Fast deep-tissue multispectral optoacoustic tomography (MSOT) for preclinical imaging of cancer and cardiovascular disease

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ABSTRACT

Optoacoustic imaging has enabled the visualization of optical contrast at high resolutions in deep tissue. Our Multispectral optoacoustic tomography (MSOT) imaging results reveal internal tissue heterogeneity, where the underlying distribution of specific endogenous and exogenous sources of absorption can be resolved in detail. Technical advances in cardiac imaging allow motion-resolved multispectral measurements of the heart, opening the way for studies of cardiovascular disease. We further demonstrate the fast characterization of the pharmacokinetic profiles of light-absorbing agents. Overall, our MSOT findings indicate new possibilities in high resolution imaging of functional and molecular parameters.

Keywords: photoacoustic imaging, optoacoustic imaging small animal imaging

1. INTRODUCTION

Optical imaging at macroscopic and mesoscopic scales has emerged over recent years as a powerful set of methods for probing the underlying biology of living organisms, thereby enabling more efficient drug discovery and improved diagnostic capabilities. In particular, studies of cancer[1] and cardiovascular disease[2, 3] frequently see the application of optical imaging techniques. However, optical imaging at these scales suffers from the strong scattering of light in tissue: spatial resolution degrades rapidly with increasing depth[4]. Optoacoustic imaging offers a way around this problem[5]. Optical absorption, which provides rich contrast in tissue, is resolved by means of the broadband ultrasound waves emitted due to thermal expansion. This allows optical contrast at a higher resolution, since the scattering of ultrasound in tissue is orders of magnitude less than that of light.

Parallel detection of ultrasound waves by transducer arrays, as employed in routine medical ultrasonography, provides optoacoustic imaging at high-rates in real-time. Apart from the clear advantages that these fast imaging capabilities bring in terms of temporal resolution, high frame rates can additionally allow advances motion correction techniques, since each frame provides a meaningful snapshot of the morphology being imaged. The heartbeat and respiration are sources of significant motion in imaging and dealing with such motion is an important topic in biomedical imaging in general. Particularly because the primary advantage of optoacoustic imaging is the high spatial resolution, degradation due to motion represents a serious challenge, and correcting for the effects of motion is therefore an important part of optoacoustic imaging.

By means of multi-wavelength illumination of the tissue under examination, multispectral optoacoustic tomography (MSOT) enables spectroscopic characterization of the various tissue absorbers producing optoacoustic signals[6, 7]. This separation of contrast sources according to their spectral signatures can be applied to resolve various significant absorbers intrinsic to tissue, such as the different oxygenation states of hemoglobin, and can additionally provide information on the distribution of exogenous optical contrast agents. Combined with high-rate capabilities, this provides a tool to track the fate of exogenous agents in-vivo at a high temporal resolution.

In this paper, we investigate the different parts of an MSOT imaging platform that, as a whole, is capable of high-rate, real-time, multichannel, in-vivo, motion-resolved, high resolution imaging of endogenous and exogenous sources of

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contrast. We demonstrate this in studies on mice and discuss the potential of this imaging platform for clinical applications.

2. MATERIALS AND METHODS

2.1 Experimental MSOT imaging system

The most important specifications of the experimental MSOT imaging system we employed (Fig. 1) are wavelength-tunable illumination, sufficiently high pulse energy to provide meaningful images from each pulse, and parallel multi-element ultrasound detection focused on a two-dimensional slice. The system has been described in detail previously[8, 9]. Briefly, a Q-switched Nd:YAG laser with a pulse repetition rate of 10Hz pumps an optical parametric oscillator (OPO) to provide a source of illumination with a wavelength tunable in the range 700nm-950nm and a maximum output pulse energy around 100mJ (Opotek). A fiber bundle assembly with 10 output arms delivers the light to the animal being imaged, spreading the energy over the surface of the region of interest to keep the fluence below maximum permissible exposure values. Detection of the optoacoustic signals is provided by a 64-element ultrasound transducer array with the center frequency at 5MHz (Imasonic), providing an axial resolution around 150-200μm. The elements are arranged in one row on an arc covering approximately 180°, which is focused (focal length 4cm) on a selected transverse slice through the animal. The signals are recorded in parallel by a custom acquisition system at 40Msamples/s. Overall, this setup provides 10 two-dimensional transverse slice images per second.

