

Cellular-resolution *in vivo* tomography in turbid tissue through digital aberration correction

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Noninvasive tomographic imaging of cellular processes *in vivo* may provide valuable cytological and histological information for disease diagnosis. However, such strategies are usually hampered by optical aberrations caused by the imaging system and tissue turbidity. State-of-the-art aberration correction methods require that the light signal be phase stable over the full-field data acquisition period, which is difficult to maintain during dynamic cellular processes *in vivo*. Here we show that any optical aberrations in the path length difference (OPD) domain can be corrected without the phase stability requirement based on maximum intensity assumption. Specifically, we demonstrate a novel optical tomographic technique, termed amplitude division aperture synthesis optical coherence tomography (ADAS-OCT), which corrects aberrations induced by turbid tissues by physical aperture synthesis and simultaneously data acquisition from sub-apertures. Even with just two sub-apertures, ADAS-OCT enabled *in vivo* visualization of red blood cells in human labial mucosa. We further demonstrated that adding sub-apertures could significantly scale up the aberration correction capability. This technology has the potential to impact a number of clinical areas where noninvasive examinations are preferred, such as blood count and cancers detection.

1. INTRODUCTION

Imaging pathophysiological processes at the cellular level is critical for understanding and diagnosis of human diseases, which is routinely realized through cytological or histological examinations following biopsies in clinical settings. Because biopsy is associated with sampling errors and complications due to its invasive and destructive nature, it is not suitable for screening a large population or imaging over a large tissue area. Moreover, cytology and histology procedures are time consuming which may result in delayed diagnosis. Tomographic imaging modalities are the mainstay of noninvasive diagnostic tools, including ultrasound imaging [1], computed tomography [2], magnetic resonance imaging [3] and positron emission tomography [4]. Although these existing tools have fundamentally changed clinical practice and basic research, cellular-level cytological and histological information is still not available in tomograms *in vivo* due to the limited spatial resolution.

Optical tomography techniques are capable of achieving cellular-level spatial resolution in thin tissue sections or semi-transparent tissues [5-7]. However, imaging through thicker (>300 μm) tissues *in vivo* is still highly challenging because the spatial resolution is degraded by optical aberrations, which can be roughly classified into system aberrations and sample-induced aberrations. System aberrations are

generally inherent effects arising due to the focusing optics, such as defocus and inevitable imperfections in the optics used in endoscopic applications [8, 9]. Sample-induced aberrations in turbid tissues generally vary spatially and temporally. They must be corrected before clear images of cells can be obtained *in vivo*.

The straightforward way to correct these aberrations is by using hardware-based adaptive optics (HAO), which measures the wavefront distortion and cancels it by modulating the incident light wavefront. With an HAO correction, a set of correction parameters is valid only over a sufficiently small volume and over a sufficiently short period of time during which the sample-induced aberrations are constant. Consequently, it is time consuming to screen a large tissue volume [10, 11]. From a practical point of view, the system complexity and cost also impede its applications.

In OCT, optical aberrations result in not only the suboptimal coupling of signals at the detection pinhole but also interferometric wavefront distortions, which compromise both the coherence gating and the transverse focusing. Fortunately, the optical aberrations are recorded as the interferometric wavefront distortions that are detected optoelectronically, which opens up the possibility of correcting the aberrations digitally. Wide-field computational techniques, such as sub-aperture correlation [12, 13], computational adaptive optics [14-16], and model-based methods [17-19] have been developed to overcome the above-mentioned system complexity and

speed issue with HAO. However, these techniques usually require high phase stability over the full-field acquisitions, which is challenging for imaging dynamic cellular processes *in vivo*, especially in endoscopic scenarios.

To overcome the limitation in phase stability, synthetic aperture OCT has been developed and demonstrated to be effective in digitally correcting the spherical aberration of the focusing optics [20]. However, it divides the aperture central-symmetrically by wavefront [20] or sequentially by time [21, 22] so that it is not possible to correct the sample-induced aberrations. In this study, we report a novel technique, ADAS-OCT, which corrects both the sample-induced aberrations and spherical aberrations without any phase stability requirement. We show that ADAS-OCT can deliver clear images of rapidly moving blood cells by restoring the spatial resolution degraded by the aberrations caused by the human mucosa *in vivo*.

2. METHODS

A. Working principle of aberration correction in ADAS-OCT

In typical point-scanning confocal optics, when the wavefront of the optical beam is distorted by aberrations, destructive interference may occur at the detection pinhole, resulting in a broadened three-dimensional (3D) point-spread function (PSF) with a lower peak intensity compared with the diffraction-limited PSF (Fig. 1). Assuming the full aperture can be segmented into a number of sub-apertures, the aberrations in the optical path difference (OPD) domain can be digitally corrected if the OPD of light rays encircled in each sub-aperture can be recorded separately in the electrical signals. In spectral-domain OCT (SD-OCT), recording of the OPD is achieved by interference between the sample light with the reference light as governed by the following simplified formulation:

$$I(k) = 2S(k) \int_{-\infty}^{\infty} \sqrt{I_r(k)I_s(k)} \cos(2kz) dz \quad (1)$$

where $I(k)$ is the spectral interference signal detected by the spectrometer, $S(k)$ is the spectral distribution of the light source and will be omitted in the following derivations, $I_r(k)$ and $I_s(k)$ are the electric field reflectivity from the reference arm and the sample arm at the depth of z , respectively. In the sample arm, one can synthesize the aperture of the objective lens by dividing the illumination beam by its amplitude and displace them evenly along the radial direction (Fig. 1). In the pupil plane, each beamlet creates a sub-aperture with distinct center spatial frequency, which defines both an illumination light path and a detection light path. The OPD of each distinct round-trip path can be shifted by a fixed length using an optical path encoder (Fig. 1) so that the corresponding interferometric signals do not overlap with each other in the OPD domain. In such an ADAS-OCT system, the m -th ($m=1, 2, \dots, 2n-1$, represents the round-trip path number) depth-encoded cross-correlation term is given by the expression

