Optical coherence refraction tomography

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Optical coherence tomography (OCT) is a cross-sectional, micrometre-scale imaging modality with widespread clinical application. Typical OCT systems sacrifice lateral resolution to achieve long depths of focus for bulk tissue imaging, and therefore tend to have better axial than lateral resolution. Such anisotropic resolution can obscure fine ultrastructural features. Furthermore, conventional OCT suffers from refraction-induced image distortions. Here, we introduce optical coherence refraction tomography (OCRT), which extends the superior axial resolution to the lateral dimension, synthesizing undistorted cross-sectional image reconstructions from multiple conventional images acquired with angular diversity. In correcting refraction-induced distortions to register the OCT images, OCRT also achieves spatially resolved refractive index imaging. We demonstrate greater than threefold improvement in lateral resolution as well as speckle reduction in imaging the tissue ultrastructure, consistent with histology. With further optimization in optical designs to incorporate angular diversity into clinical instruments, OCRT could be widely applied as an enhancement over conventional OCT.

Optical coherence tomography (OCT) is a cross-sectional, micrometre-scale imaging modality based on coherence gating for depth resolution that has become the clinical standard of care for pathological diagnosis and treatment monitoring in several medical specialties. For long-depth-of-focus OCT systems typical of those in clinical settings, the axial resolution is dominated by the coherence length of the light source and can be sub-micrometre in scale. On the other hand, the lateral resolution of OCT relies on confocal gating of a focused Gaussian beam, characterized axially by its depth of focus. Specifically, there is a tradeoff between a Gaussian beam’s waist diameter (which determines the OCT lateral resolution) and its depth of focus (which limits the OCT imaging depth field of view). Thus, conventional implementations of OCT commonly accept anisotropic resolutions, in particular with inferior lateral resolutions on the order of 10 μm or greater, in order to obtain long depths of focus on the order of hundreds of micrometres to millimetres for bulk tissue imaging. Previous studies have addressed this tradeoff between the lateral and axial resolution, using focus techniques such as digital refocusing, beam shaping (or both of these combined) and dynamic focusing, but in all cases the lateral resolution was still limited by the diffraction limit. Furthermore, none of these methods explicitly addressed the anisotropic resolution, which can obscure ultrastructural features depending on their orientations with respect to the optical axis.

Another limitation of conventional OCT is that images are distorted due to the spatially inhomogeneous refractive index (RI) distributions in tissue. Previous attempts to correct such RI-induced distortions required a priori information on both the sample geometry and RI values, which severely limits the generalizability of such methods.

To address both shortcomings of conventional OCT, we present a technique called optical coherence refraction tomography (OCRT), which uses multiple OCT cross-sectional images (‘B-scans’) acquired at a diversity of angles to reconstruct isotropic, high-resolution, cross-sectional images with the superior axial coherence gating of conventional OCT extended to the lateral dimension. In the absence of refraction, light rays travel in straight lines and the reconstruction procedure is analogous to that of X-ray computed tomography (CT). However, due to refraction and optical path delays in the sample, OCT B-scans acquired at multiple angles are distorted differently and hence need to be dewarped and registered. Thus, in correcting for sample-induced refraction, OCRT is simultaneously a novel method for estimating the spatially resolved RI distribution of the sample, which is aligned with the high-resolution reconstruction. Previously published multi-angle RI tomography techniques for thick samples accounted only for path delay (not changes in ray direction), required access to the other side of the sample and were not applied to biological samples. Focal shift-based OCT methods for depth-resolved RI measurement typically require experimental repositioning of the focus and physical depth scanning in the sample or reference arm. However, OCRT can be implemented using any conventional OCT system, requiring only a method to rotate the sample or an appropriate scanning protocol.

