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Estimation of Oxygen Saturation in Microvessels Using Photoacoustic Microscopy with Two-Wavelength Laser

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1. Introduction

Angiogenesis occurs in inflammatory diseases such as rheumatoid arthritis, and microvessels develop in the early stages of inflammation. It has been reported that this hemodynamic change is accompanied by an increase in oxygen consumption at inflammatory sites, resulting in changes in oxygen saturation (SaO_2) in the blood, especially in venous blood.[1)] Therefore, highly sensitive detection of changes in SaO_2, as well as vascular structure, is important for early detection of inflammation, and is useful for planning better treatment strategies.

SaO_2 is the ratio of oxyhemoglobin to the total concentration of oxy- and deoxyhemoglobin.[2)] Due to the different optical absorption coefficients of oxy- and deoxyhemoglobin, SaO_2 can be calculated by using the difference in optical absorbance of laser light of two different wavelengths. Currently, point SaO_2 measurements using commercially available near-infrared spectroscopy-based pulse oximeters are widely used to obtain average SaO_2 values.[3)] However, this technique cannot simultaneously assess the relationship between SaO_2 and blood hemodynamics. Noninvasive functional magnetic resonance imaging (fMRI) can also image SaO_2 changes, but with a spatial resolution of only several millimeters.[3)]

Photoacoustic imaging (PAI), in contrast, can selectively visualize blood vessels and simultaneously visualize SaO_2 by using two wavelengths of laser light. PAI is an imaging technique that is based on the photoacoustic effect, in which a material absorbs a laser light and thermally expands, producing ultrasound waves. Because PAI is based on optical absorption, the specificity of optical imaging techniques is retained. In addition, however, information on the absorber is carried out of the tissue by ultrasound, allowing deeper tissue imaging than optical imaging while providing higher image resolution.

Our research group has developed an acoustic-resolution photoacoustic microscope (AR-PAM) system equipped with two wavelengths of laser light with a resolution of tens of micrometers.[4)] The AR-PAM system can visualize vascular structures and their SaO_2 maps at once from the signals obtained by irradiating 532 nm and 556 nm laser light. The system can also measure the optical output at each measurement point when irradiating the laser lights, and can compensate for differences in optical fluence, which causes problems in SaO_2 estimation, thus providing highly reliable results. In this study, two-wavelength PAI was performed on rats with arthritis to investigate the estimated SaO_2.

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2. Material and Methods

2.1 Experiment setup and data acquisition

The AR-PAM system used in this study has been reported in a previous paper.[4)] The four-element annular array transducer (center frequency 60 MHz, detection bandwidth 24 MHz) used as the sensor had a concave surface with a geometric focus of 6 mm and a central hole (ϕ 1 mm) for the laser outlet. The Nd:YAG lasers were 532 nm with a pulse width of 1.2 ns and 556 nm with a pulse width of 7 ns.

The PA transmitter/receiver unit was raster-scanned with an XY stage while repeatedly emitting laser pulses and receiving PA signals. In each transmission event, the two light sources were irradiated at a repetition rate of 1 kHz. The optical output at each measurement point during laser pulse irradiation was detected by a photodiode. The received signals were processed by the DAS method.[4)]

The data were visualized in C-mode using the maximum amplitude projection (MAP) method. The image size and step widths of the vertical and horizontal axes were 9.0 × 9.0 (x × y) mm and 60 × 30 (x × y) μm, respectively. Smoothing by linear interpolation was performed to suppress image distortion caused by the difference in step width between the vertical and horizontal axes, and the pixel size of the resulting C-mode MAP image was 30 × 30 μm. All signal processing and visualization were performed on MATLAB (Ver. 2024a, Mathworks).

2.2 Estimation of oxygen saturation

SaO_2 in PAI using two-wavelength laser light $\lambda_{1,2}$ is estimated as follows,[2)]

$$SaO_2 = \frac{\frac{p_1}{p_2} \cdot \frac{\Phi_2}{\Phi_1} \cdot \mu_2 - \mu_1}{\frac{p_1}{p_2} \cdot \frac{\Phi_2}{\Phi_1} \cdot \delta\mu_2 - \delta\mu_1},$$

where $p_{1,2}$ represents the photoacoustic signals produced by each laser light, $\Phi_{1,2}$ is optical fluence at each wavelength, $\mu_{1,2}$ is optical absorption

coefficient of Hb at each wavelength, and $\delta\mu_{1,2}$ is difference between the optical absorption coefficients of Hb and HbO_2. As shown in the equation, SaO_2 can be estimated by PAI using the difference in optical absorption coefficients of oxy- and deoxyhemoglobin at each wavelength. The optical absorption coefficients were based on the values publicized by the Oregon Medical Laser Center (Scott Prahl, Optical Absorption of Hemoglobin, 1999). The fluence ratio was calculated using measured optical output ratios.

