Photoacoustic power azimuth spectrum for microvascular evaluation

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A R T I C L E  I N F O

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A B S T R A C T

The tubular structures and dendritic distributions of blood vessels emit anisotropic photoacoustic (PA) signals with different intensities and frequency components at different angles. Therefore, spectral analysis of PA signals from a single angle cannot accurately determine the physical characteristics of microvessels. This study investigated the feasibility of using the PA power azimuth spectrum (PA-PAS) method to evaluate microvessel structures. We mapped the acoustic power spectrum of the PA signals along the azimuth direction. Based on a frequency-domain analysis of the broadband PA signal, we calculated the spectral parameter power-weighted mean frequency (PWMF). The results demonstrate that the PA signal information of the microvessel is mainly concentrated in the direction of its width. In addition, the PWMF decreases linearly with the microvascular size. The experimental findings exhibit good agreement with the simulation results, thus demonstrating that this approach can effectively differentiate the sizes of microvessels.

1. Introduction

The occurrence and development of numerous diseases are closely associated with changes in the morphology and function of microvasculature, including size and density \cite{1–7}. Quantitatively, these changes can provide insights into the dynamics of tumor angiogenesis, which is important for clinicians diagnosing and treating cancer.

Many well-established clinical detection modalities, such as optical coherence tomography (OCT), ultrasonography, and magnetic resonance imaging (MRI), have been adopted for vascular imaging. Owing to the strong scattering of light in biological tissues, OCT can provide high-resolution images; however, its imaging depths are limited \cite{8,9}. Although ultrasonography can measure blood flow velocity at a satisfactory imaging depth, it is not suitable for microvessels, the diameter of which is less than 150 \textmu m \cite{10,11}. MRI can provide blood volume, perfusion, permeability, and other vascular information; however, its resolution is insufficient for analyzing microvessels \cite{12,13}. At present, immunohistochemical staining of the vascular endothelium is still the conventional method for identifying microvessels in clinical practice \cite{12}; however, it is invasive and severely painful for patients. Therefore, noninvasive diagnosis of microvascular diseases has proved extremely challenging.

Photoacoustic tomography (PAT) is a new type of noninvasive biomedical imaging modality that combines the advantages of high-contrast optical imaging with the advantages of high-resolution ultrasound imaging in deep biological tissues \cite{9,14–16}. Because of the high optical contrast between the hemoglobin in blood and the surrounding tissue \cite{17}, PAT has been widely used in vascular detection \cite{18,19}. Photoacoustic computed tomography (PACT) is based on wide-field light illumination and unfocused ultrasonic detection at multiple locations; it relies on an inverse algorithm to reconstruct the image showing light absorption distribution and can achieve penetration depths up to 70 mm in living tissue \cite{20}. The delay-and-sum reconstruction algorithm \cite{21,22} is a basic and widely used method in PACT. Most of the previous PACT studies on microvessels were focused on either imaging the distribution of microvessels \cite{23–25} or quantifying hemoglobin oxygenation \cite{26–28}. They were mainly interested in the amplitude of the time-domain photoacoustic (PA) signals. However, the imaging resolution in PACT is limited by the center frequency and bandwidth of the transducer \cite{29}. Research has revealed that the frequency components of PA signals are related to the sizes and contents of the absorbers \cite{30,31}. PA frequency analysis has been widely applied in examining
fatty livers [32], bones [33], and blood cells [34]. Moreover, frequency analysis has proved feasible in detecting absorbers with sizes smaller than the system resolution [31,35], which is extremely advantageous for analyzing microvascular structures. To extract the main characteristics of PA signals in the frequency domain, the power spectrum of PA signals is calculated. Then the spectral parameter, namely the slope, can be obtained via linear regression fitting of the normalized power spectrum, representing the microstructure of biological tissue [33,35,36].

