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Accurate detection of low signal-to-noise ratio neuronal calcium transient waves using a matched filter

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HIGHLIGHTS

- We develop a matched filter for multi-unit calcium event detection in neurons.
- We tested the detector on simulated and experimentally recorded calcium imaging data.
- The detector had near perfect performance on simulated data with SNR as low as 0.2.
- The detector also performed well on experimentally recorded data.
- The detector is written in MATLAB and freely available.

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ABSTRACT

Background: Calcium imaging has become a fundamental modality for studying neuronal circuit dynamics both *in vitro* and *in vivo*. However, identifying calcium events (CEs) from spectral data remains laborious and difficult, especially since the signal-to-noise ratio (SNR) often falls below 2. Existing automated signal detection methods are generally applied at high SNRs, leaving a large need for an automated algorithm that can accurately extract CEs from fluorescence intensity data of SNR 2 and below.
New method: In this work we develop a Matched filter for Multi-unit Calcium Event (MMiCE) detection to extract CEs from fluorescence intensity traces of simulated and experimentally recorded neuronal calcium imaging data.
Results: MMiCE reached perfect performance on simulated data with SNR $\geq$ 2 and a true positive (TP) rate of 98.27% ($\pm$ 1.38% with a 95% confidence interval), and a false positive(FP) rate of 6.59% ($\pm$ 2.56%) on simulated data with SNR 0.2. On real data, verified by patch-clamp recording, MMiCE performed with a TP rate of 100.00% ($\pm$ 0.00) and a FP rate of 2.04% ($\pm$ 4.10).
Comparison with existing method(s): This high level of performance exceeds existing methods at SNRs as low as 0.2, which are well below those used in previous studies (SNR $\simeq$ 5–10).
Conclusion: Overall, the MMiCE detector performed exceptionally well on both simulated data, and experimentally recorded neuronal calcium imaging data. The MMiCE detector is accurate, reliable, well suited for wide-spread use, and freely available at sites.uci.edu/aggies or from the corresponding author.
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1. Introduction

Functional multineuron calcium imaging (fMCI) has been particularly successful as a means of studying the interactions of multiple (often hundreds) of neurons in large populations (Takahashi et al., 2007) with specific applications both *in vitro* (Cossart et al., 2003; Ikegaya et al., 2004; Sasaki et al., 2006, 2007, 2008), and *in vivo* (Kerr et al., 2005; Takahashi et al., 2012). Regardless of the details

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in fluorescence imaging, or focus on either synaptic or somatic calcium waves, calcium imaging in neurons often suffers from low signal-to-noise ratios (SNR). This makes signal detection very difficult, prompting the development of many automated or partially automated detection approaches.

Pioneers in the field first represented calcium imaging data as fluorescence changes ($\Delta F/F$), and established a link between somatic calcium transient waves and action potentials (APs) (Smetters et al., 1999; Kerr et al., 2005). Subsequent studies linked the amplitude of calcium transient waves to AP firing patterns and rates (Yaksi and Friedrich, 2006; Moreaux and Laurent, 2007; Greenberg et al., 2008). Although it is now universally accepted that relative amplitudes of fluorescent CEs are indicative of the frequency or number of APs fired by the cell, algorithms back-calculating AP firing rates are difficult to generalize. The amplitude of a given CE depends on the amount of fluorescent calcium indicator taken up by the cell, as well as the proximity of the region of interest (ROI) to the focal imaging plane. When performing imaging population studies, such as those using fMCI (Takahashi et al., 2007), it is impractical and often impossible to calculate the exact distance from the focal plane and to quantify the amount of indicator present in each cell or ROI. Comparison of CE amplitudes across ROIs or data sets is therefore unreliable. Furthermore, low-SNR signals where CEs are generated from 5 or fewer APs often fail to be detected (Moreaux and Laurent, 2007). When they are detected, the low-SNR CEs often lack the necessary resolution for AP firing rate estimation. Although Sasaki et al. overcame many of these issues with their support vector machine approach, they still fell victim to rapid performance degradation at low SNR (Sasaki et al., 2008). It may therefore be more advantageous to sacrifice precision in AP firing rate estimation for better accuracy in CE detection at low SNR.

Other approaches to CE detection less focused on AP firing rates include thresholding the time derivative of fluorescent changes in given ROIs (Ramdya et al., 2006; Ikegaya et al., 2004), Hanning filters (Cossart et al., 2003), and matched filters (Kerr et al., 2005). These approaches work well in high-SNR settings (SNR $\geq$10) but their performance is either not reported at low SNRs or quickly degrades. Since a large fraction of CEs fall below SNR 2, there is a pressing need to develop automated methods for low-SNR CE detection. Some denoising algorithms have been developed to enhance the SNR of calcium imaging data (Joucla et al., 2013; Malik et al., 2011). However, they are not applied or tested as detection tools, but rather as post hoc analyses. Due to the various shortcomings of these methods, as well as availability and implementation hurdles, manual CE detection remains the primary means of scoring calcium imaging data even though it is both prone to bias and incredibly laborious.

