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Rapid functional optoacoustic micro-angiography in a burst mode

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Optoacoustic microscopy (OAM) can image intrinsic optical absorption contrast at depths of several millimeters where state-of-the-art optical microscopy techniques fail due to intense light scattering in living tissues. Yet, wide adoption of OAM in biology and medicine is hindered by the slow image acquisition speed, small field-of-view (FOV), and/or lack of spectral differentiation capacity of common system implementations. We report on a rapid acquisition functional optoacoustic micro-angiography (FOMA) approach that employs burst-mode laser triggering scheme to simultaneously acquire multi-wavelength 3D images over an extended FOV covering 50 mm by 50 mm in a single mechanical overfly scan, attaining 28 μm and 14 μm resolution in lateral and axial dimensions, respectively. Owing to an ultrawideband low-noise design featuring a spherically-focused polyvinylidene difluoride (PVDF) transducer, we demonstrate imaging of human skin and underlying vasculature at up to 3.8 mm depth when using per-pulse laser energies of only 25 μJ without employing signal averaging. Overall, the developed system greatly enhances the performance and usability of OAM for dermatologic and micro-angiographic studies. © 2020 Optical Society of America

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Human skin is a difficult target for non-invasive investigations, primarily owing to its optically heterogeneous and highly scattering nature. Optical dermoscopy is a standard tool in dermatology for inspecting skin lesions via classification and categorization of microscopic surface features inaccessible by the naked eye [1]. In human skin, strong light scattering from the rough and curved Stratum Corneum and underlying layers limits the effective imaging depth of optical microscopy methods to just a few hundred micrometers [2], making lesions like melanoma with average breslow thickness of 0.72 mm only partially accessible by optical imaging techniques [3]. High definition optical coherence tomography (OCT) has also been shown

to discriminate between skin malignancies, yet its clinical application is similarly limited to imaging of superficial skin layers further suffering from lack of functional and molecular contrast [4]. Overall, the lack of appropriate *in vivo* imaging methodologies leads to extensive use of biopsies in dermatology [3].

Optoacoustic (OA) imaging has recently evolved as a promising approach attaining rich optical absorption contrast and high spatial resolution in deep tissues by detecting laser-generated broadband ultrasound (US) waves [5]. Several OA microscopy and mesoscopy systems have been studied as a viable noninvasive alternative to histopathological analysis of biopsied skin [6–10]. Clinical progress is mainly hindered by the limited technical performance of existing implementations that lack adequate penetration depth, field-of-view (FOV), and/or imaging speed to allow for practical *in vivo* applications. Furthermore, most existing systems operate at a single or narrow wavelength range while chiefly focusing on imaging vascular anatomy. On the other hand, multiwavelength solutions often require successive mechanical scanning at each wavelength, resulting in long data acquisition times and severe motion artifacts further inhibiting *in vivo* applicability [11].

We developed a rapid acquisition functional optoacoustic micro-angiography (FOMA) approach to overcome limitations of existing optoacoustic microscopy (OAM) systems. In particular, using a burst-mode trigger scheme, large skin areas of up to 50 mm by 50 mm can be imaged at several optical wavelengths within a single overfly scan.

A schematic representation of the imaging system is shown in Fig. 1. Three nanosecond pulsed laser sources running at up to 100 kHz pulse repetition frequency (PRF) are employed to illuminate the sample. Two of the sources have fixed wavelengths of 532 nm and 1064 nm (Onda 532 nm and 1064 nm, Bright Solutions, UK) while the third source is a tunable dye laser (Credo, Sirah Lasertechnik, Germany) whose wavelength was set at the 578 nm isosbestic point of hemoglobin in the current study. The per-pulse-energy (PPE) of each beam is adjustable through a half wave plate followed by a polarizing beam splitter. Two dichroic mirrors (DMLP550 and DMSP805, Thorlabs, USA) combine the three beams into a single optical axis. A beam sampler (BSF10-A, Thorlabs, USA) redirects approximately 1 % of the combined

Fig. 1. Schematic diagram of the system for rapid functional optoacoustic micro-angiography (FOMA) in a burst mode. FOV covering 50 mm by 50 mm area is scanned in a zig-zag pattern using a continuous overfly scan. Three laser sources at 532 nm, 578 nm, and 1064 nm are combined and coupled into a multimode fiber (MMF). The position of the fast moving stage is monitored with 200 nm accuracy by reading out its quadrature encoder signal. Following each pre-defined incremental stage movement, all three lasers are triggered in a cascade with a short 6 μ s delay to rapidly acquire multi-wavelength data free of motion artifacts.

beam into a photodiode (PD) to monitor PPEs and delays of the individual light pulses (DET10A2, Thorlabs, USA). Light is coupled into a MMF (0.2 NA, 200 μ m core diameter, FG200LCC, Thorlabs, USA) using a 100 mm focusing lens (AC254-100-A-ML, Thorlabs, USA) and a two-dimensional adjustable stage (CX11, Thorlabs, USA).

