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Imaging multi-scale dynamics in vivo with spiral volumetric optoacoustic tomography

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ABSTRACT

Imaging dynamics in living organisms is essential for the understanding of biological complexity. While multiple imaging modalities are often required to cover both microscopic and macroscopic spatial scales, dynamic phenomena may also extend over different temporal scales, necessitating the use of different imaging technologies based on the trade-off between temporal resolution and effective field of view. Optoacoustic (photoacoustic) imaging has been shown to offer the exclusive capability to link multiple spatial scales ranging from organelles to entire organs of small animals. Yet, efficient visualization of multi-scale dynamics remained difficult with state-of-the-art systems due to inefficient trade-offs between image acquisition and effective field of view. Herein, we introduce a spiral volumetric optoacoustic tomography (SVOT) technique that provides spectrally-enriched high-resolution optical absorption contrast across multiple spatio-temporal scales. We demonstrate that SVOT can be used to monitor various *in vivo* dynamics, from video-rate volumetric visualization of cardiac-associated motion in whole organs to high-resolution imaging of pharmacokinetics in larger regions. The multi-scale dynamic imaging capability thus emerges as a powerful and unique feature of the optoacoustic technology that adds to the multiple advantages of this technology for structural, functional and molecular imaging.

Keywords: Optoacoustic tomography, photoacoustic tomography, multiscale imaging, multiscale dynamics, real-time imaging, whole-body imaging.

1. INTRODUCTION

Advancements in life sciences are directly related to the feasibility to non-invasively observe the behavior of living organisms at different levels. In this regard, imaging modalities play an essential role in biological discovery as well as in the diagnosis and treatment monitoring of many human diseases.[1] Traditionally, the standard and most widely accepted approach to interpret an image consists in resolving structural shapes. Increasing the spatial resolution comes however generally at the expense of other important features that yield alternative information. For example, molecular imaging methods enable sensing specific targets associated to cellular and sub-cellular processes,[2] whose importance stems from the fact that molecular changes precede structural changes in disease evolution and treatment response. On the other hand, the temporal resolution is also an essential feature for *in vivo* imaging, where the understanding of many biological processes requires dynamic visualization of samples. Considering a limited data acquisition rate, the temporal resolution is inversely proportional to the spatial resolution and the field of view, and a trade-off is generally established depending on the particular interest.

The importance of the trade-off between different imaging features is manifested in the wide use of optical microscopy, which remains a workhorse tool in biological research despite its limited spatial resolution as compared e.g. with electron microscopy techniques.[3] Indeed, optical imaging provides unique advantages to image dynamics of living samples non-invasively as well as to visualize sub-cellular structures with protein-specific contrast. However, the limited penetration of ballistic photons within biological tissues represents a strong barrier in optics. Although diffuse-optics-based techniques can still provide valuable molecular information at depths up

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to a few centimeters in tissues, the very limited spatial resolution achieved prevents structural imaging beyond the optical diffusive limit (≈ 1 mm).[4]

In the last decade, high (ultrasound) resolution imaging of optical contrast in the diffuse regime has been enabled with optoacoustic (OA, photoacoustic) imaging techniques,[5,6] while optical-resolution microscopic imaging systems have also been suggested by using this technology.[7,8] OA then offers the unique capability to bridge the gap between the microscopic and macroscopic realms with the same type of contrast.[9] On the other hand, the multidimensionality of OA data allows for different means to independently extract information from a sample. Anatomical structures can e.g. be identified in the volumetric (three-dimensional) maps of light absorption.[10–12] Also, the wavelength dimension in multispectral OA systems provides the specificity required for molecular imaging,[13,14] where any substance absorbing light can potentially be used as a contrast agent. Finally, high-frame-rate imaging of fast dynamic events is possible by simultaneously acquiring a sufficient number of signals with each laser pulse.[15] Recently, it was shown that the real-time imaging capability can be preserved in three-dimensional multispectral OA imaging with fast wavelength-tunable lasers, so that the five dimensional (3 spatial dimensions + wavelength + time) imaging capacity can be fully exploited.[16] The temporal resolution of OA tomographic imaging systems can then be significantly higher than in other macroscopic imaging technologies, with the additional advantage of multispectral specificity.

