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Multiple aperture synthetic optical coherence tomography for biological tissue imaging

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Abstract: An inherent compromise must be made between transverse resolution and depth of focus (DOF) in spectral domain optical coherence tomography (SD-OCT). Thus far, OCT has not been capable of providing a sufficient DOF to stably acquire cellular-resolution images. We previously reported a novel technique named multiple aperture synthesis (MAS) to extend the DOF in high-resolution OCT [Optica 4, 701 (2017)]. In this technique, the illumination beam is scanned across the objective lens pupil plane by being steered at the pinhole using a custom-made microcylindrical lens. Images captured via multiple distinctive apertures were digitally refocused, which is similar to synthetic aperture radar. In this study, we applied this technique for the first time to image both a homemade microparticle sample and biological tissue. The results demonstrated the feasibility and efficacy of high-resolution biological tissue imaging with a dramatic DOF extension.

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OCIS codes: (170.4500) Optical coherence tomography; (170.3880) Medical and biological imaging; (120.3890) Medical optics instrumentation.

References and links

1. Introduction

Optical coherence tomography (OCT) is a versatile cross-sectional and three-dimensional imaging technique capable of producing non-invasive, high-speed and high-resolution images through highly scattering samples [1, 2]. In classical optics, the depth of focus (DOF) is proportional to the square of the transverse resolution in the OCT sample arm. A compromise must be made between transverse resolution and DOF. Various approaches to extending the DOF have been proposed and studied in the past decade. Generally, these methods can be classified into three main categories: 1) Bessel beams with axicon optics employed for aperture microscopy [3–5], phase [6–8] or amplitude [9, 10] apodization; 2) adaptive optics [11–13]; 3) and digital refocusing [14–17]. Axicons generate a narrow focal line along the optical axis that can be approximately regarded as a zero-order Bessel beam, which preserves the same transverse distribution over a larger DOF than the Rayleigh range but at the expense of diminished sensitivity and the introduction of sidelobe artifacts [3–5]. Compared with axicon optics, phase or amplitude apodization is popular because of its easy fabrication and miniaturization; however, phase or amplitude apodization suffers a signal loss of ~10 dB for both the illumination and the detection beams [6, 10]. Although these problems can be mitigated by dark-field Gaussian detection, the fundamental problem of a suboptimal coherence transfer function must still be resolved [3–5, 18, 19]. Adaptive optics using pupil segmentation or active phase modulation of the pupil can recover near-diffraction-limited performance from various biological and non-biological samples exhibiting aberrations large
or small and smoothly varying or abruptly changing. However, this approach requires a phase-stabilizing setup and is very time consuming [11–13]. The digital refocusing approach has drawn considerable research interest because it only requires minute changes to the hardware used for conventional Fourier-domain OCT (FD-OCT). A widely reported digital refocusing approach is interferometric synthetic microscopy (ISAM), which computationally reconstructs volumes with resolution in all planes identical to that achieved at the focal plane in conventional FD-OCT by solving the inverse scattering problem for interference microscopy. However, this approach requires great phase stability when capturing consecutive images at different depths and suffers from heavy computation costs [14]. Another method named multi-focus [20–23] requires parallel acquisition of multiple cross-sectional images focusing at different depths. While the DOF extension in this technique is limited by the number of parallel channels.

In a previous study, we developed a novel technique named multiple aperture synthesis (MAS) to address the fundamental problem of limited DOF in high-transverse-resolution FD-OCT [19]. In the present study, we applied MAS for the first time to biological tissue imaging and achieved substantial DOF extension performance that is comparable to that in a microparticle sample. The results demonstrate the efficacy and potential of MAS to extend DOF in biological tissue imaging.

2. Materials and methods

2.1 Multiple aperture synthesized optical coherence tomography

![Multiple Aperture Synthesized SD-OCT System](image_url)

Fig. 1. Schematic of the multiple aperture synthesized SD-OCT system. SLD, super-luminescent diode; OC1-2, 90:10 fiber optic coupler; L1-L6, collimating and focusing lens; L7, camera lens; NDF, neutral density filter; RM, reference mirror; BS, beam splitter; GS, galvo scanner; G, transmission diffraction grating; IMAQ, image acquisition; C, computer; PC, polarization controller; SMF, single mode fiber; MCL, microcylindrical lens; PZT, piezoelectric transducer.

