Best Paper Award

Cardiac function and perfusion dynamics measured on a beat-by-beat basis in the live mouse using ultra-fast 4D optoacoustic imaging

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ABSTRACT

The fast heart rate (~7 Hz) of the mouse makes cardiac imaging and functional analysis difficult when studying mouse models of cardiovascular disease, and cannot be done truly in real-time and 3D using established imaging modalities. Optoacoustic imaging, on the other hand, provides ultra-fast imaging at up to 50 volumetric frames per second, allowing for acquisition of several frames per mouse cardiac cycle. In this study, we combined a recently-developed 3D optoacoustic imaging array with novel analytical techniques to assess cardiac function and perfusion dynamics of the mouse heart at high, 4D spatiotemporal resolution. In brief, the heart of an anesthetized mouse was imaged over a series of multiple volumetric frames. In another experiment, an intravenous bolus of indocyanine green (ICG) was injected and its distribution was subsequently imaged in the heart. Unique temporal features of the cardiac cycle and ICG distribution profiles were used to segment the heart from background and to assess cardiac function. The 3D nature of the experimental data allowed for determination of cardiac volumes at ~7-8 frames per mouse cardiac cycle, providing important cardiac function parameters (e.g. stroke volume, ejection fraction) on a beat-by-beat basis, which has been previously unachieved by any other cardiac imaging modality. Furthermore, ICG distribution dynamics allowed for the determination of pulmonary transit time and thus additional quantitative measures of cardiovascular function. This work demonstrates the potential for optoacoustic cardiac imaging and is expected to have a major contribution toward future preclinical studies of animal models of cardiovascular health and disease.

Keywords: Cardiac Imaging, Mouse Heart, Optoacoustics, Optoacoustic Imaging, Contrast Agents, Indocyanine Green

INTRODUCTION

Mice are widely used as models of human cardiac diseases, largely due to the ability to modify the genes associated with cardiac disease in humans. Transgenic mouse models are used to better understand a wide variety of inheritable cardiac diseases, and surgical methods are used to model coronary artery disease and myocardial infarction[1,2]. As more primary causes of cardiovascular disease and therapeutic agents are discovered, the need to characterize their effects is growing. Functional cardiac imaging in mouse models allows for robust characterization of the effects of disease-related gene and protein alterations, and testing the efficacy of potential therapeutics. As a result, much of our understanding of the mechanisms underlying human cardiac health and disease come from studies of the murine heart. However, delineating correlations and causations of cardiac disease requires investigating the link between function on the molecular and organ levels, preferably in studies of the same heart.

Optoacoustic imaging holds multiple prospective advantages for cardiac imaging in that it: 1) provides high spatial and temporal resolution; 2) possesses optical contrast, and hence can be used in combination with biologically-targeted contrast agents, allowing for characterization of molecular-level dysfunction, and; 3) has the advantage of deeper penetration, allowing for imaging of cardiac function truly in-vivo in the intact heart. Herein, we present a volumetric optoacoustic tomographic system capable of cardiac imaging at never-seen-before time resolution of 50 volumetric frames per second. Furthermore, we demonstrate its applicability to approximate blood oxygenation levels in the heart chambers and to track blood flow in the heart by visualizing the distribution of intravenously-administered indocyanine green.

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METHODS

Experimental setup

The layout of the experimental setup is depicted in Fig. 1a, and is based on a custom-made spherical array of 256 piezoelectric elements (Imasonic, SaS, Voray, France), designed to efficiently detect optoacoustic signals generated in a region close to the center of the sphere[3]. Optical illumination is provided by means of a fiber bundle inserted in a central cavity of the array. A short-pulsed wavelength-tunable laser with a pulse repetition rate of 50 Hz was used as light source (Innolas Laser GmbH, Krailling, Germany). The 256 optoacoustic signals were simultaneously acquired, providing a signal length of 2030 samples at a rate of 40 MSPS, using a custom-made data acquisition system (Falkenstein Mikrosysteme GmbH, Taufkirchen, Germany). The size and orientation of the transducer elements provided suitable signal to noise ratio (SNR) at this pulse repetition rate, even at laser energy levels below laser safety standards[4]. Agar and ultrasound gel were used to efficiently couple the mouse skin to the transducer elements. Additionally, both agar and the ultrasonic gel are transparent in the illumination wavelengths used (730-850nm), thus ensuring efficient laser light transmission to the heart.