Herein, we describe an OA imaging approach that can offer the unique capability to bridge the existing gap in the observation of dynamic biological events occurring at different time scales. This approach, termed spiral volumetric optoacoustic tomography (SVOT) enables efficiently coregistering dynamic processes at multiple time scales by scanning a spherical piezoelectric transducer array around the sample to be imaged. The temporal resolution of the system inversely scales with the size of the scanned region, while imaging at a frame rate determined by the pulse repetition rate of the laser is possible for an area corresponding to a given position of the transducer. The feasibility of imaging multiscale dynamics is showcased in different biological processes.

2. MATERIALS AND METHODS

2.1 Spiral volumetric optoacoustic tomography set-up

A lay-out of the suggested spiral volumetric optoacoustic tomography (SVOT) set-up is depicted in Fig. 1. Basically, a spherical transducer array was used to simultaneously collect 256 optoacoustic signals, which allow rendering a volumetric (three-dimensional) image of a specific region with a single laser pulse as described in Ref. 17. In SVOT, the transducer is scanned following a spiral trajectory by means of a rotation stage with 360° angular coverage (IAI Inc., Japan) and a translation stage with 15 cm range (IAI Inc., Japan).[18] In the experiments, the center of the spherical array was positioned at a distance of approximately 5 mm from the rotation axis. The elements of the transducer array have a central detection frequency of 4 MHz and a -6 dB bandwidth of 100%. Their size (3×3 mm^2) ensures an acceptable signal-to-noise ratio of the acquired signals, providing an almost isotropical resolution of 200 μm over a field of view of approximately 1 cm^3.[17] The optoacoustic signals were digitized at 40 MSPS with a custom-made data acquisition system (Falkenstein Mikrosysteme GmbH, Taufkirchen, Germany) triggered with the Q-switch output of the laser. Both the data acquisition and the motorized stages were controlled with a PC using MATLAB. On the other hand, an optical parametric oscillator (OPO)-based laser was used as an optoacoustic excitation source. The laser provides short pulses (≈ 10 ns) with energy per pulse of approximately 30 mJ and a pulse repetition frequency up to 100 Hz. The wavelength of the laser can be tuned on a per-pulse basis within the range $700 - 900$ nm. The light beam was guided through a custom-made fiber bundle (CeramOptec GmbH, Bonn, Germany) inserted into a central cavity of the array. The output of the bundle provides an approximately Gaussian illumination profile at the tissue surface with a full-width at half maximum of approximately 10 mm and ≈ 20 mJ energy. The imaged mice were placed in a specifically-designed holder attached to the bottom of a water tank. During the measurements, the mouth of the mouse being imaged was inserted in a gas anesthesia mask attached to the holder. The water in the tank ensures optimum ultrasound transmission.

2.2 Image reconstruction and unmixing

Image reconstruction of individual frames was performed with a graphics processing unit (GPU) implementation of a back-projection reconstruction formula as described in Ref. 19. Image formation of a large region of

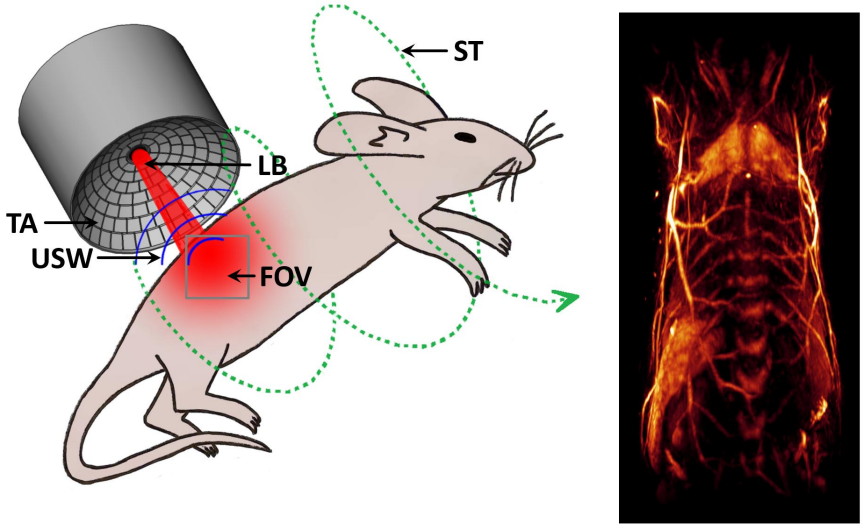

Figure 1. SVOT imaging concept. Data acquisition is performed with a spherical array following a spiral (helical) scanning trajectory surrounding the sample (mouse). TA - transducer array, LB - laser beam, USW - ultrasound waves, FOV - field of view, ST - spiral trajectory. The whole-body image obtained by combining all three-dimensional images acquired during the scan is also shown.