$$I_m(k) = \sqrt{I_r(k)I_s(k)} [\exp(i2kz_m) + C.C.] \quad (2)$$

where $C.C.$ stands for complex conjugate. Fourier transform ($\mathfrak{F}$) of these cross-correlation terms give the standard SD-OCT images obtained through individual paths

$$i_{SD-OCT,m}(z) = \mathfrak{F}[I_m(k)] \quad (3)$$

Correction of aberrations in the OPD domain can be simply achieved by eliminating the phase delay α_m of m -th cross-correlation term I_m with regard to I_1 . Assuming that the aberration free condition is met when the local intensity of coherently summed image reaches its maximum, the problem is equal to finding α_m^{coh} which is the best

estimate of α_m so that the local intensity $\max \{i(z)|_{z \in [z', z'']}\}$ reaches its maximum, where $i(z) = \sum_{m=1}^{2n-1} \mathfrak{F}[I_m(k) \cdot \exp(i\alpha_m)]$ is the coherent summation of the Fourier transform of the cross-correlation terms denoted by Eq. (2), $[z', z'']$ is the local depth range with $1 \leq z' \leq z'' \leq N$, and N is the length of Fourier transformation. Then the resultant aberration corrected A-line is given by

$$i_{ADAS}(z) = \sum_{m=1}^{2n-1} \mathfrak{F}[I_m(k) \cdot \exp(i\alpha_m^{coh})] \quad (4)$$

While the interferometric signals from the local scatterer ($z'' - z' = 10$ coherence length) are coherently summed, those from the out-of-focus scatterers are suppressed due to OPD mismatches between light paths through different sub-apertures. Thus, this digital aberration correction mechanism can restore both the spatial resolution and the peak signal intensity degraded by aberrations. To produce the aberration-uncorrected control A-line, we incoherently summed the Fourier transform of the cross-correlation terms denoted in Eq. (2) so that $i(z) = \sum_{m=1}^{2n-1} |\mathfrak{F}[I_m(k) \cdot \exp(i\alpha_m)]|$, and then found the optimal phase value for α_m so that global intensity $\max \{i(z)|_{z \in [1, N]}\}$ reaches its maximum value. Therefore, the aberration-uncorrected control A-line is given by,

$$i_{Control}(z) = \sum_{m=1}^{2n-1} |\mathfrak{F}[I_m(k) \cdot \exp(i\alpha_m^{incoh})]| \quad (5)$$

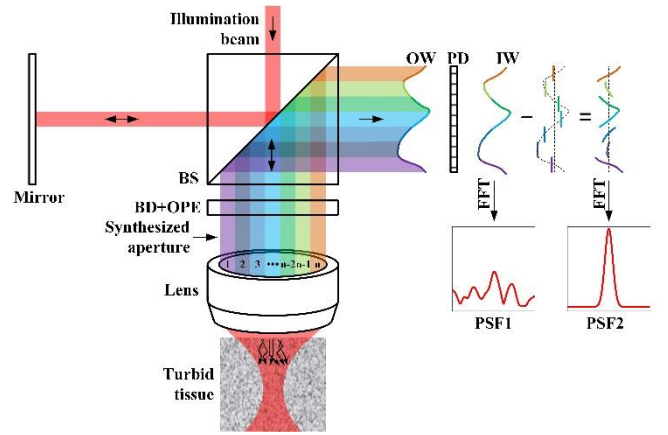


Fig. 1. Schematic depicting the amplitude-division aperture synthesis method and interferometric wavefront distortion correction. Aperture-division detection is achieved by dividing the sample beam by the amplitude and displacing the divided beams over the plane of the objective-lens pupil. Detection of spatial-frequency division enables the distortions of the optical wavefront to be recorded as the interferometric wavefront distortions pass through the pinhole detector. The digital correction of the interferometric wavefront distortions can restore both the PSF and the signal amplitude. PSF1: PSF degraded by aberrations; PSF2: restored PSF; BS: beam splitter; BD: beam displacer; OPE: optical pathlength encoder; 1- n , represents the sub-aperture numbers; OW: optical wavefront; PD: pinhole detector; IW: interferometric wavefront and FFT: fast Fourier transform.

B. ADAS-OCT imaging system

We developed an ADAS-OCT system on an SD-OCT platform (Fig. S1). Specifically, we created two sub-apertures using a birefringent beam displacer (Fig. 2b and Table. S1), with which we obtained three cross-correlation terms with the aid of a quarter-wave plate (Fig. 2c): $I_1(k)$,

$I_2(k)$, and $I_3(k)$ are from the *oo*-path, the *oe/eo*-path, and the *ee*-path, respectively (Fig. 2d). By adding a second birefringent beam displacer (Fig. 2e, Fig. S2 and Table. S2), we were able to create three sub-apertures and adjust the displacement between them, with which we could tailor the aberration correction performance.

A light source with a spectral bandwidth of 180 nm centered at 800 nm was used to achieve an axial resolution of 1.50 μm in water (refractive index = 1.33). Meanwhile, the effective numerical aperture of the focusing optics of the SD-OCT platform was set to 0.13 (1% power level) to achieve a transverse resolution of 2.75 μm (full width at half maximum, FWHM) and a depth of focus of 30 μm (confocal parameter). This spatial resolution allowed us to clearly visualize cellular structures under the aberration-free condition. Details of the system construction and signal transduction are provided in the Supplement 1.