In-vivo mouse handling

An adult wild-type male CD1 mouse was used in the experiments. Prior to the experiment, the imaging area was shaved using a razor and depilation cream to optimize artefact-free ultrasound transmission. The mouse was anesthetized during the experiments using a mixture of isoflurane and medical air (2% isoflurane) at a flow rate of 0.8 L gas per min. While under anesthesia, two measurements were made: one multispectral experiment, and another indocyanine-green injection experiment. In the first multispectral experiment, the mouse heart was imaged at four different wavelengths (730, 760, 800, and 850 nm), corresponding to different absorption values of oxygenated and deoxygenated hemoglobin. In a second experiment, cardiac imaging was performed during an intravenous tail-vein injection of 100 nmol indocyanine green (ICG). Several seconds of data were acquired at a single wavelength (800 nm) corresponding to the peak absorption of ICG. The experiments were performed according to institutional and the District Government of Upper Bavaria regulations regarding animal care.

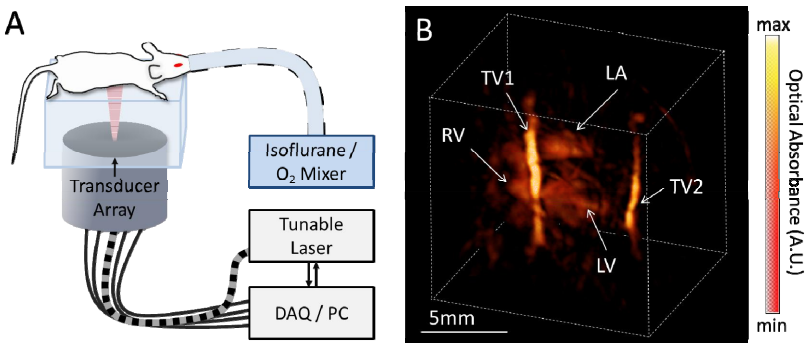

Figure 1: Schematic of the experimental setup (A), and representative 3D optoacoustic image of the mouse heart (B). Important structures are identified and annotated in the optoacoustic image: RV, right ventricle; LV, left ventricle; LA, left atrium; and TV1 and TV2, thoracic vessels 1 and 2.

Data processing

The 256 optoacoustic signals corresponding to each 256 detector elements (each with central frequency 4 MHz) were band-pass filtered using cut-off frequencies between 0.1 and 7.5 MHz. Three-dimensional images were reconstructed on a frame-by-frame basis for a volume of interest of 14x14x12 mm^3 using a back-projection formula, as described elsewhere[5]. A representative volumetric frame of the mouse heart is shown in Fig. 1b.

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RESULTS AND DISCUSSION

Multispectral imaging of the heart

Fig. 2a shows optoacoustic images acquired at each illumination wavelength (730, 760, 800, and 850 nm) used in this study. The images are represented as maximum intensity projections (MIP) along the z-direction for the three-dimensional data. These wavelengths were chosen based on the differences in the absorption by oxy- and deoxygenated hemoglobin, thus allowing for distinction between highly oxygenated or deoxygenated cardiac structures. In fact, the regions marked LA and TV1 in Fig. 2a markedly increase in optical absorbance as illumination wavelength increases, which correlate to the absorbance spectra of oxygenated hemoglobin (spectrum not shown). Thus, the data indicate that the LA and TV1 are structures containing highly-oxygenated blood. Thus, while anatomical and dynamic information are available in single-wavelength images (Fig 1b), further functional information was derived from multi-wavelength images (Fig 2a).

