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Structural and Functional Small Animal Imaging Using Hybrid-Focus Optoacoustic Biomicroscopy

Johannes Rebling[a,b], Héctor Estrada[a], Sven Gottschalk[a], Daniel Razansky[a,b]
[a]*Institute for Biological and Medical Imaging (IBMI), Helmholtz Zentrum München, Neuherberg 85764, Germany;*
[b]*Technical University of Munich, Munich 81675, Germany;*
Author e-mail address:dr@tum.de

Abstract: We present an optoacoustic microscope, combining structural and functional optical-resolution optoacoustic and ultrasound pulse-echo imaging. The system was applied to image Zebrafish larvae and complex vascular networks in the murine brain and ear.
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1. Introduction

Optoacoustic (OA) imaging with its combination of rich optical absorption contrast and ultrasound (US) depth penetration has emerged as a widely applicable biomedical imaging modality. The OA effect enables the *in-vivo* imaging of endogenous chromophores and exogenous contrast agents in small model organisms [1–4]. OA microscopy (OAM) has been employed for the imaging of mouse vasculature [5,6] and was successful in the visualization of zebrafish [7,8]. However, optical-resolution OAM is impeded by a limited field-of-view (FOV) and depth of focus [9,10] while acoustic-resolution OAM suffers from slow scanning speeds, insufficient spatial resolution and requires time-consuming reconstructions [11,12].

Here, we present a hybrid ultrasound and dual-wavelength optical-resolution optoacoustic microscope for in-vivo imaging of small model organisms such as zebrafish and mice. The microscope is capable of fast, in-vivo morphofunctional imaging of small model organisms. HFOAM is based on the coaxial alignment of the optical and acoustic foci within a fast moving scan-head that is continuously scanned over the sample by means of fast linear stages, providing both rapid 3D images while not sacrificing a large FOV. HFOAM is also capable of imaging in ultrasound pulse-echo mode, allowing the extract additional anatomical data. We display in-vivo morphological and functional imaging of Zebrafish larvae, as well as the mouse ear and mouse brain.

2. Methods

A schematic of the HFOAM biomicroscope is depicted in Fig. the system employs a fast moving scan head with a spherically focused US transducer which is coaxially aligned with a gradient-index lens which focuses nanosecond pulsed laser light into the sample, as show on the left in Fig 1.

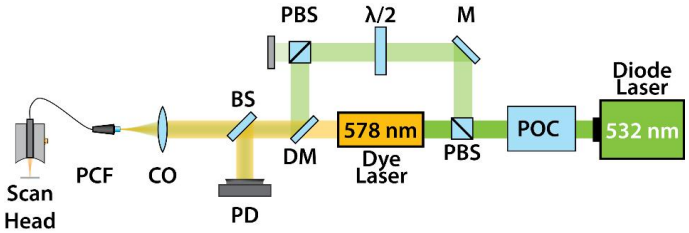

Fig. 1. Schematic illustration of the hybrid-focus optical-resolution optoacoustic microscope (HFOAM) with a fast moving scan head shown on the left and the dual-wavelength optics shown on the right. POC - Pockels cell, PBS - polarizing beam splitter, λ/2 - wave plate, DM - dichroic mirror, BS - beam sampler, CO - collimating lens, PD - photodiode.

Optoacoustic signals are generated by local absorbers in the sample (e.g. hemoglobin or melanin) and the generated signals are recorded by the US transducer and digitized and processed using a custom MATLAB script. Fast wavelength switching is performed using a Pockels cells and polarizing optics, as shown on the right of Fig. 1. The switching of wavelengths between an isosbestic point of hemoglobin at 532 nm and a non-isosbestic point at 578 nm enables functional imaging of the vasculature [13,14]. The scan head can acquired 3D images over a FOV of 10x10 mm² with a spatial resolution of 12 µm within less than 2 minutes [6,15]. The HFOAM system was used to obtain *in-vivo* morphological images of 6-day-post-fertilization (dpf) wild type Zebrafish larvae as well as images of 6 and 14 week old nude-Foxn1nu mice. The 6 dpf zebrafish larvae were anesthetized in MS-222, mounted in 0.5% low melting agarose prepared with fish water and were imaged in a Petri dish filled with fish water for acoustic coupling. The nude mice were

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anaesthetized with isoflurane and fixed in a stereotactic holder. The mouse brain was imaged in both OA and US mode with the scalp removed but with the skull intact. All procedures involving animals and their care conform to the institutional guidelines and with approval from the Government of Upper Bavaria.

3. Results and Discussion

Figure 2 showcases the ability of the HFOAM system to accurately image small model organisms such as 6 dpf zebrafish larvae. Figure 2a) shows a conventional bright-field microscope image, stitched from three separate images while Fig. 2b) shows the corresponding OA maximum amplitude projection (MAP). It is evident from Fig. 2) that the HFOAM system is capable of high-resolution structural imaging of the absorbing melanocytes in the zebrafish, as the acquired OA image in Fig. 2b) closely resembles the bright-field image shown in Fig. 2a). The HFOAM system furthermore provides 3D structural information and hence yields information about the volumetric distributions of the melanocytes, which cannot be acquired with conventional bright-field microscopy.

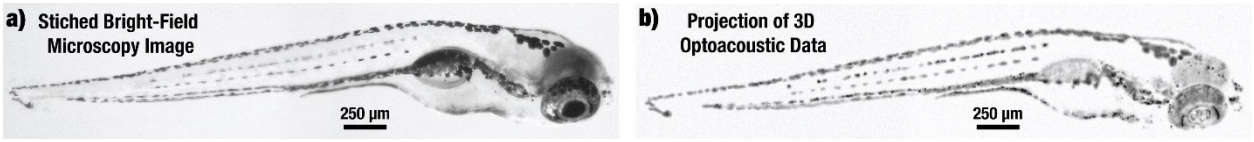

Fig. 2. Comparison of morphological bright-field and optoacoustic microscope images of a 6 days-post-fertilization wild type zebrafish larva. a) Bright-field microscope image created by stitching three overlapping images recorded using an epi-illumination USB microscope. b) Two-dimensional maximum amplitude projection of the three dimensional optoacoustic data recorded using the HFOAM biomicroscope.

