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Optoacoustic imaging in five dimensions

X Luís Deán-Ben, Sven Gottschalk, Thomas F Fehm and Daniel Razansky *

Institute for Biological and Medical Imaging, Technical University of Munich and Helmholtz Center Munich, Ingolstädter Landstraße 1, 85764 Neuherberg, Germany.

ABSTRACT

We report on an optoacoustic imaging system capable of acquiring volumetric multispectral optoacoustic data in real time. The system is based on simultaneous acquisition of optoacoustic signals from 256 different tomographic projections by means of a spherical matrix array. Thereby, volumetric reconstructions can be done at high frame rate, only limited by the pulse repetition rate of the laser. The developed tomographic approach presents important advantages over previously reported systems that use scanning for attaining volumetric optoacoustic data. First, dynamic processes, such as the biodistribution of optical biomarkers, can be monitored in the entire volume of interest. Second, out-of-plane and motion artifacts that could degrade the image quality when imaging living specimens can be avoided. Finally, real-time 3D performance can obviously save time required for experimental and clinical observations. The feasibility of optoacoustic imaging in five dimensions, i.e. real time acquisition of volumetric datasets at multiple wavelengths, is reported. In this way, volumetric images of spectrally resolved chromophores are rendered in real time, thus offering an unparallel imaging performance among the current bio-imaging modalities. This performance is subsequently showcased by video-rate visualization of in vivo hemodynamic changes in mouse brain and handheld visualization of blood oxygenation in deep human vessels. The newly discovered capacities open new prospects for translating the optoacoustic technology into highly performing imaging modality for biomedical research and clinical practice with multiple applications envisioned, from cardiovascular and cancer diagnostics to neuroimaging and ophthalmology.

Keywords: Hand-held probe, three-dimensional imaging, real-time imaging, clinical imaging, graphics processing unit.

1. INTRODUCTION

Despite the ancient discovery of the optoacoustic effect, it was not until a relatively recent time when it was first applied to biomedical imaging, with a very strong development in the last decade.^{1,2} More than one century ago, Alexander Graham Bell first detected pressure waves generated by an intensity-modulated beam of sunlight.³ The measurement of such acoustic waves, excited by light absorption in tissue chromophores, is the basis of optoacoustic imaging. Being time-resolved signals, they provide one dimensional depth profiling similarly to A-mode visualizations in ultrasound imaging.¹ Then, optoacoustic microscopy setups have been suggested based on raster scanning a focused light beam and/or an ultrasonic transducer along the imaging sample, yielding three-dimensional (volumetric) images of the absorption distribution by stacking up the set of measured signals.⁴⁻⁶

Optoacoustic microscopy presents however some limitations. For example, when optical resolution (light focusing) is used, the penetration depth is limited by the photon ballistic propagation distance in biological tissues (approximately 1 mm in the near-infrared). For deeper imaging, photons undergo strong scattering and focusing can only be achieved acoustically. Thereby, the frequency-dependent sensitivity field of the transducer limits the imaging performance. In fact, a three dimensional region is inevitably illuminated in deeper locations so that signals from a set of locations covering a large solid angle must be collected to accurately image absorbers with different sizes and orientations. In this way, optoacoustic tomographic setups based on acquisition of optoacoustic signals at positions surrounding the imaging sample have been suggested for small animal imaging.^{2,7-9} In both cases, optoacoustic microscopy and optoacoustic tomography, a large number of signals are generally required in order to achieve the desired image quality. Thereby, the time resolution is limited by the number of scanning

*E-mail: dr@tum.de

positions and the pulse repetition rate of the laser employed, being generally low. A different tomographic approach based on simultaneous acquisition of optoacoustic signals with an array of cylindrically focused ultrasonic transducers have been suggested,^{10,11} allowing high resolution cross-sectional imaging in real time (a few Hertz). Then, a new imaging dimension (time) is provided at the expense of the third spatial dimension, which can however be recovered by scanning if time resolution is not needed. More recently, the feasibility of four dimensional imaging (three-dimensional imaging in real time) was showcased with systems based on simultaneous acquisition of signals on locations distributed on a spherical surface,^{12–14} and developments in laser excitation methods and data acquisition systems allow further imaging very fast dynamic processes.^{15,16}

