mathbf{b}^{(\tau+(n-1)(M+1))} = \sum_{t=1}^{T} y_t \sum_{\mathbf{z}_t\, s.t. z_t[\tau]=n} \gamma(\mathbf{z}_t) \tag{18}$$

$$\mathrm{A}_{\tau+(n-1)(M+1),\tau'+(n'-1)(M+1)} = \begin{cases} \sum_{t=1}^{T}\sum_{\mathbf{z}_t\, s.t. z_t[\tau]=n} \gamma(\mathbf{z}_t) & , \tau = \tau', n = n' \\ 0, \tau = \tau', n \ne n' \\ \sum_{t=1}^{T}\sum_{\mathbf{z}_t\, s.t. z_t[\tau]=n, z_t[\tau']=n'} \gamma(\mathbf{z}_t) & , \tau \ne \tau' \end{cases} \tag{19}$$

σ was updated as

$$\sigma = \frac{1}{T}\sum_{t=1}^{T}\sum_{\mathbf{z}_t} \gamma(\mathbf{z}_t)\left(y_t - \sum_{\tau=1}^{M+1} \mu_{z_t[\tau],\tau}\right)^2 \tag{20}$$

The number of neurons N was chosen by minimizing Bayesian information criterion (BIC).

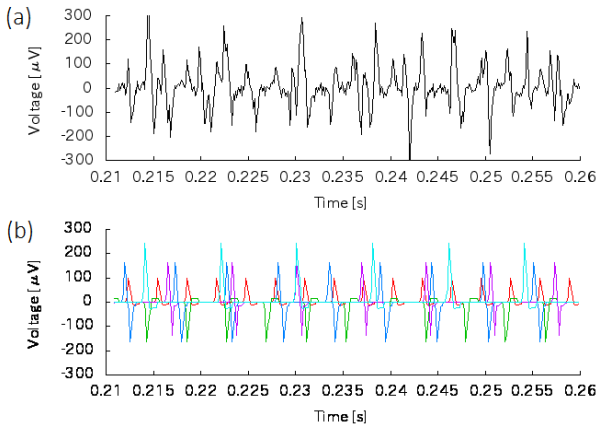

Figure 1. A part of the simulated signal and spike waveforms contained in the signal. Almost all spikes were overlapped and there were complex overlaps of more than three neurons. (a) Simulated signal. (b) Spike waveforms contained in the signal.

$$\mathrm{BIC} = -2\log p(y_{1:T}|\Theta) + \log \mathrm{T}\ (\mathrm{N}(\mathrm{M}+1)+2) \tag{21}$$

$$\log p(y_{1:T}|\Theta) = \sum_{t=1}^{T} \log c_t \tag{22}$$

These procedure needed too high computational cost because of high dimensionality of $\mathbf{z}_t$. To reduce the computational cost, $\hat{\alpha}(\mathbf{z}_t)$ smaller than a given threshold p_{th} was approximated by zero and we calculated all the probabilities for only $\left\{\mathbf{z}_t^{(i)}\right\}_{1\le i\le I_t}$, nonzero instances of z_t. I_t was the number of nonzero instances at sampling time t.

III. Experiments and Discussion

A. Assessments using Simulated Signals

We assessed the performance of our method by applying it to the 0.3-second simulated signal containing a lot of complex overlaps of spikes (shown in Fig. 1). In simulation, spike shapes shown in Fig. 2(a) were present in the signal whenever indicated by the timings shown in Fig. 3(a). The signal was additionally corrupted by adding Gaussian noise. The standard deviation of noise was 15 μV and the sampling rate was 10 kHz. We applied our method by setting the parameter values to $\phi = 0.01, p_{\mathrm{th}} = 1\times10^{-15}, M = 1.5\mathrm{ms}$ and detected spikes when the probability $\gamma(\mathbf{z}_t)$ of spiking exceeded the value of 0.5.

As the result, all the spike templates were appropriately estimated as shown in Fig. 2(b) and complexly overlapped spikes shown in Fig. 1 were all detected. However, 2 positive errors and 25 negative errors were found as shown in Fig. 3(b) (The number of spikes in simulation (Fig. 3(a)) was 302). One of reasons of these non-negligible negative errors was canceling out of positive and negative spikes. Note that the numbering of neurons was arbitrary and not same between simulation and in estimation.