Results

OCRT and CT are Fourier synthesis techniques. As OCRT can be described as a Fourier synthesis technique from multi-angle illumination, it is useful to compare it to CT. A single one-dimensional (1D) projection in CT can be considered an image resulting from the convolution of the 2D scene with a point-spread function (PSF) that is infinitely wide in its projection dimension (x) and narrow in z (Fig. 1a). Thus, in Fourier space, the product of the 2D Fourier transform of the 2D scene and the kernel transfer function is a delta function in the x direction, and of finite width in the z direction (Fourier slice theorem). Hence, by taking projections at multiple directions, the entire 2D Fourier spectrum of the object can be synthesized up to the frequency cutoff defined by the resolution of the 1D detector and the beam size. The inverse 2D Fourier transform then reconstructs the image with isotropic high resolution. In practice, a backprojection algorithm can be employed, in which each 1D projection is uniformly smeared in the direction of the X-rays, and summed across all angles, which is mathematically equivalent to the Fourier synthesis technique in a continuous framework. Filtered backprojection (FBP) applies a filter to the 1D projections to account for the fact that the centre is over-represented, the ideal filter being the ramp filter, H(f) = |f|, which arises from the Jacobian of the transformation between cartesian and polar coordinates.
in. However, because OCT already has some lateral frequency support, OCRT requires data from fewer angles than CT. The angular spacing (assumed equal) depends on the axial-to-lateral resolution ratio and the amount of tolerance on the gaps in the Fourier space at intermediate angles (Fig. 1b,c). As full angular coverage at ±90° is approached, isotropic resolution in the image plane, x−z, is achieved (Fig. 1d).

**Refraction correction.** Although the RIs of biological tissue in the X-ray regime are close to 1, in the optical regime a spatially varying RI distribution distorts OCT images by changing the path lengths and the directions of the rays, preventing rigid-body registration among the multi-angle B-scans. If the RI distribution were known, the rays could be propagated accordingly to dewarp the image before FBP application to generate a high-resolution reconstruction. To infer the unknown RI distribution, in OCRT we solve the inverse problem using the ray equation as the forward model, which in 2D (x and z) is

\[
\frac{d}{ds} (n_A \frac{dx}{ds}) = \frac{\partial n_A}{\partial x} \quad \frac{d}{ds} (n_A \frac{dz}{ds}) = \frac{\partial n_A}{\partial z}
\]

(1)

where \(n_A(x,z)\) is the RI distribution parameterized by A and s is the position along the 1D ray trajectory.\(^a\) The parameterization we chose was a sum of a regularly spaced grid of Gaussian kernels such that \(n_A(x,z)\) is differentiable everywhere and minimizes the effects of the ‘staircase’ artefacts stemming from discretization onto a Cartesian grid. In particular, we chose the index distribution given by the Nadaraya–Watson kernel parameterization.\(^b\) This parameterization also avoids having to use finite differences to compute spatial gradients, as analytic expressions are available.

Because, in general, there is no closed solution to the ray equation, we employed numerical integration (fourth-order Runge–Kutta).\(^c\) To specify the optical path delay, we used a constant step size for the numerical solver, which was scaled by the inverse of the RI value at the current position of the ray. To initiate ray propagation, we specified the initial conditions, which were the initial ray positions and directions. Assuming telecentric scanning and uniform sampling of A-scans within each B-scan, the initial directions were all parallel, and the initial positions were equally spaced for each angle. In this way, we propagated the A-scans from each B-scan from each angle as individual rays, which caused the overall B-scan image to dewarp. In theory, if the optimal RI distribution were found, the images would be perfectly registered. We assumed that the group and phase indices are approximately the same in biological tissue, such that a common index distribution governs both path trajectory and delay. Thus, in this Article, we use the terms group (refractive) index and RI interchangeably.

**Optimization by joint registration, isotropic, high-resolution reconstruction and RI map estimation.** To provide feedback on the accuracy of \(n_A(x,z)\) to aid its optimization, we required a differentiable metric that quantifies the degree of joint registration among all the B-scans. Here, we describe an intensity-based metric, with which we computed the mean squared error (MSE) between the raw B-scans and a forward prediction of the B-scans based on the estimated high-resolution reconstruction (Fig. 2). The forward model started with the current estimate of the high-resolution reconstruction based on FBP along the ray trajectories governed by the current estimate of the RI distribution. For each B-scan orientation, that current estimate was then rotated, warped according to the same trajectories, blurred by the OCT PSF, depthwise attenuated, and locally intensity-rescaled according to the orientation of the structure to the illumination. The result constituted the forward prediction of the B-scan, the MSE between which and the raw B-scan data was to be minimized with respect to the forward model parameters.
We also included regularization on the index distribution to impose spatial smoothness, stabilizing the solution and optimization. After registration, we optimized the filter for FBP. More details about the forward model are provided in Supplementary Section 1.