A custom-made, 9 μ m foil polyvinylidene difluoride (PVDF) spherically-focused transducer (7 mm focal distance and 0.43 numerical aperture) detects the optoacoustically-generated waves (Fig. 2 A, B) using custom-made 34 dB impedance-matching amplifier with 1 MHz to 100 MHz bandwidth (-6 dB). The MMF is guided through a central hole in the transducer allowing concentric alignment of light cone and acoustic axis [12]. The detected OA signals are amplified in the transducer housing followed by additional off-site amplification positioned in a Faraday cage (24 dB, ZFL-500LN, Mini-Circuits, USA). A dual-channel, 16 bit resolution data acquisition card (DAC) (M4I.4420-X8, Spectrum Systementwicklung Microelectronic, Germany) simultaneously digitizes transducer and PD signal at 250 MSps for each transducer position and wavelength. The transducer is held by two perpendicularly arranged stages. During imaging, a fast moving stage (DDSM50-M, Thorlabs, UK) continuously oscillates between the edges of the imaged area with 0.5 m/s peak velocity and 3.5 m/s² acceleration, while the second stage (M683, Physik

Instrumente, Germany) moves incrementally following each B-scan, thus resulting in a zig-zag-scan pattern. A microcontroller (Teensy 3.6, PJRC, USA) reads the quadrature encoder signal of the fast moving stage, detecting incremental stage movements down to 200 nm. An internal counter tracks relative stage position and generates a trigger signal depending on the step size defined prior to scanning through a serial based MATLAB interface. A second microcontroller (Teensy 4.0, PJRC, USA) translates the position trigger into a burst-mode cascade by shooting all three lasers shortly after each other with 6 μ s temporal delay sufficient to avoid a cross-talk between OA data recorded at each excitation wavelength. Laser and data acquisition are triggered for each wavelength and stage position resulting in a minimal memory consumption, a regularly spaced volume grid, and no laser pulse overshoot near the edges of the imaged area where the fast stage is accelerating or decelerating.

Fig. 2. Characterization of imaging performance. (A) Raw OA signal emitted by a 10 μ m sphere embedded in agar. (B) Microsphere signal spectrum before (blue) and after (red) correcting for frequency-dependent US attenuation in water [13], exhibiting a useful detection bandwidth beyond 100 MHz. (C) Reconstructed cross-sectional OA slices through microsphere revealing full width half maximum (FWHM) of (28 ± 8) μ m and (14 ± 2) μ m in lateral and axial directions, respectively. (D) Example of raw OA waveforms detected from human skin at the three wavelengths and the corresponding PD signals.

The recorded OA responses are bandpass filtered between 0.5 MHz and 120 MHz using a first order Butterworth filter. PPE is calculated by integrating the PD signal after subtracting the noise level. The precise instants of the laser shots are further extracted by identifying maxima in the PD curves. The recorded OA waveforms are then individually corrected for laser delays and energy fluctuations. A synthetic aperture focusing technique (SAFT) was applied to the preprocessed data where each reconstructed point is rated by the wavefront coherence to reduce smearing in lateral dimension [14–17]. After reconstruction, the volume is deconvolved with a point spread function extracted from reconstructed microsphere measurements to further improve lateral resolution and signal-to-noise ratio (SNR) [18]. By employing a graphics processing unit (GPU)

(CUDA 10.0, RTX2070S, NVIDIA, USA), datasets containing 2×10^9 voxels over $20 \text{ mm} \times 20 \text{ mm} \times 5 \text{ mm}$ are reconstructed within 10 min. Phantom measurements using $10 \text{ }\mu\text{m}$ to $20 \text{ }\mu\text{m}$ diameter microspheres (BKPMS-1.2, Cospheric, USA) sparsely distributed in agar allowed for an automatic determination of the exact transducer's focal distance (6.85 mm) and speed-of-sound in the medium (1484 m/s). The measurements further revealed an ultrawideband sensitivity of the PVDF transducer exceeding 100 MHz (Fig. 2 A,B), which was determined by also taking into account frequency dependent absorption of US waves in deionized water. The effective lateral and axial spatial resolutions of the system were measured to be $(28 \pm 8) \text{ }\mu\text{m}$ and $(14 \pm 2) \text{ }\mu\text{m}$, respectively (see Fig. 2 C). Fig. 2D further shows an example of raw OA waveforms detected from human skin at the three wavelengths and the corresponding PD signals.

