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Motion correction for cellular-resolution multi-photon fluorescence microscopy imaging of awake head-restrained mice using speed embedded HMM

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ABSTRACT

Multi-photon fluorescence microscopy (MFM) captures high-resolution fluorescence image sequences and can be used for the intact brain imaging of small animals. Recently, it has been extended from anesthetized and head-stabilized mice to awake and head-restrained ones for *in vivo* neurological study. In these applications, motion correction is an important pre-processing step since brain pulsation and body movement can cause motion artifact and prevent stable serial image acquisition at such high spatial resolution. This paper proposes a speed embedded Hidden Markov model (SEHMM) for motion correction in MFM imaging of awake head-restrained mice. The algorithm extends the traditional Hidden Markov model (HMM) method by embedding a motion prediction model to better estimate the state transition probability. The novelty of the method lies in using adaptive probability to estimate the linear motion, while the state-of-the-art method assumes that the highest probability is assigned to the case with no motion. In experiments we demonstrated that SEHMM is more accurate than the traditional HMM using both simulated and real MFM image sequences.

1. Introduction

Mammalian brain microscopy plays an important role in studying the electrical and biochemical processes in neurons. Previously, charge-coupled device (CCD) imaging had been used to study mouse brain [1], in which a single fiber-optic bundle is placed in direct contact in the barrel cortex of awake mouse to image the cortical activities in conjunction with voltage sensitive dye. Since fiber-optic bundle has intrinsic low spatial resolution limited by the size of fiber core, this kind of imaging system does not reach cellular-resolution but allows researchers to obtain cortical activation map with relatively high temporal resolution. Recently, multi-photon fluorescence microscopy (MFM) imaging has been used to capture neural images from anesthetized mice at higher spatial and temporal resolution [2–5]. In [2,5], Ca^{2+} transients were quantified using MFM in mouse models of Alzheimer's disease (AD). Unfortunately, animals under anesthesia cannot demonstrate the neural dynamics sufficiently because overall brain activities are suppressed. Therefore, more MFM imaging studies of awake mice were carried out [6–9], and two-photon microscope (TPM), a special variant of the MFM, had demonstrated as a superior alternative due to its deeper tissue penetration, efficient light detection, and reduced phototoxicity [3].

There are two models for TPM in awake mice: head-mounted [6,7] and head-restrained [8,9]. A head-mounted microscope requires innovative engineering to mount a miniaturized microscopy on the head of a freely moving animal. For example, the miniature scanner, the photomultiplier tube, and the objective holder need to be designed and mounted on the animal head [8]. On the other hand, head-restrained TPM [8,9] uses a standard microscope system to visualize simultaneous Ca^{2+} dynamics while restraining the head of the animal under the microscope and allowing it to walk or run freely on a stationary exercise ball [10]. Both techniques are capable of imaging the brain of alive animals, but because of the mice's movement the relative subtle position shifting between the microscope and the brain could lead to line shifting artifacts and affect image reconstruction and quantification of the functional dynamics. Thus, a motion correction algorithm is needed for quantifying brain motion and aiding in stabilizing cell displacements within a region of interest (ROI) in order to reduce the number of brain-motion-related fluorescence transients.

In the literature, two methods were recently proposed for motion correction of TPM in awake mice [8,9]. Greenberg and Kerr [9] proposed a method based on the Lucas–Kanade algorithm [11,12] to estimate the offsets by minimizing the squared intensity differences between a reference frame and the subsequent frames using the gradient descent method. In [8], a Hidden Markov model (HMM)-based method was proposed to calculate the motion using

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a probabilistic HMM [13]. The advantage of the HMM approach over the squared intensity difference minimization is that HMM applies a temporal motion consistency statistical constraint based on the Markov model, whereas the latter may be stuck in local minima due to the use of a gradient descent algorithm. Additionally, the HMM algorithm generates more temporally stable results due to better characterization of local motion deviations, which acts as a "temporal smoothness" constraint. In HMM, statistical constraints are used for estimating the motion of each line in the video sequences, and the highest probability is given to the "no motion" status of each line. "No motion" here is referred to the case that the position of the current line is predicted as if the mouse does not move. A standard exponential model (or distribution) is used to describe this assumption. In fact, the no motion assumption is only realistic when the mice are standing still or relatively still (the relative position for scanning beam and mice's local neural region of interest stays constant), but this situation is not very common because mice motion is disordered and random and includes rapid changes of speed all the time during the procedure. Thus, in HMM, due to the inherent drawback for predicting the motion, although the state transition model works effectively for the resting stage, it might fail and yield wrong estimation during the running stage.

In this paper, we extend the HMM algorithm by incorporating a speed estimation into the state transition model for more accurate motion correction, called speed embedded Hidden Markov model (SEHMM) algorithm. The major difference between the HMM and SEHMM algorithms is that SEHMM first uses an exhaustive searching method to obtain a preliminary speed estimation result and then embeds the speed estimation into the final HMM-based motion prediction. By embedding speed estimation in HMM, the highest probability is given to the motion vector estimated from the previous frames.

