Proceedings of the 2005 IEEE
Engineering in Medicine and Biology 27th Annual Conference
Shanghai, China, September 1-4, 2005

Combined Photoacoustic and Molecular Fluorescence Imaging In Vivo

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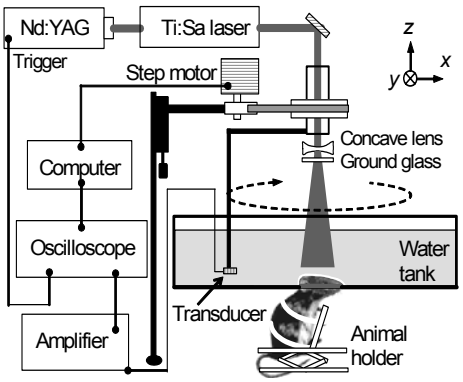
***Abstract*- Because of the overwhelming scattering of light in biological tissues, the spatial resolution and imaging depth of conventional fluorescent imaging is unsatisfactory. Therefore, we present a dual modality imaging technique by combining fluorescence imaging with high-resolution noninvasive photoacoustic tomography (PAT) for the study of an animal tumor model. 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 $\alpha_v\beta_3$ integrin expression. An integrin $\alpha_v\beta_3$–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 structure and the angiogenesis associated with tumors in mice. The coregistration between the PAT and fluorescence images was used to visualize tumor location, angiogenesis, and brain structure simultaneously.**

I. Introduction

Photoacoustic tomography (PAT) is an emerging imaging modality that takes advantage of both high optical contrast and good ultrasound resolution [1]. It is a nonionizing imaging modality based upon the different absorption rates at which different biological tissues absorb electromagnetic waves. In PAT, an electromagnetic wave in the visible or Near–infrared (NIR) region irradiates a biological sample to generate photoacoustic waves according to the thermoelastic mechanism. The acoustic waves can be detected by highly sensitive piezoelectric devices. With the detected photoacoustic signals, the distribution of electromagnetic absorption in the sample can be reconstructed. PAT has been shown to be a promising tool for biomedical applications, such as the monitoring of oxygenation in blood vessels, epidermal melanin measurement, angiography, and breast tumor detection [2].

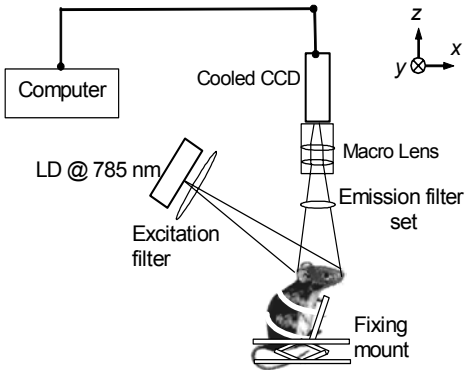
Fluorescence results from a three–stage (excitation, excited–state Lifetime, and fluorescence emission) process that occurs when certain molecules (generally polyaromatic hydrocarbons or heterocycles), called fluorophores, or fluorescent dyes, absorb light. The absorption of light by a population of these molecules raises their energy level to a brief excited electronic singlet state. As they decay (typically within 1–10 nanoseconds) from this excited state, they emit fluorescent light. Fluorescence imaging is highly sensitive, and it can 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 metastasis [5][6]. Fluorescence imaging shows strong potential for the diagnostic imaging of tumors.

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. PAT and near-infrared (NIR) fluorescence imaging are noninvasive techniques that can both provide complementary structural information about angiogenesis as well as detect tumors. Here, we present the results from the coregistration of PAT and molecular fluorescence imaging obtained with a mouse tumor model. *In vivo* experiments were performed to render this dual modality imaging method. The experimental setup is described below.



(A)

Fig. 1. (A) Experimental setup of *in vivo* photoacoustic tomography of a small animal head.



(B)

Fig. 1. (B) Experimental setup of *in vivo* fluorescence imaging of a small animal head with a peptide–ICG contrast agent.

II. Materials And Methods

A. Photoacoustic Tomography

The setup for non–invasive *in vivo* PAT of the nude mouse head is shown in Fig. 1(A). A tunable Ti:Sapphire 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 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 reduced by a concave lens and ground glass until it was less than 2 mJ/cm2, which is much less than the maximum value allowed by the ANSI standard. An 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°. The data acquisition time for one image was ~15 minutes. An electronic shutter system was employed to reduce the duration of the mouse head exposure to the pulse laser light. Since the mouse head was exposed to the laser light only during the data acquisition rather than during the whole scanning circle, the decay of the peptide–ICG conjugated contrast agent was reduced. In order to couple the photoacoustic signals, the transducer was immersed in a water tank. The mouse was fixed by a homemade mount with its head protruding into the water tank through a hole in the bottom of the 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].

The PAT was realized by a circular scan from a single element transducer. Images obtained by this system represent a distribution of averaged optical absorption during the period of data acquisition. In the future, when an ultrasonic transducer array is employed, we will utilize real–time monitoring and achieve more accurate PAT images.