Three-dimensional imaging of the murine cardiac cycle

The potential for visualizing fast cardiac cycle dynamics was further demonstrated by acquisition of optoacoustic images at each wavelength at 50 Hz. Fig. 2b depicts volumetric images of four time points acquired within one mouse cardiac cycle at 800 nm wavelength. The time points chosen for Fig 2b iterate at every other imaging instance for the purpose to show an entire cardiac cycle. In an attempt to quantify cardiac dynamics, the optoacoustic signal was monitored over time at spatial coordinates corresponding to the left atrium and left ventricle, respectively. Fig. 2b showcases the fast time profile of the optoacoustic signal at P1 and P2, as indicated by red and blue data traces, respectively. The high temporal resolution of the cardiac dynamics can also be qualitatively appreciated by the several time points analyzed per cardiac cycle, and in the movies showcased at the congress. Fig. 2c depicts the optical absorption of points located on the left ventricle (P1), or left atrium (P2). Of particular interest was that the periodicity of the cardiac cycle, and asynchronous motion and flow of blood into the ventricle and atrium was readily observed using optoacoustic imaging. These findings demonstrate the ability for volumetric optoacoustic imaging to identify the cardiac cycle and quantify functional, based on contrast alone, on a beat-by-beat basis.

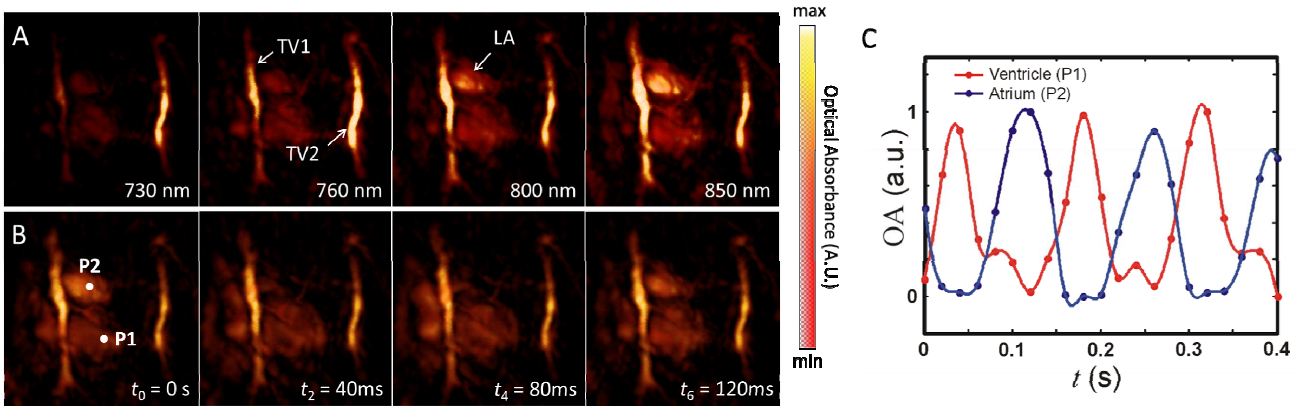

Figure 2: Multispectral optoacoustic images of the heart (A), revealing highly oxygenated structures (TV1 and LA) based on the increased optical absorption with increasing wavelength. Imaging was performed at a fast 50 volumetric frames per second, and panel B shows volumetric reconstructions at every other time point to show multiple phases of one complete cardiac cycle. Optical absorptions at points located at the left ventricle and left atrium (P1 and P2, respectively), are plotted over time (C), demonstrating the potential for robust determination of cardiac function parameters - at high temporal resolution - based on the absorbance profiles in each chamber.