In experiments, a quantitative validation was performed to compare the HMM and SEHMM algorithms based on both simulated and real data. First, simulated image sequences were generated to mimic various real motion situations, and we applied different dynamic amplitudes to make the validation more realistic and reasonable. The accuracy of both HMM and SEHMM can then be compared because the ground truth of the motion was known. For real data, we showed the comparative results to demonstrate the performance of SEHMM. The results indicate that using SEHMM, higher estimation accuracy can be obtained to recover the motion vectors, and more accurate image alignment results were achieved as compared with HMM, especially in the running stages of the image sequences.

The remainder of the paper is organized as follows. Section 2 exposes the problem we need to solve. Section 3 proposes the SEHMM algorithm in detail, including initialization and SEHMM itself. The experimental results and validation are presented in Section 4. We conclude the work and discuss the future work in Section 5.

2. Problem formulation

A two-photon microscope captures a series of images by passing the focus of laser excitation repeatedly over a rectangular or square region of fluorescently labeled tissue and collecting the resulting photons via a photon multiplier tube (PMT) [14]. Because of the subtle relative motion between the probe and mouse, to generate stable microscopy videos a motion correction tool is necessary to temporally align the image sequences. Generally, although the mouse motion is in 3-D, shifts along the Z-axis (depth) is less than 1 μm for a scanning speed of 2 ms/line, much lower than the X (medial-lateral direction along the raster scan line) and Y (rostral–caudal direction across the raster scan line) directions

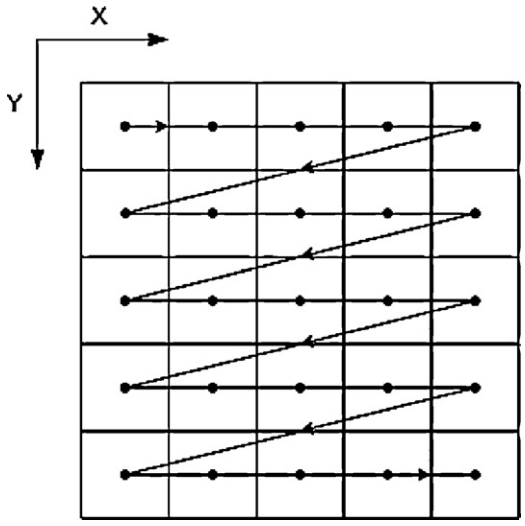

Fig. 1. Raster scanning with a zigzag pattern.

(10–40 μm). Therefore, our task is to estimate the motion in X and Y directions. According to the raster scan pattern shown in Fig. 1, if there is no motion we can obtain the pixel position as,

$$\begin{cases} X_i^k(t) = \left\{ \dfrac{t}{(\tau/N)} \right\} \cdot N \\ Y_i^k(t) = \left[\dfrac{t}{(\tau/N)} \right] \end{cases}, \tag{1}$$

where $X_i^k(t)$ and $Y_i^k(t)$. indicate the location of the laser focus of the ith pixel $i \in \{1, 2, 3, \ldots, N\}$ of line k, $k \in \{1, 2, 3, \ldots, N\}$, and the size of each frame is $N \times N$. $[\cdot]$ represents the integer operation, and $\{\cdot\}$ denotes the fractional operation. It can be seen that the laser focus moves in a zigzag pattern in the X direction and in a step function in Y direction [9]. Denoting t_{pixel} as the duration between any consequent pixels for the scan, $\tau = N^2 t_{\text{pixel}}$ is the estimated scanning time for a frame, and $\tau_{\text{line}} = N\tau_{\text{pixel}}$ is the estimated scan time for a line. Given that I is the current image and R is the reference image, which will be used as the baseline to which all the remaining frames can be aligned, if the scanning laser beam is at the ideal position $(X_i^k(t), Y_i^k(t))$ and if there is no motion, the image intensity $I_i^k = I(X_i^k(t), Y_i^k(t))$ should be similar to the intensity of the corresponding location in the reference image, i.e., $R(X_i^k(t), Y_i^k(t))$. Due to the mice motion during the raster scan progression, the relative motion of each line can be written as,

$$\begin{cases} X'^k_i = X_i^k + \delta_x^k \\ Y'^k_i = Y_i^k + \delta_y^k \end{cases}, \tag{2}$$

where (δ_x^k, δ_y^k) is the offset of line k from the ideal position (X_i^k, Y_i^k), and (X'^k_i, Y'^k_i) is the actual position where the scanning laser beam is focused on. Therefore, once the offset of each line is estimated the microscopy video can be re-aligned according to the reference frame, producing a relatively stable video sequence. Our goal is thus to estimate the offset values (δ_x^k, δ_y^k) from the serial image input. Herein, we chose a line-by-line motion correction algorithm by assuming that each line has the same relative displacement. The reason why a line-by-line strategy is chosen is that the shifts for all the pixels within a line do not get beyond one-pixel in Y direction and are very tiny in X direction [8] given the scanning speed of 2 ms/line, and considering the requirement for future real-time application, a common solution is that a line-based motion correction is sufficient in this application [8].