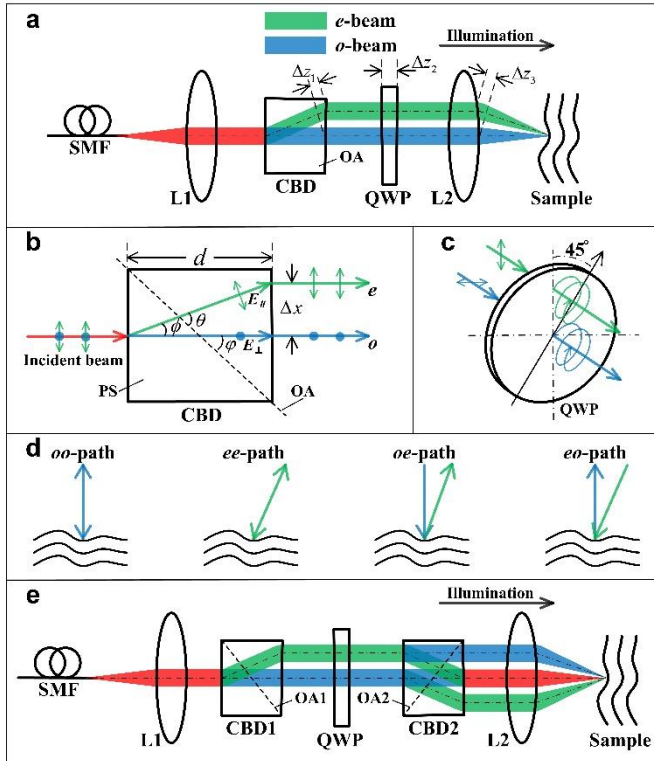


Fig. 2. Schematic of the sample arm optics of ADAS-OCT. (a) Sample arm optics with a beam displacer and a quarter-wave plate, where the red beam is the combination of the *o*- and *e*-beams, the green beam is the *e*-beam, and the blue beam is the *o*-beam. SMF: single-mode fiber; L1: collimating lens; CBD: calcite beam displacer; OA: optical axis of the CBD (in plane of paper); QWP: quarter-wave plate; L2: focusing lens; *o*: ordinary beam; *e*: extraordinary beam; Δz , the single-trip OPD caused by the CBD between the *o*- and *e*-beams. (b) Illustration of birefringent beam displacement. PS: principle section; d : length of the CBD; Δx : separated distance of the *o*- and *e*-beams perpendicular to the direction of propagation; ϕ : angle of separation of the *e*-beam in the CBD; φ : angle between the slow axis and OA of the CBD; θ : angle between the fast axis and OA of the CBD; $E_{\parallel}$ and $E_{\perp}$, transverse and vertical electric field, respectively. (c) Each of the displaced beams was circularly polarized on the round trip to maximize the coupling back to the fiber pinhole. (d) The four round-trip optical paths taken by the light. (e) The number of sub-apertures can be scaled up through the use of cascading beam displacers, where the yellow beam is the combination of the *oo*- and *ee*-beams.

3. RESULTS

A. Correction of sample-induced aberrations *ex vivo*

To demonstrate the ability to correct sample-induced aberrations, we first imaged a simplified tissue phantom that consisted of a collection of polystyrene microparticles with a mean diameter of 6 μm and that were uniformly suspended in agarose gel. We focused the sample light to the upper half of the phantom through a chicken breast tissue with thickness of 289 μm in air (Fig. 3a), resulting in broadened transverse and axial profiles of the particles in the control image (Fig. 3c, e, g) compared with an representative image obtained without the tissue on the phantom (Fig. 3b). In the aberration corrected image, both the transverse and axial profiles of the microparticles were significantly restored (Fig. 3d, f, h). For quantitative comparison, the FWHM values showed a mean improvement of 24.1% and 16.0% in the transverse and axial directions, respectively (Fig. 3i and Fig. 3j). In addition, a mean improvement in the peak intensity of 53.7% was obtained in favor of the signal-to-noise ratio (Fig. 3k). The image contrast and clarity of the chicken breast tissue were also significantly improved (boxes in Fig. 3c, d).

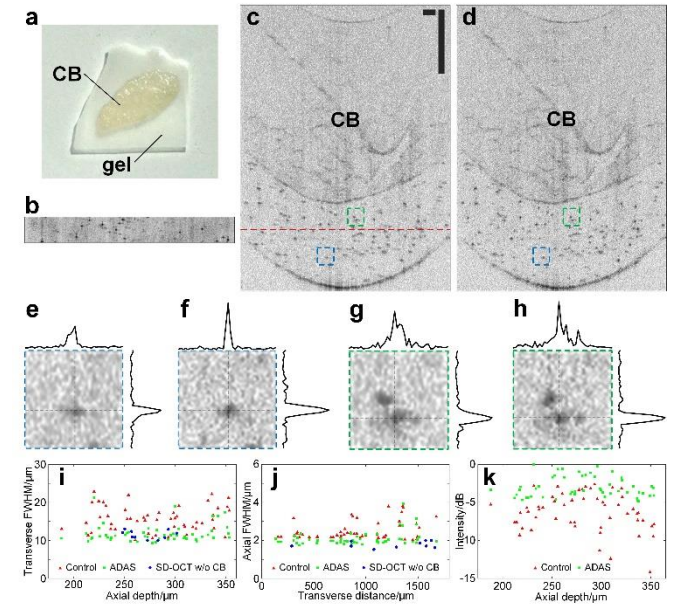


Fig. 3. Correction of sample-induced aberrations. (a) Photograph of the tissue phantom. CB: chicken breast. (b) An image of microparticles acquired without chicken breast tissue. (c) A representative control image. This was obtained using Eq. (4) with $[z', z'']$ being the full depth range. The red dashed line indicates the focal plane. (d) The corresponding aberration corrected image. (e), (g) Six-fold magnified view of two insets in (c) show blurred microparticles. (f), (h) The corrected images corresponding to (e) and (g), respectively. The transverse and axial profiles are shown at the top and on the right of panels (e)-(h), respectively. The profiles in (e) & (g) are normalized to the peak values in (f) and (h), respectively. (i) and (j) The FWHM of the transverse and axial profiles are restored in the ADAS-OCT image (ADAS, green rectangle) with respect to the control image (Control, red triangle) and the SD-OCT image (SD-OCT w/o CB, blue circle). (k) Signal intensity is also restored in the ADAS-OCT image (green rectangle) with respect to the control image (red triangle). Transverse and vertical scale bars: 100 μm .