2.1. 2D numerical simulation model

To evaluate the feasibility of the PA-PAS method, we first implemented numerical simulations on models with a single microvessel in biological tissue. The PA signal from each model was simulated using the MATLAB k-wave toolbox (R2019b, MathWorks, Natick, MA) [37]. We extracted the typical vascular structural characteristics that affect the PA power spectrum for the simulation and simplified the model to a two-dimensional entity without loss of physical meaning. To simulate different sizes of blood vessel in biological tissue, the simulated microvessel were scaled to the actual microvascular size. The widths of the simulated microvessels were scaled to 100, 200, and 300 μm, respectively. The length of the simulated microvessel was scaled to 4 mm. According to the simulation setting in k-wave, the white pixels in Fig. 2 (a) represent the microvessel and were designated as PA sources with the initial acoustic pressure of 1, while the black ones were designated as the other biological tissues without initial acoustic pressure. The speed of sound was set at 1500 m/s for the simulated area, and a 72-point annular acoustic sensor array around the model with an angle interval of 5° was used to receive the PA signals, as shown in Fig. 2(a). The distance of the acoustic sensors to the center of the simulated model was set to 1 cm.

2.2. Gelatin phantom materials

The phantoms were prepared from porcine gelatin (Gelatin, Sangon Biotech, Shanghai, China) using a mixture of 8 % gelatin and 92 % water; the acoustic impedance of the phantoms is identical to that of biological tissue. The phantoms were disk-shaped with radii and thicknesses of approximately 1 and 0.8 cm, respectively. Fishing lines were used to simulate blood vessels in the experiment. The fishing lines were cut into 5-mm segments and embedded into each phantom to simulate the single microvessel in biological tissue.

2.3. Chick embryo preparation

The feasibility of the proposed PA-PAS method for evaluating microvessels in vivo was verified using a chick embryo model, which is considered an important model for studying tumor angiogenesis [38,39]. The fertilized chicken eggs were incubated for 3–5 d and the blood vessel diameters were approximately 100–300 μm. Then, the embryos were removed from the eggshells and placed on a petri dish with plastic membrane. In order to simulate the in vivo detection of animal models, petri dishes were kept as the acoustic scattering interface to obtain PA signals with low signal-to-noise ratio (SNR). There were two groups of samples, Group-1 (n = 3) and Group-2 (n = 3). The mean sizes of the blood vessels in the two groups measured microscopically (KH-7700, Hirox, Shanghai, China) were 200 and 250 μm, respectively. Fig. 1(b) presents the microscopic images and measured sizes of the blood vessels.

2.4. System setup and PA measurements

Fig. 2(b) presents the schematic of the experimental setup. An optical parametric oscillator system pumped by an Nd:YAG laser (PhocMo Mobile, OPOTEK, Carlsbad, CA) was used to provide laser pulses with a repetition rate of 10 Hz and a pulse width of 5.5 ns. At a 750-nm wavelength, the samples were illuminated by a laser beam of 1-cm diameter, which generated PA signals. These signals were received by a needle hydrophone (HNC1500, ONDA Corp., Sunnyvale, CA) with a bandwidth of 1–10 MHz, but it could cover frequencies up to 20 MHz. The energy incident on the sample was 10 mJ, which was below the safety limit specified by the American National Standards Institute. Driven by a computer-controlled stepper motor (TBR 100, Zolix, Beijing, China), the hydrophone was used to scan the samples in a circular motion with a step size of 5°; the total number of steps was I = 72. An ultrasonic coupling agent was used as the acoustic transmission medium between the samples and hydrophone. After 25-dB amplification by an amplifier (5072PR, Olympus Corp., Tokyo, Japan), the PA signals were recorded using a digital oscilloscope (HDO6000, oscilloscope, Teledyne Leckroy, USA) at a sampling rate of 2500 MHz.

2.5. PA-PAS analysis

The simulation and experimental data were processed using MATLAB. To extract the main characteristics of the PA signals in the frequency domain, the power spectra of the PA signals were computed using the Welch’s approach with a 5-μs moving Hamming window and 60 % overlapping. Then the power spectra of the PA signals were unfolded along the radius direction and the spectra acquired at all the angles were combined along the angle axis to form a PA-PAS map, as shown in Fig. 2(c). The radius axis gives the ultrasonic frequency distribution and represents the structural size of the microvessel; the angle axis represents the detection angles, showing the structural growth direction of the microvessel. Owing to the differences in the structural growth direction and size of the microvessels, each microvessel should possess a unique PA-PAS map.

