DVD pickup head based optical resolution photoacoustic microscopy

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ABSTRACT

Optical resolution photoacoustic microscopy (OR-PAM) has been shown as a promising tool for label-free micro-vascular and single-cell imaging in clinical and bioscientific applications. However, most OR-PAM systems are realized by using a bulky laser for photoacoustic excitation. The large volume and high price of the laser may restrain the popularity of OR-PAM. In this study, we develop a low-cost and compact OR-PAM system based on a commercially available DVD pickup head. We showed that the DVD pickup head have the required laser energy and focusing optics for OR-PAM. The firmware of a DVD burner was modified to enable its laser diode to provide a 13-ns laser pulse with 1.3-nJ energy at 650 nm. Two excitation wavelengths at 650 and 780 nm were available. The laser beam was focused onto the target after passing through a 0.6-mm thick DVD transparent polycarbonate coating, and then aligned to be confocal with a 50-MHz focused ultrasonic transducer in forward mode. To keep the target on focus, a scan involving auto-tracking procedure was performed. The lateral resolution was verified via cross-sectional imaging of a 6-μm carbon fiber. The measured -6 dB width of the carbon fiber was 6.66 μm which was in agreement with optical diffraction limit. The proposed OR-PAM has potential as an economically viable and compact blood screening tool available outside of large laboratories due to its low cost and portability. Furthermore, a better spatial resolution could be provided by using a blue ray DVD pickup head.

Keywords: photoacoustic microscopy, DVD pickup head, micro-vascular imaging

1. INTRODUCTION

Because of the desire for photoacoustic images with higher spatial resolution, Maslov et al. promoted photoacoustic microscopy (PAM) from conventional acoustic resolution to optical resolution[1]. As an emerging branch of photoacoustic imaging, optical resolution PAM (OR-PAM) has advantages of conventional acoustic resolution PAM (AR-PAM) while forsaking the penetration depth to achieve micrometer-level or even submicrometer-level lateral resolution. By strongly focusing the excitation source, e.g. laser pulses, into the superficial structure of a sample, the lateral resolution of PAM system is dominated by the laser focusing spot size. This strategy provides a unique opportunity for label-free microvascular imaging including ophthalmic angiography[234], single cell detection[56], transcranial brain imaging[78], and functional imaging, etc., which are difficult to AR-PAM because the attenuation of ultrasound is unacceptable under such a high frequency. Recently, OR-PAM systems show their fantastic capability of spatial resolution ranging from 0.2 ~ 8 μm according to different applied optical wavelength and numerical aperture (NA). In comparison with optical microscopy, higher image contrast and label-free imaging capability are provided by OR-PAM. Because the contribution of contrast comes from the absorption of photons; a specific excitation wavelength could be chosen for the microvasculature or even the measurement of blood oxygen saturation in a single capillary. Owning such an adaptive imaging capability, OR-PAM has a great potential to apply to clinical blood screening without sample-dependent and complex staining process. However, most of the current OR-PAM systems are realized by using a bulky or fiber laser for photoacoustic excitation. The high cost and huge volume of these lasers obstruct the popularity of OR-PAM for clinical applications.

In this study, we worked on developing a low-cost and compact OR-PAM system based on a commercially available DVD pickup head by replacing the conventional excitation sources. A DVD pickup head provides at least two laser wavelengths, 650 and 780 nm, covers from visible (VIS) to near infrared (NIR) region which can be adaptive to various targets such as blood, nanoparticles, and specific pigment in tissues. Its compact size and PC-compatibility also make it a perfect excitation source with great portability and facile operation. Thanks to the need for high-concentration data

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storage and fast accessing rate on the DVD market, the DVD pickup head has its own intrinsically compact and accurate focusing assembly and provides the potential of high pulse repetition frequency (PRF) for real-time photoacoustic imaging. One last and also the most important thing we concern about is if a DVD pickup head can provide enough power to excite detectable photoacoustic waves. A recent study indicates that the energy of 6.5 nJ/pulse at a 7-ns pulse width should be good enough to generate detectable photoacoustic waves from a 6-μm carbon fiber. This energy level can be approached when current DVD pickup head is able to provide about 100-mW mean power within dozens of nanoseconds.