B. Assessments using the Real Neural Signal

We also assessed our method using the eight-second signal recorded from neurons cultured on the Multi-Electrode Array (MEA). Cortical tissues were isolated from Wistar rat embryos (embryonic days 18) and dissociated by digestion with 0.1% trypsin-EDTA. The dissociated cells were plated on MEA substrates coated with polyethylenimine at a density of 1.7×10^3 cells/mm^2. The MEA substrate used in the experiment was MED545A (Alpha MED Scientific Inc.), which had 8×8 indium–tin–oxide (ITO) electrodes coated with platinum black. Each electrode had a surface of 50μm square and the distance between electrodes was 450μm. The culture medium consisted of Dulbecco's modified eagle's medium (DMEM), 5% fetal bovine serum, 5% horse serum, 2.5μm/mL insulin and 5-40 U/mL penicillin streptomycin. The cultures were maintained in an incubater at 37 °C, 5% CO_2, and in a water-saturated atmosphere. We recorded voltage signals from one of the electrodes for 10 minutes after 45 days in vitro. The signals were amplified to 1000 times and filtered to cut frequency under 100 Hz. The sampling rate was 10 kHz. A part of the recorded signal is shown in Fig. 4(a). We applied our method to the signal with the setting of parameters same as the simulation experiment.

As the result, eight spike templates were created as shown in Fig. 4(d) and the spike timings were estimated as shown in

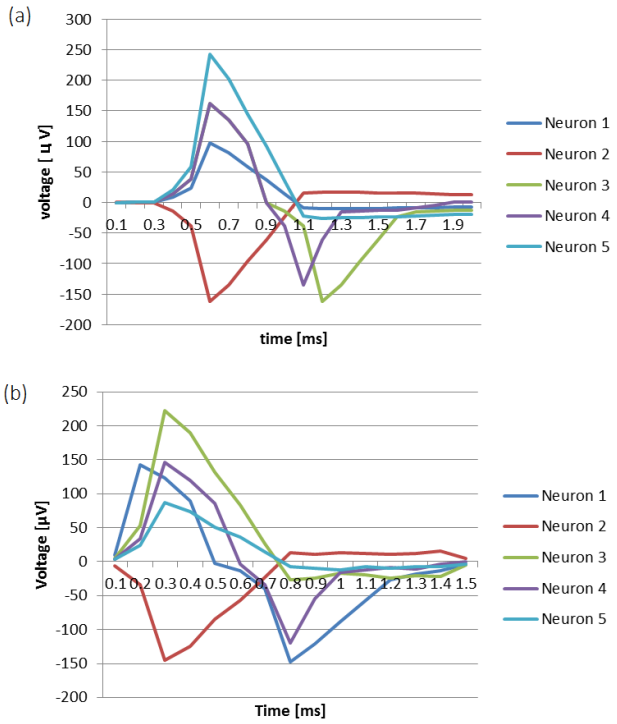

Figure 2. Five spike templates used in the simulation and spike templates estimated by our method. (a) Spike templates used in the simulation. (b) spike templates estimated by our method

