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Photoacoustic Tomography and Molecular Fluorescence Imaging: Dual Modality Imaging of Small Animal Brains In Vivo

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ABSTRACT

We present a dual modality imaging technique by combining photoacoustic tomography (PAT) and near-infrared (NIR) fluorescence imaging for the study of animal model tumors. PAT provides high-resolution structural images of tumor angiogenesis, and fluorescence imaging offers high sensitivity to molecular probes for tumor detection. Coregistration of the PAT and fluorescence images was performed on nude mice with M21 human melanoma cell lines with αvβ3 integrin expression. An integrin αvβ3–targeted peptide–ICG conjugated NIR fluorescent contrast agent was used as the molecular probe for tumor detection. PAT was employed to noninvasively image the brain structures and the angiogenesis associated with tumors in nude mice. Coregistration of the PAT and fluorescence images was used in this study to visualize tumor location, angiogenesis, and brain structure simultaneously.

Keywords: Photoacoustic tomography, fluorescent imaging, molecular imaging, contrast agent

1. INTRODUCTION

The development of noninvasive diagnostic imaging techniques is an area of great clinical interest. Such imaging tools are critical for the study of tumor detection and tumor physiology, such as angiogenesis. Photoacoustic tomography (PAT) and near-infrared (NIR) fluorescence imaging are noninvasive techniques that can provide complementary structural information about angiogenesis as well as detect tumors. Photoacoustic tomography (PAT) is an emerging imaging modality that takes advantage of both high optical contrast and good ultrasound resolution.[1] PA signals are induced by pulsed light illumination. When light energy is absorbed by biological tissues, the resulting thermal expansion of the tissues generates ultrasonic waves. The ultrasonic waves can be detected by an ultrasonic transducer and then used to reconstruct the optical absorption distribution inside the tissues. PAT has been shown to be a promising tool for biomedical applications, such as the monitoring of oxygen saturation in blood vessels, epidermal melanin measurement, angiography, and breast tumor detection.[2] Fluorescence imaging has high sensitivity, and it can also be used to image a variety of molecular properties due to its versatile fluorescent probe design.[3, 4] Recently, Li and Ke et al. developed an integrin $\alpha v\beta_3$–targeted peptide conjugated with ICG as a fluorescent molecular imaging contrast agent for the noninvasive *in vivo* visualization of M21 human melanoma tumors with $\alpha v\beta_3$ integrin expression where the integrin $\alpha v\beta_3$ plays an important role in the tumor angiogenesis and metastsis.[5, 6] Fluorescence imaging shows strong potential for the diagnostic imaging of tumors.

Here, we present the results from the coregistration of PAT and molecular fluorescence imaging as obtained with an *in vivo* nude mice tumor model. *In vivo* experiments were performed to render this dual modality imaging method. The experimental setup is described below.

2. MATERIALS AND METHODS

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2.1 Photoacoustic tomography

The experimental setup for the *in vivo* PAT of a nude mouse is shown in Fig. 1(A). A tunable Ti:Sa nanosecond pulse laser (LT–2211A, Lotis T II, Minsk, Belarus) pumped by an Nd:YAG laser (LS–2137/2, Lotis T II, Minsk, Belarus) was employed to provide laser pulses with a pulse repetition rate of 10 Hz and a wavelength of 785 nm. The incident energy density of the laser beam on the surface of the mouse head was controlled to < 2 mJ/cm^2. A unfocused ultrasonic transducer (XMS–310, Panametrics) with an active element of 2 mm in diameter, a center frequency of 10.4 MHz, and a -6dB fractional bandwidth of 100% was used to detect the photoacoustic signals. A computer–controlled step motor drove the transducer to circularly scan the cortical surface of the mouse brain with a radius of 3.5 cm and a step size of 1.5^o. The data acquisition time for one image was ~15 minutes. The mouse was fixed by a homemade mount with its head protruding into the water tank through a hole in the bottom of the water tank. The hole was sealed with a piece of polyethylene membrane. The mouse head surface was covered with a thin layer of ultrasonic coupling gel. The detected photoacoustic signals were amplified and then digitized by an oscilloscope. The digitized signals were transferred to a computer, and the distribution of the optical absorption in the imaging plane (x-y plane) was reconstructed using a modified back-projection algorithm after a full view scanning.[7]