B. Correction of sample-induced aberration *in vivo*

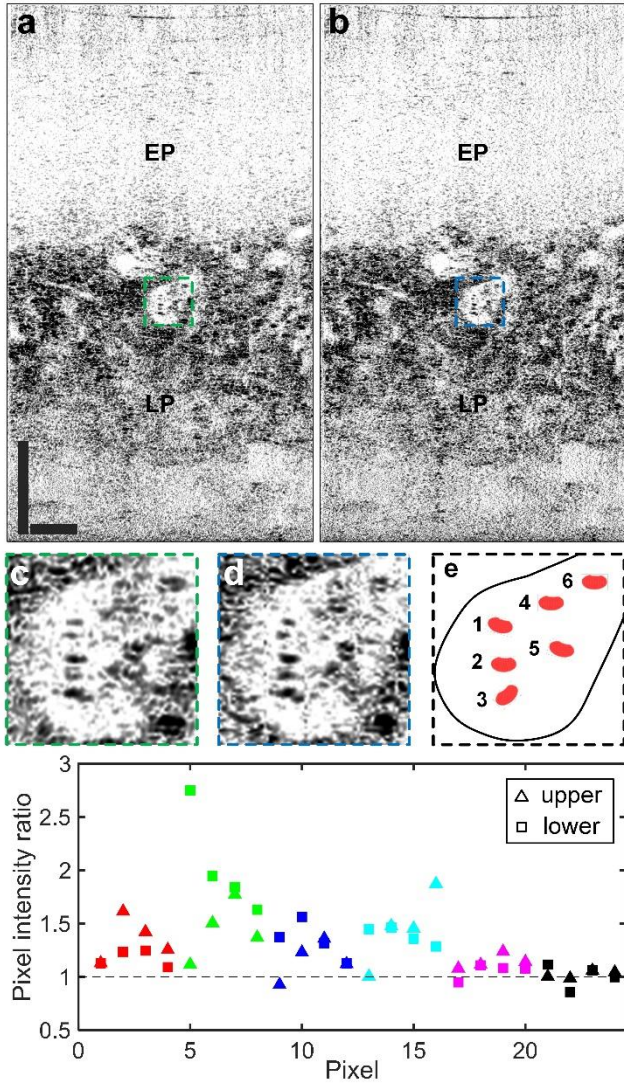


Fig. 4. Correction of sample-induced aberration in humans *in vivo*. (a) A representative control image. This was obtained using Eq. (4) with $[z', z'']$ being the full depth range. (b) Corresponding aberration-corrected image. (c) Three-fold magnified view of the blood vessel in the control image (green dashed box in (a)). (d) Four-fold magnified view of the corresponding aberration-corrected image (blue dashed box in (b)). (e) Ratio of the peak pixel (signal) intensity of the aberration-corrected RBC image to that of the control image. Upper and lower denote the upper and lower boundaries of cells, respectively. EP: epithelium; LP: lamina propria. Transverse and vertical scale bars: 100 μm .

RBCs are ideal image phantoms due to their characteristic axial scattering profiles and spatial sparsity in blood vessels. According to our previous observations in zebra fish larvae, a typical OCT image of an RBC consists of two coherence-length limited reflection signals from the upper and lower boundaries of the cell's biconcave disk [23]. Under the aberration free condition, RBCs should be easily identified in OCT images since the platelets and white blood cells are much fewer and present very different scattering characteristics [24, 25]. We imaged the labial mucosa of human volunteers with the focal region aligned to the lamina propria. The optical thickness (refractive index = 1.33) was approximately 300 μm from the surface of the stratified squamous epithelium to the RBCs in focus. In a representative aberration-corrected ADAS-OCT image (Fig. 4b, d), we could clearly identify six pairs of reflectance signals, presumably six RBCs (Fig. 4e). In contrast, in the corresponding control image (Fig. 4a, c), RBCs numbered 4-6 could not be readily recognized due to aberrations. It is obvious that

aberration correction significantly enhanced the peak signal intensity of RBCs as compared with the control image (Fig. 4f), which contributed to the visibility of RBCs. In one of the other images, we could recover signals of RBCs even when one or both reflection signals were lost due to aberrations (Figs. S3 and S4).

C. Scalable imaging performance

The performance of the ADAS-OCT technique can be enhanced by scaling up the number of sub-apertures and increasing the displacement between them. Using the dual beam displacer setup (Fig. 2e and Fig. S2), we obtained three equally displaced sub-apertures: *oo*, *ee*, and *oe/eo*. The displacement between the centers of the sub-apertures Δx was adjusted in terms of R by rotating the second displacer ($R = 1.3 \text{ mm}$ is the radius of the illumination beam measured at the 1% power level).

This scalability was demonstrated by correcting defocus, which is the simplest aberration but represents a general problem in microscopy and endoscopy. As before, we used the tissue phantom consisting of 6- μm -diameter microparticles suspended in agarose gel. At a sample location 250 μm above the focal plane, the mean transverse FWHM of the particles was degraded to 24.1 μm , 25.6 μm , and 27.1 μm in the control images (Fig. 5a-c and red line in j-l).

