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Optical Incoherence Tomography: a method to generate tomographic retinal cross-sections with non-interferometric adaptive optics ophthalmoscopes

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ABSTRACT
We present Optical Incoherence Tomography (OIT): a completely digital method to generate tomographic retinal cross-sections from en-face through-focus image stacks acquired by non-interferometric imaging systems, such as en-face adaptive optics (AO)-ophthalmoscopes. We show how to use OIT to guide focus position in cases where the user is “blind” focusing, such as autofluorescence imaging of the Retinal Pigment Epithelium (RPE). We also demonstrate that OIT can produce distinctive cross-sectional views of the retina using back-scattered, multiply-scattered or even fluorescent light, making it a complementary technique to OCT.

Keywords: Ophthalmology; Retinal imaging; OCT; adaptive optics; Split detection

1. INTRODUCTION
Optical Coherence Tomography (OCT)$^1$ is ubiquitous. It is one of the most prescribed medical imaging procedure, and it has revolutionized retinal imaging by producing so-called b-scans, i.e. cross-sectional images. These B-scans allow to reveal the laminar structure of the retina, and to document its abnormalities in many retinal pathologies.

However, OCT is not the universal imaging tool for retinal imaging, and is challenged by incoherent imaging modalities on several aspects. For starters, the highest lateral resolution is currently obtained with incoherent modalities: Structured Illumination Microscopic$^2$ photon reassignment$^3$ or sub airy confocal imaging$^4$ techniques are so far restricted to non-interferometric modalities. Secondly, some non interferometric techniques such as autofluorescence or off-axis detection techniques$^5,6$ yield better (or different) contrast than OCT on specific structures with non-ballistic photons. For instance, high resolution vessel structures, or more generally retinal objects with a static phase signature, are best revealed with dark field techniques.$^6$ These imaging modalities make use of multiply-scattered light which are filtered out in OCT because they weakly interfere with the reference beam.

Producing cross-section images with these modalities would therefore be highly valuable, either for focus-guiding autofluorescence users, who are mostly “blind” focusing since displayed images present a weak signal-to-noise ratio (SNR) and a low contrast; or simply for visualizing the axial arrangement of the retinal features which are best seen with incoherent modalities.

Here, we intend to fill these gaps by introducing Optical Incoherence Tomography (OIT): a completely digital method to generate tomographic retinal cross-sections from en-face through-focus image stacks acquired by non-interferometric imaging systems, such as en-face adaptive optics (AO)-ophthalmoscopes. We show how to use OIT to guide focus position in cases where the user is “blind” focusing, such as autofluorescence imaging of the Retinal Pigment Epithelium (RPE). We also demonstrate that OIT can produce distinctive cross-sectional views of the retina using back-scattered, multiply-scattered or even fluorescent light, making it a complementary technique to OCT.

1
2. METHODS

2.1 The Optical Incoherence Tomography procedure

The OIT method is composed of three steps (see fig. 1):

**Z-stack acquisition:** Acquisition of *en-face* images from different focal planes, forming a Z-stack (or a fly-through movie) in the z direction. Here, the Z-stacks were acquired in a step-wise manner, by manually changing the focus position and acquiring images at each plane. An entire Z-stack was acquired in around 20 minutes.

**En-face image filtering:** Each *en-face* image from the Z-stack is high-pass filtered.

**OIT Bscan computation:** For each filtered image from the z-stack, we compute the energy map (by taking the squared modulus), and we average this map on an $n \times m$ pixel window. We slide this window along the y axis to produce one strip of the OIT Bscan.

An open source Matlab GUI allowing to load a Z-stack and generate its OIT cross-section in a few seconds is available. A detailed description of the image processing procedure can be found in.

2.2 Data acquisition

The Z-stacks necessary to generate OIT cross-sections were obtained using the PARIS AO-FIO and a modified version of the MAORI (multimodal adaptive optics retinal imager) AO-SLO (Physical Sciences, Inc., Andover, MA, USA). Both systems were described in detail elsewhere. The Z-stack from the PARIS AO-FIO was obtained by translating the imaging camera parallel to the optical axis with a constant step of 30 µm in the retinal plane. AO-SLO Z-stacks were obtained by adding constant defocus values to the deformable mirror. The equivalent axial displacement was approximately 20 µm for Fig. 3 and 15 µm for Fig. 4. The aperture of the PARIS AO-FIO and the MAORI AO-SLO were respectively limited to 5 mm and 7 mm diameter at the pupil plane, giving a theoretical depth of field at the diffraction-limit of around 50 µm and 25 µm respectively.

The AO-loop rates of the PARIS AO-FIO and MAORI AO-SLO were set to 50 Hz and 25 Hz respectively.

Image acquisition was performed on two healthy subjects aged 25 and 38. Research procedures followed the tenets of the Declaration of Helsinki. Informed consent was obtained from subjects after the nature and possible outcomes of the study were explained. The study was authorized by the appropriate ethics review boards (CPP and ANSM (IDRCB numbers: 2016-A00704-47 and 2019-A00942-55)). Before the acquisition, pupil dilation and accommodation paralysis were performed by introducing one drop of each Tropicamide and Phenylephrine 10%.
3. RESULTS

3.1 Proof of concept
To demonstrate the axial sectioning capability of the OIT, we applied the OIT procedure to a Z-stack obtained from a USAF target using the PARIS AO-FIO. Figure 2 presents the area of one en-face image where the OIT cross-section was generated, and the corresponding OIT cross-section.