We used a modified version of gradient descent called Adam\(^33\) to minimize this regularized MSE, which jointly registered the B-scans and estimated both the RI distribution and the high-resolution, isotropic image. We computed the gradient of the intensity-based registration metric through the numerical differential equation solver with respect to the forward model parameters using TensorFlow\(^34\), a software library that employs automatic differentiation, a widely used technique in the deep learning community requiring only the specification of the differentiable forward model. Based on this forward model, the gradient was computed through recursive application of the chain rule.

In summary, the OCRT optimization procedure simultaneously registers all B-scans, generates an undistorted, isotropic, high-resolution reconstruction and a co-aligned estimate of the RI distribution of the sample. An overview of this intensity-based implementation of OCRT is given in Fig. 2. A more detailed description of this implementation is provided in Supplementary Section 1.

**Experimental results.** To validate the isotropic resolution of OCRT, we imaged 560-nm polystyrene beads embedded in a 2% (wt/vol) agarose gel inside a glass microcapillary tube (Fig. 3). We used a custom-designed inverted rotation stage (Supplementary Fig. 4) to rotate the tube sample, which was immersed in water to reduce

![Fig. 2 | Overview of the iterative OCRT reconstruction algorithm.](image)

Fig. 2 | Overview of the iterative OCRT reconstruction algorithm. Multi-angle B-scans served as the input, which were backprojected along trajectories derived from ray propagation (ray propag.) according to the current estimate of the RI distribution to form an estimate of the high-resolution reconstruction. The same trajectories sampled the current high-resolution estimate to generate forward predictions of the B-scans. The MSE between the B-scan data and B-scan estimates was iteratively minimized with respect to the RI distribution. For a more detailed description, see Supplementary Section 1.

![Fig. 3 | Experimental validation of the isotropic resolution and RI estimates of OCRT. a, b, OCT B-scan of sub-resolution polystyrene beads, averaged 1,200 times (a, also averaged across 18 frames in the out-of-plane dimension) and histogram-matched to the OCRT reconstruction (b). The two sets of enlarged views (red and blue boxes; dashed, averaged B-scan; solid, OCRT reconstruction) show clear lateral resolution enhancement. The arrows in the enlarged views indicate beads resolved in the OCRT reconstruction, but not in the averaged B-scan. c, Bar plots of the 2D Gaussian width (full-width at half-maximum, FWHM) fits (median, with 90% data coverage intervals after filtering out poor fits; numerical labels are medians) demonstrate a greater than threefold enhancement in lateral resolution and show that the spatial resolution of OCRT is isotropic. For OCRT, the x and z axes were defined arbitrarily. d, The left column shows the OCRT RI map for the polystyrene bead sample embedded in a 2% agarose gel reconstructed in b. The right column shows the RI map for a separate polystyrene bead phantom, which was instead embedded in PDMS, which has a higher RI. The estimated RI values for the embedding media fall within the uncertainty range (dotted lines represent 1 s.d.) estimated using OCT (see Methods). Scale bars, 100 μm.

![Fig. 3](image)
the RI contrast with the glass tube wall. Because the index contrast was still large, our optimization procedure started with a gross estimation of the index distribution by first assuming a circular tube geometry and optimizing with respect to only the index of the medium and the glass, with the tube diameter, wall thickness and centre calibrated in a separate step (Supplementary Sections 1 and 2). The subsequent optimization step refined this gross estimate by allowing the entire 2D index distribution to vary arbitrarily, as well as small translations of the OCT B-scans. Excision of tissue samples and their insertion into cylindrical tubes were done in this first report to aid in sample mounting and rotation, but these steps are not in general required for OCRT (see Discussion).

To quantify the resolution of OCRT, we localized the beads of both the reconstruction (Fig. 3b) and the averaged volume (Fig. 3a), and fit a 2D Gaussian function with axes oriented with the cartesian coordinate system, where z and x are the axial and in-plane lateral dimensions, respectively (for the reconstruction, the x and z axes are arbitrary). The resulting distributions of the PSF width fits in x and z (Fig. 3c) demonstrate a factor of >3 enhancement in the lateral resolution, and confirm that the resolution afforded by OCRT is isotropic, given by the axial resolution of the original OCT images.

