Opto-ultrasound imaging in vivo in deep tissue

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Abstract. It is of keen importance of deep tissue imaging with high resolution in vivo. Here we present an opto-ultrasound imaging method which utilizes an ultrasound to confine the laser pulse in a very tiny spot as a guide star. The results show that the imaging depth is 2mm with a resolution of 10um. Meanwhile, the excitation power we used is less than 2mW, which indicates that our methods can be applied in vivo without optical toxicity and optical bleaching due to the excitation power.

1. Introduction

Microscopy imaging technology has been confirmed as the key to the “gate of micro-world” since Leeuwenhoek invented the first microscope in 1665 and no one would deny its contribution to myriads of fields up to now. However, since the biological tissue usually has strong scattering properties, it is difficult for the light to pass through and therefore it is difficult for the general microscope to “look” through and present out. To explore the secret inside brain, which is the most mysterious part in biological tissue, a section technology called MOST[1] is used to image every thin slice first and then reconstructed to 3D images. This method can get the structure information of the whole brain in vitro with high resolution of about 300nm. However, since the tissue is first sliced preparing for imaging, it cannot be use in vivo. Therefore, to explore the function of special biological tissue in living body, it is necessary to solve the problem of imaging in deep tissue. This request promotes another approach called two-photon fluorescence microscopy (TPM), to be widely used in biological science. The advantages of TPM lies on the long wavelength beam employed has much deeper penetration depth, meanwhile only the fluorescence on the focus can be excited. The general penetration depth with TPM can reach at 600um. To further increase the depth penetration, the most common strategy used is increasing the laser power exponentially to maintain adequate ballistic excitation power at relatively larger focal depths. However, this neglects the fact that femtosecond-pulsed laser are power limited. Moreover, increasing the excitation power can lead to saturation, photobleaching and photodamage.

Here we introduce an opto-ultrasound imaging system, which can expend the penetration depth to 2mm with a high resolution of 10um [2,3]. Meanwhile, the excitation power is restricted to 2mw, which is a safe energy and allows the applications in most biological tissue in vivo.

2. Theory and Methods

The opto-ultrasound imaging system combines single-cycle pulsed ultrasound modulation and digital optical phase conjugation.
Figure 1. Experiment set-up. BS, non-polarizing beamsplitter; BE, beam expander; M, mirror; BP, bandpass filter; LP, long-pass filter; L1, f=35 mm lens; L2, f=50 mm lens; D, fluorescence detector; stage, three-axis motorized translation stage.

The pixel size of both the SLM and the CMOS camera is 8um. Distance from sound focus to SLM=305 mm.

The system set-up is demonstrated in Fig. 1. We use single-cycle focused ultrasound pulses and tightly synchronized near-infrared laser pulses to achieve a near-isotropic three-dimensional confined interaction volume. The pulsed light and pulsed sound waves are precisely synchronized so that the light wave illuminates the sample only when the single-cycle ultrasound pulse propagates through its spatial focus. Accordingly, the sound modulation zone is confined to 40um in the transverse direction by the sound focusing element, and to 40um in the axial direction by the temporal profile of the single-cycle sound pulse convolved with the temporal profile of the laser pulse. To provide sufficient and also durable optical power for fluorescence excitation, we used DOPC to perform phase conjugation.

3. Results

The fluorescence imaging of the opto-ultrasound imaging system is provided in Fig. 2. Figure 2a shows the fluorescence pattern (hole array) which will be sandwiched by two 2 mm thick high scattering media. In such a condition, as a result of random scattering the structural information of the fluorescence pattern was completely lost using direct wide-field fluorescence image. Figure 2b presents an original fluorescence image of the hole array with tissue phantoms around it using our methods. Figure 3c shows the image of Fig 2b using bicubic interpolation.
Acknowledgment
This work is supported by National Basic Research Program of China (973 Program) (2015CB352005) and by the Fundamental Research Funds for the Central Universities.

References
[1] Li, Anan, et al. "Micro-optical sectioning tomography to obtain a high-resolution atlas of the mouse brain." Science 330.6009 (2010): 1404-1408.
[2] Si, Ke, et al. "Fluorescence imaging beyond the ballistic regime by ultrasound-pulse-guided digital phase conjugation." Nature photonics 6.10 (2012): 657-661.
[3] Si, Ke, et al. "Breaking the spatial resolution barrier via iterative sound-light interaction in deep tissue microscopy." Scientific reports 2 (2012).