the mouse was subsequently performed by combining the volumetric images for each position of the array. For this, the position and orientation of the spherical array with respect to the rotation axis were accurately calibrated prior to each experiment by performing a rotation scan of an agar phantom containing a 100 μm polyethylene microsphere.[18] A clustering approach was further applied to remove the influence of breathing-associated artefacts in the whole-body images. For this, 50 frames were acquired for each position of the spherical array and the correlation matrix of the frames series was calculated. A k-means sorting algorithm was applied to the thresholded correlation matrix, so that only 35 frames were removed, which are not affected by respiratory motion. On the other hand, unmixing of specific chromophores was performed by least-square fitting the reconstructed optical absorption at multiple wavelengths to the molar extinction coefficients of the chromophore of interest and oxygenated and deoxygenated hemoglobin as described in Ref. 20. While cross-talk is generally produced with this approach due to wavelength-dependent optical attenuation, it yields robust results for the concentration of the probes used in this work.

2.3 Imaging experiments

Two experiments in female athymic nude-Foxn1^{nu} mice (Harlan Laboratories LTD, Switzerland) are showcased in this work in order to illustrate the multi-scale dynamic imaging capability of SVOT.[21] The mice were imaged according to institutional guidelines of the Helmholtz Center Munich regarding animal handling and with approval from the Government District of Upper Bavaria. The mice were anesthesized with isoflurane (2-3% by volume, 0.9 l/min gas flow) and the eyes were covered with vet ointment (Bepanthen, Bayer AG, Germany) to prevent dehydration and to ensure protection from the laser. The water temperature in the tank was kept at 34°C to stabilize the body temperature of the mouse during data acquisition.

In a first experiment, the real-time capacity of the SVOT system was showcased by positioning the focus of the spherical array approximately in the heart region. 200 frames were acquired at this position for a 100 Hz pulse repetition frequency of the laser, where the wavelength of the laser was set to 800 nm. A whole-body scan of the mouse at the same wavelength was subsequently performed.

The ability of SVOT to observe slower pharmacokinetics in a larger region was further demonstrated by imaging the renal clearance of a small-molecule-based contrast agent, which occurs on a timescale of seconds to minutes. Specifically, 10 nmol of AlexaFluor 750 diluted in 200 μl of phosphate-buffered saline (PBS) were

injected in the tail vein of a mouse and the unmixed bio-distribution in a relatively large region of the mouse abdomen containing both kidneys was monitored. Unmixing for AF750 was performed with images acquired at 710, 730, 745, 765, 800, 850 and 900 nm.

3. RESULTS

Fig. 2 displays maximum intensity projections (MIPs) of the reconstruted heart images for consecutive time points. The heart images are superimposed to the whole-body image of the mouse, which serves as a valuable anatomical reference. The high-frame-rate available for a single position of the spherical array enables the identification of different phases of the heart cycle, which is challenging with other imaging modalities for murine heart rates of approximate 400-600 beats per minute.

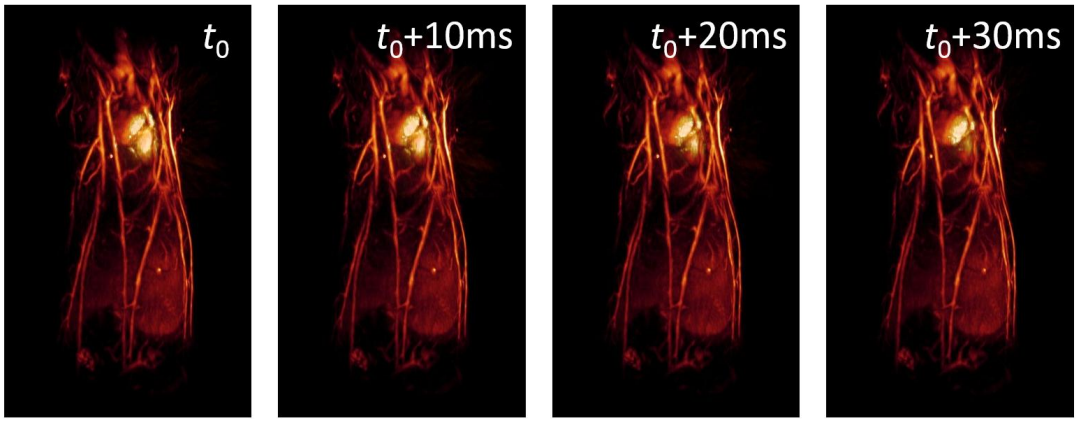

Figure 2. Real-time monitoring of the murine cardiac cycle. Four consecutive frames are shown superimposed to the whole-body image at 800 nm.

