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Detecting cells using non-negative matrix factorization on calcium imaging data

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ABSTRACT

We propose a cell detection algorithm using non-negative matrix factorization (NMF) on Ca^{2+} imaging data. To apply NMF to Ca^{2+} imaging data, we use the bleaching line of the background fluorescence intensity as an a priori background constraint to make the NMF uniquely dissociate the background component from the image data. This constraint helps us to incorporate the effect of dye-bleaching and reduce the non-uniqueness of the solution. We demonstrate that in the case of noisy data, the NMF algorithm can detect cells more accurately than Mukamel's independent component analysis algorithm, a state-of-art method. We then apply the NMF algorithm to Ca^{2+} imaging data recorded on the local activities of subcellular structures of multiple cells in a wide area. We show that our method can decompose rapid transient components corresponding to somas and dendrites of many neurons, and furthermore, that it can decompose slow transient components probably corresponding to glial cells.

1. Introduction

Ca^{2+} imaging techniques enable us to measure the temporal variation in the intracellular Ca^{2+} concentration (Grynkiewicz, Poenie, & Tsien, 1985). In the case of nerve cells, the intracellular Ca^{2+} concentration is closely related to the membrane potential of cells because Ca^{2+} is recruited inside through voltage-dependent Ca^{2+} channels whose conductivities depend on the membrane potential. Therefore, the instantaneous elevation of the intracellular Ca^{2+} concentration gives us important information on the time of action potential generation. Many research groups have developed multi-cellular Ca^{2+} imaging systems to record individual cellular activities of a cell assembly in vitro and in vivo. For example, Ikegaya et al. (2004), Ikegaya, Le Bon-Jego, and Yuste (2005) recorded the spike times of hundreds of cortical neurons in in vitro Ca^{2+} imaging and discovered a repeated firing sequence from particular groups of neurons. Dombeck, Harvey, Tian, Looger, and Tank (2010) recorded in vivo hippocampal CA1 neurons of a moving rat and showed the spatial distribution of place cells. Furthermore, our group has developed a multi-cellular Ca^{2+} recording system able to record from dendritic tufts as well as somas (Maeda et al., submitted for publication, 2012). Thus, Ca^{2+} imaging can also be used to record the activities of subcellular structures of multiple cells in a wide area.

The first step of a typical analysis of multi-cellular Ca^{2+} imaging data is the identification of the positions of individual cells as regions of interest (ROIs) within the image; this step is called 'cell detection' (Lutcke & Helmchen, 2011). In almost all of the related studies, experimenters searched for cells by inspecting the movie data with the naked eye, and they manually identified ROIs in the movie frames. This manual method is very effective but it needs of a lot of time and effort. The rapid progress of imaging systems has made it possible for us to record high spatial and temporal resolution imaging data for a long time (Ziv et al., 2013). However, the rapid increase in the data volume makes it very difficult for us to analyze the imaging data manually. This has meant that automatic or semi-automatic methods of cell detection have become increasingly necessary.

To overcome this issue, many research groups have devised sophisticated statistical algorithms (Junek, Chen, Alevra, & Schild, 2009; Miri et al., 2011; Miri, Daie, Burdine, Aksay, & Tank, 2011; Mukamel, Nimmerjahn, & Schnitzer, 2009; Ozden, Lee, Sullivan, & Wang, 2008; Reidl, Starke, Omer, Grinvald, & Spors, 2007; Valmianski et al., 2010; Vogelstein et al., 2010). Some research

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groups have demonstrated the effectiveness of independent component analysis (ICA) on high spatial and temporal resolution imaging data (Mukamel et al., 2009; Reidl et al., 2007). In particular, Mukamel et al. (2009) proposed an automated image segmentation method based on ICA that provides a fast and efficient strategy for analyzing large-scale calcium imaging data sets (Lutcke & Helmchen, 2011). ICA was first used to detect cells in low-resolution imaging data about a decade ago (Brown, Yamada, & Sejnowski, 2001). A revival in interest in ICA began with Mukamel's work (Dombeck et al., 2010; Ziv et al., 2013). This algorithm initially performs the principal component analysis (PCA) to reduce the dimensions of the data, and after that it executes a joint maximization of two objective functions, spatial skewness and temporal skewness. The performance of this algorithm depends on the number of principal components and the parameter for tuning the priorities between the two objective functions, and users need to fine-tune them.

The aim of this study is to establish a reliable method that detects the position of cells automatically from such a high-resolution multi-cellular Ca^{2+} imaging data. Our cell detection algorithm based on non-negative matrix factorization (NMF), which is a low rank matrix decomposition method that restricts the component matrices to have non-negative values (Lee & Seung, 1999). NMF is a well-known algorithm that is useful for separating image data into constitutive parts (Hoyer, 2004). To apply it to calcium imaging movie data, we introduced a background constraint: the bleaching line of the background fluorescence intensity, which can be estimated directly from the imaging data, is given as an a priori background constraint to uniquely dissociate the background component from the image data. This constraint helps to reduce the non-uniqueness of the solution, which is known to be a big problem of factorization (Benzi, 2002). The advantages of this method are that (1) it involves no parameter that needs tuning, except for the number of cells, (2) model order selection can (in principle) be used to determine the number of cells, and (3) it can incorporate the effect of dye-bleaching as a result of being given the bleaching line of the background as a constraint.

