Light excitation methods for five dimensional optoacoustic imaging

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ABSTRACT

Multispectral optoacoustic tomography offers unprecedented capabilities in biological research and newly-developed systems prompt the clinical translation of this modality. By exciting tissues at multiple optical wavelengths, the distribution of spectrally-distinctive absorbers can be resolved with high resolution in deep tissues, thus enabling reading important biological parameters such as blood oxygenation or the biodistribution of photo-absorbing agents. Multispectral three-dimensional optoacoustic imaging generally comes at the expense of slow acquisition times, which limits the dynamic imaging capabilities of this modality. Recently, the feasibility of multispectral three-dimensional imaging in real time (five dimensional imaging) has been showcased. Two different illumination strategies can be used for this purpose. The first approach is based on tuning the wavelength of the laser on a per-pulse basis, which enables acquisition of large multispectral datasets on a very short time. The second approach is based on properly synchronizing the light beams from two (or more) laser sources. The performances of these two approaches are compared and discussed herein based on experiments with mice and human volunteers.

Keywords: Three-dimensional imaging, real-time imaging, multispectral imaging, five dimensional imaging.

1. INTRODUCTION

The feasibility of optoacoustic (photoacoustic) imaging biological tissues was showcased more than a decade ago[1,2] and the ongoing technological development of this modality increasingly attracts the biological research community due to the unique advantages offered. Indeed, high-resolution imaging of optical absorption at depths beyond the diffusive limit of light allows visualizing otherwise invisible anatomical structures.[3–5] More importantly, specific endogenous and exogeneous absorbing molecules can be spectroscopically isolated from images taken at multiple optical wavelengths, which opens new possibilities in functional and molecular imaging.[6,7] Overall, it is possible to resolve the spatial (three-dimensional) and temporal distribution of optical absorbers as well as their spectral absorption profiles, so that biological tissues can be interrogated in five independent imaging dimensions. However, the need to scan the ultrasound transducer(s) employed or to change the imaging wavelength generally leads to slow acquisitions, which have prevented fully exploiting the time dimension.

The feasibility of real-time multispectral optoacoustic imaging was demonstrated by means of parallel acquisition of signals excited with a tunable laser having wavelength-switching capacity on a per-pulse basis.[8] Thereby, by using a spherical transducer array, it was shown that five dimensional (three spatial dimensions + real-time + wavelength) optoacoustic imaging is possible. Specifically, it was shown that multispectral datasets can be acquired at a rate of $50/n$ three-dimensional images per second, being n the number of wavelengths employed.[9] New prospects in optoacoustics are then open, where several important applications are anticipated in biological research with small animals[10] as well as for the clinical translation of this technology.[11–13]

Real-time acquisition of multispectral datasets imposes significant technological challenges when considering relative motion between the optoacoustic probe and the sample. This can be due to intrinsic motion in living subjects, e.g. due to breathing or heartbeat,[14] or it can occur when operating optoacoustic imaging devices in a hand-held mode. In many cases, the fast wavelength-tuning approach can avoid motion-related artefacts when using a high pulse repetition rate of the laser. However, the substantial (millisecond level) time delay between

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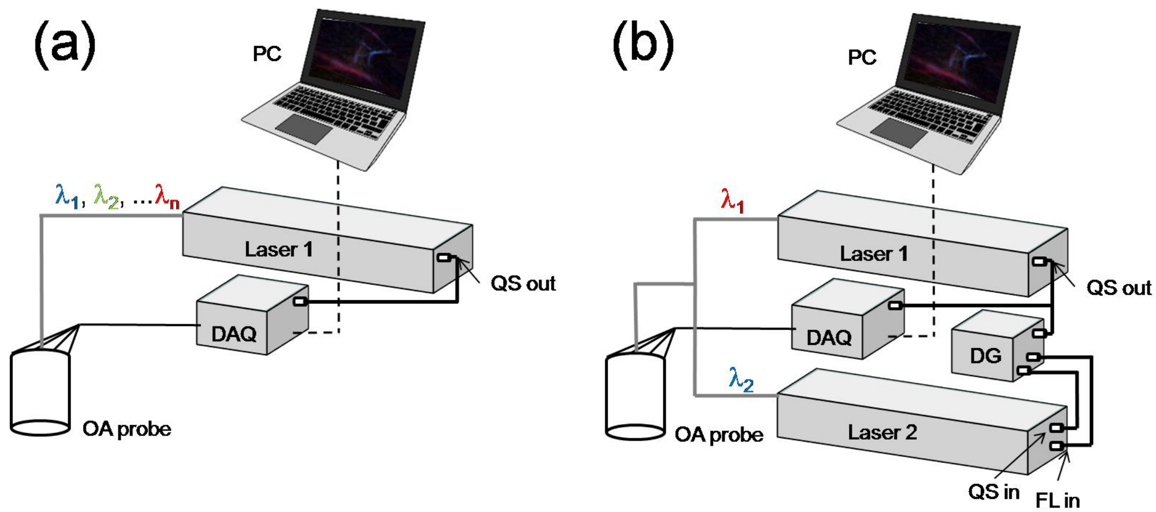