To validate our RI distribution estimates, we created another bead phantom substituting the agarose gel for polydimethylsiloxane (PDMS). Figure 3d shows that the OCRT index estimate is in good agreement for both agarose and PDMS, falling within one standard deviation of the bulk RI estimated independently from OCT path-length measurements (see Methods).

To demonstrate the performance of our method, we imaged and applied OCRT to a variety of ex vivo biological samples, including several mouse organs (vas deferens, femoral artery, bladder and trachea), a human donor cornea and a mars cran fly (Tipula oleracea) leg. All samples were inserted into microcapillary tubes for convenience during imaging, and haematoxylin and eosin (H&E)-stained histological sections of neighbouring tissue samples were obtained for comparison. Masson’s trichrome stain was additionally obtained for the artery sample. Overall, we observed that lateral resolution was significantly improved in the OCRT reconstruction across all samples imaged, and that the features in the RI maps matched features in the OCRT reconstructions. In the first vas deferens sample (Fig. 4a–b), the structures of the adventitia, smooth muscle, lamina propria and the transitional epithelium are clearly resolved. For example, it is apparent in the OCRT reconstruction but not in the B-scan (Fig. 4a,b) that the smooth muscle layer consists of more circumferential structure, in contrast to the adventitia, which has a different texture; this is consistent with the histology (Fig. 4d).

Furthermore, the lamina propria is very prominent in the OCRT reconstruction as a darker, more strongly scattering layer, unlike in the histology where the delineation is more subtle, and the collagen-shaped transitional epithelium is much more recognizable in the OCRT reconstruction than in the B-scan. In the second vas deferens sample (Fig. 4i–p), in addition to the features in the first vas deferens sample, we observe clear evidence of resolution enhancement in the artefactual ribbon-like detachments of the adventitia, consistent with the histology (Fig. 4l).

In the mouse femoral artery sample (Fig. 4q–x), the smooth muscle layer and the external and internal elastic lamina are much more apparent in the OCRT reconstruction, but essentially invisible in the B-scan. Note in the OCRT reconstruction the undulatory pattern of the internal elastic lamina (Fig. 4v,x), consistent with the Masson’s trichrome-stained histology, which accentuates the elastic lamina with a blue colour (Fig. 4t, bottom slice). The internal elastic lamina is lined by nuclei in the OCRT reconstruction, appearing as dark dots (Fig. 4x), which is consistent with the H&E-stained histological slide (Fig. 4t, top slice).

In the mouse bladder sample (Fig. 5a–h), the layered structures of the smooth muscle are much clearer in the OCRT reconstruction than the B-scan, where they are less apparent due to the lower lateral resolution and the presence of speckle noise. The connective tissue in the lamina propria is also better resolved in the OCRT reconstruction. In particular, a comparison of the images in Fig. 5e,g,h,l reveals connective tissue features of the lamina propria present in the OCRT reconstruction, but not in the B-scan. In the mouse trachea sample (Fig. 5i–p), the basement membrane and ciliated epithelium are well resolved as dark lines across the entire sample, but only partially visible in the B-scan where they are near normal to the optical axis, suggesting that the anisotropic resolution of the B-scan is the culprit. The round cartilaginous structures are also clearer in the OCRT reconstruction.

A human donor cornea sample is shown in Fig. 6a–h. The lamellar structures of the corneal stroma are parallel to the front and back surfaces of the cornea. Thus, the features of the stroma are largely axial and hence the increase in resolution for OCRT over the B-scan is not immediately appreciable; rather, the visibility of the lamellae increases due to speckle reduction (Supplementary Fig. 5). More interestingly, the RI maps generated by OCRT range from 1.38 to 1.40 (Supplementary Fig. 6), which agrees with previous bulk group index measurements at similar near-infrared wavelengths, considering the influence of dehydration on the index.