On the other hand, by imaging at different optical wavelengths, multispectral optoacoustic tomography (MSOT) provides absorption specificity as a new imaging dimension. As a large variety of substances present a characteristic absorption spectrum in the visible and near infrared, the versatility of MSOT for molecular imaging probably overcomes any other imaging modality, with applications in small animal research continuously emerging. Furthermore, the intrinsic and specific haemoglobin-based contrast of blood opens new promising prospects in selected anatomical and functional clinical imaging applications.^{17–19} Multiwavelength illumination comes however at the expense of a reduced time resolution due to the wavelength-tuning capabilities of the lasers employed. New developments in optical-parametric-oscillator (OPO) laser technology allow now per-pulse wavelength tuning capability for a 50 Hz pulse repetition rate laser, so that acquisition of the generated optoacoustic signals with a custom-made spherical ultrasonic array permits volumetric multispectral imaging at frame rates up to 25 Hz, providing for the first time five dimensional optoacoustic imaging, i.e., three-dimensional imaging of specific absorbers in real time.²⁰ The application of the system for hand-held in human visualization of vessels having different oxygenation levels is presented herein.

2. MATERIALS AND METHODS

2.1 Experimental setup

The set-up of the experimental system is depicted in Fig. 1. The five dimensional imaging capability is enabled with an optical parametric oscillator (OPO)-based laser (Innolas Laser GmbH, Krailling, Germany) having wavelength tuning capability in a per-pulse basis. The pulse repetition rate of the laser is set to 50 Hz, so that optical excitation with several pulses at arbitrary wavelengths ranging from 700 to 900 nm delayed by 20 ms is possible. The light beam is guided through a custom-made fiber bundle (CeramOptec GmbH, Bonn, Germany) towards the part to be measured, reaching the skin with a spot size of approximately 1 cm. A matching fluid (water) contained by a transparent membrane is used as a coupling medium for the generated optoacoustic waves. Its transparency also guarantees efficient illumination without energy loss. The maximum permissible exposure imposes limitations in the per-pulse energy and hence in the achievable signal-to-noise ratio (SNR) with single shot illumination, in a way that an efficient design of the ultrasonic detection probe is essential to obtain high quality images with each laser pulse. In the spherical array used for collecting the signals, the array elements have a relatively large size ($3 \times 3 \text{ mm}^2$) and are conveniently oriented towards the center of the sphere in order to provide a good sensitivity in this imaging region of interest, so that a good SNR in the reconstructed images is rendered even with the relatively low energy per pulse. The pressure signals at the 256 locations of the array elements are collected at 2030 instants with a sampling frequency of 40 megasamples per second. A specifically design data acquisition system (Falkenstein Mikrosysteme GmbH, Taufkirchen, Germany) allows data acquisition at the pulse repetition rate of the laser and subsequent transmission to a personal computer through an ethernet connection.

2.2 Hand-held scanning

The performance of five dimensional optoacoustic imaging was first tested in a hand-held scanning experiment in a human healthy volunteer.²⁰ The experiment was done in full accordance with work safety regulations of Helmholtz Center Munich. The pulse repetition rate of the laser and the per-pulse energy were selected to fulfill laser safety standards for clinical measurements at all times. The in-vivo experiments were conducted in the wrist region of a healthy volunteer. Prior to the experiments, hair was removed around the imaged region in order to avoid image artifacts due to strong optoacoustic signals. During imaging, the hand-held probe was gently moved along the skin surface with at a relatively low scanning speed while acquiring the multi-spectral volumetric data.