In order to further quantitatively analyze the structural size of microvessel, the spectral parameter PWMF was calculated through the power spectrum of the PA signal as follows:

$$PWMF = \frac{\int f (P(f)) df}{\int P(f) df}.$$  

where $P(f)$ is the power spectral density at each frequency. PWMF represents the main frequency component of the microvessel, reflecting its main size in the detection area. For determining the acoustic power spectra of the PA signals, the lowest spectral range of ≤1 MHz was discarded because of the poor frequency response of the hydrophone below 1 MHz. Therefore, the power spectra were analyzed in the range of 1–10 MHz, covering a sound level of approximately 25 dB.
3. Results

3.1. Simulation results of a single microvessel

Fig. 3(a) shows the waveforms of the PA signals recorded at $i = 0^\circ$ and $90^\circ$. Then, the power spectrum of the PA signal was analyzed, as shown in Fig. 3(b). Fig. 3(a) and (b) show that the tubular structure of the blood vessel emits anisotropic PA signals with different intensities and frequency components at different angles. Therefore, spectral analysis of PA signals from a single angle cannot accurately analyze the physical characteristics of microvessels. This frequency anisotropy caused by the vascular structure should be considered in PA spectral analysis.

The PA-PAS method mapped the acoustic power spectra of the PA signals along the azimuthal direction. Fig. 4 (a)-(c) show the PA-PAS maps of single microvessels of three different sizes, whose diameters are 100, 200, and 300 μm. The ultrasonic frequencies are shown along the radius axis, the detection angles are shown along the angle axis, and the amplitudes of the power spectra shown in pseudo-color. As observed in the PA-PAS maps, there are obvious PA signals along the width direction of microvessels, and the spectral amplitude along the width direction is considerably greater than that in the length direction of the microvessels. In addition, the 100-μm-diameter microvessel comprises more high-frequency components than the 200- and 300-μm-diameter microvessels. With more high-frequency components, the power spectrum of the microvessel appears more extended along the radius axis in the width direction of the microvessel. Further, to differentiate the microvascular sizes, the PWMF was calculated. As shown in Fig. 4(d), the PWMF increases from the length direction (0°) to the width direction (90°) of the microvessel. Compared to the microvessels with diameters of 200 and 300 μm, the one with a diameter of 100 μm shows higher PWMF values in the width direction; however, no difference is observed...
in the length direction. The simulation results show that the PA power spectra of the microvessels exhibit significant anisotropy at different angles. The PA signal in the width direction of the microvessel has a higher amplitude and more high-frequency components than that in the length direction. The PWMF values of the microvessels with different sizes differ significantly in the width direction of the microvessels but not in the length direction. Therefore, the power spectrum along the width direction of the microvessel can better characterize the microvascular size. To quantify the size of the microvessel more accurately, the detection direction of the transducer should be along the width direction of the microvessel. Then, the PWMF values in the width direction of the microvessels were extracted to characterize the microvascular sizes, as shown in Fig. 4(e). The PWMF value is linearly related to the vascular diameter, as indicated by the green dashed line. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

