Cerebral blood flow recorded at high sensitivity in two dimensions using high resolution optical imaging

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1. ABSTRACT

Knowledge about sensory-evoked blood-flow changes is essential for constraining hemodynamic response models used to interpret functional brain imaging signals, such as fMRI. Here, we extracted 2-dimensional blood-flow and its temporal modulations from high-resolution optical imaging data in the awake monkey. Optical imaging allows to track moving erythrocytes (or small clusters thereof), thus providing, albeit noisy, information about their velocity in individual blood vessels, across the whole imaged area. Here, we illustrate the algorithms that allowed us to extract, at the single microvessel level, red blood cell (RBC) motion information from the noisy optical signals. For this purpose, we developed an algorithm that is both robust and computationally efficient, using the structure tensor, known to detect an average direction of image intensity gradient. This structure tensor tool is applied to detect trajectory directions in the spatio-temporal data. Since blood-flow modulation by the cardiac pulsation was clearly detected, our method should be applicable also to study blood-flow modulations by neuronal activity, and their spatio-temporal patterns.

2. INTRODUCTION

Baseline blood flow and its sensory-evoked changes are commonly measured by the Laser Doppler technique. This method, however, allows only single-point measurements. Furthermore, blood flow values are integrated over a relatively large volume (> 1 mm³), hampering the resolution of blood flow dynamics in single vessels. Such a resolution can be attained using 2-photon fluorescent microscopy [1, 2], but, due to finite scanning times, this method is applicable only to one vessel at a time. Using reflection-based optical imaging, Grinvald et al. [3] succeeded to measure blood flow in large portions of the human retina. However, they reported only baseline values but no modulations. We extended this latter approach to image baseline blood flow and its modulations in the awake monkey primary visual cortex, using high-resolution optical imaging (~15 micron/pixel, 5 ms/frame).

As motion detection needs to be carried out in every selected vessel and for every individual trial, we developed an algorithm that is both robust and computationally efficient. To test the method’s sensitivity, our primary goal here was to detect blood-flow modulation by heart pulsation, which can be easily verified. We achieved this by using the structure tensor tool. The structure tensor determines a local mean orientation for intensity gradient in an image [4]. As a control for our method, we recorded blood flow also by a Laser-Doppler probe simultaneously with imaging, from the same piece of cortex. The signals obtained by the two measurements were consistent (up to a phase shift) with respect to the blood flow modulation caused by the heart-beat, thus validating our approach. Moreover, the two measurements were also consistent with respect to small blood flow fluctuations superimposed on the cardiac pulsation, suggesting that the SNR attained by the present method should be sufficient to resolve also blood-flow modulations known to be caused by local neuronal activation [5].

The very same imaging data recorded here contain also high spatio-temporal resolution 2-dimensional information about changes in blood-volume [6] (or oximetry, depending on the wavelength used for illumination). Changes in blood volume (or oxygenation) can thus be imaged simultaneously with the blood flow signal, allowing a detailed characterization of the interplay between the various hemodynamic processes, selectively in the different microvascular compartments. Such information should prove useful to adequately constrain hemodynamic models, as used to model fMRI responses.

3. METHOD

3.1. Data preprocessing

Blood is not a homogeneous fluid; rather, hemoglobin travels through blood vessels, packed in red blood cells (RBC)s. When the vasculature is imaged at sufficiently high resolution, these RBCs (or clusters thereof) are visible in the recorded images as small contrast changes that shift along the vasculature with time. However, the large differences in reflected light intensity between blood vessels and parenchyma...
are much larger than the small intra-vascular light-intensity differences due to the presence or not of RBCs. Thus, raw images do not allow to pick out single RBCs (one example is shown in Fig. 1, top left). These large differences can be eliminated by normalizing for the static light intensity gradient among different parts of the image, dividing, on a pixel-by-pixel basis, each frame by its mean value over an acquisition sequence, and allowing thereby to “flatten the image”. The contrast can then be largely enhanced and it becomes possible to track single clusters of RBCs moving along single vessels (Fig. 1 bottom left and zooms).

Individual vessels were segmented by hand, and then light-intensity time courses in each of them were extracted from the images as 2D arrays \( n_t \) by \( n_x \) spatial points along the vessel. Figure 2 (left) shows such light-intensity time courses. The main observed feature were large horizontal “waves”. Those pulsations do not result from changes in the RBCs’ velocity. Rather, they result from the well known overall light absorption changes, due to blood-volume oscillations caused by the heart-beat (~0.5 Hz for our monkey, as measured independently by a pulse-oximeter).