2.3 Rat imaging

One knee joint of a Wistar rat was inflamed and examined after 8 weeks. Hair on the surface were shaved before imaging, and the images were taken under anesthesia, with the approval of the Ethics Committee for Animal Experiments of the Support Center for Laboratory Animal and Gene Researches, Tohoku University. SaO_2 was estimated at the point where the amplitude of the photoacoustic signal was greater than 500 (a.u.) in the obtained C-mode MAP image. Linear interpolation was performed on the estimated SaO_2 map to remove local variations in the values.

3. Results and discussion

Fig. 1(a) shows the photoacoustic image obtained, and Fig. 1(b) shows the estimated SaO_2 map. Wavelength-dependent scattering could cause errors in the calculation of SaO_2 values, but the wavelengths used in this system were so close that the path length differences were negligible.

The estimated SaO_2 values were approximately reliable. However, part of the large vessels showed a significant decrease in SaO_2 values. Because SaO_2 values are usually around 90% not only in arteries but also in veins, further improvement is required for the current method.

Validation of the estimated SaO_2 values need to be evaluated in the future. This will be examined using a blood phantom with adjusted SaO_2. Furthermore, because the thickness of the epidermal and dermal layers of the skin varies from subject to subject, ultrasound imaging is needed to measure their thicknesses so that optical propagation and acoustic attenuation can be corrected in the future.

4. Conclusion

In this study, we investigated the estimation of SaO_2 by PAI using two-wavelength laser lights to evaluate the SaO_2 of the microcirculation. The estimated SaO_2 of the microcirculation in a 9 × 9 mm area of the knee joint of rats was approximately reliable. Further improvement of the estimation accuracy will be possible in the future when light propagation and acoustic attenuation are considered. Establishment of a high-accuracy SaO_2 estimation method is expected to provide new insights into the variability of SaO_2 in inflammatory diseases.

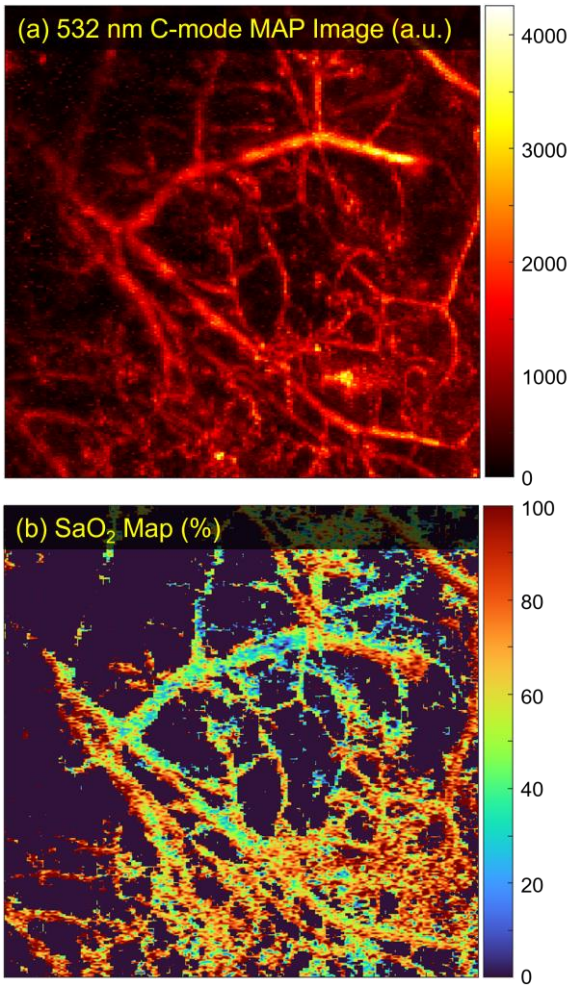

Fig. 1 (a) Photoacoustic C-mode MAP image obtained at 532 nm. (b) Estimated oxygen saturation map.

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References

1) C. A. Hitchon, and H. S. El-Gabalawy, Arthritis Res. Ther., **6**(6), 265 (2004).
2) V. Perekatova, P. Subochev, M. Kleshnin, and I. Turchin, Biomed. Opt. Express, **7**, 3979 (2016).
3) R. Bulsink, K. A. J. Mithun, X. Marvin, M. Srivalleesha, S. Wiendelt, and J. F. Kalloor, Sensors, **21**(1), 283 (2021).
4) R. Suzuki, R. Shintate, T. Ishii, and Y. Saijo, Jpn. J. Appl. Phys., **61**, SG1047 (2022).