3.2. Microvessel phantom experimental results

Fig. 5(a)-(c) are the plots of the PA signals generated by the microvessels with diameters of 100, 200, and 300 μm, respectively. There is no difference between the time-domain signals from the three differently sized microvessels. However, Fig. 5(d) shows that there are significant differences in the PA power spectra of the different microvessels. The acoustic power spectrum of the 100-μm-diameter microvessel comprises more high-frequency components than the acoustic power spectra of the 200- and 300-μm-diameter microvessels. The PA power spectra at all angles are graphically displayed through PA-PAS maps in Fig. 6(d)-(f). For reference, the images obtained through PACT with the delay-and-sum reconstruction algorithm [21,22] are illustrated in Fig. 5(a)-(c). As observed, the PACT images present several ring artifacts and the PACT images of microvessel with different sizes are very similar; therefore, it is impossible to visually distinguish...
the differences in microvascular sizes. However, such differences can clearly be observed when using the PA-PAS method. The 100-μm vessel [Fig. 6(d)] shows more high-frequency components than the 200-μm [Fig. 6(e)] and 300-μm [Fig. 6(f)] vessels. Compared with the PACT method, the PA-PAS method can better demonstrate the size differences among the microvessels. The PA-PAS maps show that the PA power spectra of the microvessels have significant anisotropy at different angles. There are obvious PA signals in the width direction of the microvessels and the PA spectral amplitudes are larger than that in length direction. The PWMF values were extracted from the power spectra of the PA signals for further quantitative analysis. Fig. 6(g) plots the PWMF-angle curve. The PWMF values in the width direction are higher than those in the length direction. In addition, the smaller the microvessel size, the higher the PWMF value. The experimental results verified the correctness of the simulation results, showing that the information on the microvessels is mainly concentrated in the width direction of the microvessels. The ultrasonic frequencies in the width direction can better reflect the structural sizes of the microvessels. The angular direction corresponding to the spectral amplitude peak is considered the width direction of the microvessels. The PWMF values for signals within 30° of the peak spectral amplitude were calculated to further analyze the microvascular size quantitatively. As shown in Fig. 6(h), the experimental results are in good agreement with the simulation results showing that the PWMF value tends to decrease linearly with the microvessel size, as indicated by the green dashed line. There are statistical differences between the PWMF values obtained for different microvessel sizes. To obtain the accurate sizes of microvessels in deep tissues using the PA-PAS method, curve fitting was performed using the PWMF values. The quantitative relationship between the PWMF values and microvascular sizes was established by linear fitting of the PWMF values obtained for blood vessel sizes of 200 and 300 μm. Thereafter, the PWMF value of the 100-μm-diameter microvessel, which is difficult to detect in deep tissues, was predicted using this curve. The predicted mean PWMF for the 100-μm microvessel was 3.0 MHz and the experimentally determined mean PWMF was 3.1 MHz. Hence, the experimental results were in good agreement with the predicted values. These results indicate that the accurate size of the microvessel can be obtained using the quantitative relationship between the PWMF values and microvascular sizes.

![Fig. 5. Representative PA signals recorded by the detector in the width direction of microvessels with diameters of (a) 100, (b) 200, and (c) 300 μm. (d) Power spectra of the PA signals in (a)-(c). The PA power spectra were normalized using the maximum amplitude for directly comparing the power spectra obtained for different vascular sizes. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).](image)

3.3. In vivo experimental results

The PA-PAS maps of two detection areas in the two groups of chick embryos are shown in Fig. 7(a) and (b). The two detection areas display an apparent distinction. Compared to that of Group-2, the power spectrum of Group-1 appears more extended along the radius axis in the width direction of the microvessel, indicating that the diameters of the blood vessels in Group-1 are smaller than those in Group-2. The PWMF-angle curve is shown in Fig. 7(c). The PWMF value at the peak position of the PA spectral amplitude, namely in the width direction of the microvessel, was extracted to quantitatively characterize the sizes of the microvessels in the detected area, as shown in Fig. 7(d). The PWMF values obtained for Group-1 are higher than those obtained for Group-2, suggesting that the average sizes of the blood vessels in Group-1 are smaller than those in Group-2. The PA-PAS results are consistent with the microscopic observations [Fig.7(e)]. We also applied Welch’s t-test to the two groups of PWMFs. The statistical results show significant differences. The proposed PA-PAS method is capable of quantifying structural changes in microvessels having sizes smaller than the system resolution.

