High-resolution imaging of mouse anatomy with a multi-purpose optoacoustic tomography system.

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ABSTRACT

Optoacoustic imaging can noninvasively provide visualization of vasculature structures with optical contrast well below the skin surface. For meaningful biological investigations, high-resolution at mesoscopic penetration depths and therefore thorough adaption of the systematic arrangement to the object of interest is required. A suitable modular optoacoustic tomography system is presented here and its performance is demonstrated in three exemplary studies on imaging agar phantoms, mouse head vasculature and mouse tumor vasculature ex-vivo.

1. INTRODUCTION

Pre-clinical studies on pharmaceuticals have shown the need to visualize vasculature with high resolution for manifold applications like investigations on the nervous system, tumors or the cardiovascular system itself. [1] Currently visualization of complex biological processes like tumor growth in vivo is difficult due to the various technological demands. Unlike in ex-vivo high-resolution imaging, which can be done via methods based on x-ray computed tomography (XCT) or selective plane illumination microscopy (SPIM), in vivo the tumor must stay intact. As one of the few imaging methods to cope with this, optoacoustic tomography provides it by avoiding the scattering of the information before it is detected. Based on the conversion of ultra short high energy light pulses to pressure waves and the therewith associated reduction of scattering effects, it can overcome the high-resolution imaging depth of purely optical imaging methods. Not only has it been accepted as a powerful method for imaging the anatomy and physiology in mice but it also provides molecular imaging capabilities like the detection of chromophores. [2,3]

In this work we present a modular optoacoustic system which is well-suited for imaging not only superficial vasculature in mice, especially demonstrating its suitability for head vasculature and subcutaneous tumor vasculature imaging in ex-vivo experiments. Going beyond the experimental options of the systems described in [4] and [5], this system allows fast adaption of illumination and ultrasound detection to various sample geometries by its modular conception.

2. EXPERIMENTAL SYSTEM

The samples are excited by nanosecond light pulses from an optical parametric oscilator (OPO) laser (OPOTEK, Carlsbad, CA, USA) at a repetition rate of 20Hz, and apart from the pump beam at 532nm the OPO can tune in the 700-900 nm range, making it attractive for molecular imaging by multispectral optoacoustic tomography (MSOT). The beam size is firstly adjusted to the region of interest by lenses aligned as a Galilean telescope. All lenses are anti-reflection coated and the light paths in water are kept as short as possible to reduce losses in pulse energy.

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Considering a tradeoff between out-of-plane illumination, projection-loss artifacts and the area accessible for illumination of the sample, two types of illumination turn out to be the best suitable:

1. bilateral coplanar illumination for out-of-plane artifact-free images at 532nm.

   The beam has to be split in two arms - then it enters the water tank vertically to the water surface (minimal reflectivity).

2. single spot: a top illumination in the NIR and for high surface sensitivity at 532nm.

   Especially where the region of interest is not accessible laterally, like in imaging head vasculature, the illumination must occur from the top.

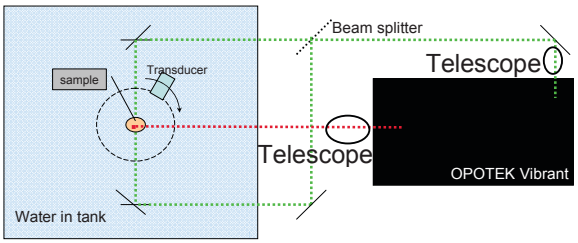

Image 1: Top view schematic of the setup showing two common illuminations

The ultrasonic detection is provided by a high-frequency cylindrically focused transducer (Panametrics NDT V319). Its resonance frequency, bandwidth, focal length and element diameter are 15.28MHz, 47.85% of the central frequency, 0.757in. and 0.5in. respectively, leading to a numeric aperture of 1.51. At the central frequency, the -6db length and width of its focal zone value 1.75mm and 0.2mm and both can be further decreased by filtering the signal for higher frequencies which is made possible by the large bandwidth. Due to the full tomographic view, the in-plane resolution is given by the frequency band and values 32µm.[5] Acoustic signals are recorded by a digital acquisition card (GaGe Octopus) at a sampling frequency of 100MHz via Labview.