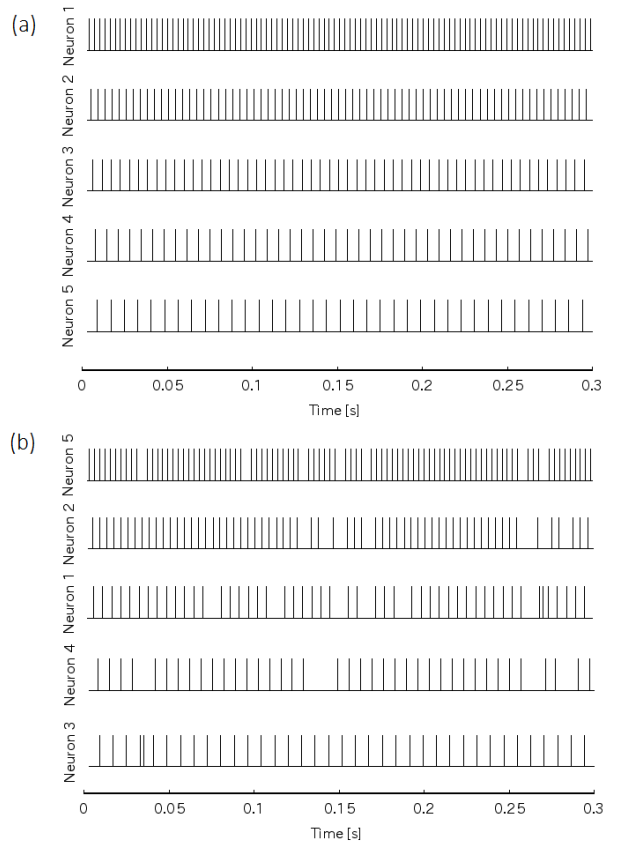

Figure 3. Spike timings used in the simulation and spike timings estimated by our method. (a) Spike timings used in the simulation. (b) spike timings estimated by our method

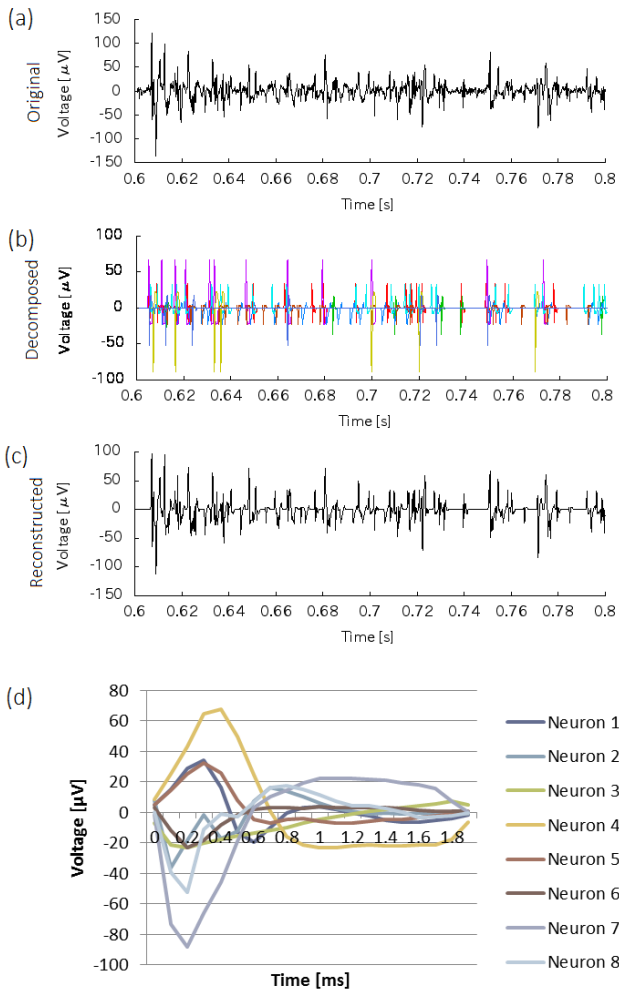

Figure 4. The result of estimation from the signal recorded from cortical neurons cultured on the Multi-Electrode Array (MEA) by our method. Eight templates were obtained and complexly overlapped spikes could be decomposed and the original signal could be appropriately reconstructed from estimated templates and spike timings. (a) The original signal recorded from cultured neuron. (b) Spike waveforms detected from the recorded signal by our method. (c) The signal reconstructed from estimated templates and timings. (d) Eight spike templates obtained from the recorded signal by our method.

Fig. 4(b). In Fig. 4(b), more than three or four spikes were superimposed at the same position, which means complex overlaps were decomposed. We reconstructed the signal from estimated timings and templates using the recording model (1) and obtained the signal similar with the original (Fig. 4(c)). It means that the decomposition of complexly overlapped spikes was appropriate. However, it was uncertain whether these spikes were "neural" spikes or artifacts. The extracellular voltage signal with simultaneously recorded the intracellular voltage signal will be needed for more accurate validation.

IV. Conclusion

In this paper, we developed the method to estimate spike templates and timings from the signal containing overlaps of the arbitrary number of spikes. In our method, the inference based on HMM with the probabilistic penalty was efficiently calculated by approximation. We assessed the performance of the method and showed that it could appropriately decompose the simulated and real signals containing complexly overlapped spikes. Our method will help more accurate analysis of highly synchronized neural activity such as bursting [9] or responses evoked by electrical stimuli [10].

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