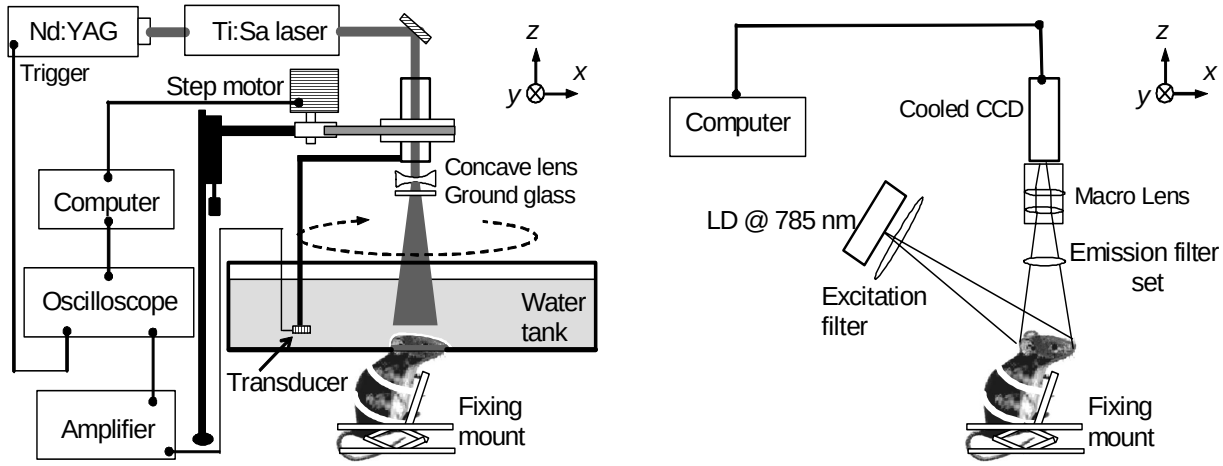

Figure 1. (A) Experimental setup of *in vivo* photoacoustic tomography of nude mice. (B) Experimental setup of *in vivo* fluorescence imaging of nude mice with peptide–ICG contrast agent.

2.2 Near-infrared flurescence imaging

Fig. 1(B) shows the *in vivo* fluorescence imaging setup for nude mice with a peptide-ICG contrast agent. The mouse head was illuminated with light from a laser diode (785 nm, 80mW) expanded to ~5 cm diameter circular area. A macro lens, coupled to a cooled CCD camera (BU401–BR, Andor Technology, CT), collected the emitted fluorescent light from the conjugated ICG molecules. The lens was fitted with a holographic notch–plus filter (785–nm center wavelength, Kaiser Optical Systems, Inc., Ann Arbor, MI) and a bandpass filter (830–nm center wavelength).

2.3 Animal Protocols

Nude mice weighing about 20 grams were used in our *in vivo* animal experiments. A dose of 87 mg/kg Ketamine plus Xylasine 13 mg/kg, administered intramuscularly, was used to anesthetize the mice during the experiments. M21 human melanoma tumor cells were inoculated subcutaneously on the head of the nude mice. When the tumor grew to a size of about 5 mm diameter, the peptide–ICG conjugated contrast agent was injected, with an estimated dosage of ~17 µg per

body weight, into the circulatory system of the mice via tail vein injection. The fluorescence imaging and PAT were conducted 24 hours after the ICG injection.