Superimposed on those large horizontal waves, one can also observe roughly straight, oblique lines through the image: Those are the spatiotemporal trajectories of RBCs in the vessel (they move right when time increases), which contain the RBCs’ motion information. The slope of these lines is indeed directly linked to RCB velocity: if a hemoglobin packet moves from position \( x \) to position \( x' \) between times \( t \) and \( t + 1 \), then its velocity along the vessel direction is \( x' - x \).
The differences between vasculature images:

The flow estimation was carried out for each individual trial. Heart pulsation is correctly recovered. Differences in phase between vessels indicate delays in blood transfer. The amplitude decreases because inter-trial heart-beat synchronization deteriorates progressively.

This formula for RBC speed, however, cannot be applied directly because of noise in the image. It is necessary to stabilize the algorithm by using a neighborhood averaging. The idea is to find an average direction of trajectories over a small neighborhood (we used a neighborhood of about 30 time points by 5 voxels length), which can be achieved with the structure tensor [4].

Let us define the following degenerate symmetric matrix:

\[
A(x, t) = \begin{pmatrix}
I_x^2(x, t) & I_x(x, t)I_t(x, t) \\
I_x(x, t)I_t(x, t) & I_t^2(x, t)
\end{pmatrix}.
\]

The first eigenvector of \(A\) is the gradient direction \((I_x, I_t)\) and its second eigenvector (with zero eigenvalue) is our trajectories’ direction \((-I_t, I_x)\). The structure tensor is defined by averaging matrix \(A\) over a small neighborhood:

\[
S(x, t) = \sum_{(x', t') \in \mathcal{V}(x, t)} A(x', t').
\]

Then the first eigenvector of \(S\) gives the direction in which image intensity varies the most; the second one gives the direction in which image intensity varies the least, which is actually the direction we are looking for, and the inverse of its slope is the estimated RCBs velocity.

### 3.3. Vessels coregistration

The flow estimation was drawn by hand on one raw image (using Fig. 1, left panel). However, it happens that, because of the animal’s movements, the whole vasculature shifts slightly from one trial to the next. To average, it is thus necessary to register these rigid motions. This was accomplished by finding the isometry (a rotation plus a translation) that minimizes the differences between vasculature images:

\[
(\hat{\theta}, \hat{\mathbf{t}}) = \arg\min_{\theta, \mathbf{t}} \| I_2 - \mathcal{T}_\mathbf{t} \circ \mathcal{R}_\mathbf{t}(I_1) \|^2.
\]

This fact is supported by the similarity between all the 3 obtained time courses shows that our algorithm can also capture signal fluctuations that are superimposed on heart pulsation, such as those resulting from neuronal activity, evoked by sensory stimulation.

### 3.4. Results

To increase SNR of the recovered heart response, data were averaged over trials (n=144; data acquisition was synchronized with heartbeat). Figure 3 shows the obtained velocity time courses for several vessels, well matching with the simultaneous Laser-Doppler measurements.

Finally, we wanted to test the sensitivity of our method with respect to small, non-heart-beat-related blood-flow changes (Fig. 4). First, we divided the trials into two independent sets. We then averaged the velocity estimations over each of the two sets. We then calculated the ratio between those two averaged signals, as is customary done to extract sensory-evoked hemodynamic responses [5, 6], and compared it to (i) the same ratio obtained from the simultaneously acquired Laser Doppler and from (ii) blood volume recordings. The similarity between all the 3 obtained time courses shows that our algorithm can also capture signal fluctuations that are superimposed on heart pulsation.

### 3.5. Experimental setup

**Preparation:** Experiments were performed on an awake adult male macaque monkey (M. mulatta, 6-7 Kg), with a transparent cranial window chronically implanted onto a ~2x2cm aperture in the skull above the V1/V2 border, at about 2.5° eccentricity of the visual field. For a detailed description of all surgical and maintenance procedures see [8]. All animal procedures were in accordance to NIH guidelines.

**Experimental paradigm:** Data acquisition was synchronized with the heartbeat, recorded independently by a pulse-oximeter. A trial started when the monkey began fixating on a fixation point (0.08-0.1°), displayed on a CRT screen. The monkey had to continue to fixate until the fixation point disappeared, for a total fixation period of about 4s. An isoluminant uniform, grey screen, was shown between the trials. Inter-trial interval was at least 10s. To avoid visually-evoked noise, data from trials where the monkey broke fixation were rejected.

**Optical recordings:** We imaged 3.5x3.5 millimeters of cortex, continuously over 4 seconds, illuminating at a wavelength that is isosbestic for oxy- and deoxy-hemoglobin (570nm) to avoid oximetric artifacts. Images were acquired at 200Hz using a commercial, CCD-based, imaging system (Imager
obtained by blood volume and oximetry, as well as important constraints for hemodynamic response models, as those used in modern functional brain imaging techniques.

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6. REFERENCES


