High-resolution imaging of retinal cells in the living eye

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Abstract

During diseases such as age-related macular degeneration, glaucoma, or retinal degeneration, visualizing and counting retinal cells would be of interest to diagnose early stages and to determine the short-term progression rate of these conditions. This is of major interest to optimize neuroprotective/regenerative therapies in these slowly progressive diseases. Such cellular imaging is already feasible in the eye of laboratory animals. In the near future, it is likely that visualization of individual neuronal cells in humans will become a routine clinical procedure, thanks to continuous technological improvements in optical imaging technologies. In mice, commercially available confocal scanning laser ophthalmoscope (cSLO) allows imaging of axons, capillaries, as well as dye-labelled cells (such as gfp-expressing cells). In humans, commercially available optical coherence tomography (OCT) allows routine imaging of retinal layering, but not of individual cells. Several research groups (including ours) are working in the highly competitive area of retinal cellular-imaging in humans. Different systems of adaptive optics, SLO, and OCT (in spectral domain a-mode or *en face* mode) and second harmonic generation imaging are under evaluation. These techniques have demonstrated cellular-level imaging capabilities of photoreceptors, of the nerve fibre layer and of capillaries. The main challenges that these systems face are biological (determination of the origin of the signal), medical (the clinical pertinence of the information) and technical (optimization of hardware, development of user interface).

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Introduction

Imaging the fundus *in vivo* in experimental models of retinal diseases is of great interest for screening purposes, for characterization of the

natural history of retinal diseases or for monitoring therapeutic effects. In slowly progressing diseases, accurate evaluation of the progression of neuronal cell death is crucial to evaluate neuroprotective/regenerative strategies. Yet, currently available techniques, either morphological or functional, do not allow to reliably estimate the progression rate of the disease in an individual patient in the short term.

In mice, confocal scanning laser ophthalmoscopy (cSLO) allows high-resolution fundus imaging.¹ Indeed, the optical set-up of the cSLO is in such a way that the smaller the eye, the higher the resolution, thus resolution is higher in the mouse eye than in the human eye. The Heidelberg Retina Angiograph (Heidelberg Engineering, Dossenheim, Germany) is a digital cSLO equipped with four laser wavelengths (488, 514, 788, and 820 nm), with filters for fluorescein and indocyanine green (ICG) fluorescence. The reflectance imaging associates reflection from nerve fibre bundles and vessel wall, and absorption by haemoglobin and melanin. Representative examples of cSLO fundus imaging in mice are shown in Figure 1a. Several planes of optical sectioning within the retina permits tridimensional viewing of the microvascular array by fluorescein angiography, and accurate morphologic and spatial characterization of fluorescently labelled cells such as gfp-expressing cells or ICG-labelled ganglion cells (Figure 1b).² Finally, retinal and choroidal haemodynamics of fluorescently labelled cells can be appreciated.^{3–6} An interesting development has been reported by Cordeiro *et al.*⁷ who showed that apoptotic cell death of retinal ganglion cells in rats can be detected by SLO after intravitreal injection of dye-labelled antibodies against annexin. Thus, the metabolism of retinal neurons can be appreciated *in vivo*, given that the appropriate fluorescent marker is available and that it can be delivered to the cell. An emerging and promising technique is *en-face* optical coherence

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Figure 1 Left, illustration of cSLO imaging in a pigmented mouse by 514 nm laser. Note the visibility of the nerve fibre layer axons. Centre and right, visualization of ICG-labelled ganglion cells in a rat (the right image is a magnification of the centre image).

tomography (OCT). Compared to ‘conventional’ (a-mode) OCT, *en-face* OCT uses white light, which has a very narrow coherence band and thus allows potentially higher definition than diode illumination. Moreover, the technique is relatively simple in its conception, since a XY image is acquired by a single shot; scanning is necessary only for acquisition of volumetric data. In *ex vivo* experiments, this technique has shown promising perspectives (Figure 2).⁸ *In vivo* experiments are ongoing. Another promising technique is that of second-harmonic generation, which is of particular interest for imaging structures such as axons.⁹

In humans, several research groups including ours are working in the highly competitive area of neuronal imaging. OCT, which is now part of routine clinical examination, does not yet truly perform cellular imaging, since only the retinal layering can be resolved. To progress further toward true cellular imaging, techniques such as adaptive optics, SLO,¹⁰ high-resolution a-mode or *en face* OCT alone or in combination, are being developed. Fundus imaging techniques using adaptive optics have already shown the potential to resolve individual cells, essentially cone photoreceptors. The principle used in our lab is based on the analysis of the fundus reflection of a light spot, whose deformation by cornea and lens is corrected through the use of a deformable mirror; once the optical aberrations are corrected, a flash image is acquired. Using this system, the perifoveal cone photoreceptor array is visible (Figure 3). The imaging capabilities are improving owing to technological improvements of deformable mirrors, especially the number of actuators and their amplitude, that is, their aptitude to compensate high-degree aberrations.

These techniques have demonstrated cellular-level imaging capabilities of photoreceptors, of the nerve fibre layer and of capillaries. Moreover, capillary

Figure 2 *En-face* optical coherence tomography imaging of the pigment epithelium and of the sclera *ex vivo*. This transverse image is a reconstruction of 100 *en-face* images. The retina has been stripped off before imaging procedure.

Figure 3 Imaging of the perifoveal cone array by adaptive optics in a human subject.

haemodynamics can be appreciated since the alternation of red cells and plasma in capillaries, and thus the circulation in the capillary meshwork can be detected noninvasively and with a higher precision than by conventional fluorescein angiography. A clinically pertinent objective would be to obtain imaging of the retinal pigment epithelium array, which to our knowledge has not yet been achieved.

It can be suggested that the specific contribution of cellular imaging will be early diagnosis and determination of short-term progression rate during age-related macular degeneration, glaucoma, retinal degeneration among other diseases. Thanks to progress in optical imaging technologies, it is only a matter of time before cellular imaging will enter the clinical practice. However, to become clinically useful tools, several issues must be addressed, such as optimization of the hardware, but also development of an ergonomic user interface, the determination of the origin of the signal and of the clinical pertinence of the information. Indeed, it is likely that the huge amount of information generated will take time to be replaced in the context of their specific medical interest. It is likely that several competing systems will coexist. It can be suggested that retina specialists will soon face the same challenge as neurologists did with the advent of magnetic resonance imaging.

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