To facilitate imaging of any required slice, the imaged subject, most commonly a mouse, can be translated through the imaging plane by a linear stage. The subject is positioned in a custom holder that includes a mask for Isofluorane anesthesia. In this holder, the underneath of the animal is covered by a thin, transparent polyethylene membrane. This is then submerged in a temperature-controlled water-filled chamber for imaging, thus providing a medium for the generated ultrasound waves to reach the detectors.

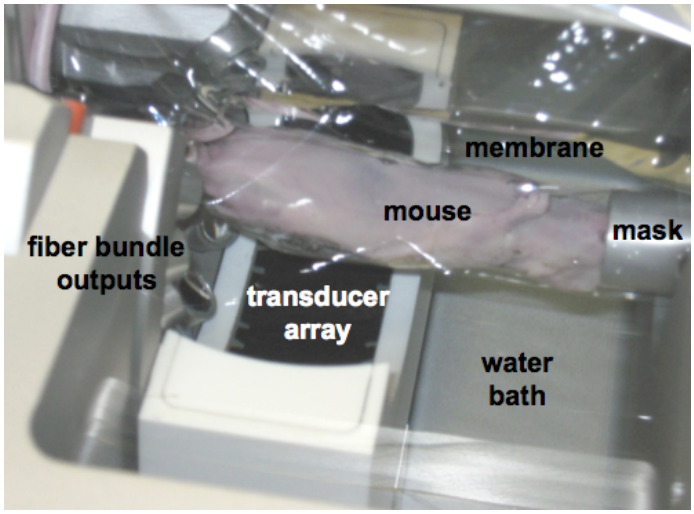

Fig. 1: Photograph of the real-time MSOT imaging setup.

2.2 Image reconstruction

We reconstruct the optoacoustic images using either one of two techniques. For the live display of images on the system during acquisition, we use a delay-and-sum method that requires less than 100ms, ie. less than the time between laser pulses, for reconstruction. For offline reconstruction we apply a model-based inversion, which is capable of providing quantitative images provided that all significant system characteristics are taken into account in the model[10]. These reconstruction methods are performed on each frame, yielding one image per laser pulse.

2.3 Motion correction

Images formed from individual laser pulses in our implementation have an effective acquisition time of approximately 33μs—the time taken for signals from a field of view of 2cm diameter centered around the array's 4cm focal length to

reach the detection surface. We assume that motion during this time will not significantly degrade spatial resolution by blurring.

Since the ultrasound transducer array is read out in parallel and no mechanical scanning is performed to capture multiple projections, consecutive frames (laser pulses) would be identical in the absence of motion in the subject. Therefore, motion taking place between frames can be resolved on a frame-by-frame basis. The need for motion correction in multi-wavelength imaging is clear: since different wavelengths are applied at different times, there can be significant motion between wavelengths, causing motion in the resulting spectrally resolved images.

The most significant sources of motion in biological imaging subjects are heartbeat and respiration. Since both of these cause almost periodic motion, the task of correcting their effects can be accomplished by sorting individual frames into the phases of heartbeat or motion which they represent, for example diastole or systole in the case of heartbeat. This classification of frames prior to spectral unmixing can be performed manually, by inspecting the images[9], or automatically, for example using a clustering algorithm like *k*-means[11].

2.4 Spectral unmixing

A common approach taken to spectral unmixing in MSOT is to use the known absorption spectra of absorbers assumed to contribute significantly to the optoacoustic contrast to build a system of linear equations, which can then be solved for the concentration of each spectral source per pixel. In situations where the source components are well-known, such as images dominated by combinations from oxy- and deoxyhemoglobin and a possible exogenous agent, this method can work, as shown in our results in this work. However, in situations where exogenous agents are to be detected in the presence of unknown tissue absorbers, or where wavelength-dependent light attenuation strongly distorts the detected optoacoustic spectra, blind source separation techniques like principle components analysis or independent components analysis have shown much promise[12]. Therefore the selection of spectral unmixing technique depends on the given imaging scenario.