We confirm that the sectioning ability of OIT is limited by the depth of field (DOF) at the diffraction limit, which can be defined as $\frac{2\lambda}{NA^2}$ (where $\lambda$ is the illumination source wavelength and $NA$ the numerical aperture)\(^{12}\).

3.2 Using OIT Bscans to guide autofluorescence imaging
Figure 3(a,c) presents OIT tomographic retinal cross-sections acquired at 7° Nasal using the AO-FIO and confocal AO-SLO. We compare both OIT cross-sections with an OCT image acquired at the same retinal location with Spectralis OCT (Heidelberg Engineering, Germany, Fig. 3(b)). Although OCT can achieve a far better axial resolution than OIT (i.e., not limited by DOF but by the light source bandwidth), most of the retinal layers commonly resolved in OCT can nevertheless be identified in both AO-SLO and AO-FIO OIT cross-sections, namely: 1) the NFL (blue arrow), which gets thicker as eccentricity increases in the OCT image (Fig. 3(b)); 2) two intermediate layers labeled, IPL (Inner Plexiform Layer) and OPL (Outer Plexiform Layer); 3) inner/outer segment junction (IS/OS, green arrow) and cone outer segment tips (COST) (orange arrow). Note that layer appellations are assigned based on comparison with OCT, and as such follow OCT nomenclature.

We used the AO-SLO OIT to precisely adjust the focus position of the system to the COST layer and new images were acquired using confocal AO-SLO and autofluorescence AO-SLO. While confocal AO-SLO en-face images present photoreceptors (Fig. 3(d)), the detection of the autofluorescence signal in AO-SLO reveals the RPE\(^{10}\) (Fig. 3(d,e)). Indeed, since the axial resolution of the AO-SLO is not sufficient to separate COST and RPE, a sharp image of the RPE autofluorescence is produced at the COST focal position. Peaks of cone and RPE densities are consistent with previous studies for 7° eccentricity.\(^{10,14}\) The axial distance between the IS/OS junction and the COST for OIT cross-sections of both AO-SLO and AO-FIO was approximately 30 µm, in accordance with in-vivo OCT and AO-OCT data.\(^{15}\)

We have shown here how we can use an OIT cross section built from confocal SLO images to guide the acquisition of an autofluorescence image. In the next section, we present OIT cross sections using multiply-scattered photons which cannot be used in OCT.

3.3 Split detection Bscans and 3D microvasculature
Figures 4(a-c) present a comparison between OIT cross-sections generated through confocal AO-SLO, nonconfocal split detection and motion-contrast AO-SLO at 7° Nasal. White dashed rectangles indicate the area of the en-face image where OIT cross-sections were generated.

Two main differences can be noticed when producing split detection OIT cross-sections compared to those generated from confocal images. The first difference concerns the NFL layer which seems to disappear in split detection OIT, indicating, as previously stated, that NFL becomes mostly transparent in multiply scattered light modalities.\(^6\) Secondly, retinal layers in the inner retina get brighter with split detection OIT. By producing the OIT corresponding to a Z-stack of perfusion map images (Fig. 4(c)), we can deduce that these layers correspond to vascular plexuses, of which we expect there to be four at 7° Nasal.\(^{16,17}\) Using the split-detection OIT to precisely position the focal plane, we were able to extract perfusion maps of each of the four vascular plexuses (Fig. 4(d)), named according to:\(^{16}\) radial peripapillary capillary plexus (RPCP), superficial vascular plexus...
Figure 3.  

(a-c) Tomographic retinal cross-sections generated by, respectively, AO-SLO OIT, OCT, and AO-FIO OIT for the same subject and retinal location, where the main retinal layers can be identified (IPL and OPL stand for Inner and Outer Plexiform Layer). Red arrows: vessel location.  

(d,e) En-face retinal images obtained using respectively confocal and autofluorescence AO-SLO. The AO-SLO focal plane was adjusted to the COST focal position prior to acquisition using the previously generated OIT cross-section (a).  

(a,b,c) Log scale. Scale bar: 50 µm. Adapted with permission from © The Optical Society. (SVP), intermediate vascular plexus (IVP) and deep vascular plexus (DVP). Owing to the precise location of focus position, one can generate depth-color coded perfusion maps with ease, revealing the 3D organization of the vascular network (Fig. 4(e)).

4. DISCUSSION

We have introduced Optical Incoherence Tomography (OIT), a completely digital method enabling the generation of in-vivo tomographic retinal cross-sectional images from through focus en-face image stacks. We showed that the OIT procedure can be used on any high numerical aperture adaptive optics incoherent imager, whether scanning or full field, confocal or nonconfocal, making use of back-scattered, multiply-scattered or even fluorescent light, without any hardware modification. Although the axial resolution capacity of the OIT method, limited by the numerical aperture, is far from what can be achieved using OCT when applied to the retina, we would like to discuss some interesting assets unique to the OIT method that are complementary to OCT.