We imaged skin of a healthy volunteer and revealed vasculature over its full thickness reaching up to 3.8 mm depth when using $25 \text{ }\mu\text{J}$ at 532 nm and 578 nm and $100 \text{ }\mu\text{J}$ at 1064 nm , respectively, note that no averaging was used for phantom or skin imaging. Due to broadband sensitivity of PVDF, vascular networks containing vessels with diameters ranging from $10 \text{ }\mu\text{m}$ up to 2 mm can be clearly resolved (Fig. 3A). By bandpass filtering the detected OA signals at low (5 MHz to 25 MHz) and high (30 MHz to 120 MHz) frequencies, differently sized vessels can be readily differentiated in the side-view projection. When inspecting the multi-wavelength data, the images manifested several vascularized skin layers known as subpapillary, cutaneous, and deep plexus (Fig. 2C). Since no vessels pass through the dermal-epidermal junction, the epidermal layer is avascular formed by 95% of keratinocytes mixed with melanocytes, Langerhans' cells, and Merkel cells [19]. Since melanin has a broad optical absorption spectrum, the OA signal at the 1064 nm excitation wavelength in the infrared (IR) is mainly concentrated in the outermost epidermal layer. Arterioles are surrounded by elastic tissue which contains smooth muscle cells, elastin, and collagen fibers [19], thus further providing signal in the IR. The 1064 nm wavelength is also absorbed in the uppermost layer of the epidermis (Stratum Corneum) revealing fibrous like orientation along the skin surface.

While our current FOMA implementation can image very large FOV of 20 mm by 20 mm at multiple wavelengths within an unprecedentedly short duration of 2 min, the imaging speed could be further improved by combining two mechanical stages with a microelectromechanical system (MEMS) scanner oscillating over shorter distances, at the trade-off of smaller FOV, increased complexity, cost, and weight. Furthermore, mounting of stages, transducer, and fiber on a passive robotic arm may extend usability to less accessible regions of the human body [6]. The use of laser diodes would allow more compact optics and therefore bed-side imaging in clinical settings, potentially also reducing the overall cost. Nevertheless, intense pulses with ultrashort durations in the few nanosecond range are difficult to achieve with laser diode technology, resulting in reduced spatial resolution or otherwise insufficient PPE, low SNR, and penetration depth [20]. In terms of image reconstruction, model based inversion schemes allow for a more accurate representation of the measurement geometry, thus could aid to mitigate limited-view artifacts and achieve better image quantification performance [21].

In conclusion, we developed a functional optoacoustic micro-angiography (FOMA) system incorporating a burst-mode trigger scheme which allows multi-spectral image acquisition in a single rapid mechanical overfly scan without employing signal

Fig. 3. Large-area microvascular network in a human palm acquired at three wavelengths within 2 min. (A) maximum intensity projection of the 3D FOAM image at 578 nm showing vasculature of the plexus [P] and capillary loops in the upper dermal layer [C]. (B) Single wavelength image at 578 nm bandpass filtered in different acoustic frequency bands to distinguish small capillary loops in superficial layers from larger vessels in the plexus. (C) Complication of cross-sectional images at three wavelengths: melanin is present in the epidermis [E] while vasculature is limited to the dermis [D] and subcutaneous tissue [S]. Note that vasculature was resolved down to 3.8 mm depth in a highly scattering and absorbing tissue.

averaging, thus averting motion between the consecutive wavelengths. The system's performance was showcased by imaging vasculature over the full human skin depth. Large volumes over 50 mm by 50 mm area were scanned at resolutions down to

the capillary level within several minutes. The laser triggering speed is adjusted according to the precise positions read from the stage quadrature encoder, which allows to render a uniform image grid and avoid pulse overshoot and excessive laser exposure at the stage turning points. The system allows imaging at a broad spectral window spanning from 532 nm to 1064 nm that can be used for distinguishing different anatomical structures or perform functional imaging of e.g. oxygen saturation. Note that the 1064 nm wavelength is less absorbed by the skin as compared to visible light. Besides deeper penetration into biological tissue, this wavelength range is recently becoming attractive due to development of new contrast agents tailored for the second near-infrared window [22]. All in all, the developed system overcomes several drawbacks of the existing implementations and represents an important step towards wider adoption of OA microscopy and mesoscopy for biomedical and clinical applications.

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DISCLOSURES

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