Figure 1. Lay-out of the experimental set-ups used for five dimensional optoacoustic tomographic imaging. (a) Five dimensional optoacoustic imaging based on a fast wavelength-tuning laser. (b) Five dimensional optoacoustic imaging based on synchronization of two lasers. PC - personal computer, DAQ - data acquisition system, DG - delay generator, OA - optoacoustic, QS - Q-switch, FL - flash lamp.

laser pulses at different wavelengths can limit the applicability of this approach. Also, increasing the pulse repetition rate of the laser implies reducing the energy per pulse in order to conform to laser safety standards regarding mean light power density, which in turn affects the signal-to-noise ratio of the images. Thereby, an anternative approach based on introducing a microsecond-range delay between two short-pulsed lasers operating at different wavelengths has been suggested for five dimensional optoacoustic imaging.[15]

Herein, we describe the two optical excitation strategies used for five dimensional optoacoustic tomography, namely fast wavelength tuning and synchronization of different laser sources. The performances, advantages and limitations of both approaches are discussed for small animal imaging and hand-held scanning measurements.

2. FIVE DIMENSIONAL OPTOACOUSTIC TOMOGRAPHY

Five dimensional optoacoustic tomography refers to three dimensional optoacoustic imaging at multiple wavelengths in real-time. We describe in this section two possible light excitation approaches that can be employed for this purpose. The first one is based on a laser with wavelength tuning capability on a per-pulse basis and the second one consists in the synchronization of lasers operating at different optical wavelengths.

2.1 Fast wavelength-tuning laser

Five dimensional optoacoustic tomographic imaging with a tunable laser has been suggested in Ref. 9. Basically, an optical parametric oscillator (OPO)-based laser is used as a light excitation source. The laser allows tuning the output wavelength on a per-pulse basis at a pulse repetition rate of 50Hz, so that acquisition of the generated optoacoustic signals with a custom-made spherical array permits volumetric multispectral imaging at frame rates up to 25Hz (two laser wavelengths). The set-up of the experimental system is depicted in Fig. 1a. The output beam of the laser is coupled to a custom-made fiber bundle that delivers light to the surface of the tissue. The detected signals at the piezoelectric elements of the spherical array[16] are simultaneously acquired with a custom-made data acquisition system, and subsequently transmitted to a personal computer where three-dimensional images are reconstructed on a graphics processing unit (GPU).[17] More details on the set-up employed can be found in Ref. 9.

2.2 Synchronized laser sources

An alternative approach for five dimensional optoacoustic tomography consists in the synchronization of light pulses from different laser sources as suggested in Ref. 15. The set-up of the experimental system used in this approach is depicted in Fig. 1b. Basically, two OPO-based lasers tuned at two different wavelengths are used. The output laser beams are coupled to the two inputs of a fiber bundle having a common output. The individual fibers at the output of the bundle are randomly distributed with respect to the two inputs so that both beams create approximately the same light profile at the surface of the tissue. The flash-lamps and Q-switch of one of the lasers are externally triggered with delayed signals obtained from the Q-switch output of the other laser. In this way, the two laser pulses are delayed by approximately 17μs. Much like in the fast wavelength-tuning approach, the signals at the elements of the spherical array are simultaneously acquired with the data acquisition system and subsequently transmitted to the personal computer. More details on the set-up employed can be found in Ref. 15.

3. EXPERIMENT DESCRIPTION

We illustrate the capabilities of the two described light excitation approaches with two representative experiments.

In the first experiment, an eight-week old female nude mouse was anesthetized with 2% isoflurane and positioned in a supine position with the head on top of the spherical array. Indocyanine green (ICG) (50nmol diluted in 50ml of saline) was injected intravenously and the head of the mouse was imaged by tuning the laser wavelength between 730 and 850nm (30nm step) on a per-pulse basis. The pulse repetition rate of the laser was set to 50Hz, so that multispectral datasets consisting of 5 different wavelengths were collected at a rate of 10Hz. The experiment was conducted in conformity with institutional guidelines and with approval from the Government of Upper Bavaria. A more detailed description of this experiment can be found in Ref. 9.

In the second experiment, the spherical array was hand-held scanned on the palm of a healthy volunteer. Two lasers with wavelengths set to 760 and 850 nm and delayed by approximately 17μs were simultaneously coupled to the fiber bundle. The displacement d of the probe between two consecutive laser pulses is given by $d = c/PRF$, where c is the scanning velocity and PRF is the pulse repetition frequency of the laser. In the experiment performed, the scanning velocity was estimated to be between 1 and 10mm/s. A more detailed description of this experiment can be found in Ref. 15.

4. RESULTS

The results of the two experiments described are displayed in Fig. 2.