For the group of two sub-apertures (*oo* and *ee*), the transverse FWHM was found to improve by 9.1% (for displacement between sub-apertures, $\Delta x = 0.2R$), 25.8% (for $\Delta x = 1R$) and 57.2% (for $\Delta x = 1.8R$) (Fig. 5d-f and green line in j-l). Larger displacement results in better correction also held true in the group of three sub-apertures (*oo*, *ee*, and *oe/eo*) (Fig. 5g-i and blue line in j-l). In the largest displacement of 1.8R between the *oo* sub-aperture and the *ee* sub-aperture, the mean transverse FWHM was 9.2 μm with three sub-apertures, which was 69.4% better than that of the control image data (Fig. 5j), while the mean transverse FWHM was 11.6 μm with two sub-apertures. It is suggested that the number of sub-apertures is related to the improvement of the transverse FWHM.

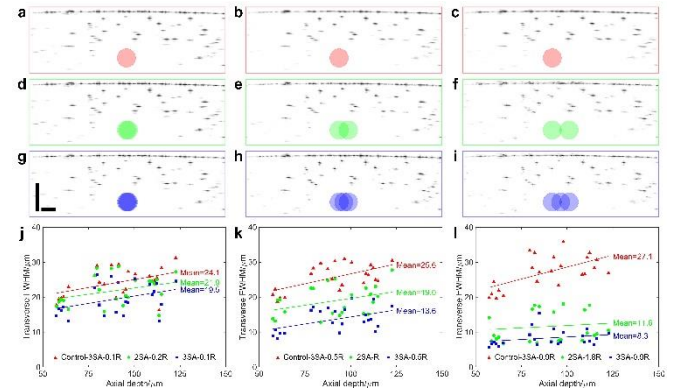


Fig. 5. Improving the transverse performance of the defocus correction by increasing the number of sub-apertures. (a-c) Control images of microparticles located 250 μm above the focal plane. The transverse resolution did not vary significantly with sub-aperture displacement in the control images. (d-f) Corresponding aberration-corrected images using two sub-apertures: *oo* and *ee*. (g-i) Corresponding aberration-corrected images using three sub-apertures: *oo*, *ee*, and *oe/eo*. Insets: illustrations of sub-apertures. (j) Comparison of the transverse FWHM of the microparticles on going from the control image (a) to the corrected image with two sub-apertures (d) to the corrected image with three sub-apertures (g). (k) Comparison of the transverse FWHM of the microparticles on going from the control image (b) to the corrected image with two sub-apertures (e) to the corrected image with three sub-apertures (h). (l) Comparison of the transverse FWHM of the microparticles on going from the control image (c) to the corrected image with two sub-apertures (f) to the corrected image with three sub-apertures (i). Mean transverse FWHMs of each plot are

indicated in (j-l). SA, sub-apertures; R, radius of the illumination beam. Transverse and vertical scale bars: 100 μm .

4. DISCUSSION

ADAS-OCT offers a relatively simple route to cellular-level resolution tomography through turbid tissues *in vivo*. Our approach avoids the phase stability issue encountered by existing computational-based aberration-correction methods. Current computational formulations require full-field data acquisition, during which motion-induced phase instability is inevitable. ADAS-OCT is inherently insensitive to motion-induced phase variations or spatially variant wavefront distortions because it collects scattered signals from all sub-apertures simultaneously. Therefore, to the best of our knowledge ADAS-OCT is the first method that can correct tissue induced aberrations during a rapid cellular process, such as blood flow.

This technical advance may enable noninvasive blood count, which will potentially address many challenges in diagnosis, management, and treatment of diseases. Neonatal blood sampling represents one type of clinical challenges where blood volume and accessible veins are limited for needle puncture, and capillary skin puncture may be associated with increased risk of infection, injury, pain, and maternal anxiety [26, 27]. While, blood count monitoring during chemotherapy represents another type of challenges where the venipuncture-based blood test may lead to complications, and delays and/or dose reductions of subsequent courses of chemotherapy [28, 29]. A non-invasive blood count device enabled by ADAS-OCT could potentially reduce the number of blood samples from neonatals and allow rapid tailoring the dosage and improve the life quality of patients undergoing chemotherapy.

ADAS-OCT is based on the same depth-priority point-scanning mechanism as the most of current endoscopic and intracoronary OCT systems, so that it can be potentially developed into an endoscopic technology that performs high-speed circumferential and helical scanning. In contrast, to maintain phase stability high enough to guarantee coherent aperture synthesis, existing computational-based aberration-correction methods have to rely on special scanning mechanisms such as line-field scanning or time-domain lateral priority scanning [14–16], which are not suitable for endoscopic applications in tubular organs. An ADAS-OCT endoscopic probe could be realized in practice by incorporating a beam displacer and a polarizer into a classical fiber-optic design [30] (Fig. S5). Using a calcite beam displacer, the two-beam separation angle could be 2.2° with a probe diameter of 2 mm and a rigid length of 21.2 mm, based on optical simulation results using Zemax software (Zemax, LLC, Kirkland, Washington, USA). This probe diameter should be compatible with the instrument channel of a standard video endoscope [31] or in a tethered capsule endomicroscope [30, 32].

Once developed, such an ADAS-OCT endoscope may enable tomographic diagnosis of mucosal abnormalities at the cellular basis in internal organs. Potential clinical benefits may include the early detection of intraepithelial neoplasia and metaplasia in the gastrointestinal tract. Such early detection can be missed by standard video endoscopy and random biopsy procedures if the lesions are focally and heterogeneously distributed over a large mucosal area, leading to a lack of morphological features at the mucosal surface. ADAS-OCT could potentially improve screening and diagnostic outcomes by capturing the cellular hallmarks of the precancerous lesions invisible to standard-of-care tools, such as goblet cells in Barratt's esophagus and specialized intestinal metaplasia in the stomach.