In order to create precise tomographic images with high resolution, the speed of sound and therefore the water temperature must be kept as constant as possible. This is addressed by a two-tank technology with continuous flow where the tanks are separated in height. The water is heated at the lower level and pumped to the measurement tank at higher level from where it can flow back to the lower one. The bottom of the measurement tank is covered by a threaded aluminum plate for easy fixation of additional optics, devices and samples.

The optoacoustic images are reconstructed with a back-projection algorithm [5], after bandpass filtering the acoustic signals in the 2-30 MHz range. For a region of interest that is up to 10 mm and a resolution of 300x300 pixels it is possible to resolve sub 30µm structures.

3. RESULTS AND DISCUSSION

1. Imaging Phantoms

To evaluate the performance of the system, multiple phantom measurements with tissue-like absorption and scattering are conducted. An agar phantom with a hexagonal insertion is prepared (Agar powder: Sigma-Aldrich) containing Intralipid and black india ink leading to a reduced scattering coefficient of $\mu_{s'}$=10/cm and absorption coefficient values of μ_a=0.2/cm for the background and μ_a=1/cm for the insertion. The illumination geometry used in this experiment is bilateral at 532 nm and a typical reconstructed image is shown in Fig. 2.

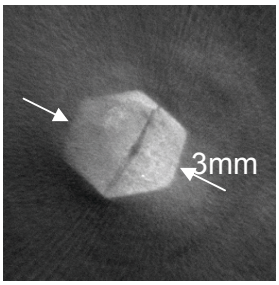

Figure 2: Tissue-mimicking phantom. The insertion in shape of a hexagonal key is five times more absorbing than the background. Fine substructures inside the insertion can be seen.

2. Imaging Head Vasculature

This experiment shows the different penetration of red and green light into tissue aiming at the visualization of mouse head vasculature with a setup arrangement similar to Ref. [4]. A CD1 nude mouse has been euthanized and positioned so the brain axis is oriented horizontally. The light at both wavelengths (700nm and 532nm) has been arranged to illuminate the head from the top. The results in Fig. 3, show the vasculature down to the level of the skull. In red illumination, the deeper positioned mid-cranial artery appears in strong contrast whereas microvasculature of subcutaneous vessels can be visualized in the green. By unweighted overlay of both images, a projection can be generated.

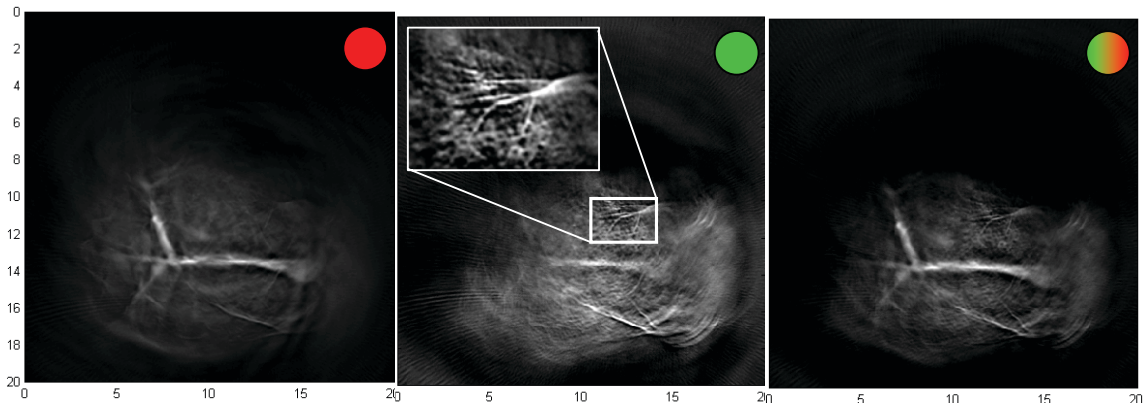

Figure 3: Head vasculature of a mouse at 700nm (left) and 532nm (center) light wavelength and an unweighted sum overlay of both.