The HFOAM system is capable of accurately imaging the large and intricate vascular networks present in both the mouse brain an ear, as demonstrated in Fig. 3a) to c) where images of a 14 week old nude mouse are presented. The images in Fig. 3 were acquired *in-vivo* and without exogenous contrast agents. The murine pial neurovasculature can be observed in Fig. 3a) with a more detailed section shown in Fig 3b). Figure 3c) demonstrates the massive FOV of the HFOAM system of 10x10 mm² applied to imaging an entire mouse ear.

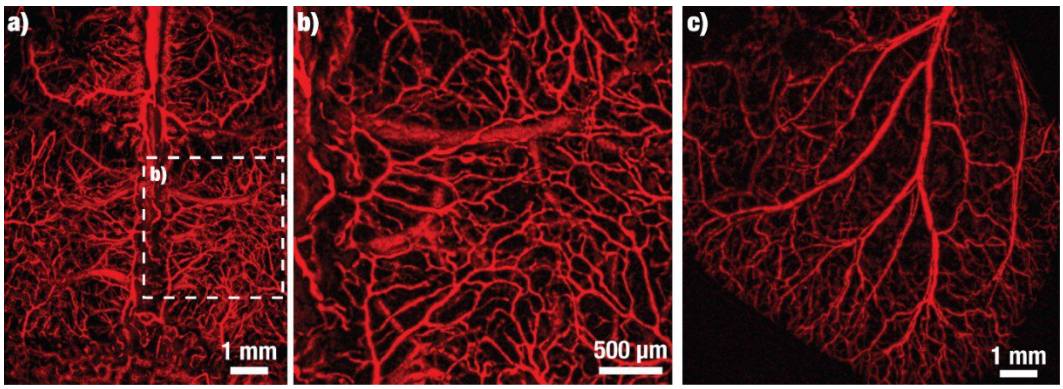

Fig. 3. Large-scale high resolution structural optoacoustic images acquired *in-vivo* and without exogenous contrast agents with the HFOAM biomicroscope. a) Entire mouse brain imaged through the intact skull. b) A detailed scan of the area indicated by the dashed box in a), showing large arteries and veins as well as fine capillaries and c) Image of the entire mouse ear.

Since the HFOAM system is equipped with a focused US transducer, it is able to acquire both US and OA images as shown in Fig. 4a) and b). Figure 4a) presents a pulse-echo US image of the intact skull of a 6 week old nude mice while Fig. 4b) displays an OA scan of the brain vasculature. Using the dual-wavelength capability, the HFOAM system is furthermore capable to acquire detailed functional images of the cerebral vasculature, as shown in Fig. 4c) where the oxygen saturation in the brain is shown.

4. Conclusion

A hybrid-focus optical-resolution optoacoustic biomicroscope (HFOAM) capable of structural small animal imaging was successfully applied to the imaging of 6-dpf Zebrafish larvae and nude mice. Using the dual-wavelength capabilities, we further demonstrated functional imaging of the murine brain *in-vivo*. Both structural and functional imaging were performed without external contrast agents, solely relying on endogenous chromophores such as melanin and hemoglobin. The fast-moving and highly sensitive scan head in combination with the scalable resolution of optoacoustic microscopy allows the high-speed investigation of areas as large as 100 mm² while still capturing intricate details. The

HFOAM system holds great promise in bridging the gap between purely optical microscopy techniques and macroscopic tomographic imaging as it can help understand the link between single cells, tissues, organs and whole organisms.

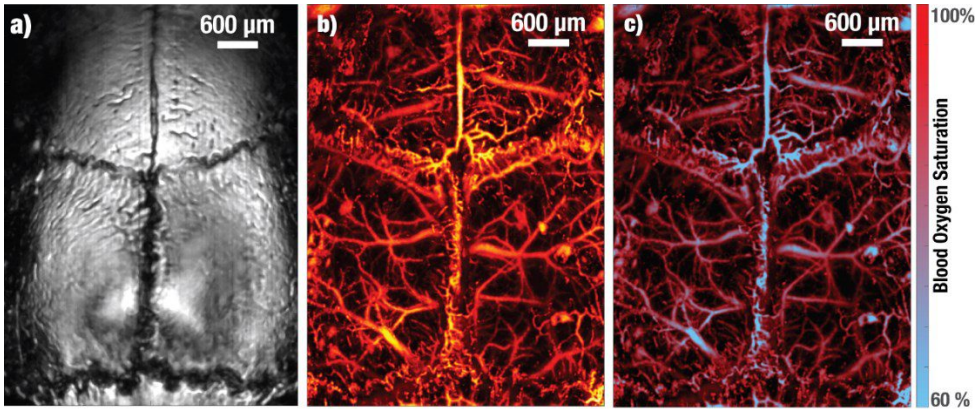

Fig. 4. Structural and functional *in-vivo* imaging brain imaging of a 6-week old nude-mouse acquired with the HFOAM biomicroscope. a) Ultrasound pulse-echo scan of the intact mouse skull. (b) Structural imaging of the cerebral vasculature over the same FOV as a) imaged trough the intact skull and without exogenous contrast agents. c) Functional brain imaging visualizing the blood-oxygen saturation in the brain.

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