Focus-guidance for AO-ophthalmoscopes  Owing to the fact that the axial dimension in OIT cross sections is given as a function of defocus, OIT can be used to precisely position the imaging focal plane in the retina and facilitate non-interferometric im’aging of a specific retinal layer. We demonstrated that OIT cross-sections can also be applied to precisely position the focal plane in imaging modalities where users are mostly “blind” focusing because of inherent weak SNR and low contrast, as in the case of autofluorescence (Fig. 3(a)), split-detection (Fig. 4(b)), and motion contrast (Fig. 4(c)) techniques.

Bscans for super-resolution imaging methods  Let us consider small objects, unresolved laterally by OCT, and sparsely distributed in the retina ( i.e. not forming a uniform layer that would have a strong signature in an
Figure 4. (a-c) Tomographic retinal cross-sections generated by, respectively, confocal, split detection, and motion contrast techniques in AO-SLO for the same subject at 7° Nasal where the NFL is dense and four vascular plexuses can be seen. (d) En-face retinal images from the original Z-stack obtained when precisely positioning the focal plane at the layers labeled RPCP, SVP, IVP and DVP, with the help of OIT method, using motion contrast technique. (e) Composite perfusion map image, revealing the 3D organization of the retinal vascular network. White-dashed rectangle: Area of the en-face image where OIT cross-sections were extracted. (a) Log scale. (b,c) Linear scale. Scale bar: 100µm. Adapted with permission from © The Optical Society.

OCT bscan). Such objects would be almost invisible in OCT, but would have a high contrast on super-resolution en face images (obtained with SIM, ISM or sub-airy confocal imaging). An OIT cross section built from such super-resolution modalities would have a clear signature of these sub-diffraction sparse objects, invisible in OCT.

**Elucidating the origin of some dark field signatures** Comparing different modalities, along with their en-face images, may help to elucidate the cellular origin of the observed features on en-face images. For example, whether split-detection signal comes from the structure itself, or from light refracted by the structure onto an other layer, is still debated. To illustrate how OIT could help sorting out hypotheses, we show in Fig.5 an OIT built from Paris AO-FIO images, including zones outside the illuminated patch (indicated by green rectangles). The contrast obtained in these non-illuminated zones is very similar to AO-SLO offset aperture, as we previously documented. This OIT cross-sectional image allows to visualize on the same image bright field zones -involving ballistic photons- and dark field zones - involving multiply-scattered photons. Similarly to what was shown in Figs.4(a) and (b), some layers (e.g. the nerve fiber layer) have a lower contrast in dark field, as indicated by orange arrows. But there is no line break at the bright-dark interface, which tends to indicate that the axial position of a layer is the same in both modalities.

**Using multiply scattered light to generate retinal cross-sections** Multiply scattered light (or nonconfocal) imaging modalities such as dark-field, offset aperture and split detection, widely applied in AO-SLO, and recently introduced for AO-FIO, provide excellent contrast for blood vessels, mural cells and translucent retinal structures, which are poorly or not visualized in back-scattered light imaging systems. Unlike OCT, in which the coherent detection limits the use of multiply scattered light, we showed that OIT can make use of incoherent light, generating, for the first time to our knowledge, a retinal cross-sectional view using multiply-scattered light, here in split-detection mode (Fig. 4(b)). We present in Fig.6 a first attempt to build a multimodal...
OCT-OIT bscan. This result emphasizes how different the contrast of most retinal features is with these two modalities, thus showing the complementarity of OCT and OIT.

The use of multiply-scattered light can also be extended to other techniques, such as offset aperture, multi-offset and dark-field techniques. Moreover, fluorescent light could also be used to generate a cross-section, which does not produce any signal in OCT. This specificity of OIT could prove to be decisive when imaging patients with diseases for which the presence or absence of translucent structures is a biomarker. For instance, micro cystoid spaces in a primary open-angle glaucoma have been shown to have a much better contrast in split detection modality than in OCT. A split-detection OIT cross-section for these patients would certainly be highly informative and complementary to an OCT Bscan.

5. CONCLUSION

We have introduced Optical Incoherence Tomography (OIT), a completely digital method enabling the generation of in-vivo tomographic retinal cross-sectional images from through focus en-face image stacks. We showed that the OIT procedure can be used on any high numerical aperture adaptive optics incoherent imager, whether scanning or full field, confocal or nonconfocal, making use of back-scattered, multiply-scattered or even fluorescent light, without any hardware modification. We explored the focus sensitivity of OIT to guide focus position in cases where the user is “blind” focusing, e.g. in autofluorescence. Most importantly, we believe we have demonstrated that OIT is complementary to OCT, showing the axial position of structures invisible when using only coherent ballistic photons. OIT and OCT shall therefore be used together to provide a genuine multimodal Bscan, which may play an important role in clinical in-vivo retinal imaging, shining a new light on retinal diseases.
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