A schematic of the multiple aperture synthesized SD-OCT system is shown in Fig. 1 (additional details may be found in [19]). The power source is a super-luminescent diode (SLD) array (Superlum Broadlighters T-850-HP) with a 3-dB bandwidth of 165 nm centered at 850 nm. In brief, the incident light is split into the sample arm (90%) and the reference arm (10%) by a 2 × 2 coupler (TW850R2A2, Thorlabs, Newton, NJ, USA). In the reference arm,
the light is collimated by lens L1 (AC050-010-B-ML, Thorlabs, Newton, NJ, USA) and focused by lens L2 (M Plan Apo NIR 20 × , Mitutoyo, Takatsu-ku, Kawasaki, JP) onto reference mirror RM (PF10-03-P01, Thorlabs, Newton, NJ, USA). The light beam in the sample arm is collimated by lens L3 (AC050-010-B-ML, Thorlabs, Newton, NJ, USA) and focused by objective lens L4 (M Plan Apo NIR 20 × , Mitutoyo, Takatsu-ku, Kawasaki, JP). A beam splitter BS (BS017, Thorlabs, Newton, NJ, USA) is positioned in the sample arm to differentiate the illumination and detection beams. The backscattered light from the sample is focused by lens L5 (AC050-010-B-ML, Thorlabs, Newton, NJ, USA), guided to the other 2 × 2 coupler (TW850R2A2, Thorlabs, Newton, NJ, USA), recombined with the light from the reference arm, and finally directed into the spectrometer. The spectrometer consists of a 1765 lines/mm diffraction grating (PING-Sample-020, Ibsen Photonics, Farum, Denmark), a camera lens (85 mm, f/1.2, Canon Inc., Tokyo, JP), and a 4096-pixel CCD camera (AViiVA EM4, e2V, Chelmsford, UK). The detected spectrum is digitized at 12-bit resolution and transferred to a computer via an image acquisition board (KBN-PCE-CL4-F, Bitflow, Woburn, MA, USA). Two-directional transverse scanning is implemented by two galvanometer-mounted mirrors driven by a saw-tooth pulse generated by a 16-bit analog output of a data acquisition (DAQ) board (PCI-6221, National Instruments, Austin, TX, USA). Image acquisition and galvo mirror scanning are synchronized by the external trigger from a DAQ digital output. Discrete Fourier transform is performed on each frame of 1024 A-lines obtained by the CCD to resolve the axial depth profile of the sample.

To extend the DOF using MAS, a microcylindrical lens (MCL) is positioned 10 μm from the tip of the sample fiber, with an axisymmetric configuration along the optical axis. The MCL is linearly shifted along a transverse direction perpendicular to the clinical axis by a piezoelectric transducer (PZT, AE0505D16F, Thorlabs, Newton, NJ, USA). As shown in Fig. 1, the five positions PZT shifted along are indicated by numbers 1–5. Because of the transversely shifted MCL, the optical path difference (OPD) is segregated into two parts: one part is induced by the transverse shift of the MCL, and the other part is caused by the variation of the local wavefront. The MAS algorithm is designed to correct these OPDs by coherently synthesizing five cross-sectional images (B-scans) captured from five distinctive apertures into a new B-scan, which preserves the diffraction-limited transverse resolution over an extended DOF. Because we scan the illumination beam across the objective lens pupil plane along one transverse dimension (e.g., x-dimension), this refocusing technique can only be used for B-scans and perform digital refocusing in one transverse dimension.

2.2 Multiple aperture synthesis algorithm

As derived in [19], the original cross-correlation (CC) term from the n-th aperture is given by

\[ I_n(k) = \exp(i\alpha_n) \cdot \sqrt{I_r(k)I_s(k)[\exp(i2kz_n)\exp(i\beta_n)]} + C.C. \]

(1)

where the subscript \( n \) represents the sequence number of distinctive apertures, \( I_r(k) \) and \( I_s(k) \) are the electric field reflectivity from the reference arm and the sample arm at the depth of \( z_n \), and C.C. is the abbreviation for the complex conjugate. This equation includes a constant phase \( \alpha_n = -k_0 \cdot \Delta z_n \) and an oscillation phase \( \beta_n = -\Delta k \cdot \Delta z_n \) induced by transverse shifts of the MCL and the local wavefront variation between two transverse shifts, where \( k = k_0 + \Delta k \).