Finally, OCRT reconstructions of the titia and femur cross-sections of a crane fly (Fig. 6i–j) exhibit superior resolution of the outer walls (cuticle) and the ultrafine features that surround them. The cuticle, apparent as a thin orange structure in the H&E, manifests at the bottom of femur as two thin dark lines in the OCRT reconstruction. Note also that although the larger hair-like structures (setae) present in the B-scan and OCRT reconstruction are not visible in the H&E (probably lost during tissue processing), the much finer setae lining the wall in the H&E are still visible in the OCRT reconstruction. The circumferential orientation of the internal longitudinal muscle fibre is also much more apparent in the OCRT reconstruction than in the B-scan. Furthermore, the RI map indicates that the lumen of the titia was filled with air (n ≈ 1) while the lumen of the femur was filled with water. Although this disparity is probably a consequence of the sample preparation procedure, it is a feature not at first apparent from the B-scan. However, on closer inspection, we note that the region indicated by the green arrow in

Fig. 4 | OCRT of mouse vas deferens and femoral artery. a,b: B-scan of mouse vas deferens (a), averaged 1,200 times and histogram-matched to the OCRT reconstruction (b). c: RI map that registers the multi-angle B-scans. d: Histology of vas deferens from a separate animal. e–h: Enlarged views of regions of interest in a and b. i–p: The same information as in a–h but for another vas deferens sample from a different animal. In both samples, structures in the adventitia (Ad) are better resolved in the OCRT reconstruction, particularly where the adventitia is artefactually detached (labelled d). The adventitia has a texture that is different from the smooth muscle layer (SM) in b, consistent with the histology. The transitional epithelium (TE), lamina and lamina propria (LP) are more apparent in the OCRT reconstruction. q–x: The same information as in a–h but for a collapsed mouse femoral artery. In t, two adjacent histological slices of femoral artery from a separate animal are shown. The top slice was stained with H&E and the bottom slice with Masson’s trichrome stain to accentuate the internal and external elastic lamina (IEL and EEL) lining the SM. Unlike in the B-scan, in the OCRT reconstruction, the IEL and EEL are clearly resolved, the former exhibiting undulatory patterns, consistent with histology. Scale bars, 100 μm.
Fig. 5 | OCRT of mouse bladder and trachea. a, b. B-scan (inverted) of a mouse bladder sample (a), averaged 1,200 times and histogram-matched to the corresponding high-resolution OCRT reconstruction (b), which shows the layers of the SM more clearly than in the B-scan. Furthermore, connective tissue structures in the LP are much better resolved. c. RI map that registers the multi-angle B-scans, which exhibits two distinct layers corresponding to the SM layer and the TE. d. H&E-stained histological section from the same animal. e–h. Enlarged views of regions of interest of the OCT B-scan and OCRT reconstruction in a and b. i–p. The same images as in a–h but for mouse trachea (B-scan also inverted). The histology in l was obtained from a separate animal. The basement membrane (BM) and ciliated epithelium (CE) are well resolved throughout the entire sample, but only partially for the averaged B-scan. The cartilaginous (C) structures are also clearer in the OCRT reconstruction. The adventitia (Ad) is delineated by another dark line that is not readily apparent in the B-scan. Scale bars, 100 μm.
Fig. 6 | OCRT of human cornea and crane fly leg reveals additional RI information. a, b, B-scan of human cornea (a), averaged 1,200 times and histogram-matched to the high-resolution OCRT reconstruction (b). c, The RI map, further analysed in Supplementary Fig. 6, agrees with literature values (RI \approx 1.38–1.40). d, Histology from the same sample. e–h, Enlarged view of regions of interest in a and b. The lamellar structures of the stroma (St) are more clearly defined in the OCRT reconstruction and are consistent with histology. The epithelium (Ep) and Descemet's membrane (DM) are also apparent, although partially damaged from processing (Ep damage is consistent with the histology). i–l, Information analogous to that of a–d for crane fly femur (Fem) and tibia (Tib). The outer wall of the femur (cuticle, Cu) is apparent in the OCRT reconstruction, consistent with the histology (j). Small hair-like protrusions of the cuticle (setae, Se) are apparent in the OCRT reconstruction (l). The circumferential orientation of the longitudinal muscle fibres (LMFs) are clearer in the OCRT reconstruction than in the B-scan. The RI map (i) shows an index close to 1 inside the tibia, but close to that of water inside the femur. The lower index inside the tibia is supported by the apparent upward displacement of the regions below the lumen in the B-scan (k), as indicated by the green arrow. Scale bars, 100 \mu m.
multiple angles to overcome only the limited depth of penetration of OCT.

