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Photoacoustic tomography of rat brain *in vivo* using multibandwidth ultrasonic detection

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ABSTRACT

Photoacoustic tomography employs short laser pulses to generate acoustic waves. The photoacoustic image of a test sample can be reconstructed using the detected acoustic signals. The reconstructed image is characterized by the convolution of the sample structure in optical absorption, the laser pulse, and the impulse response of the ultrasonic transducer used for detection. Although laser-induced ultrasonic waves cover a wide spectral range, a single transducer can receive only part of the spectrum because of its limited bandwidth. To systematically analyze this problem, we constructed a photoacoustic tomographic system that uses multiple ultrasonic transducers, each at a different central frequency, to simultaneously receive the induced acoustic waves. The photoacoustic images associated with the different transducers were compared and analyzed. The system was used to detect the vascular system of the rat brain. The vascular vessels in the brain cortex were revealed by all of the transducers, but the image resolutions differed. The higher frequency detectors with wider bandwidths provided better image resolution.

Keywords: photoacoustic tomography, medical imaging, biological tissue, brain imaging, spatial resolution, reconstruction.

1. INTRODUCTION

Photoacoustic tomography is a nonionizing imaging modality for visualizing biological tissues with optical contrast and ultrasonic resolution. The induced acoustic signals from the biological tissues exhibit the heterogeneity of the optical absorption in the samples and reveal the structure of tissues. For some specified wavelength of light, the absorption coefficient of blood can be 10 times higher than that of its surrounding tissues.¹ Because fast developing tumors consume more blood, most malignant tumors have higher optical absorption. For example, the absorption contrast between a breast tumor and normal breast tissue can be as high as 300% at the 1064-nm wavelength.² Another example is tumors of the nervous system, which in general are well vascularized, especially the most malignant ones such as glioblastoma multiforme (GBM). GBM is characterized by an increased vascular proliferation, cellular heterogeneity and necrosis. These characteristics reflect a rich and distorted vasculature within the tumor. Intra-tumoral arteriovenous shunting and early filling of draining cerebral veins are commonly seen due to tumor-related neovascularization.³

Photoacoustic imaging is based on ultrasonic propagation and detection, which means that it has the same good spatial resolution as pure ultrasound imaging. The geometry of the region over which the ultrasonic transducer is scanned determines the imaging mode and the reconstruction algorithms. For the detection of layered structures of tissues or tumors under human epidermis, the ultrasonic transducer must be placed on the skin along the laser beam to accomplish detection *in vivo*. Karabutov and Oraevsky *et al.*^{4,5} presented photoacoustic imaging with axial resolution up to 15 μm . The imaging of small deeply embedded tumors, such as breast tumors, was also studied by Esenaliev *et al.*⁶ and Oraevsky *et al.*^{2,7} Hoelen *et al.* employed a transducer array on a plane to detect induced signals from vascular systems.⁸ The image was reconstructed using a delay-and-sum algorithm, where the depth resolution was better than 20 μm , and the lateral resolution was better than 200 μm . The detector can also scan over a circular track and the reconstruction algorithms have been developed to use the signals from the scan to form an image. Examples of current reconstruction algorithms include the weighted delay-and-sum method,⁷ the optimal statistical approach,⁹ and the Radon

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transform in the far-field approximation.¹⁰ Only recently have exact reconstructions been studied theoretically and reconstruction algorithms for various regular geometries derived.^{12–15}

The generation, propagation, and detection of induced acoustic signals can be described by thermal expansion, wave equations and bandwidth filtering, respectively.¹⁶ By directing the laser pulse on a test sample, the absorbed optical energy in tissue is transformed into thermal energy, which is then, due to thermo-elastic expansion, converted into mechanical stress. If the energy deposition occurs rapidly in a time period that is much less than the thermal relaxation time and the stress relaxation time, the local pressure rise after heating can be derived as $\Delta p = \frac{\beta c^2}{C_p} E_a$, where Δp is the

pressure rise; β is the isobaric volume expansion coefficient; c is the speed of sound; C_p is the specific heat; and E_a is the absorbed optical energy density. The behavior of photoacoustic waves has been studied by Diebold and Gusev *et al.* based on the following non-homogenous wave equation:^{17,18}

$$\nabla^2 p(\mathbf{r}, t) - \frac{1}{c^2} \frac{\partial^2 p(\mathbf{r}, t)}{\partial t^2} = -\frac{\beta}{C_p} \frac{\partial H(\mathbf{r}, t)}{\partial t}, \quad (1)$$

where $p(\mathbf{r}, t)$ is the acoustic pressure at time t and position $\mathbf{r}$ and $H(\mathbf{r}, t)$ is the heat function of the optical energy deposited in the tissues per unit volume per unit time, which can be expressed as

$$H(\mathbf{r}, t) = A(\mathbf{r})I(t), \quad (2)$$

where $A(\mathbf{r})$ describes the optical energy deposition within the tissues at position $\mathbf{r}$ (structure of tissues); and $I(t)$ describes the shape of the irradiation pulse, which can be further expressed as $I(t) = \delta(t)$ for impulse heating.