Fig. 3 shows the unmixed bio-distribution of AF750 (green) superimposed to the anatomical image (acquired at 800 nm) of the same area. The AF750 signal in the renal artery reaches a maximum value within 5 minutes post injection and rapidly decays. On the other hand, the AF750 signal in the kidney cortex also increases up to a maximum value in the first 5 minutes after injection, but decays at a slower rate. Finally, the AF750 signal in the renal pelvis the signal starts increasing after 5 minutes post-injection and subsequently reaches a plateau, which is consistent with the elimination of the agent towards the ureter.[21]

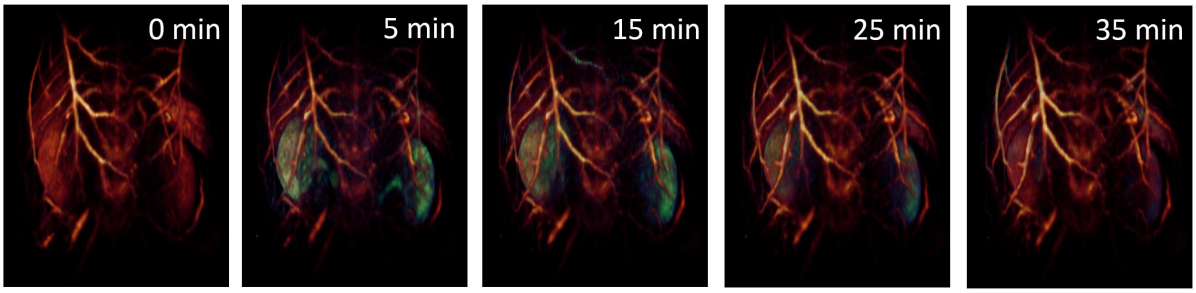

Figure 3. Renal clearance of AlexaFluor 750. The unmixed bio-distribution of the agent is displayd in green superimposed to the three-dimensional image at 800 nm.

4. DISCUSSION AND CONCLUSIONS

The new spiral volumetric optoacoustic tomography (SVOT) described herein has been shown to provide consistent high-resolution imaging performance in living organisms across multiple spatio-temporal scales using the

same optical absorption contrast. This represents a significant advantage with respect to commonly applied imaging paradigms relying on different imaging modalities to provide information at different spatio-temporal scales. However, combinations of different modalities are often associated with inefficient trade-offs in terms of effective field of view, temporal resolution and contrast mechanims rendered with each specific system. The presented experiments in mice have showcased a wide range of multi-scale imaging capabilities for the SVOT method, from beat-to-beat visualization of the murine heart to slower contrast agent kinetics in selected areas covering the entire renal system. Whole-body tomographic imaging with SVOT is also improved with respect to e.g. cross-sectional imaging systems strongly affected by out-of-plane artefacts.[22] Multi-scale imaging of dynamics is of importance in many areas of biological research. For example, an essential issue in cancer research is to dynamically monitor the physiologic microenvironment of neoplastic lesions during the evolution of the disease. Understanding brain function implies dynamic observations of the complex structural and functional connectivity networks between the distinct units of the nervous system, so that multi-scale dynamic OA imaging. Hence, multi-scale dynamic OA imaging can also greatly impact neuroscience research. The feasibility of imaging dynamics at multiple time scales adds to the multifarious advantages provided by the OA technology that drive the strongly-growing interest towards this modality. The OA technological development is heavily fostered by the promising prospects towards clinical translation.[6, 23–25]

In conclusion, the ability to deliver high resolution images at the whole-body scale along with high-frame-rate three-dimensional imaging capability in smaller regions with the same type of contrast and the same set-up is a unique feature of SVOT with respect to other existing pre-clinical imaging methods. In addition, as opposed to non-optical imaging methods, such as MRI, CT or ultrasound, SVOT has the powerful contrast advantages of optical interrogation methods, including the ability to visualize rich functional and molecular information on blood oxygenation as well as targeted delivery of contrast agents and genetic labels.[4] However, contrary to optical microscopy, SVOT does not suffer from spatial resolution degradation in deep tissues associated to scattering of light. Overall, it is anticipated that further technological developments will evolve in parallel with the onset of new biomedical applications, and the growing use of this technology by researchers and physicians is poised to revolutionize biology and medicine.

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