2. MATERIALS AND METHODS

Constrained to the 3T-11T limitation, the excitation form of the DVD pickup head for a DVD reader/writer can not directly apply to photoacoustic imaging. Therefore, the firmware of DVD pickup head controller of a commercially available DVD reader/writer was properly modified to be able to generate a repetitive nanosecond pulse in a microsecond cycle; the PRF can be up to kHz correspondingly. Figure 1 shows the block diagram of proposed DVD pickup head based OR-PAM system. The sample is placed in a water tank which is motorized by a three-dimensional (3D) motorized stage with precision of 1 μm. The DVD pickup head focuses the laser pulses on the sample through a 0.6-mm DVD cover layer, and then the excited photoacoustic waves will be detected by a 50-MHz ultrasound transducer (focal length: 9.2 mm) in forward mode. To achieve the optimal signal-to-noise ratio (SNR), the ultrasound transducer is aligned to be confocal with the DVD pickup head. The detected photoacoustic signal will be pre-amplified by a low-noise amplifier, amplified and filtered by a pulser/receiver (MODEL 5900PR, OLYMPUS), digitized by an oscilloscope (TDS5034B, Tektronix), and then stored to the PC for further processing. In the scanning process, a customized Matlab program synchronizes all the devices and manages a raster route for two-dimensional (2D) scanning to acquire the 3D image. An auto-focusing procedure is also involved to compensate the displacement during the scanning.

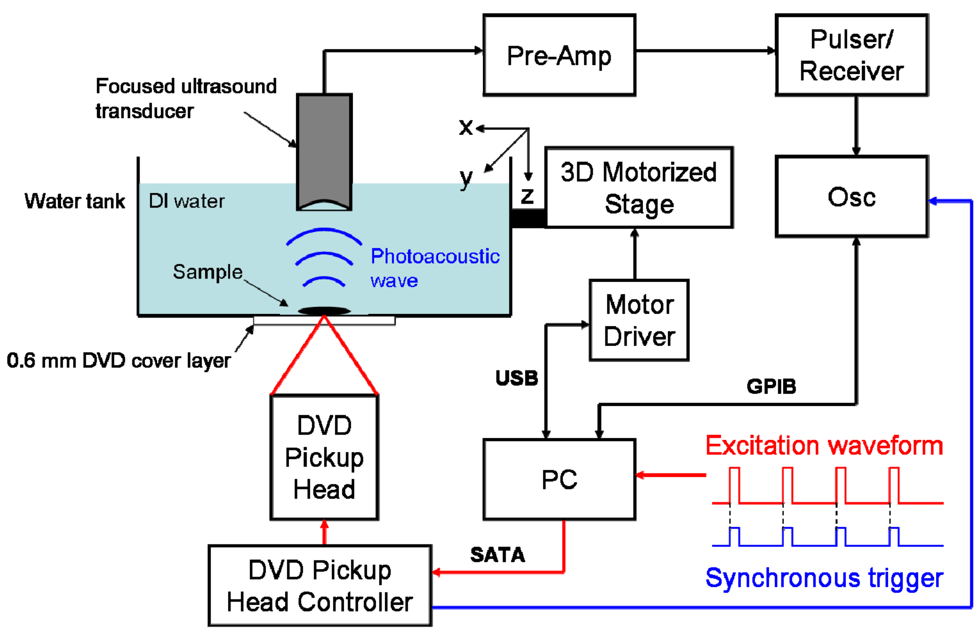

Figure 1. OR-PAM system block diagram.

3. EXPERIMENTAL RESULTS AND DISSCUSIONS

To verify the feasibility of photoacoustic excitation generation, a black film made of $CaCO_3$ with strong optical absorption at the VIS region was used as the sample. 13-ns laser pulses with energy of 1.3 nJ/pulse at 650 nm were provided for photoacoustic excitation. One hundred times signal averaging was performed to increase the SNR. Figure 2(a) shows the photoacoustic A-line signal of the black film. Because the black film was tightly attached to the cover layer, the photoacoustic wave (within the rectangular ROI in Figure 2(a)) of the film was followed by a strong reflection at the bottom of the water tank. The spectrum of photoacoustic signal of the film shown in Figure 2(b) indicates that the center frequency and bandwidth of the signal are both in accordance with the specifications of the ultrasound transducer which means a broadband signal is successfully excited by the DVD pickup head.

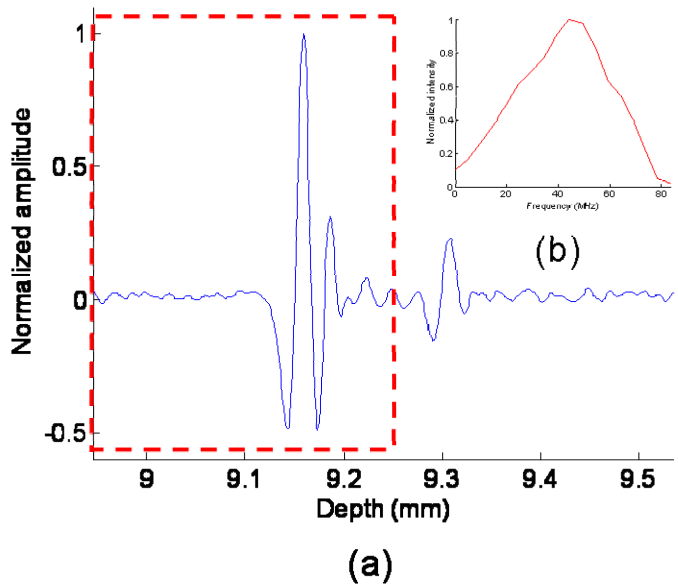

Figure 2. (a) Photoacoustic A-line signal of the black film. (b) Spectrum of the signal within the ROI in (a).