The purpose of photoacoustic tomography is to reconstruct the distribution of the optical absorption $A(\mathbf{r})$ in the tissues from a set of measured acoustic signals $p(\mathbf{r}, t)$. For a cylindrical scanning configuration, the exact inverse solution can be derived. In most practical image reconstructions, the algorithm can be simplified to shorten the reconstruction time. If the detection radius r_0 is much longer than the wavelength that corresponds to the central frequency of the detection device (far-field detection), we can assume $|k|r_0 \gg 1$ and simplify the exact inverse solution to the form of¹⁹

$$A(\mathbf{r}) = -\frac{\beta}{2\pi c^4 C_p} \iint_{S_0} dS_0 (\cos \Theta) \frac{1}{t} \frac{\partial p(\mathbf{r}_0, t)}{\partial t} \bigg|_{t=|\mathbf{r}_0-\mathbf{r}|/c}. \quad (3)$$

Fig. 1. Simulated photoacoustic spectra of spherical sources of different diameters

where S_0 is the surface over which the detectors are scanned; Θ is the angle between the normal line of the detector and the vector from the detector to the reconstruction point.

The laser-induced thermal expansion of an absorptive structure in tissue creates acoustic waves by a thermoelastic mechanism. Accordingly, the photoacoustic signal is characterized by the convolution of the tissue structures in the optical absorption and the pumping profile. Applying the Fourier Transform to the temporal photoacoustic signal gives the photoacoustic spectrum. So, the spectrum depends on the profile of the laser pulse and the geometrical shape and size of the object. For example, if the laser pulse is in Gaussian profile with

width of 6.5 ns, if we assume the two optical absorptive objects are in the spherical shape, then one has a diameter of 200 μm , the other of 60 μm . The simulated spectra of these two photoacoustic sources are shown in figure 1. The spectrum of the smaller sphere is broader with more high frequency components.

Generally, the induced acoustic waves range widely within the frequency spectrum. However, a single ultrasonic transducer can only respond to part of the spectrum because of its limited bandwidth.²⁰ Most transducers with a higher central frequency detect acoustic signals with a wider bandwidth, but the signal is weaker. Sometimes choosing the ultrasonic detector is a dilemma since a trade-off between imaging resolution (bandwidth) and sensitivity may be necessary. In this paper, we demonstrate a photoacoustic imaging experiment that simultaneously uses four transducers with the 2.25-MHz, 3.5-MHz, 10-MHz and 20-MHz central frequencies, respectively. Images associated with the four transducers are compared and analyzed.

2. EXPERIMENTAL SETUP

The experimental setup for the photoacoustic tomographic system employing multiple ultrasonic transducers is shown in Fig. 1. A Q-switched pulsed Nd:YAG laser (Brilliant B, BigSky), operating at the second harmonic wave with a wavelength of 532 nm, is used as the pumping source. The laser pulse width is 6.5 ns and the pulse repetition rate is 10 Hz. The laser beam is expanded by a concave lens and homogenized by a ground glass and then delivered onto the test sample. The pulse energy density is controlled at $\sim 20 \text{ mJ/cm}^2$, which is the maximum permissible exposure (MPE) for human skin to a laser beam at a wavelength of 532 nm.²¹

Fig. 2. Experimental setup of photoacoustic tomography employing multiple ultrasonic transducers, each at a different central frequency.

Four ultrasonic transducers (V323/2.25MHz, V383/3.5MHz, XMS-310/10 MHz, and V316-N/20MHz, Panametrics) are used as detectors to receive the induced acoustic signals. The diameters of the active areas are 6 mm for the 2.25MHz, 10 mm for the 3.5 MHz transducer, 2 mm for the 10 MHz transducer and 3 mm for the 20 MHz transducer, respectively. The transducers are located in the same horizontal plane and their distances from the scanning center (detection radius) are 38.3 mm for the 2.25MHz, 39.0 mm for the 3.5 MHz transducer, 41.7 mm for the 10 MHz transducer and 28.8 mm for the 20 MHz transducer, respectively. These commercial transducers have nominal bandwidths of 50% to 80% of their central frequencies.