3. Imaging tumor vasculature

A subcutaneous mouse cancer model has been imaged to evaluate the performance of the system to resolve microvasculature. Here, 0.5x106 4T1 cells have been implanted centrally on the back of a CD1 nude mouse and imaged ten days after when the diameter of the tumor has valued ca. 8 mm. The mouse has then been euthanized and positioned so that the tumor is illuminated from the top and carefully placed in the center of the field of view of the transducer. The tumor has been imaged using 532 nm excitation to exploit the high intrinsic contrast of hemoglobin in the blood vessels. Acoustic signals have been acquired at 900 angular projections and for multiple slices at a step size of 150 µm. Representative reconstructions at three slices (top, middle, bottom) are shown in Fig. 4. In the reconstructed images the microvasculature of the tumor is shown at an ultra high resolution.

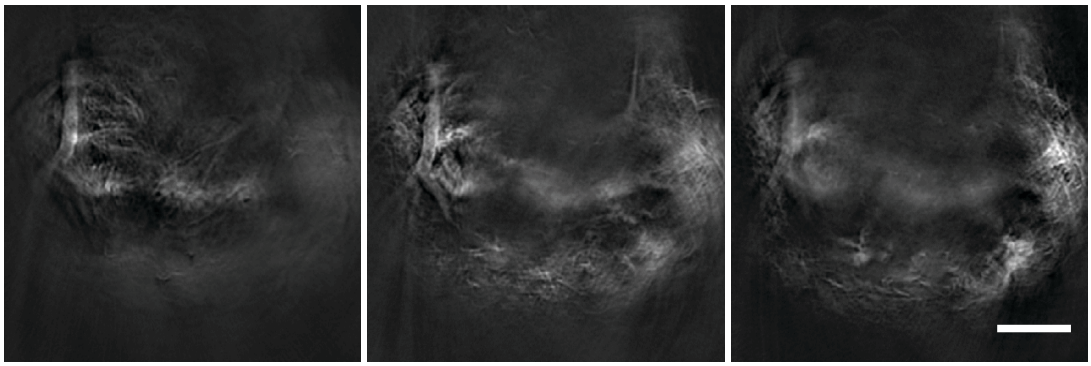

Figure 4 Optoacoustic images of a 4T1 tumor centered on the back of a mouse.
Vascular structures that are located below the skin are visualized at different levels (top, center, bottom). Scale bar: 2mm

4. CONCLUSION

In this work we have built and evaluated the performance of an optoacoustic tomography system capable to image vascularization in high resolution. Such a system can significantly aid research imaging studies in functional and molecular imaging of diseases and the corresponding drug development. By thorough adaption of illumination and detection geometry to tumors, e.g., significantly thinner slices can be recordable, opening the gates for simultaneous isotropic resolution and detection of multiply sized anatomical and pharmaceutical structures.

REFERENCES

[1] Maslov et al., „Optical-resolution photoacoustic microscopy for in vivo imaging of single capillaries“, Opt. Lett. 33, 9 (2008)

[2] Bühler et al., „Video-rate optoacoustic tomography of mouse kidney perfusion“, Opt. Lett. 35, 14 (2010)

[3] Taruttis et al., „Real-time imaging of cardiovascular dynamics and circulating gold nanorods with multispectral optoacoustic tomography“, Opt. Exp. 18, 19 (2010)

[4] Wang et al., „Noninvasive laser-induced photoacoustic tomography for structural and functional in vivo imaging of the brain“, Nat. Biotech. 21, 7 (2003)

[5] Ma et al., „Multispectral optoacoustic tomography (MSOT) scanner for whole-body small animal imaging“, Opt. Exp. 17, 24 (2009)