2.5 Animal handling and experimental protocol

All procedures concerning the handling of animals were approved by the Government of Upper Bavaria. Mice (CD-1) were under isofluorane anesthesia for the duration of experiments. Hair was removed from the imaged region by cream prior to MSOT imaging. For imaging of the heartbeat, mice were placed in a prone position and translated so that a transverse slice through the ventricles was imaged. 100 frames at 860nm were then recorded. In the case of imaging contrast agent kinetics, a catheter was inserted into the tail vein prior to imaging. Mice were placed in the prone position and translated to image a slice through the upper thorax where blood vessels were visible. During imaging, 0.3mg/kg of Indocyanine green (ICG) was injected. Continuous imaging at multiple wavelengths (725nm-850nm in steps of 25nm) with 100 frames captured per wavelength allowed 10 complete multispectral data points to be recorded in a time span of under 20 minutes.

3. RESULTS

3.1 Motion resolved cardiac imaging

The optoacoustic images through the ventricles of the mouse heart (Fig. 2) give a detailed representation of the anatomy. At the imaged wavelength, 860nm, oxyhemoglobin displays stronger absorption than deoxyhemoglobin. Therefore, in the images, we can distinguish the brighter areas representing the blood pools inside the heart chambers from the less oxygenated, and therefore less bright myocardium. The myocardium of the left ventricle is particularly well resolved. To investigate automatic clustering (*k*-means) for resolving motion, we applied the algorithm for separation into two motion states on the 100 frames captured through the ventricles. This resulted in one cluster representing diastole and another representing systole, as can be inspected on the resulting mean images (Fig. 2): the diastole image (Fig. 2a) displays a larger area inside the left ventricle when compared to the systole image (Fig. 2b), and the image obtained by averaging all 100 frames, that is, the uncorrected case, shows a visibly blurred combination of the two phases (Fig. 2c). Since other common sources of motion in imaging subjects are, like in this case, almost periodic (heartbeat, pulse, respiration), we believe that this method has general applicability.

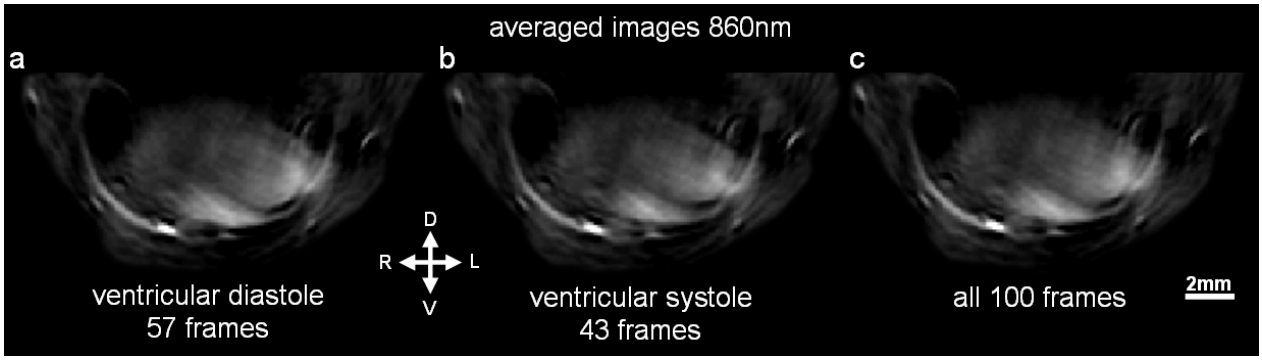

Fig. 2: Averaged images with and without clustering, taken at 860nm. a: Averaged image over cluster corresponding to diastole. b: Averaged image over cluster corresponding to systole. c: Averaged image over all 100 frames.

3.2 Kinetic profiles of exogenous agents

The combination of high-rate imaging with spectral unmixing of absorption sources enables fast characterization of the kinetic profiles of exogenous contrast agents. As is evident in the MSOT images taken through the upper thorax (Fig. 3), blood vessels are clearly resolved due to the dominant absorption of hemoglobin in tissue. ICG is known to remain within blood vessels until it is removed from the circulation by the liver. As expected, MSOT detects the ICG within blood vessels which are visible in the images (Fig. 3). By imaging at multiple wavelengths during the i.v. injection of ICG we were able to characterize the kinetics of that agent in the circulation. A half-life of approximately 2 minutes was obtained by fitting an exponential function to the data, a value which agrees with the well-known characteristics of the agent.