Looking towards the goal of tomographic diagnosis at the cellular level, future efforts will focus on the development of a clinically viable endomicroscopic ADAS-OCT system. The diameter and rigid length of the probe may be further reduced by using materials with larger birefringence, such as yttrium orthovanadate (YVO4). Space constraints could be further mitigated by employing a tethered capsule that can accommodate larger-diameter optics. The ranging depth of

the spectrometer could be extended through the use of spectral combination [23], linearization [33], and circular interferometric ranging [34].

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See Supplement 1 for supporting content.

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Supplement 1

1. The ADAS-OCT imaging system

ADAS-OCT assumes the basic set-up using in SD-OCT, with broadband light delivered to the input port of a Michelson interferometer and the spectral interference signals detected using a spectrometer (Fig. S1). A supercontinuum light source (SuperK Extreme OCT, NKT Photonics A/C, Birkerød, Denmark) provided illumination with a bandwidth of 180 nm centered at 800 nm through a single-mode fiber (780 HP, Nufern, USA). The input light was collimated by a lens L1 (AC050-010-B-ML, Thorlabs, Newton, NJ, USA) and split into the reference arm and sample arm by a 50:50 beam splitter BS (BS011, Thorlabs, Newton, NJ, USA). The light in the reference arm was focused by a lens L2 (M Plan Apo NIR 20X, Mitutoyo, Takatsu-ku, Kawasaki, JP) onto the reference mirror M. The light in the sample arm was guided by a pair of galvanometer mirrors (GVS002, Thorlabs, Newton, NJ, USA) to an objective lens L3 (M Plan Apo NIR 20X, Mitutoyo, Takatsu-ku, Kawasaki, JP). The light backscattered or reflected from both arms was coupled by a lens L4 (AC050-010-B-ML, Thorlabs, Newton, NJ, USA) into an SMF (780 HP, Nufern, USA) connected to the spectrometer. The spectrometer consisted of a 1500 lines/mm diffraction grating (PING-1500, Ibsen Photonics, Farum, Denmark), a camera lens (Canon EF 85 mm, f/1.2, Tokyo, JP), and a 4096-pixel linear camera (AViVA EM4, e2V, Chelmsford, UK). This spectrometer provided a ranging depth of 1.87 mm (6-dB roll-off) (Fig. S6). The detected interference signal was digitized at 12-bit resolution and transferred to a computer via an image acquisition board (KBN-PCE-CL4-F, Bitflow, Woburn, MA, USA). Image acquisition and galvanometer mirror scanning were digitally synchronized. Dispersion correction [1] and a discrete Fourier transform were performed on each axial line (A-line) to obtain the axial depth profile of the tissue.

The core of our ADAS-OCT system was a custom-designed CBD (see Table. S1 for details of the design) and a QWP (AQWP05M-980, Thorlabs, Newton, NJ, USA) at the infinite space of the sample arm, which together served as the amplitude beam divider and the optical path encoder (Fig. 2b, c). The collimated beam from the light source was split into doubly-refracted beams – the ordinary beam (*o*-beam) and the extraordinary beam (*e*-beam) – due to the anisotropic refractive index of the CBD (Fig. 2b). For clarity, we defined the sub-aperture corresponding to the ordinary ray illumination as the *o*-sub-aperture and the sub-aperture corresponding to the extraordinary ray illumination as the *e*-sub-aperture. The two beams were laterally displaced by Δx and axially shifted by Δz in the OPD with respect to the reference light. Backscattering resulted in four round-trip paths of the detected light (*oo*-, *ee*-, *oe*- and *eo*-paths; Fig. 2d).

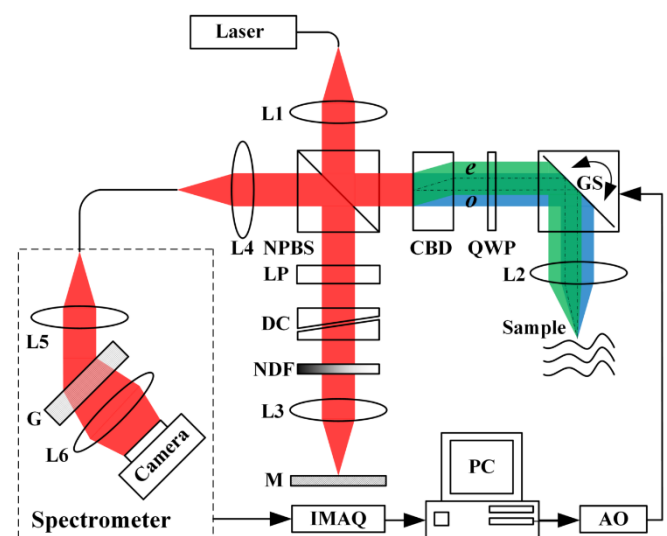


Fig. S1. Schematic of the ADAS-OCT system. L1-5: optical lenses; L6: camera lens; G: transmission diffraction grating; NPBS: non-polarizing beam splitter; CBD: calcite beam displacer; QWP: quarter-wave plate; GS: two-dimensional galvo scanner; LP: linear polarizer; DC: dispersion compensator; NDF: neutral density filter; M: mirror; IMAQ: image acquisition system; PC: personal computer; AO: analog output; o - and e -, the ordinary beam and the extraordinary beam.

It should be noted that the oe - and eo -paths share the same OPD with respect to the reference light, such that three interference signals from a scatterer were encoded in the depth domain. The number of sub-apertures was increased from two to three by adding another beam displacer (Fig. 2e and Fig. S2; see Table. S2 for details of the design). In addition, a linear polarizer LP (LPNIRE 100-B, Thorlabs, Newton, NJ, USA) was placed in the reference arm for polarization management.