Fig. 2a shows the maximum intensity projections (MIP) of the three-dimensional images corresponding to the unmixed distribution of ICG in the mouse brain. The time after ICG injection is indicated in each image. The unmixed images are obtained at a frame rate of 10 Hz, corresponding to the acquisition of multispectral datasets. In order to be able to efficiently isolate (unmix) the biodistribution of a spectrally-distinctive absorber from the endogenous absorbers, a relatively large number of wavelengths is required. Considering that the dynamic changes in the biodistribution of the contrast agent injected could generally be resolved at frame rates lower than 10 Hz, the fast wavelength tuning method represents a suitable approach for five dimensional optoacoustic imaging.

On the other hand, Fig. 2b displays the results of the hand-held scanning experiment. Specifically, two consecutive MIPs of the unmixed distributions of oxygenated (HbO2) and deoxygenated (Hb) hemoglobin are displayed. If blood is the dominant endogenous substance, Hb and HbO2 can be unmixed from images at two different wavelengths. As a reference, a point labeled '1' is displayed in the two images to illustrate the motion between consecutive frames, so that the fast wavelength tuning approach would lead to motion-associated artefacts in this case.

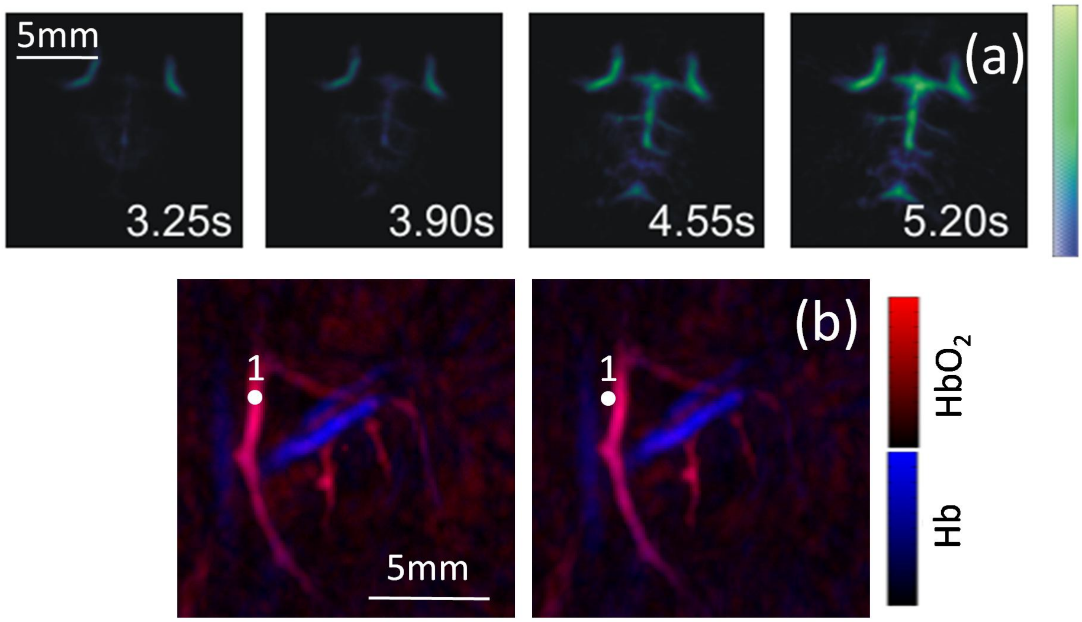

Figure 2. Experimental results for the fast wavelength-tuning experiment (a) and the two-laser experiment (b). (a) Unmixed distribution of ICG in the mouse brain for four different instants after injection of the agent. The time after injection is indicated in the images. (b) Unmixed distribution of oxygenated and deoxygenated hemoglobin for two consecutive instants. The point labelled '1' is shown as a reference to illustrate the motion between the frames.

5. CONCLUSIONS

The feasibility of real-time optoacoustic imaging in three dimensions with multispectral specificity enables independently interrogating a tissue in five different imaging dimensions. In this way, it is possible to resolve spectrally-distinctive optical absorbers in living organisms with high spatial and temporal resolution, which opens unprecedented prospects in anatomical, functional and molecular imaging. Important applications are then anticipated in clinical practice as well as in pre-clinical research with small animals. We have shown herein that five dimensional optoacoustic imaging can be performed with two illumination strategies offering different advantages. On the one hand, multispectral illumination by tuning the wavelength of the laser on a per-pulse basis enables to acquire an entire multispectral dataset over a broad spectral range in a very short time, which is needed to efficiently resolve specific optical biomarkers from strong endogeneous contrast associated to substances such as hemoglobin or melanin. On the other hand, by properly delaying the light pulses from two (or more) laser sources, it is possible to spectrally unmix different absorbers even in the presence of intrinsic tissue motion, such as cardiac or breathing movements, and also when fast hand-held scanning is performed. This approach scales in price and complexity with the number of wavelengths employed. However, the signal-to-noise ratio of real-time multispectral imaging is maximized while maintaining safety exposure limits regarding energy per pulse and average power. This is of particular relevance for the clinical translation of the optoacoustic technology. Overall, the demonstrated five dimensional imaging capacity of optoacoustics represents a new technological development that adds to the many advantages of this modality.

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