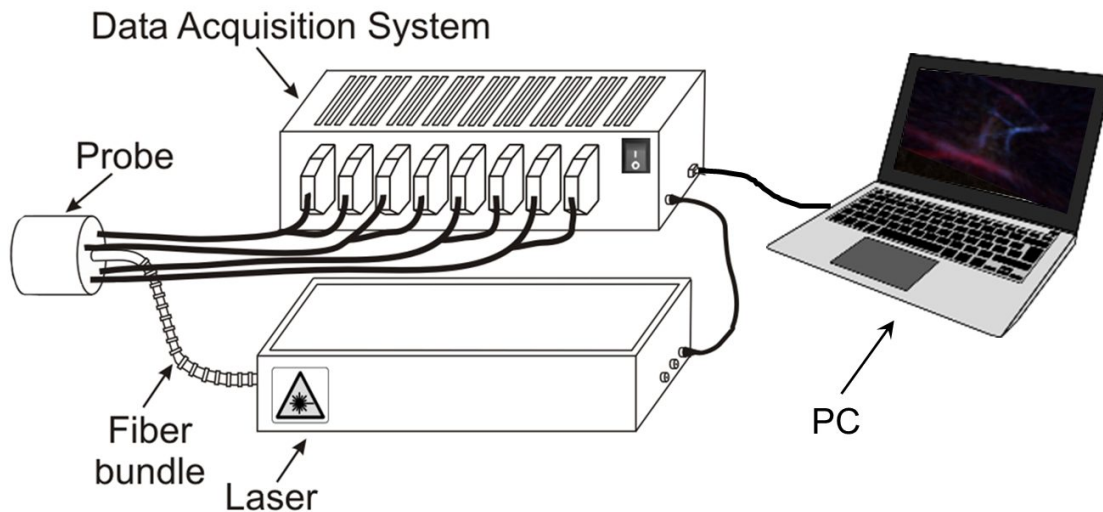


Figure 1. (a) Lay-out of the experimental system.

2.3 Hemodynamic changes in the mouse brain

The mouse brain for an animal subjected to oxygenation stress was imaged to demonstrate the capability of the five dimensional imaging approach to monitor multiple hemodynamic parameters.¹⁶ Specifically, an eight week old Female athymic nude-Foxn1nu mice (Harlan Laboratories LTD, Itingen, Switzerland) was imaged in full compliance with institutional guidelines of the Institute for Biological and Medical Imaging and with approval from the Government District of Upper Bavaria under animal protocol reference number 55.2.1.54-2532-95-12. The mouse was anesthetized with isoflurane (1.5-2.5% v/v) in 100% O₂. During data acquisition, the body temperature was kept constant and continuously monitored along with other physiological parameters such as blood oxygenation and heart rate. The experiment was initiated under hyperoxic conditions (100% O₂) and the inhaled gas was changed every two minutes between medical air (20% O₂) and oxygen (100% O₂). The isoflurane level was kept constant and the gas mixture was controlled manually using a multi-gas flowmeter.

3. RESULTS

The real-time multispectral imaging capacity for the hand-held scanning experiment is showcased in Fig. 2. The measurements were performed by scanning the probe on the wrist area of a human healthy volunteer. Blood vessels having different oxygen saturation levels were present in this area, along with a skin pigmentation rich in melanin. The laser wavelength was tuned to multiple wavelengths between 730 and 850 nm with 30 nm step (5 wavelengths). In this wavelength range, the absorption of melanin monotonically decreases, the absorption of oxygenated hemoglobin monotonically increases and there is a characteristic peak in the absorption of deoxygenated haemoglobin. The entire multispectral dataset is acquired in 100 ms due to the fast-tuning capacity of the laser. Real-time visualization during the experiment is enabled with a graphics processing unit (GPU) implementation of back-projection reconstruction. The maximum intensity projection (MIP) images along the depth direction for 3 different wavelengths corresponding to a given position of the hand-held probe are displayed in Fig. 2a. In this way, the yellow spot in Fig. 2 represents the skin pigmentation whereas red and blue structures most likely correspond to arteries and veins.

On the other hand, 750 multispectral frames were acquired for the mouse experiment, which corresponds to 10 minutes. A three-dimensional view of the reconstructed brain region for a wavelength of 800 nm is displayed in Fig. 3a, where two volumes of interest (VOI) are indicated. From each multispectral frame, the distribution of oxygenated (HbO₂) and deoxygenated (Hb) hemoglobin are unmixed by least square fitting on a per-pixel

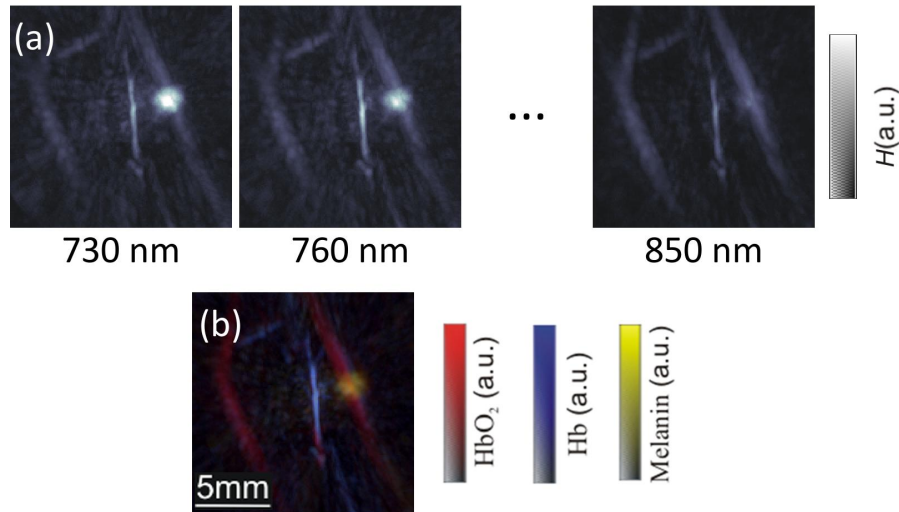


Figure 2. (a) Maximum intensity projection along the depth direction of optoacoustic images for three different wavelengths corresponding to three consecutive pulses for the laser being operated at 50 pulses per second. (b) Spectrally unmixed images showing distribution of oxygenated (red) and deoxygenated (blue) hemoglobin and melanin (yellow).