Real-time volumetric tracking of blood perfusion in the cardiac chambers

Volumetric tracking of blood perfusion was visualized by injection of indocyanine green (ICG), as described in the methods section. Optoacoustic images at four representative time instances corresponding approximately to the same phase of the cardiac cycle are shown in Fig. 3a. Absorption values for two voxels of interest in the right ventricle (V1) and left ventricle (V2) are plotted as a function of time in Fig. 3b. An increase in absorption is first perceived in the right side of the heart (left side of the image), whereas the maximum absorption in the left side of the heart (right side of the image) is delayed by several cardiac cycles. As shown in Fig. 3b, the difference in the time (Δt) to peak values of ICG absorbance in the RV and the LV is ~1.2 seconds. Physiologically, this parameter estimates the time for blood to travel through the pulmonary circulation (from the RV to the LV) and is referred to as pulmonary transit time.

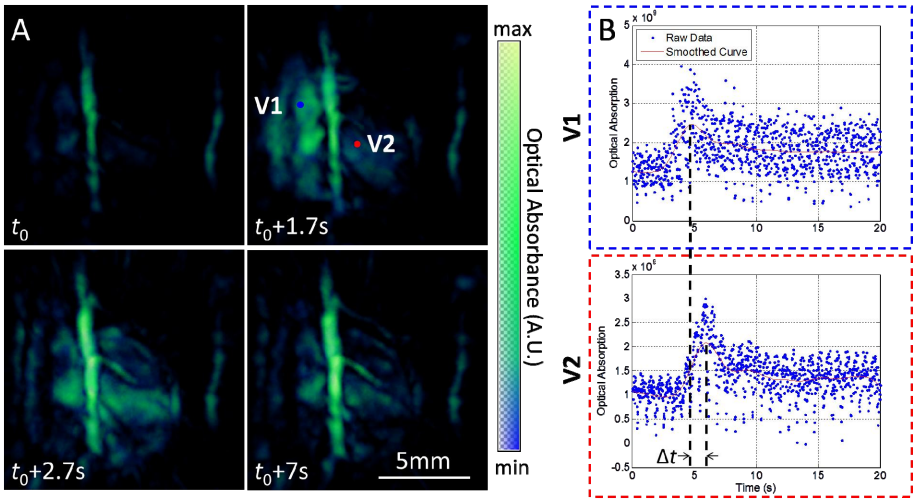

Figure 3: Real-time tracking of blood-flow dynamics by visualizing the time-series of optoacoustic absorbance in the heart following ICG administration (A). ICG can be seen first entering the right ventricle (indicated by point V1), and later reaching the left ventricle (indicated by point V2). The time profile of ICG distribution was smoothed and plotted (B), from which the difference in time to peak in ICG absorbance from RV to LV (Δ*t*) was used to estimate pulmonary transit time.

CONCLUSIONS

The results presented herein showcase the unique capability of four-dimensional optoacoustic tomography for studies of cardiac dynamics with high spatio-temporal resolution necessary for characterizing fast beating mouse heart in vivo. Versatility of the system in resolving functional and, potentially, molecular contrast is enabled via fast acquisition of images at multiple excitation wavelengths. In this way, basic cardiac functional parameters were derived here from the intrinsic spectral dependence of oxy- and deoxy-hemoglobin as well as the dynamics of the extrinsically-administered ICG contrast agent. Left and right cardiac chambers were distinguished based on spectral dependence of the optoacoustic signals in the respective regions of interest. In addition, high temporal-resolution tracking of blood flow in the heart was demonstrated by visualizing distribution of the ICG optical probe following intravenous injection and allowed for further distinction of left and right cardiac chambers. Due to the adequate penetration of optoacoustics, these measurements were possible non-invasively, without the need to open the chest of the animal. In conclusion, we have shown the capabilities of volumetric optoacoustic cardiac imaging at unprecedented time resolution. In combination with its unique advantages of high spatial resolution and functional and molecular optical contrast, the presented cardiac imaging approach anticipates a broad range of applications that require fast dynamic visualization of entire volumes currently unfeasible with other imaging modalities.

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