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Phase contrast imaging to detect transparent cells in the retinal ganglion cells layer

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Abstract. The eye is an optical window giving access to neural networks in a non-invasive way. It is possible to find in the retina biomarkers informing about the pathological state of other parts of the human body, and in particular of the brain. Neurodegenerative diseases could thus be diagnosed early and monitored by high-resolution imaging of the retina. However, a large part of the neurons in the retina are too transparent to be detected by existing techniques. At the Quinze-Vingts hospital, we have a unique retinal imaging platform in which ophthalmologists, neurologists and engineers participate. We implemented a technique based on scanning laser ophthalmoscopy (SLO) to capture the fine variations in refractive index between retinal cells. In this project we aimed at imaging and monitor cellular changes on transparent cells in the retinal ganglion cells layer in vivo on healthy participants and patients with neurodegenerative diseases.

1 Introduction

Adaptive optics technology has been introduced into clinical tools and paved the way for high-resolution imaging of the retina. This technology corrects the wavefront aberrations of the light induced by the eye lens, which cause the lack of resolution in existing clinical systems [1]. These aberrations are calculated by an analyser and then compensated by a deformable mirror allowing the system to reach a resolution of only a few microns. There are two types of clinical cameras corrected by adaptive optics, the full field ophthalmoscope [1], where an extended source is projected over the entire field of the image; and the scanning ophthalmoscope (AOSLO), where a light source is scanned to generate the image [2]. In addition, AOSLO can be equipped with a confocal pinhole that filters out all photons from layers outside the image plane. Using adaptive optics, highly reflective cells, such as photoreceptors, have been revealed [1,2]. However, the confocal configuration turned out to be inefficient in the case transparent cells such as certain neurons or capillaries. To meet this challenge, AOSLO was made sensitive to the fine phase variations of the light passing through these cells by modifying the detection. The AOSLO was optimized by integrating phase contrast imaging techniques from scanning microscopy known as “off-axis” techniques which rely on the displacement of the confocal pinhole of the AOSLO to block the confocal signal reflected by highly reflective structures [3-6]. We can then detect the light backscattered by the deep layers of the retina, which will pass through the transparent cells and therefore illuminate them “by transmission”. When crossing these transparent cells, the light is deflected with an angle that depends on the refractive index of the tissue crossed. The displaced confocal pinhole will therefore detect more or less light depending on this angle, generating phase-dependent intensity variations on the detector. A range of modalities based on this off-axis detection already exist. At University of Pittsburgh we developed an optimal detection pattern which consists in a radial combination of multiple offset pinholes allowing for the first time to obtain *in vivo* phase contrast images of microglial and ganglion cells on healthy participants [7]. We implemented this “multi-offset” detection in an AOSLO at the Quinze Vingts hospital in order to image the transparent cells in the retinal ganglion cells layer on healthy subjects as well as patients.

2 Phase contrast Adaptive Optics Scanning Laser Ophthalmoscope (AOSLO)

We modified our AOSLO system previously described [8] at the detection plane to reproduce optimal multi-offset detection configuration determined before [7]. We added a rotation stage to the current detection fiber bundle. The latter is a fiber bundle composed of two opposite fibers that act as offseted pinholes placed at opposite side of a central confocal pinhole. It can be manually rotated to complete the multi-offset pattern as shown in Fig.1. Four acquisitions of 100 frames at 24 Hz need to be recorded at the same region and layer.

The multi-offset technique enables us to convert the phase signal of the photons going through the transparent cells into intensity at the detection plane. The light, which has previously been multiply-scattered by deep layers of the retina, goes through the cells where it is deviated differently depending on the refractive index of the tissue and therefore photons from different regions of the cell do

not reach the same offset aperture leading to differences between adjacent tissues with different refractive indexes in transparent cells [7].