A two-dimensional translation of the detectors is designed; one involves circular scanning in the horizontal plane, and the other involves linear scanning along the vertical direction. A rotational apparatus supported by two bearings is driven by a step motor to implement the circular scanning. The entire horizontal scanning section is made as one piece and then connected to an uprightly placed translation stage. Hence, the section of the circular scan can be impelled vertically. The transducers are attached to the rotational apparatus to complete circular scanning around the test sample.

By combining the two movements, the transducers can scan in a cylindrical contour around the test sample.²² In this experiment, we are interested in the vascular system of the rat cortex so the vertical translation is used to adjust the transducers situated in the plane that best detects the vessels in the rat brain. Typical scanning parameters in our experiment are 240 steps for a full view of 2π circular scanning ($1.5^\circ/\text{step}$).

The transducers convert the acoustic energy into electrical signals that are amplified by amplifiers (ZFL-500LN, Mini-Circuits). An oscilloscope with four channels (TDS-540A, Tektronix) is employed to monitor and record the acoustic signals. A LabView program controls the scanning of the transducers and the data acquisition according to the following sequence: first, start the step motor to move the transducers to the arranged position; secondly, fire the laser and trigger the data acquisition; third, average the time-dependant signals on the oscilloscope to improve the SNR; finally, save the time-dependent data at this scanning stop. After that, the transducers are moved to the next scanning stop and the above procedure is repeated.

For coupling the induced acoustic signals, the transducers are immersed in water in a container. The rat (Sprague Dawley, 50 ~ 100 g) is first anaesthetized by the IP injection of Ketamine and Xylazine. Then, the hair on the rat head is removed gently with hair remover lotion to improve light penetration and decrease the photoacoustic interference generated by the hair. The head of the rat that is to be imaged is popped into the water tank, but isolated from the water by a thin plastic film. We build a rat mount and breathing mask to hold the rat firmly in the correct position and provide it with oxygen.

3. RESULTS AND DISCUSSION

The in-vivo photoacoustic images of the rat brain obtained by the four ultrasonic transducers are shown in Figs. 3(a)–(d), respectively. The images are compared with an open-scalp photograph of the rat brain shown in Fig. 3(e) that was taken after the photoacoustic imaging. The vessels in the upper cerebral cortex provide the primary photoacoustic source because the laser light is directed to the top of the rat head and the blood in the vessels has a high optical absorption coefficient at this wavelength. The skull and scalp, as well as the other brain tissues around the upper cerebral vessels, have much smaller optical absorption coefficients. Light attenuation further reduces the absorbed optical energy density below the cortex. On the detection side, the photoacoustic sources in the imaging plane are preferentially detected. The diameters of the ultrasonic transducers are greater than their detected ultrasonic wavelengths and cause receiving directionalities; as a result, the effective zone of detection in the imaging area is ~4 mm along the vertical axis. The combined effects of the optical and ultrasonic selectivities produce clear photoacoustic images of the upper cerebral blood vessels.

By comparing the photoacoustic images with the anatomical photograph, some image properties that correlate with the detection spectra become evident. First, the main vessels on the cortex are disclosed by all four photoacoustic images. The photoacoustic signal covers a broad spectrum and the signal with a limited bandwidth does succeed in recording some of the characteristics of the object. Secondly, the detected signal from the lower-frequency transducer is stronger, so the SNR of the image is better; but its resolution is poorer. In the images obtained by the 2.25-MHz and 3.5-MHz transducer, every trait shows a feature in the rat brain although the detailed structure is blurred. Thirdly, higher-frequency detectors provide better image resolution as expected. For an identical vessel, the images obtained by the 2.25-MHz and 3.5-MHz transducers are thicker than those obtained by the two higher-frequency transducers. The tiny vessels in the vascular system and some vascular ramifications spreading from the main vessels are perceptible in the images obtained by the 10-MHz and 20-MHz transducers but can hardly be seen in the images obtained by the 2.25-MHz and 3.5-MHz transducers. The subtle vessels are totally blurred in the images obtained by the lower frequency transducers. The image of the vessels obtained by the 20-MHz transducer is not finer than that from the 10-MHz transducer. That can be explained by the detection distance and the aperture effects on the resolution in the following context. Another possible explanation is that the higher frequency components of the photoacoustic signal experience more attenuation when the signal propagates through the tissue to reach the detector.