OCRT is advantageous over other techniques that address the depth-of-focus tradeoff5–8 because it completely replaces the lateral resolution dependence on diffraction with the axial resolution dependence on coherence. For example, recent work in applying OCT to the extreme ultraviolet regime has yielded axial resolutions on the order of tens of nanometres, but with lateral resolutions three orders of magnitude lower9. We also note that, unlike these other techniques, OCRT, by design, renders the resolution isotropic. Furthermore, because OCRT does not require high numerical aperture (NA) objectives, much longer working distances associated with low NAs are possible, making in vivo imaging more practical. Moreover, OCRT can tolerate lateral aberrations, in particular those induced by the sample. Although the resolution of OCRT can be degraded by chromatic dispersion induced by the sample, in principle they can be corrected computationally. Another advantage of OCRT over other inverse optimization approaches such as interferometric synthetic aperture microscopy (ISAM) is that OCRT does not require phase stability10,11, which for the near-infrared wavelengths typical of OCT requires nanometre-scale stability of both the scanner and the sample over the acquisition time. On the other hand, OCRT, which employs intensity images, only requires stability on the order of the axial resolution, and even this constraint may in principle be relaxed through computational corrections during the registration step. Another advantage is improved image contrast from speckle reduction due to angular compounding of independent speckle patterns12–14. Finally, OCRT does not sacrifice signal-to-noise ratio (SNR), unlike Bessel beam-based OCT15–17, because a substantial fraction of the power in Bessel beams is contained in the side lobes, and unlike ISAM18, which suffers SNR fall off with distance from the nominal focus.

However, as a technique that relies on nonlinear, non-convex optimization, OCRT may be sensitive to initialization. For completeness, we also note three limitations stemming from the fact that this present study is a proof of concept of OCRT. First, we only varied the angles in the 2D plane, such that there was no out-of-plane (y) resolution enhancement. However, in theory this method could be extended to 3D by taking volumes at orientations described by two angles in spherical coordinates19. Second, all samples in this study were subject to destructive preparation using glass capillary tubes to assist in full 360° data collection, which led to some sample artefacts (that is, adventitial separation in the vas deferens and disruption of the corneal epithelium and endothelium). Both of these limitations could be solved by replacing sample rotation with an angularly scanning probe, as pioneered for metrology applications20. Alternatively, we have previously described OCT system designs in which dual scanners placed in planes conjugate and anti-conjugate to the sample under study allow independent control of the beam’s entry position and angle to the sample21. These approaches would make OCRT extensible to in vivo medical imaging of exposed biological surfaces such as the cornea, ocular anterior chamber, skin or luminal surfaces. Such imaging conditions would place constraints on the range of angles available, and thus future work will include extending OCRT to limited angle tomography22, which we discuss below. The third limitation is the relatively long acquisition times stemming from B-scan averaging (each of the 60 angular B-scans was 20 times averaged). However, we show in Supplementary Section 5 that 20 times averaging is conservative, specifically that even with no averaging OCRT still obtains substantial improvement over conventional OCT. Nevertheless, there are numerous avenues to speed up acquisition to make OCRT compatible with in vivo imaging. Although our OCT system operated at a 20kHz A-scan rate, multi-MHz-rate systems have been reported23 that would acquire the same number of raw B-scans (1,200) used even in our highly averaged OCRT reconstructions in <0.5s. We also note that OCRT includes a registration step such that, even at slower acquisition rates, OCRT can in principle correct for motion. Moreover, OCRT can take advantage of advanced denoising methods, which have been demonstrated to mitigate the need for averaging24. Another approach to reduce the number of averages and therefore the acquisition time is to use higher incident powers to increase the SNR, subject to appropriate laser exposure safety limitations. Finally, the number of angles from which images are acquired could be reduced, because according to Fig. 1c, we oversampled the angles required. Thus, future work will explore limited angle tomography and other ray subsampling strategies, in analogy with compressive sensing in CT25. This is analogous to the ‘missing cone’ problem26–28 in diffraction tomography, by which strictly along the axial dimension there is zero Fourier support. Nevertheless, various strategies have been employed to fill in this missing cone using regularization29–31, which could be readily adapted to limited angle OCRT.