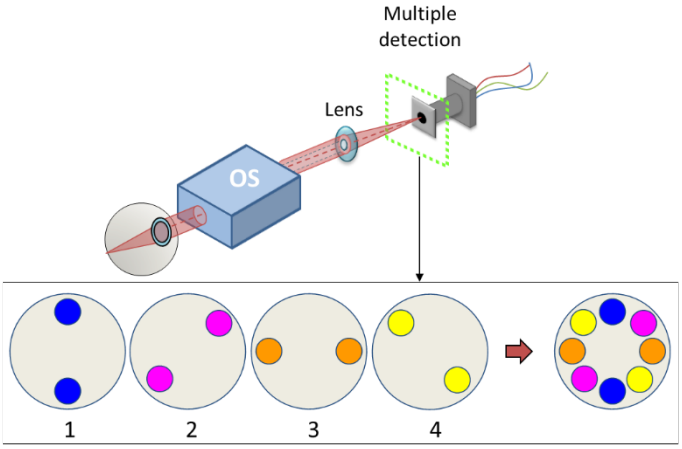

Fig. 1. Schematic drawing of the multi-offset detection configuration implemented on the Quinze-Vingt AOSLO. An arrow indicates the place of the detection fiber bundle which is placed on a rotation stage and rotated to four positions to sequentially obtain the 8 positions multi-offset pattern. OS: Optical system.

By combining the images from the multiple pinholes, we revealed the transparent ganglion cells (Fig.2 d). We also simultaneously acquired confocal images that reveal highly reflective cellular structures such as nerve fibers (Fig.2 c). Image sequences were corrected for eye motion and averaged to increase signal to noise ratio.

3 Detecting and monitoring cells in the retinal ganglion cells layer

In the retina, as in the rest of the central nervous system, immune cells play a key role in homeostasis, neuroprotection and the death of neuronal cells [9]. There is also the potential to observe ganglion cells as well as their axons (Fig.2) which are damaged over the course of neurodegenerative diseases.

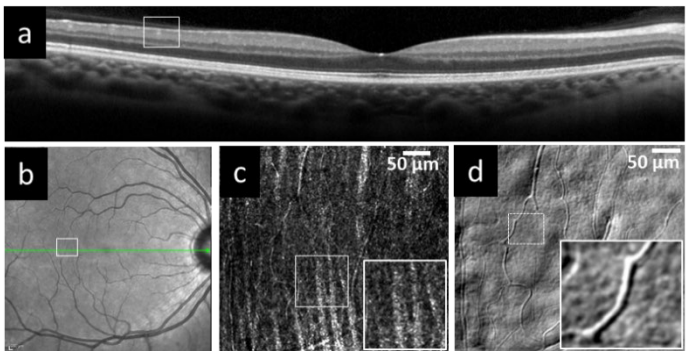

Fig. 2. (a) Optical coherence tomography (OCT) cross-section of healthy volunteer with a rectangle showing were the images were acquired. (b) SLO image of the same volunteer. (c) confocal AOSLO image of the nerve fibers (axons) of the white rectangle region in (a,b). (d) Multi-offset AOSLO images showing the ganglion cells in the same region.

Phase contrast imaging through multi-offset AOSLO has enabled us to image ganglion cells at a cellular resolution in humans in vivo. In the zoom (Fig.2 c) cells with a dark center and surrounded by a bright ring can be distinguished with a size and distribution in accordance with retinal ganglion cells characteristics. Additionally, we were also able to image patients with neurodegenerative pathologies from ongoing clinical studies at the Quinze Vingt hospital and detected putative immune cells in the ganglion cells layer. These cells could become biomarkers informing about the impact of inflammation in these neurodegenerations.

4 Conclusions and Perspectives

Using the phase contrast AOSLO imaging, we were able to detect previously invisible neural network cells as well as putative inflammatory cells specifically in the ganglion cells layer which is targeted by several neurodegenerative diseases. Future work will focus in implementing a fiber bundle detector composed of optical fibers organized according to the optimal multi-offset pattern for simultaneous acquisition of all offset pinholes. This will shorten the total acquisition time and will allow us to detect the phase signal from the same timepoint in all directions.

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