Three-Dimensional Label-Free Photoacoustic Microscopy of the Tumor Microenvironment *in vivo*

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Introduction:

Angiogenesis is a hallmark of cancer. Tumor angiogenesis produces a unique tissue microenvironment with features such as vessel dilation, perivascular detachment, and irregular vasculature. Monitoring tumor angiogenesis is important to understanding the tumor microenvironment and developing new means to treat cancer. Photoacoustic microscopy has been proven powerful for three-dimensional imaging of blood vessels with capillary resolution.

The photoacoustic microscope (PAM) relies on the photoacoustic effect and uses hemoglobin as the endogenous contrast agent to produce high-resolution images [1]. Here, we propose that photoacoustic microscopy can be used for imaging of tumor microenvironment *in vivo* and quantification of key parameters of tumor growth.

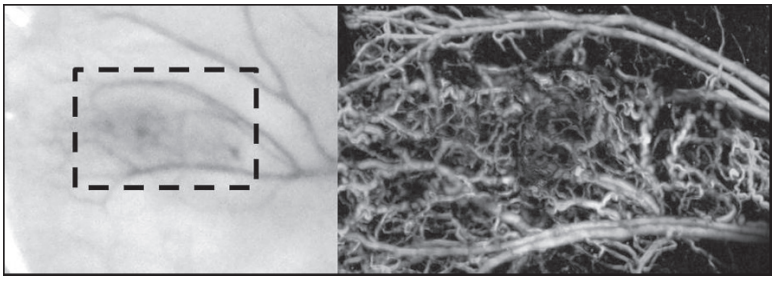

Figure 1: White light image of the tumor with the box signifying the tumor region (left), and the corresponding PA image (right).

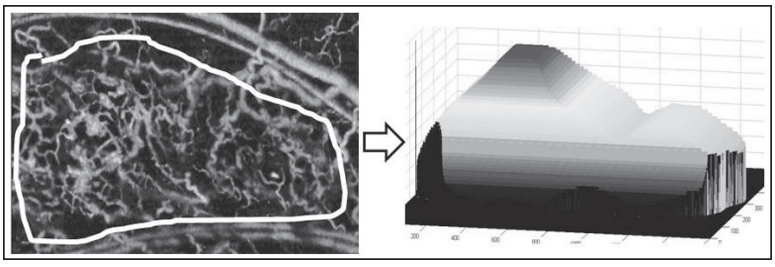

Figure 2: 2D back projections (left) were used to make the 3D region of interest (right).

Materials and Methods:

PAM System Calibration. The imaging system was first calibrated for the measurement of absolute total hemoglobin concentration. Five solutions of lysed bovine blood were measured by PAM. The calibration factor between the hemoglobin concentration and PA signal amplitude was calculated via linear fitting. Then, the system was calibrated for the measurement of absolute absorption coefficient. Five different absorbers: lysed bovine blood, red ink, blue ink, a 1:1 mixture of red and blue ink, and DQOCI were measured. The absorption coefficient of each dye at 570 nm was first measured using a spectrophotometer. Then the calibration factor between the absorption coefficient and PA signal amplitude was calculated for each individual dye.

After calibrating for absorption coefficient, a graphite phantom was made for daily system calibration. The PA signal from the graphite phantom was measured under normal system alignment, and the absorption coefficient calibration factor was calculated using DQOCI dye. This procedure was repeated under three additional system alignments.

Longitudinal Imaging of the Tumor Microenvironment. As a demonstration, a U87 glioblastoma tumor was longitudinally imaged for three weeks with white light imaging and the PAM as shown in Figure 1.

Tumor Volume and Tumor Vasculature Volume. Tumor volume and tumor vasculature volume were both quantified by back-projecting the three dimensional data onto planes at different angles to create a three dimensional region of interest as shown in Figure 2. This three dimensional region of interest was used to calculate the tumor volume and determine the tumor boundaries. The tumor vasculature

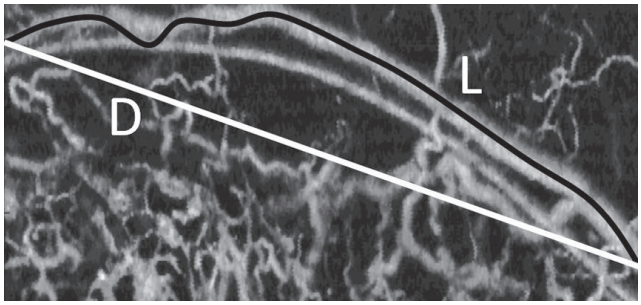

Figure 3: Tortuosity is defined as the ratio of the length of the vessel (L) to the distance between the end points of the vessel (D).

volume was calculated by computing the volume of all the blood vessels within the tumor boundaries.

Total Hemoglobin Concentration. Total hemoglobin concentration was extracted at each pixel using the hemoglobin calibration factor that was calculated during the hemoglobin calibration experiment, and then averaged across the tumor region.

Vessel Tortuosity. Vessel tortuosity was quantified as the ratio of the vessel length to the linear distance between its two endpoints as shown in Figure 3. The vessels that were visible in all images were identified using a cross-section-tracing based vessel segmentation algorithm. Tortuosity for each of these vessels was calculated and then averaged among the vessels.

Results:

System Calibration. The PAM was successfully calibrated to measure absolute hemoglobin concentration. Linear fitting revealed that the ratio of PA signal (A.U.) to hemoglobin concentrations (g/dL) was 0.027 with an offset of -0.009. The absorption coefficient calibration experiment yielded unexpected results. Each dye showed a linear relationship between the absorption coefficient and PA signal. The ratio was 0.0018 for lysed blood, 0.00021 for blue dye, 0.0042 for DQOCI, 0.00099 for the blue and red dye mixture, and 0.0018 for the red dye. Testing of the graphite phantom revealed that the relationship between absorption coefficient calibration factor and graphite PA signal is linear regardless of system alignment.

Longitudinal Imaging of the Tumor Microenvironment. The results show that, within three weeks, the tumor had grown to a size of 0.72 cm^3. Vascular volume grew to 0.03 cm^3, and vessel tortuosity increased by 25% compared with the baseline.

Conclusions and Discussion:

PAM was calibrated to measure total hemoglobin concentration and absorption coefficient. However, the absorption coefficient calibration results were different than what was expected. Assuming that the dye-water solutions all had roughly the same physical properties besides absorption coefficient, it was expected that all of the data for all of the dyes should be collinear.

The data points for each dye solution were linear, but for a given absorption coefficient each dye had a different PA signal. The explanation for these results is not yet know, but further absorption coefficient calibration experiments are being conducted. The graphite phantom proved to be a reliable way to calibrate the PAM to measure absorption coefficient. Given that there is a different relationship between absorption coefficient and PA signal for each absorber, more experiments need to be performed. In addition to the calibration experiments, PAM was used to longitudinally image a tumor for three weeks and extract the physiological parameters including hemoglobin concentration, tumor vasculature tortuosity, tumor volume, and tumor vasculature volume. These results demonstrate that PAM is a good tool for imaging the tumor microenvironment.

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References:

[1] Wang, L.V.; "Multiscale photoacoustic microscopy and computed tomography"; Nature Photonics, 3, 3-9 (2009).