Fig. 3. Photoacoustic images of a rat brain acquired with (a) 2.25-MHz transducer, (b) the 3.5-MHz transducer, (c) the 10-MHz transducer, and (d) the 20-MHz transducer. (e) Top-view photograph of the rat brain with the scalp stripped after the photoacoustic imaging. The dark area in the image represents a stronger photoacoustic signal.

The resolution of photoacoustic imaging is primarily limited by the bandwidth of the detector and the laser pulse duration. Theoretically, the structure of tissues with optical absorption contrast can in most cases be either exactly reconstructed or approximately reconstructed by Eq. (3). The pressure engaged in the equation is supposed to cover the whole spectrum; and, indeed, the induced photoacoustic signal is rather wide although only part of its spectra can be detected by a single transducer. Using signals from a limited bandwidth detector causes the reconstructed image to blur. In addition, the reconstructed structure of tissues can also be blurred by the duration of laser pulse, which is assumed to

be a delta stimulation in the derivation of the reconstruction Eq. (3). Accordingly, the reconstructed image is considered to be the convolution of the structure of tissues in optical absorption, the profile of the pumping pulse, and the impulse response of the detector:

$$I(r) = S(r) * L(t \cdot c) * D(t \cdot c), \quad (4)$$

where, convolution is denoted by $*$; $I(r)$ characterizes the reconstructed image; $S(r)$ represents the optical structure of the tissues; $L(t \cdot c)$ and $D(t \cdot c)$ are the laser pulse and transducer impulse responses converted to the spatial domain. The bell-shaped laser pulse is 6.5 ns wide in our experiments and blurs the structure of tissues by $\sim 10 \mu\text{m}$.

Detection radius and detector aperture are other two factors affecting image resolution. Except when the detector is infinitesimal in size, the detection radius and the detector aperture are two factors influencing the impulse response of the detector. In engineering, if an aperture is less than the detected ultrasonic wavelength, the detector approaches being a point detector and thus becomes omnidirectional. However, the detector's sensitivity can be augmented by increasing its active areas. The active sizes of the transducers in our experiment are not negligible and, consequently, cannot be treated as point detectors; therefore, the impulse responses depend on the position of the photoacoustic source. A heuristic understanding is that the distance between an ultrasonic source in space and a point on the ultrasonic detector with a certain aperture varies with the location of this point on the surface of the detector. This variation causes a variation in the phase of the detected photoacoustic signals which broadens the impulse response. When the transducer scans over the circle, the impulse response remains the same only for the center of the circle. When the ultrasonic source is off center, the impulse response varies as the transducer is scanned. The farther the photoacoustic source is away from the center of the circular scan, the wider the extension of the impulse response of the detector. As a result, the image resolution deteriorates with an increase in the distance of the source from the center of a circular scan. Xu and Wang deduced this aperture effect mathematically.²³ In general, the lateral blurring effect by the aperture can be approximately described by $R_C(r) \approx (r/r_0)\delta$, where r is the distance between the point source and the center of the scan circle; r_0 is the detection radius; and δ is the diameter of the detector aperture. This aperture blurring effect is obvious in Figs. 3(a)–(d), where the vessels are clear in the central area of the image and then become blurred away from the center. In addition, based on the measurement geometry of our experiments, the ratio δ/r_0 for the 20-MHz transducer is 3 mm/28.8 mm=0.104, while the ratio for the 10-MHz transducer is 2 mm/41.7 mm=0.048. Therefore, the lateral blurring effect of the 20-MHz transducer is nearly twice that of the 10-MHz transducer. In the imaging region far from the scan center, the lateral resolution of the image obtained by the 20-MHz transducer is much worse than that of the image obtained by the 10-MHz transducer.

4. CONCLUSION

Employing ultrasonic transducers of various bandwidths, two-dimensional photoacoustic tomography successfully reveals the vascular structures of the cortex in the rat brain with various traits. We demonstrated that higher-frequency ultrasonic transducers give higher resolution images, but for the configuration of a circular scan, the image resolution deteriorates with an increase in the distance of the imaged position from the center. In clinical imaging applications, malignant tumors and other tissues have complicated shapes and vary in size. Consequently, the acoustic signals have wide spectra making it difficult to set the detection bandwidth optimally in advance. Therefore, the use of multiple transducers, each differing in central frequency, is a superior method for capturing the images.

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