For the estimation of imaging capability, air force target (NT38257, Edmund) which is a popular optical resolution test target was used as the sample. There are longitudinal and lateral black bars arranged on a glass plane as shown in Figure 3(a). The pairs with different size were managed into seven groups with six element size in each group. In this case, the finest bars we imaged belonged to the group 7, element 2 which owned a 3.5-μm width for both black bars and the spacing between them. In the scanning process, the resolution test target replaced the 0.6-mm DVD cover layer as the focusing path itself with the patterned side facing toward the ultrasound transducer and a scanning size of 1 μm was employed. This was employed to avoid any unnecessary energy lost and strong ultrasound reflection on the interface of different media, e.g. the boundary between the resolution test target and the DVD cover layer. The employed laser specifications followed the description above.

Figure 3(b) shows the maximum projection (MAP) photoacoustic image of the pointed area on the resolution test target in Figure 3(a) and indicates that the 3.5-μm black bars are well-separated in both directions. In further quantitative comparison, DVD pickup head based OR-PAM shows its uniform imaging capability on the X-Y plane according the profiles along lateral and longitudinal directions presented in Figure 3(c) and 3(d). It should be noted that the edge response of the photoacoustic image of the bars is not sharp enough because the thickness of the test target is 1.5 mm which is not optimal for the pickup head focusing assembly. The “filled-in” effect can also be observed in the lower part of Figure 3(b) due to the enlargement of laser spot caused by improper focusing path.