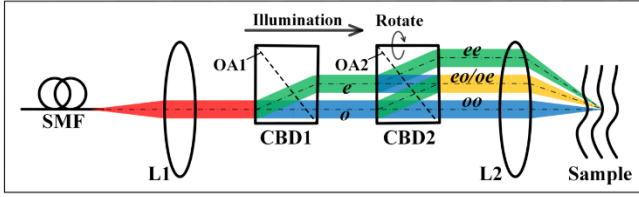


Fig. S2. Rotation of CBD2 generates maximum aperture separation. SMF: single-mode fiber; L1-2: optical lenses; CBD1-2: calcite beam displacer; OA1-2: optical axis of CBD1-2 (in plane of paper).

2. Sample preparation and visualization

Tissue phantoms were fabricated by mixing agarose solution (No. PC0701-100 g, Vivantis, Oceanside CA, USA) with polystyrene microparticles (No. 64090-15, nominal diameter 6 μ m, Polysciences Inc, Warrington, PA, USA). This mixture was stored in a vial and placed in an ultrasonic bath for ten minutes to remove residual clusters. The sample (10 g) was poured into a cell culture dish, cured for 30 minutes at 100 $^{\circ}$ C and then cured for 24 hours at room temperature before being put to use in imaging experiments.

Fresh chicken breast tissues were obtained from a local wet market on the day of slaughter. Muscular tissue was dissected after gross inspection and was sliced into sub-millimeter-thick pieces. The sample was covered by wet gauze (phosphate-buffered saline solution) to prevent dehydration before the imaging experiment.

Human labial mucosa was imaged using an air spacer mounted onto a fused silica window, which was used to maintain the working distance between the objective lens and the labial mucosa of the human subject. During image acquisition, the lower labial mucosa was in gentle contact with a polyethylene (PE) film that covered the fused silica window. Images were acquired at 20 frames/second with 1024 axial scans/second, and each image session was completed within 30 seconds. Written consent was obtained from 2 volunteers (Subject #1, male, aged 32 with no pathological changes in labial mucosa; Subject #2, male, aged 29 with no pathological changes in labial mucosa) before the experiment. The study was approved by the Institutional Review Board at Nanyang Technological University (IRB-2016-10-015).

All data processing and image visualizations were implemented using MATLAB (MathWorks, Natick, MA, USA) and ImageJ software (<https://imagej.nih.gov/ij/>).

3. Amplitude division and round-trip beam paths

In the CBD shown in Fig. 2a and Fig. S1, the separation angle between the o - and e -beams is $\phi = 2.65^{\circ}$ according to the design parameters given in Table. S1. The corresponding beam displacement Δx is given by

$$\Delta x = d \cdot \tan \phi \quad (1)$$

where $\phi = \theta - \varphi$, $\tan \theta / \tan \varphi = n_o^2 / n_e^2$. $n_o = 1.65$ and $n_e = 1.49$ are the refractive index of CBD for the o - and e -beams according to the refractive-index sphere. The thickness of the CBD was $d = 10.5$ mm; therefore $\Delta x = 0.49$ mm. These two laterally-displaced beams resulted in two divided sub-apertures in the pupil plane of the objective lens L2. The polarization axis of the QWP was 45° with respect to the linear polarization direction of the o - and e -beams. The round-trip optical path consisting of a combination of the ordinary-illumination path and the ordinary-detection path is termed the oo -path; the path consisting of a combination of the extraordinary-illumination path and extraordinary-detection path is termed the ee -path; the path consisting of a combination of the ordinary-illumination path and extraordinary-detection path is called the oe -path; and the path consisting of a combination of the extraordinary-illumination path and the ordinary-detection path is termed the eo -path.

Here, we define the single-trip OPD caused by the CBD between the o - and e -beams as Δz , which is given by

$$\Delta z = d \cdot [n_e / \cos(\phi) - n_o] \quad (2)$$

Based on the current CBD design, $\Delta z = 619$ μ m in water. Thus the round-trip CBD-induced OPD of the ee -path is 2 Δz longer than that of the oo -path, while the round-trip CBD-induced OPD of the oe -path and eo -path is Δz longer than that of the oo -path. This made it possible to encode the interferometric signals from the four paths in the OPD domain.

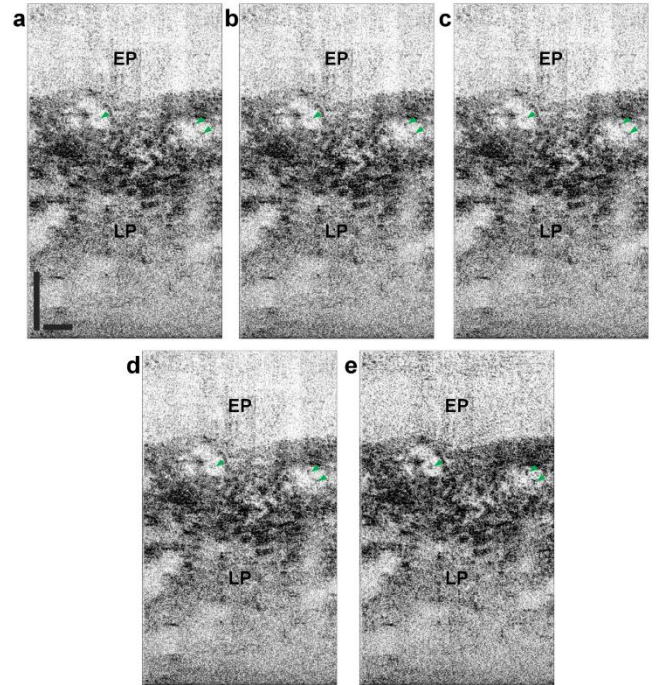


Fig. S3. One of the other representative images of RBCs *in vivo*. (a-c) m -th ($m=1-3$) uncorrected images (a-c) from the oo -path, oe/eo -path, and the ee -path, respectively. (d) The control image ($[z', z'']$ being the full depth range). In all the uncorrected images and the aberration-corrected image, the three RBCs (green arrows) were not clearly identifiable due to the lack of characteristic 'two reflection signals'. (e) Aberration corrected image, where the characteristic 'two reflection signals' are clearly visible. Transverse and vertical scale bars: 100 μ m.

