A Label-Free Functional Microscopic Technique

Photoacoustic Computed Microscopy (PACM) is a new label-free microscopic method that combines current photoacoustic microscopy technique with a model-based inverse reconstruction algorithm to provide functional images of microvasculature. This article demonstrates its in vivo imaging ability of quantifying important functional parameters at the small vessel level in a rodent model. This new technique offers a unique tool for neuroscience research and for visualizing microvasculature dynamics.

Introduction

Photoacoustic microscopy (PAM) is emerging as a powerful technique for imaging biomedical tissue at depths beyond the ~1mm depth limit associated with confocal microscopy, two- or multi-photon microscopy and optical coherence tomography. It has been shown so far to be particularly useful for imaging microvasculature associated with neural activity and tumors and for detecting optical reporters in high resolution [1, 2]. PAM, however, is essentially qualitative in nature since it measures the absorbed optical energy density, the product of the absorption coefficient and the local optical fluence rather than the absorption coefficient itself. This prevents PAM from quantitatively measuring important functional parameters including oxyhemoglobin (HbO$_2$), deoxyhemoglobin (HbR) and oxygen saturation (sO$_2$). Absolute quantification of these functional parameters will allow for correct determination of the physiological status of tissue and accurate diagnosis of pathological conditions such as tumors and neurodisorders.

Initial effort has been made to quantify optical absorption coefficient using acoustic spectra in PAM [3, 4], and a non-model-based technique was used in this effort where the optical fluence was assumed as uniformly distributed across the media, making it inevitable to negatively affect the accuracy of the results. Therefore, there is a clear need of developing new approaches to provide truly accurate microscopic photoacoustic (PA) imaging. Here we present our newly developed method, Photoacoustic Computed Microscopy (PACM), to address this critical need.
Central to PACM is a model-based inverse reconstruction algorithm, which uses the PA signals measured by a focused transducer to obtain quantitative images of HbO\textsubscript{2}, HbR and sO\textsubscript{2} [5]. In addition, assisted with an oxygen-transport model PACM permits determination of two other important functional parameters, blood flow (BF) and rate of oxygen metabolism (MRO2). Compared to the existing techniques, PACM allows the quantitative visualization of microvasculature dynamics in high spatial resolution without the use of any contrast agent.

**Imaging System**

The PACM imaging system is schematically shown in figure 1(a). Short-pulsed laser beam at 10Hz repetition rate from a Nd:YAG laser and/or a Ti:Sapphire laser are used. The laser beam from each laser is delivered to the sample via an optic fiber bundle. Both the optic fiber bundles are positioned to produce an optimal illumination at the sample surface as shown in figure 1(b). Depending on the application, either one of the lasers or both of them are used for multispectral imaging where a 50-µs time delay between the two lasers provided by the function generator is used to avoid signal overlap. The imaging probe consisting of a focused transducer and the optic fiber bundles is mounted on a two-dimensional (2D) moving stage. The sample is positioned under an open imaging window at the bottom of the water tank sealed with an ultrasonically and optically transparent membrane. Ultrasound gel is used between the membrane and the sample for acoustic transmission. At each imaging probe position, the laser-induced PA signal is received by the transducer, amplified by the amplifier and digitalized by a 8-bit data acquisition board (NI5152, National Instrument) at a sample rate of 250MS/s. One- or two-dimensional raster scanning of the imaging probe along the horizontal plane coupled with the depth-resolved ultrasonic detection forms a 2D or 3D PA image. It takes ~6s for a typical B-scan 2D imaging. For the following *in vivo* animal experiments, a focused high-frequency transducer (50 MHz, 3 mm aperture and 6mm focal length) was used, which provides a lateral resolution of 61 µm on the focal point and an axial resolution of 15µm.

**In Vivo Animal Experiments**

A rat ear experiment was first applied to validate this method and to show its possible application to functional imaging based on physiologically specific endogenous optical absorption contrasts in biological tissue. The laser applied was at a wavelength of 532nm. In the left of figure 2 we present the maximum amplitude projection (MAP) images projected along the vertical direction (z axis) to the orthogonal plane where seven orders of vessel branching, indicated by numbers 1-7, can be observed in the image. The B-scan image and the reconstructed
absorption coefficient image obtained by PACM are shown in the right of figure 2, respectively. These images are in the vertical plane (x-z plane) at the location indicated by the dash line in figure 2(left). It can be clearly seen that all of the vessel branching can be reconstructed using both the methods. And from figure 2(bottom right) we found that at most locations within the blood vessel, the value of the reconstructed absorption coefficient is in the range of 20~30mm-1, which is in good agreement with that of blood vessels at 532nm reported in the literature [6, 7].

We then demonstrate PACM’s ability of in vivo imaging HbO₂, HbR, sO₂, cerebral blood flow (CBF) and cerebral rate of oxygen metabolism (CMRO₂) [5]. In this experiment, a single blood vessel located 1.5mm below the surface of the rat brain with intact skull was scanned, and pulsed light at 1064nm from the Nd:YAG laser and at 730nm from the Ti:Sapphire laser were used for multispectral imaging. Using PACM, we obtained images of HbO₂ (figs. 3 (a-d)), HbR (figs. 3(e-h)), and sO₂ (figs. 3(i-l)) at the four selected time points, t₁=12s, t₂=24s, t₃=42s and t₄=54s, as well as at other time points. We note that the total hemoglobin concentration ([HbT]=[HbO₂] + [HbR]) of the blood vessel is in the range of 40-50 g/l, which agrees well with that described in the literature [8]. Based on these results, the reconstructed CBF and CMRO₂ images at four different times are provided in figures 4(a-d) and 4(e-h). We also see from the peak values of CBF shown in figure 4 that the recovered blood flow values (7.5-8.5ml/ml/s, or 45-51ml/100ml/min) are in good agreement with the reported CBF of rats (10-120 ml/100ml/min) and of humans (20-160 ml/100ml/min) [9, 10]. These high-resolution images not only allow for the accurate recovery of the size/shape/location of single blood vessel, but the functional parameters (indicated by the color scale on right) can also be reconstructed quantitatively over time.

**Conclusions**

PACM is capable of in vivo imaging a full set of functional parameters at the small vessel level. The spatial resolution of PACM is the same as PAM, scalable with the central frequency of the focused transducer, i.e., higher resolution at the single cell level is achievable with the sacrifice of penetration depth. The imaging speed of the current PACM is relatively slow (6s), but it can be improved considerably by using faster lasers. We expect PACM will be a valuable tool for neuroscience research where hemodynamics associated with microvasculature in response to neural activation/stimulation need to be imaged. PACM will also be applicable to visualize microvasculature dynamics involved in tumor angiogenesis and in inflammatory joint diseases.
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References

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