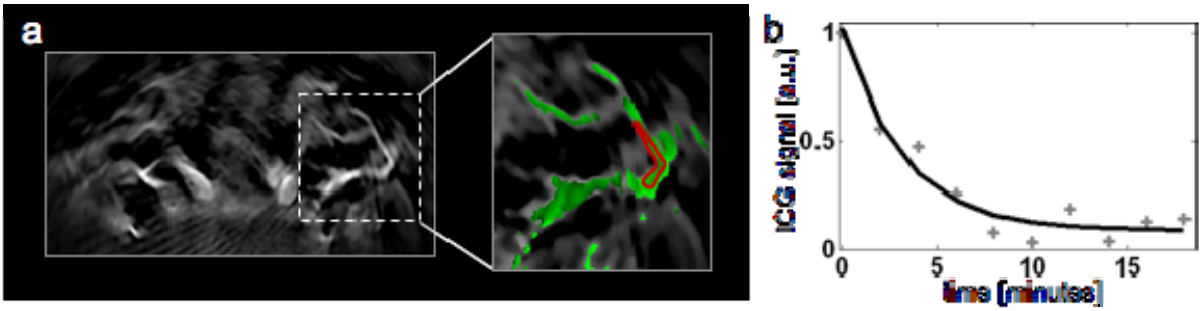

Fig. 3: Imaging the kinetic profile of an exogenous optical agent. a: Optoacoustic image: slice through upper thorax. Inset shows Indocyanine green (ICG) in blood vessel overlaid in green. Red outline indicates manually selected region of interest. b: Relative ICG intensity within the region of interest plotted over time. Crosses represent individual multispectral data points and the line is an exponential fit to those points.

4. DISCUSSION

We have demonstrated, by means of studies on mice, an MSOT imaging platform capable of resolving intrinsic tissue absorbers as well as exogenous optical contrast agents at high rates in-vivo. Motion correction techniques were demonstrated to preserve the high spatial resolution provided by optoacoustic imaging in deep tissue. In particular, the ability to image the myocardium of the left ventricle could provide a valuable tool for imaging of heart disease, especially when combined with molecular optical agents. In addition, high-rate detection of the fate of exogenous optical agents, demonstrated here by resolving ICG in the circulation, has the potential to not only to aid in the characterization

of novel pharmaceuticals, but also to assess such parameters as liver function noninvasively by measuring the plasma disappearance rates of agents taken up by the liver. In fact, since ICG is an FDA approved agent, the technique could already be applied to assessment of liver function in humans by continuously imaging a relatively superficial blood vessel after ICG injection. In general, visualization of characteristics of the vasculature, as well as distributions of exogenous optical agents, could bring valuable new insights in the fields of cancer and cardiovascular imaging. The most important components of this platform are the real-time imaging system, motion correction capabilities, quantitative reconstruction methods and spectral unmixing techniques. Real-time imaging is not only a crucial system characteristic for dynamic imaging applications, which require a high temporal resolution, but also, as shown in this work, allows correction for motion by simple sorting of individual frames into different clusters representing unique phases in the motion.

The described MSOT imaging platform is not without limitations, some of which we will attempt to resolve in future work. For high-rate dynamic imaging, demonstrated in the context of characterizing the pharmacokinetic profile of ICG in the circulation, multispectral excitation is required to isolate ICG from background tissue absorption. However, this greatly slows down the imaging rate, since wavelength tuning requires mechanical scanning in the OPO. To minimize the required acquisition time and thus obtain the best possible temporal resolution, work will be required to provide faster wavelength scanning and studies should aim to determine the minimum amount of wavelengths for robust detection of specific absorbers. A further challenge is that the current MSOT system records images in real-time from a 2D slice, which can be selected by translating the subject by a linear stage. However, in cases where entire organs or tumors should be imaged, this scanning limits the temporal resolution and complicates volumetric quantification. It is therefore of much interest in future developments to extend the real-time imaging capability to the third spatial dimension, enabling simultaneous recording of an entire volume of interest. Naturally, this would bring additional costs in terms of the number of detector elements, acquisition channels and reconstruction computation.

The MSOT imaging platform we describe here shows clear potential to be employed in a clinical setting. The transducer array we employ has elements arranged on an open arc covering around 180°, allowing access to human tissue, for example on the extremities. The real-time capabilities would be essential in this domain, allowing sonography-like live diagnostics. The optimization and assessment of MSOT imaging for clinical applications will be a topic of future work.

5. ACKNOWLEDGEMENTS

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