In summary, OCRT is a general framework that leverages multi-angle OCT images to computationally synthesize both an enhanced-resolution reconstruction and an RI map; this is not available in conventional OCT. We have demonstrated substantial improvements of OCRT over OCT in various biological samples, and found that there were structures readily apparent in OCRT reconstructions but missing in conventional OCT images. Finally, there are readily achievable multi-angle scanning strategies and extensive prior work on limited angle tomography in other domains such as CT and diffraction tomography that could realistically propel OCRT towards in vivo medical imaging applications.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, statements of code and data availability and associated accession codes are available at https://doi.org/10.1038/s41566-019-0508-1.

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Methods

OCT imaging. All OCT images were acquired using a commercial spectral domain OCT system (Bioptigen Envisu R4110 XHR SDOIS) at a 20 kHz A-scan rate, with an incident power of 1 mW, an 820 nm centre wavelength, and a nominal axial resolution of 1.2 μm in air and lateral resolution of 8.5 μm. Our measured resolution using sub-resolution beads is given in Fig. 3a. The B-scans consisted of 500 A-scans over a field of view of 1.5 mm; each A-scan had 2,048 pixels with a maximum imaging depth of 2.22 mm in air.

For each sample, we acquired 20-averaged OCT B-scans or volumes every 6° across 360°. For comparison, we acquired, registered using a previously developed subpixel registration algorithm\textsuperscript{55} and averaged 1,200 B-scans or volumes at one angle (Fig. 3a). For the polystyrene bead phantoms only, we performed out-of-plane averaging of B-scans across 18 frames spaced 3 μm apart to account for slight tube misalignment; as a result, the beads close to the wall of the tube away from the axis of rotation, because of their small size, were not present in every B-scan. We used these y-averaged B-scans as the raw data for OCRT reconstruction. For all the biological samples this was not an issue and so we did not average B-scans across the y direction.

Preparation of samples. All samples were inserted into capillary microtubes (Drummond Scientific Microcaps) with an inner diameter of 797.6 μm and immersed in phosphate buffered saline (PBS). The 560 nm polystyrene beads (Thermo Scientific) were embedded in 2% agarose gel (Sigma Aldrich) or PDMS (Dow Corning).

The animal organs used in this study were from mice (C37BL/6 wild-type) that were euthanized and discarded from unrelated experiments; they were thus not subject to Institutional Animal Care and Use Committee review. The organs were fixed in 10% neutral buffered formalin (NBF) and kept at 4 °C for 24 h. After fixation, all organs were micro-dissected using an upright dissection scope (Zeiss). The dissected tissues were inserted into the glass microtubes using dissection forceps. In some instances, the tissues were placed into the glass tube following aspiration with a 23 G truncated needle on the opposite side of the tube. This procedure allowed the specimen to gently slide into the inner wall of the glass microtube. To avoid dehydration after fixation, all specimens were transferred with the glass tube in PBS. The remaining organ tissue was used for paraffin block preparation followed by H&E staining or trichrome staining for histological examination. Histological images were acquired at ×10 or ×20 magnification using an Olympus microscope and digitally white balanced.

The donor human cornea samples were obtained from Miracles in Sight and were declared exempt from review by the Duke University Health System Institutional Review Board. The samples were stored in Optisol at 4 °C until being transferred and fixed in 10% NBF for 24 h. Cornea samples underwent the same procedure for tube embedding and histology as for the mouse tissue.

Estimating the ground truth RI of uniform embedding media using OCT. We randomly sampled n = 8 tubes and estimated their inner diameters using OCT by measuring the difference between the luminal reflections, and obtained 797.3 ± 0.9 μm (standard error of the mean), in very good agreement with the manufacturer’s specification of 797.6 μm. We then estimated the inner diameter path length of the bead samples (with either agarose or PDMS embedding media) using the same method for all angles and volume (y) slices, across which the standard deviation values in Fig. 3d were calculated.