B. Near-infrared Fluorescence Imaging

Fig. 1(B) shows the non–invasive *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 a ~5 cm diameter circular area. A macro lens, coupled with 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 notch–plus filter and a bandpass filter. Images were acquired and stored using the application that came with the CCD camera. Matlab (The MathWorks, Inc., Natick, MA) and Photoshop (Adobe Systems, Inc., San Jose, CA) were used to process and analyze the images.

C. Animal Protocols

Nude mice weighing about 20 grams were used in the *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 heads of the nude mice. When the tumors grew to a size of about 5 mm diameter, the peptide–ICG conjugated contrast agent was injected into the circulatory system of the mice via tail vein injection, leading to an estimated concentration of 10 μM in mouse blood. The fluorescence imaging and PAT were conducted 24 hours after the ICG injection.

III. Results

Fig. 2(A) shows the NIR fluorescent image of a nude mouse inoculated with an 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.

In the PAT image (Fig. 2 (B)), the nude mouse brain structure and vascular system were clearly displayed with a higher resolution. In the experiments, the mice were fixed on the same mount, and the fluorescent images were acquired form the top of the mice heads in a perpendicular direction (Fig. 1 (B)). Since the acquired PAT nude mouse

brain images also included the perpendicular direction, coregistration between the PAT images and fluorescent images became feasible.

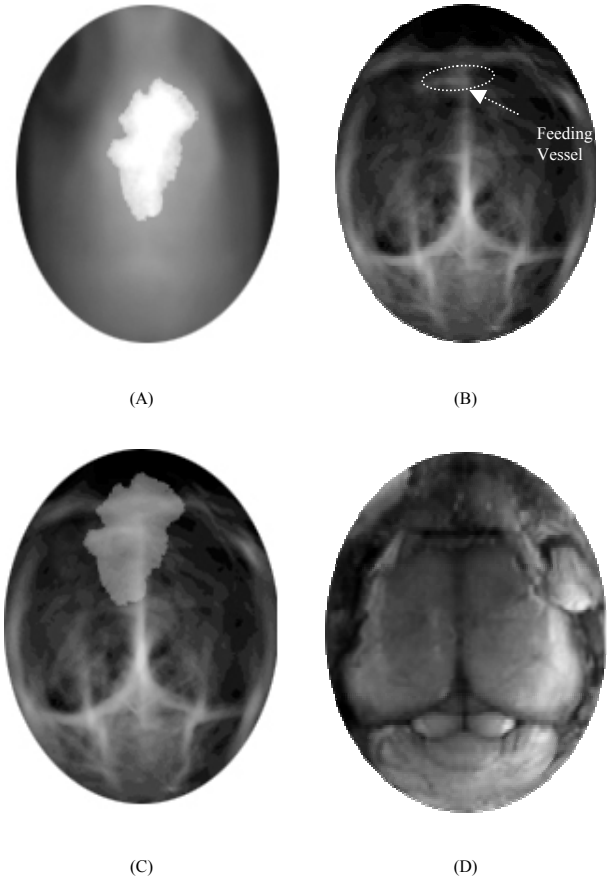


Fig. 2. (A) Noninvasive *in vivo* fluorescence image acquired 24 hours after the ICG injection, overlapped on a white-light image. (B) Noninvasive *in vivo* PAT image acquired 24 hours after the ICG injection with skin and skull intact. (C) Coregistered noninvasive *in vivo* image of PAT and fluorescence imaging. (D) Open skin and skull photograph.

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 coregistered image, the vessel marked in Fig. 2 (B) is one of the major feeding vessels of the tumor.

IV. Conclusion

A dual modality imaging method combining PAT and molecular fluorescence imaging is successfully employed to both 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 combining PAT and fluorescence imaging, the tumor location, the tumor angiogenesis, and the brain structure of the nude mouse can all be visualized at the same time.

This method offers important possibilities for combining different imaging modalities. In the future, we will study PAT molecular imaging using multiple optical wavelengths.

Acknowledgments

This study is sponsored in part by National Institutes of Health grants R01 EB000712 and R01 NS46214. Wang's email is LWang@tamu.edu.

References

[1] X. Wang, G. Ku, et al, "Non–invasive photoacoustic angiography of animal brains in vivo with NIR light and an optical contrast agent", Optics Letters, vol. 29 (7), pp. 730–732, 2004.

[2] X. Wang, Y. Xu, et al, "Photoacoustic tomography of biological tissues with high cross–section resolution: Reconstruction and experiment", Medical Physics, vol. 29 (12), pp. 2799–2805, 2002.

[3] Guilbault, *Practical Fluorescence*, 2nd ed., Marcel Dekker: New York, 1990.

[4] Lakowicz, J. R., *Principles of Fluorescence Spectroscopy*, 2nd ed., Plenum Publishing: New York, 1999.

[5] W. Wang, S. Ke, et al., "Near–Infrared optical imaging of integrin αvβ3 in human tumor xenografts", Molecular Imaging, vol. 3 (4), pp. 343–351, 2004.

[6] S. Ke, X. Wen, et al., " Near–Infrared optical imaging of epidermal growth factor receptor in breast cancer xenografts ", Cancer Research, vol. 63, pp. 7870–7875, 2003.

[7] M. Xu and L. V. Wang, "Time domain reconstruction for thermoacoustic tomography in a spherical geometry," IEEE T. Med. Imag., vol. 21, pp. 814-822, 2002.