3. RESULTS

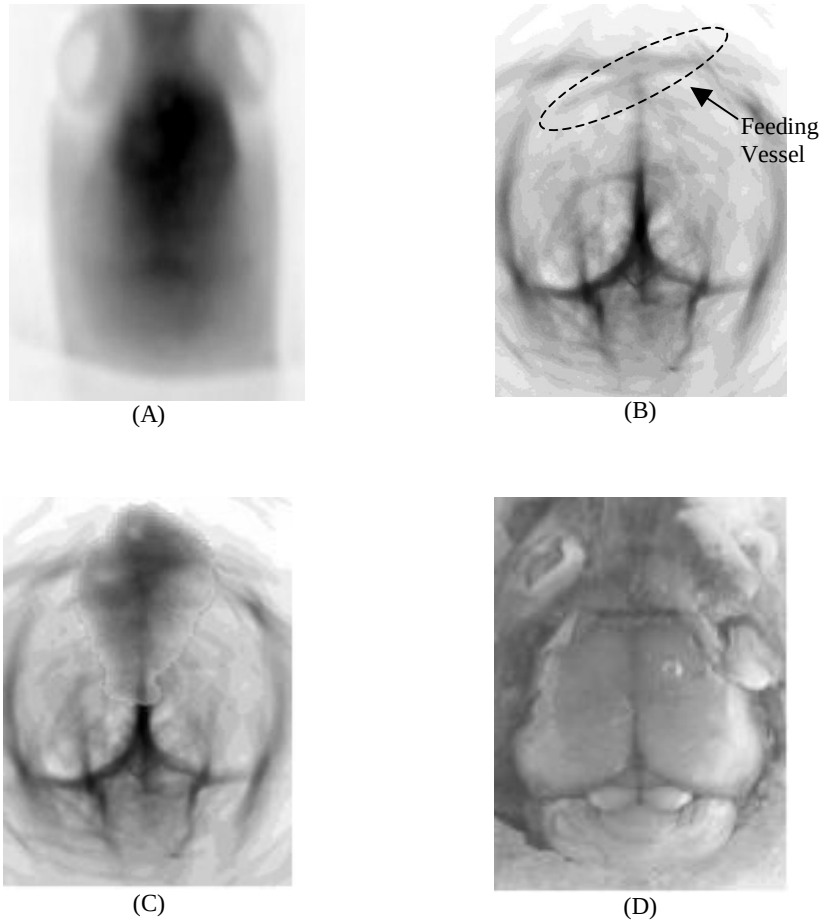

Figure 2. (A) Noninvasive *in vivo* fluorescence image acquired 24 hours after the ICG injection. (B) Noninvasive *in vivo* PAT image acquired 24 hours after the ICG injection with skin and skull intact. (C) Coregistrated noninvasive *in vivo* image of PAT and fluorescence imaging. (D) Open skin and skull photograph.

Fig. 2(A) shows the NIR fluorescent image of a nude mouse inoculated with a M21 human melanoma tumor and injected with an integrin $\alpha v\beta_3$–targeted peptide–ICG conjugated contrast agent. The signal intensity of the tumor region was significantly higher than that of background due to the uptake of the contrast agent by the $\alpha v\beta_3$ integrin receptor. This fluorescence image shows the location and shape of the tumor.

Fig. 2(B) is the PAT image. It clearly shows the brain vascular system of the nude mouse and the structure of the brain. In this experiment, the mouse was fixed on the same mount for both the PAT and fluorescence imaging, and the fluorescence images were acquired form the top of the mouse head in both cases. Hence, the coregistration between the PAT images and fluorescence images is feasible. The coregistered image of the PAT and fluorescence imaging is shown in Fig. 2(C). The fluorescence image was overlapped on top of the PAT image. Comparing Fig. 2(B) with the open skull photograph, Fig. 2(D), we find that the brain structure is well matched except for the dashed circled vessel in Fig. 2 (B), which is not shown on the surface of the brain. Based on the coregistrated image, the vessel marked in Fig. 2 (B) is one of the major feeding vessels of the tumor.

4. CONCLUSION

A dual modality imaging method combining PAT and molecular fluorescence imaging is successfully employed to image the angiogenesis of the brain containing a tumor and to detect the tumor. A tumor-targeted NIR fluorescent contrast agent is used for the tumor detection. By employing coregistration of PAT and fluorescence imaging, the tumor location, the tumor angiogenesis, and the brain structure of the nude mouse can be visualized at the same time. Future work will focus on molecular PAT imaging using spectral information derived from intrinsic and extrinsic molecular contrast.

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