The goal of the MAS algorithm is to correct \( \alpha_n \) and \( \beta_n \) stepwise, as shown in Fig. 2. Assuming that there are \( m \) transverse shifts, the optimal correct phase for \( \alpha_n \) is obtained and denoted by \( \alpha_{n}^{op} \) when \( \sum_{n=1}^{m} \Re[I_n(k) \cdot \exp(-i\alpha_n)] \) reaches its maximum value. Here we used the Fourier shift theorem. In a similar manner, the optimal correct phase for \( \beta_n \) is obtained.
and denoted by $\beta^\alpha_n(z)$ when $\max \left\{ \sum_{n=1}^{\infty} \Re \{ I_n(k) \cdot \exp(-i\alpha^\alpha_n) \} \cdot \exp[-i\beta^\alpha_n(z)] \right\}$ reaches its maximum value. Note that $\beta^\alpha_n(z)$ is both depth-dependent and contained in $\{\pi/2, \pi, 3\pi/2, \pi \}$. The digitally refocused B-scan is then given by

$$I_n = \sum_{n=1}^{\infty} \Re \{ I_n(k) \cdot \exp(-i\alpha^\alpha_n) \} \cdot \exp[-i\beta^\alpha_n(z)]$$ \hspace{1cm} (2)

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3. Results

3.1 DOF extension verification

To verify the DOF extension performance of the MAS OCT system, we conducted imaging experiments using a polystyrene microparticle calibration sample. This sample was constructed by mixing agarose solution (No. PC0701-100g, Vivantis, Oceanside CA, USA) with polystyrene microparticles (No. 64090-15, nominal size 6 µm, Sigma-Aldrich, St. Louis, Missouri, USA). This mixture was stored in a vial and placed in an ultrasonic bath for 10 min to remove residual clusters. The sample (10 g) was poured into a cell culture dish, cured for 10 min at 100 °C and then cured for 2 h at room temperature. In this experiment, five B-scans captured from distinctive apertures were used for MAS. The B-scan dispersion-compensated using the algorithm [19, 24, 25] suffered from severe defocus beyond the DOF (Fig. 3(a)). The whole depth range was 1077 µm, and the focus was located at a depth of 871 µm. Because the nominal diameter of microparticles was 6 µm, the transverse resolution should be the difference between the transverse full-width-at-half-maximum (FWHM) and the nominal diameter. As indicated, the transverse resolution degraded from 3.3 µm at the focus to 19.0 µm at a depth of 477 µm (Fig. 3(c)). Furthermore, the transverse resolution deteriorated to 51.3 µm at a depth 825 µm from the focus. By contrast, the digitally refocused B-scan had a sharper transverse FWHM and an enhanced signal-to-noise ratio (SNR) (Fig. 3(b)). The transverse FWHMs at variable depths were quantitatively compared to demonstrate the DOF extension performance (Figs. 3(c) and 3(d)). The transverse resolutions at depths of 46, 268, 477, 675, 871, and 994 µm were enhanced by factors of 7.0, 2.7, 3.1, 3.3, 1.0, and 2.2, respectively. In Fig. 3(a), the DOF was estimated to be 20 µm. In Fig. 3(b), the diffraction-
limited transverse resolution of 3.3 µm was maintained over the depth range from 675 to 871 µm, which was equal to a 9.8-fold extension of the DOF. In addition, the digital refocusing performance was distinct over the whole depth range.

3.2 DOF extension in biological tissue imaging

To demonstrate the DOF extension performance in biological tissues, we conducted two imaging experiments on fresh grapes and rat adipocytes ex vivo. Figure 4 shows the resultant images of the fresh grape sample, including the dispersion-compensated B-scan (Fig. 4(a)) and digitally refocused B-scan (Fig. 4(b)) with a size of 1747 µm (transverse) × 1069 µm (axial). Two local areas at depths of 329 µm and 760 µm from the focus were selected and magnified by a factor of two (Figs. 4(c) and 4(d)). After MAS was applied, the blurred cell walls were digitally refocused and became sharper and brighter. The transverse profiles indicated by orange and green dashed lines show that the cell-wall widths were sharpened from 27.7 µm and 23.3 µm to 5.7 µm and 7.4 µm, respectively. Anisotropy was an effective indicator of image sharpness, and increased as the blur diminished [26–28]. To quantitatively determine the image quality and assess its improvement, the anisotropy ratio of the digitally refocused B-scan to the dispersion-compensated B-scan was calculated over the whole depth range (Fig. 4(e)). Over the depth range from 130 to 920 µm, the anisotropy ratio was greater than 1 and fluctuated around 1.2, which means the transverse resolution was dramatically improved. Over the depth range from 1 to 130 µm, the anisotropy ratio was less than 1 for the
sake of strong reflection on the tissue surface. Two valleys of the anisotropy ratio at depths of 930 and 980 µm were generated for the sake of visible line artifacts in the transverse direction and limited image intensity. Over the whole depth range from 30 to 1069 µm, MAS demonstrated dramatic refocusing performance.