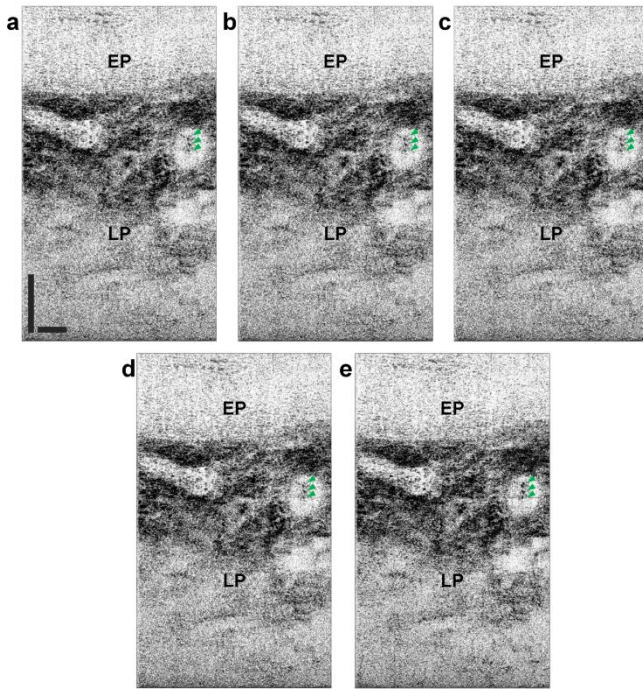


Fig. S4. Another one of the other representative images of RBCs *in vivo*. (a-c) m -th ($m=1-3$) uncorrected images (a-c) from the *oo*-path, *oe/eo*-path, and the *ee*-path, respectively. (d) The control image ($[z', z'']$ being the full depth range). In all the uncorrected images and the aberration-corrected image, the three RBCs (green arrows) were not clearly identifiable due to the lack of characteristic 'two reflection signals'. (e) Aberration corrected image, where the characteristic 'two reflection signals' are clearly visible. Transverse and vertical scale bars: 100 μm .

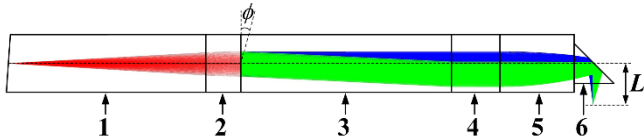


Fig. S5. Design of the ADAS-OCT probe using Zemax optical simulation software. 1: Glass spacer (BK7, diameter 2 mm, length 6.95 ± 0.05 mm). 2: GRIN lens 1 (IFRL-200-005-50, length 1.23 ± 0.005). 3: Calcite (diameter 2 mm, 7.4 ± 0.01 mm). 4: Polarization analyzer (diameter 2 mm, 1.7 ± 0.05 mm). 5: GRIN lens 2 (IFRL-200-005-50, length 1.23 ± 0.005 mm). 6: Prism (BK7, leg 0.65 mm). ϕ : Separation angle between the *o*-beam (blue) and the *e*-beam (red), with value 2.2° . The designed working distance L is 1.52 ± 0.02 mm.

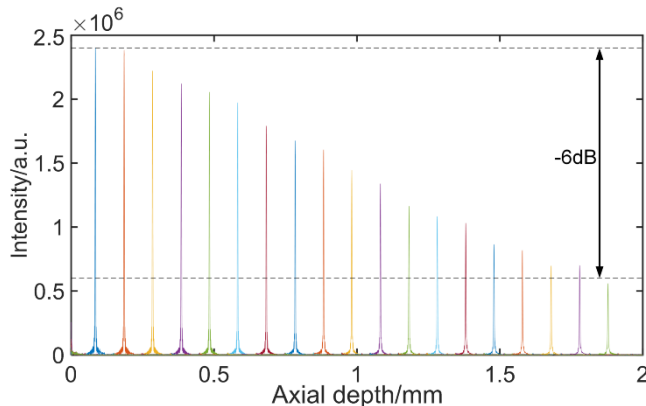


Fig. S6. Measured axial PSFs (on a linear scale). The signal intensity drops by 6 dB at a depth of 1.87 mm.

Table. S1 Design parameters of the calcite beam displacer in Fig. 2a and Fig. S1.

Dimensions	8 mm $\times$ 8 mm
Thickness d	10.5 mm
Optical axis angle φ	71.1°
Anti-reflection (AR) coating	AR / AR @700-1000 nm
Wavefront distortion	$< 1/2 \lambda$
Coating surface 1	$R < 0.5\%$ @700-1000 nm
Coating surface 2	$R < 0.5\%$ @700-1000 nm
Surface quality	20-10 scratch-dig

Table. S2 Design parameters of the calcite beam displacers in Fig. 2e and Fig. S2.

Dimensions	8 mm $\times$ 8 mm
Thickness d	12 mm
Optical axis angle φ	45°
Anti-reflection (AR) coating	AR / AR @700-1000 nm
Wavefront distortion	$< 1/2 \lambda$
Coating surface 1	$R < 0.5\%$ @700-1000 nm
Coating surface 2	$R < 0.5\%$ @700-1000 nm
Surface quality	20-10 scratch-dig

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