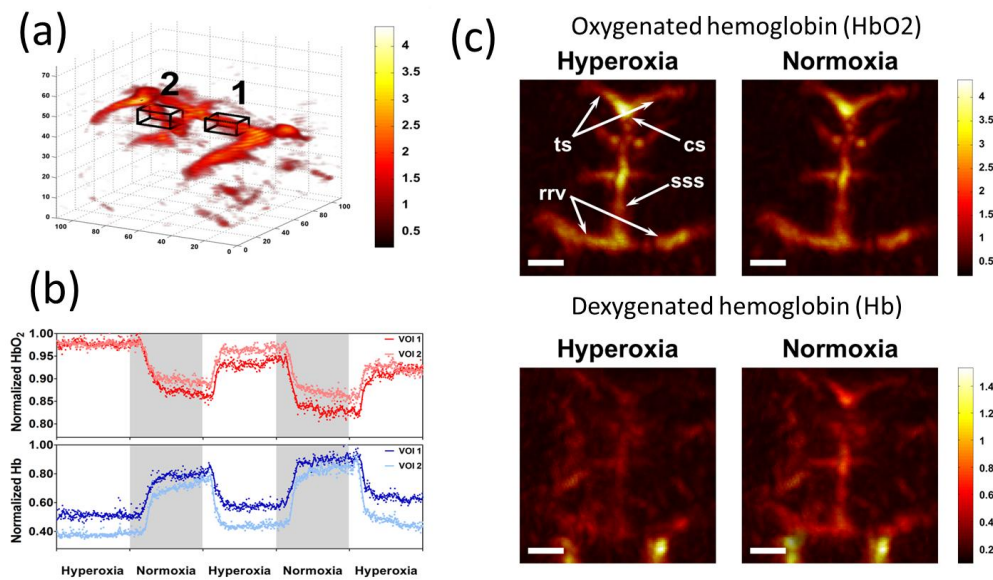


Figure 3. (a) Three dimensional view of the reconstructed brain of a mouse where two volumes of interest 1 and 2 are marked. (b) Time profiles of the unmixed oxygenated and deoxygenated hemoglobin for 1 and 2. (c) Unmixed distributions of oxygenated and deoxygenated hemoglobin for the hyperoxia and normoxia cycles.

basis of the absorbed energy to a combination of Hb and HbO₂. As an example, the unmixed distributions of oxygenated and deoxygenated hemoglobin for the hyperoxia (100% O₂) and normoxia (20% O₂) cycles are showcased in Fig. 3c. Also, the time profiles of the Hb and HbO₂ signals for the selected VOIs are shown in Fig. 3b. Multispectral acquisition of three-dimensional tomographic data in real-time enables simultaneous label-free assessment of multiple brain hemodynamic parameters such as blood oxygenation and total hemoglobin.

4. CONCLUSIONS

The presented results demonstrate the basic feasibility of optoacoustic imaging in five dimensions, i.e. rendering volumetric images of spectrally resolved optoacoustic data in real time. New prospects in optoacoustics are then

open, where several important applications are anticipated in clinical practice as well as in pre-clinical research with small animals. From the clinical perspective, the convenience of the hand-held mode combined with the real-time visualization performance allows studying functional blood parameters in patients. From the pre-clinical point of view, functional and molecular imaging applications involving dynamic visualization of specific optical biomarkers are enabled, with applications envisioned in cardiovascular and peripheral vascular disease, disorders of the lymphatic system, breast cancer, arthritis or inflammation. This opens new possibilities e.g. for in vivo cell tracking, visualization of pharmacokinetics, targeted molecular imaging studies or neuroimaging. The accuracy in the unmixing of specific substances as well as the feasibility to image multiple targets scales with the number of wavelengths employed, which however leads to a lower temporal resolution of the multispectral approach. Future work includes developing systems with a faster frame rate as well as addressing important algorithmic challenges in optoacoustic reconstruction and unmixing of specific substances.

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