To further demonstrate this DOF advantage, another experiment was conducted on a rat adipocyte sample with variable refractive index ex vivo. The resultant B-scan of application of MAS to the rat adipocyte tissue with a size of 1747 µm (transverse) × 684 µm (axial) is shown in Fig. 5(b). Over most of the depth range, the transverse resolution was improved, as indicated by cell boundaries becoming sharper and brighter, and detailed features were clearly reconstructed compared with the features in the original dispersion-compensated B-scan (Fig. 5(a)). Intact features of the adipocyte cell structures in the bottom of the B-scans were difficult to recover because of the limited laser power. Two local areas at depths of 516 µm and 453 µm above the focus were selected and magnified by four (Figs. 5(c) and 5(d)). The transverse profiles in the zoom-in area indicated with dashed lines show that the cell boundaries’ widths were sharpened from 15.3 µm and 27.7 µm to 8.5 µm and 9.2 µm,
respectively. The anisotropy ratio of the digitally refocused B-scan to the dispersion-compensated B-scan was also calculated over the whole depth range (Fig. 5(e)). Over the whole depth range, the anisotropy ratio was almost always greater than 1 and fluctuated around 1.05, indicating a transverse resolution improvement. Several valleys of the anisotropy ratio were generated for the sake of artifacts induced by strong reflection at cell junctions. This experiment demonstrated that MAS achieved good DOF extension performance when imaging the rat adipocyte sample.

![Figure 5](image_url)

**Fig. 5.** B-scans of rat adipocyte samples to verify DOF extension performance. (a) Dispersion-compensated B-scan (intensity in logarithmic grayscale) captured from one of five apertures. (b) Digitally refocused B-scan via MAS. (c-d) Two boxed areas 435 µm and 516 µm from the focus are indicated in green and orange colors and are magnified four times. The transverse profiles indicated by the dashed lines show that the defocused cell boundary was well digitally refocused. (e) Image anisotropy ratio of the digitally refocused B-scan to the dispersion-compensated B-scan.

### 4. Discussions and conclusions

We applied the MAS technique to biological tissue imaging for the first time and demonstrated dramatic DOF extension performance in high-transverse-resolution FD-OCT. A great advantage of the MAS technique is that it only required phase stability between A-lines, because five A-lines are captured to reconstruct a new A-line. The MAS technique has the potential to provide a sufficient DOF to stably acquire cellular-resolution images *in vivo* when the limitation in the current study is overcome. The spectrum passing the diffraction grating is partially confined by the camera lens (85 mm, f/1.2, Canon Inc., Tokyo, JP) resulting in a deteriorated axial resolution of 3.0 µm. A camera lens with a shorter focal length may not confine the diffracted spectrum but would result in a limited ranging depth. This limitation
can ultimately be overcome by using two spectrometers to acquire two halves of the light spectrum and then recombining them [29]. This solution imparts MAS with a sufficient ranging depth, an ~2.0 µm axial resolution and a sufficient DOF for cellular-resolution imaging. Besides, we acknowledged that there would be 3 dB detection sensitivity penalty induced by the additional beam splitter inserted in the sample arm. The MAS apparatus in this study is applicable to desktop OCT imaging systems for DOF extension; however, a substantial research effort is required to develop a miniaturized optical design for endoscopic and intravascular applications. A desktop OCT utilizing MAS could be easily developed for ocular applications. In conclusion, the MAS technique achieves dramatic DOF extension performance in complex tissues whose refractive index varies with depth (e.g., rat adipocyte tissue) and has strong potential for providing a sufficient DOF for cellular-resolution imaging in vivo.

Funding
National Research Foundation Singapore (NRF-CRP13-2014-05); Ministry of Education Singapore (MOE2013-T2-2-107); National Natural Science Foundation of China (Grant No. 61705184); National Medical Research Council Singapore (NMRC/CBRG/0036 /2013); NTU-AIT-MUV program in advanced biomedical imaging (NAM/15005).