Our work focuses on the development of a Matched filter for Multi-unit Calcium Event (MMiCE) detection specifically tailored for low-SNR CEs. Unlike the matched filter presented by Kerr et al. (2005), the algorithm developed here is completely data driven. It is also applied and tested in environments with SNR as low as 0.2, well below the range of existing algorithms. MMiCE was tested under 3 paradigms: (1) simulated somatic calcium imaging data (ground-truth available), (2) experimentally recorded simultaneous somatic fMCI and patch-clamp data (ground-truth available), as well as (3) experimentally recorded, from now on referred to as real, somatic fMCI and dendritic spine fMCI data (no ground-truth available). Although only tested on fMCI data here, due to its data-driven design, the algorithm is easily generalizable to other fluorescent neural imaging modalities, such as voltage-sensitive-dye imaging (Szymanska et al., 2015). In order to ease implementation and applicability hurdles, MMiCE was developed in Matlab with an intuitive graphical user interface. MMiCE, along with a tutorial, is openly available at sites.uci.edu/aggies/downloads or upon request from the corresponding author.

2. Methods

2.1. Tissue preparation

All animals used in this study were treated according to The University of Tokyo guidelines for the care and use of laboratory animals. All performed experiments were approved by the experiment ethics committee at the University of Tokyo, approval numbers: P24-5 and P24-8.

Acute Slices. Acute slices were prepared as described in Ueno et al. (2002), Norimoto et al. (2012). Briefly, 400 μm horizontal slices of the hippocampus from 3 week old C57BL/6J mice were prepared using a vibratome in ice-cold oxygenated cutting solution. The slices then rested in oxygenated artificial cerebrospinal fluid (aCSF) at room temperature for 1.5 h prior to imaging. For more details on the preparation and solutions used please see Ueno et al. (2002), Norimoto et al. (2012).

Ex vivo cultures. In order to facilitate the simultaneous visibility of many dendritic spines during functional multispine calcium imaging (fMCI), organotypic slice cultures were used in this study. *Ex vivo* rat hippocampal slice cultures were prepared as described in Takahashi et al. (2012) from 7 day old Wistar/ST rats. Briefly, 300 μm entorhinal-hippocampal organotypic slices were cut using a vibratome, placed on Omnipore membrane filters (JHWP02500; Millipore, Bedford, Massachusetts, USA), and incubated (5% CO_2, 37 °C) in culture medium (50% minimal essential medium, 25% Hanks' balanced salt solution, 25% horse serum, antibiotics) for 12–19 days prior to imaging. The medium was changed every 3.5 days. For more details please refer to Takahashi et al. (2012).

2.2. Dye loading

Simultaneous somatic fMCI and patch-clamp. Ex vivo slice cultures were transferred into a dish (35-mm diameter) containing 2 ml of the dye solution and were incubated for 1-h in a humidified incubator at 35 °C under 5% CO_2. The dye solution was aCSF containing 10 μl of 0.1% Oregon Green BAPTA1-AM (OGB1) dissolved in DMSO, 2 μl of 10% Pluronic F-127/DMSO and 2 μl of 5% Cremophor EL/DMSO. After being washed, the cultured slices were incubated at 35 °C for 40 min and were mounted in a recording chamber.

Somatic fMCI. Acute slices were loaded locally with OGB1 dissolved in DMSO containing 10% Pluronic F-127 to yield a concentration of 2 mM. Immediately before use, this solution was 10 diluted with aCSF and loaded into pipettes (3–5 MΩ). The tip of the pipette was inserted into the CA1 stratum pyramidale, and a pressure was applied using a 10-ml syringe pressurizer (50–60 hPa for 5 min).

Dendritic spine fMCI. CA3 pyramidal neurons were selected for spine imaging from *ex vivo* slice cultures. Selected neurons were voltage-clamped at −30 mV (MultiClamp 700B amplifier and a Digidata 1440A digitizer controlled by pCLAMP 10.4 software) and loaded with a Fluo-4 solution (97.3 $CsMeSO_4$, 42.7 CsCl, 10 HEPES, 10 phosphocreatine, 4 MgATP, 0.3 NaGTP, and 0.2 Fluo-4, all in mM). The voltage clamp facilitated channel currents mediated by NMDA receptors and calcium-permeable AMPA receptors.

2.3. Patch-clamp recording

CA3 pyramidal neurons selected for simultaneous somatic fMCI and patch-clamp recordings were voltage-clamped at 0 mV (Axopatch 700B amplifier) using a borosilicate glass pipettes (4–9 MΩ) filled with aCSF, and recorded from at a sampling frequency of 20 kHz (MultiClamp 700B amplifier and a Digidata 1440A digitizer controlled by pCLAMP 10.4 software).

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