4. Discussion

Frequency analysis is widely used in microvascular detection because of its ability to evaluate sizes smaller than the system resolution. However, owing to the tubular structures and dendritic distributions of blood vessels, the generated PA signals are inhomogeneous and exhibit anisotropy, leading to significant differences at different receiving angles. Previous studies on the power spectra of blood vessels were mainly based on comprehensive analyses of the detection regions [40,41], ignoring the power spectral differences at different angles. Hence, in this study, we developed a new method that combines the power spectra with the angles to quantify the structural changes in microvessels more accurately. The PA-PAS method maps the acoustic power spectra of the PA signals along the azimuthal direction; this reveals the physical characteristics of the microvessels, including their structural directions and sizes. Furthermore, a new spectral parameter, namely PWMF, was extracted using the power spectra of the PA signals. The amplitude of the frequency components in the power spectrum was used as the weighted coefficient; then, the main frequency of the detection area, namely the PWMF can be obtained by weighting the frequency within a predetermined interval. As the microvascular sizes in the detection area are small, there are more high-frequency components in the PA power spectra. Thus, the high-frequency weighting coefficient increases, leading to a high PWMF value. The PWMF value decreases as the
microvascular size increase. The traditional spectral parameter, namely the slope [42,43], represents the energy proportions of the high- and low-frequency components in the power spectrum, whereas the PWMF represents the main frequency component of the microvessels, reflecting the main size of the microvessels in the detection area.

The classical PACT scheme has significant advantages in determining the distribution of microvessels, but the size differences among the microvessels cannot be observed in the PACT images. In contrast, the size differences can be visually observed in the PA-PAS maps. When the vascular sizes are small, the power spectra appear more extended along the radius axis in the PA-PAS maps. Compared with the PACT method, the PA-PAS method can better distinguish microvascular sizes. Moreover, the proposed PA-PAS method has the ability of resisting low SNR than the traditional PACT method, which is very helpful for in vivo evaluation. The poor SNR in vivo detection makes it impossible to obtain clear microvascular distribution images. However, using the PA-PAS method, we can still extract the acoustic power spectrum information that can reflect the microvascular structural size at a relatively low SNR. Although, the high-frequency generated by noise increased the PWMF value in the length direction of the microvessel, resulting in the widened peak of the PWMF curve. However, the PA signal amplitude in the width direction was much larger than that in the length direction of the microvessel, which was not affected by noise; thus, the structural direction of microvessel could be identified based on the position of the maximum PA amplitude. Then the PWMF values along the structural direction could be extracted to quantify the microvascular structural sizes.

The simulation and experimental results discussed above demonstrate the feasibility of quantifying the microvascular sizes using the PA-PAS method. More experiments will be conducted to improve the robustness of the system. Additional data will be used to derive the quantitative relationship between the PWMFs and vascular sizes by linear fitting of the PWMF values obtained at different vascular diameters. Based on the fitted curve, the accurate sizes of the microvessels can be obtained. The detection areas in this study contained vessels with relatively simple dendritic distributions and no complex vascular network was involved. In future, the PA-PAS method will be used to characterize microvascular networks with multiple structural directions. In addition, the method will also be used to evaluate other physical properties of the microvessels, such as elasticity.

Fig. 6. Comparison between (a–c) PACT and (d–f) PA-PAS images. (g) PWMF–angle curves of all samples (the solid yellow boxes correspond to the width direction of the microvessels). (h) PWMF values obtained for different microvascular sizes along their width direction. The PWMF values decrease linearly with the increase in the vascular diameter, as indicated by the green dashed line. (*** p < 0.001, ** p < 0.01). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).
5. Conclusion

Considering the tubular structures and dendritic distributions of blood vessels, the PA-PAS method can characterize the microvascular structural sizes comprehensively and accurately by combining the power spectra with the detection angles. The PWMF is related to the size of the microvessels and can be used for assessing the structural changes in the microvessels that are smaller than the system resolution. The results show that the PA signal information of the microvessels is concentrated in the width direction of the microvessels. The ultrasonic frequencies in the width direction can better reflect the structural sizes of the microvessels. In addition, the PWMF values decrease linearly with the microvascular sizes. The experimental and simulation results agree well, demonstrating that the proposed approach can effectively differentiate the sizes of the microvessels.

Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

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