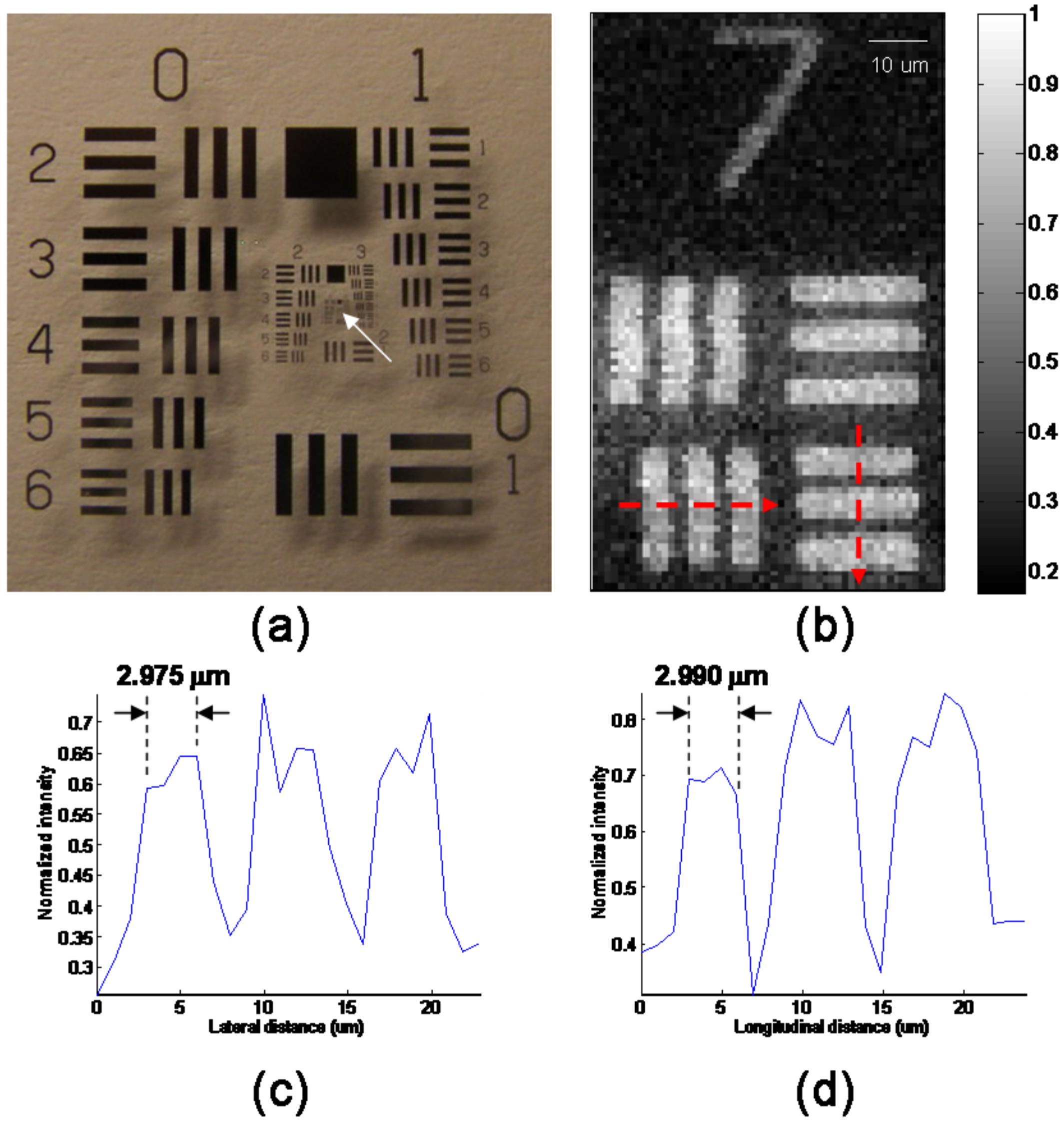

Figure 3. (a) Photo of air force resolution target. (b) Maximum projection image of air force target at group 7. (c) Profile along the horizontal dotted line in (b). (d) Profile along the vertical dotted line in (b).

A more direct way to estimate the spatial resolution is measuring the point spread function (PSF) of the imaging system by calculating the full-width-half-maximum (FWHM) or -6dB width of a point absorber. For the following confirmation, a 6-μm carbon fiber was used as the point-like absorber. The carbon fiber was attached to the 0.6-mm DVD cover layer to be fixed and focused, then was cross-sectioned as the result shown in Figure 4 in an actual aspect ratio and a linear scale. The measured FWHM in lateral and axial is 6.66 μm (it can up to 5.72 μm in the later experiment which result is not shown here) and 23.58 μm. In OR-PAM, the optical diffraction limit that defines the minimum laser spot size is constrained by the optical wavelength and NA of the pickup head. According to the wavelength of 650 nm and the 0.6 NA used in this study; the minimum laser spot size is ~ 0.66 μm. The lateral FWHM is in accordance of the width of the carbon fiber after being convoluted by the PSF. It also reveals that even though the lateral resolution is improved by the optical focusing, the axial resolution is still limited by the bandwidth of the ultrasound transducer, and it is one of the major difficulties that OR-PAM should overcome for 3D image reconstruction.

The laser energy density is also concerned for further safety issues in the biomedical applications. A rough estimation is performed under the condition that diameter of the laser spot is 0.66 μm and there is no laser energy lost. The energy density is ~ 380 mJ/cm^2 which is much larger than the ANSI safety limit of 20 mJ/cm^2. It explains why such low energy of 1.3 nJ in a single laser pulse can excite photoacoustic wave. This reason reveals that lower energy could also be used for the photoacoustic excitation to obey the safety limit. However, the low energy is detrimental to the vascular imaging because of the poor optical absorption of blood at 650 and 780 nm. To achieve the purpose without additional contrast agent, the 405-nm laser used for blue-ray DVD is a good option for label-free imaging. The blue-ray laser can provide both higher optical absorption of blood and better spatial resolution due to its shorter wavelength and higher NA value of the blue-ray DVD pickup head. When operating at the ideal laser spot size of less than 0.4 μm, a rapid auto-focusing is necessary to be involved in the scanning process.

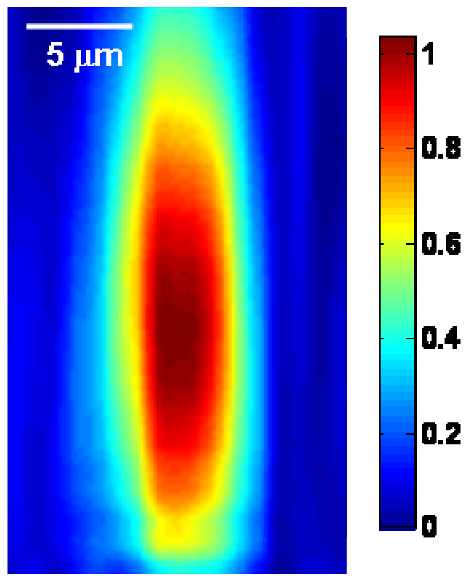

Figure 4. Photoacoustic envelope B-mode image of the cross-section of a 6-μm carbon fiber.

4. CONCLUSIONS

We successfully demonstrated the feasibility of DVD pickup head based OR-PAM to excite detectable photoacoustic waves at the laser wavelength of 650 nm. Even the applied energy is much less than the general value of current studies, good signal intensity is still available because of the high energy density at the laser focal spot. The lateral FWHM of a 6-μm carbon fiber is up to 6.66 μm at the laser wavelength of 650 nm. The spatial resolution can be still improved with a blue-ray DVD pickup head. The blue-ray laser also plays a role to realize label-free imaging for DVD pickup head based OR-PAM.

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