In this paper, we compare the performances of the NMF algorithm and the state-of-art ICA algorithm on simulated movie data. We demonstrate that in the case of noisy data, the NMF algorithm can detect cells more accurately than the optimally tuned ICA algorithm, and in the low-noise case, the NMF algorithm almost as well as the ICA algorithm. After that, we apply the NMF algorithm to high-resolution Ca^{2+} imaging data recording the local activities of subcellular structures of multiple cells in a wide area. We demonstrate that our method can decompose rapid transient components corresponding to somas and dendrites of different active neurons, and furthermore, it can decompose slow transient components probably corresponding to glial cells.

2. Materials and methods

2.1. Cell detection by non-negative matrix factorization

Here, we modify the NMF algorithm so that it can be applied to Ca^{2+} imaging data. Let us consider a case in which a two-dimensional calcium imaging movie consists of T frames in total and each frame consists of N pixels. The two-dimensional array of pixels in each frame is rearranged to form a one-dimensional column vector, and a data matrix F (N by T) is obtained. For the sake of simplicity, we assume that F is the sum of fluorescence signals from K cells labeled 1–K, background fluorescence, and noise. Moreover, assuming that cells do not spatially move during recording, the NMF generative model can be rewritten as

$$F = SA + s_b a_b + \text{noise}, \tag{1}$$

where S is a spatial component matrix (N by K) whose kth-column vector represents the shape of the kth cell, and A is a temporal component matrix (K by T) whose kth-row vector represents the time-series of fluorescence intensity fluctuations of the kth cell. s_b is a spatial component vector (N by 1) representing the spatial distribution of the background fluorescence intensity, and a_b is a temporal component vector (1 by T) representing the time course of the background fluorescence intensity. Thus, under the above assumptions, the problem we need to solve can be formulated as a decomposition of the data matrix F into four low rank matrices S, A, s_b and a_b. Since the fluorescence signals take only positive values, the matrices S, A, s_b, and a_b must be restricted to being non-negative.

Cells can be detected by estimating the matrices S, A, s_b and a_b. Assuming spatial–temporal white Gaussian noise with uniform variance across all time and space, the mean square error of the generative model, which corresponds to the log-likelihood function of the model, can be written as

$$J = \|F - SA - s_b a_b\|^2, \tag{2}$$

where $\|X\|^2$ denotes the trace of a square matrix XX^T. These matrices can be estimated by minimizing the mean square error under the constraint of non-negativity.

To improve the performance of the NMF algorithm, the time course of the background fluorescence intensity a_b is given a priori. We let a_b be a bleaching line of the background fluorescence intensity and give it the form of a decreasing linear function whose slope β represents the bleaching rate of fluorescence. The bleaching line can be estimated directly from the imaging data. This constraint helps us to incorporate the effect of dye-bleaching and reduce the non-uniqueness of the solution. The optimization under the constraints of an a priori bleaching line a_b and non-negativity of the matrices is performed by a modified version of the alternating least squares (ALS) algorithm (Berry, Browne, Langville, Pauca, & Plemmons, 2007), which is known to perform NMF efficiently. The modified ALS algorithm consists of the following steps.

1. The matrices S and A are initialized with positive random numbers before starting the matrix updates.
2. While S and A are fixed, a least squares solution for the matrix s_b is calculated using $s_b = (a_b a_b{}^T)^{-1}(F - SA)a_b{}^T$, and the negative elements of s_b are set to be zero.
3. While S and s_b are fixed, a least squares solution for the matrix A is calculated using $A = (S^T S)^{-1} S^T (F - s_b a_b)$, and the negative elements of A are set to be zero.
4. While A and s_b are fixed, a least squares solution for the matrix S is calculated using $S = (AA^T)^{-1} A(F - s_b a_b)^T$, and the negative elements of S are set to be zero. Then, all column vectors of S are normalized to avoid indeterminacy of scale.
5. Return to 2 until the maximum number of iterations (typically 100) is reached.

If all elements of s_b are set to zero, this algorithm is identical to the original ALS algorithm. We confirmed that 100 iterations are usually enough to converge to the minimum of the mean square error.

2.2. Mukamel's ICA algorithm

Here, we briefly describe the ICA algorithm (from Mukamel et al. (2009), but with slightly different notations) that we used for comparison.

Similar to the NMF algorithm, the ICA algorithm decomposes the data matrix F (N by T) into a spatial component matrix S and a temporal component matrix A. In the pre-processing for the ICA algorithm, we center the movie data. First, we normalize the signal in each pixel by dividing by its mean value over all movie time frames. We next subtract the mean fluorescence averaged