Isometric multimodal photoacoustic microscopy based on optically transparent micro-ring ultrasonic detection: supplementary material

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This document provides supplementary information to “Isometric multimodal photoacoustic microscopy based on optically transparent micro-ring ultrasonic detection”, <http://dx.doi.org/10.1364/optica.2.000169>, including fabrication details of micro-ring resonator ultrasonic detector, estimation of spatial resolution, angular dependent frequency response and sample preparation method. © 2015 Optical Society of America

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1. Fabrication of the micro-ring resonator ultrasonic detector

The optically transparent micro-ring resonator (MRR) ultrasonic detector was fabricated on a fused quartz coverslip (Ted Pella) by electron beam lithography (EBL). The coverslip was pre-cleaned in piranha solution and then spin coated (Laurell WS-650-23) with an 800 nm-thick SU-8 (MicroChem) layer on top at 800 rpm for 30 seconds. After soft-baking on a hot plate at 65 °C for 1 min and 95 °C for 1 min to evaporate extra solvent, the MRR ultrasonic detector, which consists of a circular and a matching bus waveguide, was patterned by an EBL system (FEI Quanta 600F). Followed by a post-exposure bake process at 65 °C for 1 min and then 95 °C for 1 min, the sample was immersed in SU-8 developer (MicroChem) for 1 min to dissolve the unexposed region and then cleaned with isopropyl alcohol and air-dried before using. Eventually, the structure of the MRR ultrasonic detector was characterized by scanning electron microscopy, shown in Fig. S1.

2. Optical depth of field

Equation used to calculate the depth of field is [1]

$$d = \frac{2\lambda n}{NA^2},$$

where λ is the wavelength of illuminating light; n is the refractive index of the medium between the coverslip and the objective lens; and NA is the objective numerical aperture. The depth of field of a

20X air objective lens with NA 0.45 is 5.25 μm at the wavelength of 532 nm.

3. Estimate PAM axial resolution

We estimated PAM's axial resolution through a typical time-resolved PA signal acquired from the CB thin film. The convolution of measured PA impulse responses and its time-shifted waveform was calculated using the Hilbert transformation. As show in Fig. S4, we used the minimal time delay for two convoluted impulses remain distinguishable at a contrast-to-noise ratio (CNR) of 6-dB to calculate the axial resolution [2].

4. Angular dependent frequency response

The MRR detector's angular dependent frequency response is characterized by linearly scanning the focused laser spot on the CB thin film. The time-resolved PA signals are recorded and Fourier transformed as shown in Fig. S5. The intensity was normalized to its peak value for every frequency for clarity. The black lines are the 3-dB cut-off lines.

In laser-scanning PAM, the detected PA signal amplitude and the imaging field of view (FOV) highly depend on the angular detection range, which is inversely proportional to the detection bandwidth. In our experiment, MRR detector exhibits a 7-degree in the full-width half-maximum of the angular-dependent sensitivity at 200 MHz.

5. Sample placement

A 5-axis translation stage was used to mount the specimen above the MRR for proper positioning and focusing under the excitation beam. The distance between sample and MRR is adjusted to be 0.65 mm in order to achieve sufficient FOV under broadband detection.

6. Preparation of stained RPE specimen

Human donor eye without reported vision dysfunction was obtained from an eye bank (age of the donor: 79 years) and fixed in a solution of neutral buffered formalin (10% in phosphate-buffered saline, pH 7.4). The anterior segment of the eye was removed by making an initial incision slightly posterior to the corneal limbus. The vitreous was subsequently removed, and the neural retina was carefully separated from the RPE-choroid-sclera complex by slowly peeling the tissue from several locations with forceps. The choroid was then gently separated from the sclera in a similar manner, with its connection at the optic nerve cut.

The tissue was washed with 1x PBS and fixed in paraformaldehyde (3.7% solution in phosphate-buffered saline, pH 7.4) for 5 minutes. The tissue was then permeabilized with a 0.1% solution of Triton X-100 detergent in PBS at 37°C for 10 minutes. After a second wash with PBS, the tissue was blocked with a 1% solution of bovine serum albumin (BSA) in PBS at 37°C for 10 minutes. The tissue was then transferred to a coverslip and covered with a 1:200 dilution (from methanolic stock concentration of 6.6 μM) of Alexa Fluor 488 Phalloidin. The solution was added to cover the entire coverslip and incubated at 37°C for 30 minutes. The tissue was then washed once with tris-buffered saline (TBS) with 0.05% Tween 20, and then twice with 1x PBS before being transferred to a second coverslip and laid flat. After the tissue has been air-dried, the coverslips were transferred to a clean staining jar for storage.

References

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Figures:

Figure S1. (a) SEM oblique view of the MRR ultrasonic detector. (b) High magnification top view of the gap region.

Figure S2. (a) SEM image of a patterned carbon black thin film. (b) The magnified image of the block edge.

Figure S3. (a) The edge spread function (ESF) was recorded by linearly scanning the edge of a carbon black thin film. (b) The lateral resolution of 0.73 μm was determined by the FWHM of the corresponding line spread function (LSF) obtained by calculating the gradient of the ESF. (c) Projection PAM image of group 7 on a USAF-1951 resolution test target; scale bars: 20 μm .

Figure S4. Axial resolution estimated by a shift-and-sum method.

Figure S5. Angular dependent frequency response of MRR ultrasonic detector. Black lines are the full-width half-maximum of the angular-dependent sensitivity.

Figure S6. A time domain PA signal showing the reflection from the substrate, which can be temporally separated as suggested by the blue dashed line.