Sample stage. To acquire images at multiple angles, we used a custom-built, inverted rotation stage (Thorlabs) designed to mount a single tube vertically. The tube had two orientational degrees of freedom and two translational degrees of freedom perpendicular to the axis of rotation, which we used to manually align the tube. The tube was immersed in water or PBS in a cuvette, which remained stationary during sample rotation. The entire set-up was mounted on an xyz translation stage (Newport). See Supplementary Fig. 4 for a schematic layout. Sample rotation and data acquisition were automated using a custom Python script. For all experiments, we acquired B-scans at 60 orientations, spaced evenly across 360°.

Numerical optimization. Optimization was conducted on TensorFlow 1.8 using Python 2.7 on an Intel Core i7-3930K with 48 GB of RAM or in Google Cloud Platform. Because each iteration required tens of GB of memory, we used the CPU version of TensorFlow. For all samples we ran gradient descent for 200–500 iterations with 2–3 min per iteration. Filter optimization was run for an additional 100 iterations. We used the same regularization hyperparameters for all biological samples.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

The multi-angle datasets for the biological samples in Figs. 4–6 are available at https://doi.org/10.6084/m9.figshare.8297138.

Code availability

The Python code used for generating the OCRT results in Figs. 4–6 is available at https://github.com/kevinczhou/optical-coherence-refraction-tomography.

References

55. Guizar-Sicairos, M., Thurman, S. T. & Fienup, J. R. Efficient subpixel image registration algorithms. Opt. Lett. 33, 156–158 (2008).
Reporting Summary

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For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

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Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection

The raw OCT data were collected using GUI-based commercial Bioptigen software (InVivoVue 2.2.22). Button clicking was automated using a custom Python script using pyautogui (version 0.9.36).

Data analysis

The Python code used for generating the OCRT results in Figs. 4-6 is available at https://github.com/kevinczhou/optical-coherence-refraction-tomography.

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All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
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The multangle datasets for the biological samples in Figs. 4-6 are available at https://doi.org/10.6084/m9.figshare.8297138.

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Sample size
- The sample sizes in phantom data in Fig. 3c were based on the number of beads identified in the imaged field of view (n=193 for B-scan, n=323 for OCRT). We did not perform any statistical analyses of our biological data samples.

Data exclusions
- In Fig. 3c, when computing the bar heights and 90% coverage intervals, extreme fitting results for the bead widths were excluded (i.e., if the obtained fits for the B-scan widths and OCRT widths were outside of the range [1 μm, 5.2 μm], or if the B-scan x widths were >11.8 μm).

Replication
- Including the results in the supplementary information, we applied our technique to 9 different biological samples, including repeats of the same tissue type (i.e., mouse bladder, human cornea, and mouse vas deferens). In all biological samples, we observed significant improvement of our technique, OCRT, over conventional OCT.

Randomization
- This is not relevant to our study because we did not perform statistical analysis that required allocating experimental groups.

Blinding
- As there was no experimental group allocation, blinding is not relevant to our study.

Reporting for specific materials, systems and methods

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Materials & experimental systems

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| ☒ Palaeontology |
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| ☒ Human research participants |
| ☒ Clinical data |

Methods

| n/a | Involved in the study |
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| ☒ ChIP-seq |
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| ☒ MRI-based neuroimaging |

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals
- The laboratory animal organs used in this study were from mice (C57BL/6 wild-type) euthanised and discarded from unrelated experiments.

Wild animals
- We used the legs of a single dead marsh crane fly (Tipula oleracea), captured from the wild in North Carolina.

Field-collected samples
- The dead marsh crane fly was fixed in formaldehyde.

Ethics oversight
- Since the laboratory animal organs used in this study were from mice (C57BL/6 wild-type) euthanised and discarded from unrelated experiments, our study was not subject to Institutional Animal Care and Use Committee (IACUC) review. Furthermore, insects do not fall under the purview of IACUC.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Human research participants

Policy information about studies involving human research participants

Population characteristics
- We did not have access to the characteristics of the cornea donors (the samples were de-identified), nor were they relevant to the present study.

Recruitment
- The donor corneas were procured from the Miracles in Sight eye bank in North Carolina. As the characteristics of the donors were not relevant to this study, we do not expect selection biases to impact results.

Ethics oversight
- The Duke University Health System Institutional Review Board declared this study exempt from review.

Note that full information on the approval of the study protocol must also be provided in the manuscript.