Adaptive optics for in-vivo exploration of human retinal structures

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ABSTRACT

Adaptive optics (AO)-enhanced imaging of the retina is now reaching a level of technical maturity which fosters its expanding use in research and clinical centers in the world. By achieving wavelength-limited resolution it did not only allow a better observation of retinal substructures already visible by other means, it also broke anatomical frontiers such as individual photoreceptors or vessel walls. The clinical applications of AO-enhanced imaging has been slower than that of optical coherence tomography because of the combination of technical complexity, costs and the paucity of interpretative scheme of complex data. In several diseases, AO-enhanced imaging has already proven to provide added clinical value and quantitative biomarkers. Here, we will review some of the clinical applications of AO-enhanced en face imaging, and trace perspectives to improve its clinical pertinence in these applications. An interesting perspective is to document cell motion through time-lapse imaging such as during age-related macular degeneration. In arterial hypertension, the possibility to measure parietal thickness and perform fine morphometric analysis is of interest for monitoring patients. In the near future, implementation of novel approaches and multimodal imaging, including in particular optical coherence tomography, will undoubtedly expand our imaging capabilities. Tackling the technical, scientific and medical challenges offered by high resolution imaging are likely to contribute to our rethinking of many retinal diseases, and, most importantly, may find applications in other areas of medicine.

Keywords- adaptive optics, flood illumination, scanning laser ophthalmoscope, age-related macular degeneration, arterial hypertension, photoreceptor.

1. INTRODUCTION

The fundus of the eye has been observed for more than 150 years, yet until recently most fundus structures of medical importance could not be directly observed. Indeed, the transparency of the retina and of vessels makes them invisible to conventional fundus photography. It is the advent in the 90's of optical coherence tomography (OCT) and of adaptive optics (AO)-enhanced *en face* fundus observation[1] that made the retina directly observable. These improved the visibility of the fundus by two different ways; one by detecting the faint light reflected back by the optical interfaces of the retina hence improving axial resolution, and the second one by correcting optical aberrations hence improving lateral resolution. Increasing resolution is conceivably beneficial since pathogenesis begins at the level of individual cells. Moreover, most treatment of diseases are more efficient at early stages, before tissue disorganization or destruction has occurred. Thus, understanding and detecting early steps of diseases requires cellular scale resolution. The first demonstration of the utility of AO-enhanced fundus imaging was reported in 1997 using a flood-illumination camera[1]. Later on, scanning systems were developed and currently scanning and flood illumination systems are both being used. AO is playing an increasing role as an enabling technology in several areas of basic and clinical vision science. Not only by allowing a higher transverse resolution but also by enabling finer functional studies and even measuring chemical and physiological responses

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of the retina directly. Here we will discuss the contribution of AO imaging to clinicians in the field of retinal diseases.

AO gained interest initially because it allowed the observation and quantification of cone photoreceptors. However, it rapidly appeared that AO imaging of photoreceptors faced a certain number of challenges that in combination impaired its widespread use. The combination of poor fixation, media opacities, loss of contrast, edema, pîgmentary changes and photoreceptor disarray probably account for the limited use of AO in clinical research centers. Since then, while the clinical use of OCT has been developing at an exponential pace, AO-enhanced imaging lagged somewhat behind in term of clinical applications and hence of market. Thanks to the convergence of technical maturity and better understanding of the contribution of AO imaging in areas other than retinal dystrophies it is expanding its use in research and clinical centers through the world, in ophthalmology and beyond. Indeed, the use of AO-enhanced imaging is now expanding beyond photoreceptor diseases, and this may represent a decisive step in the integration of AO-enhanced imaging in clinical practice. In the present review we will focus on the contribution of AO–enhanced imaging to the basic knowledge and management of some of the most common diseases in medical practice and point out some perspectives for improvements. We will exclude from this review some applications of AO in the field of psychophysics such as visual acuity assessment and functional measures (neurovascular coupling, intrinsic imaging). This paper will successively address photoreceptor, age-related macular degeneration, and vessels. Some technical and medical perspectives will be suggested.

2. MEDICAL APPLICATIONS OF ADAPTIVE OPTICS IMAGING

2.1. Photoreceptors

Photoreceptors were historically the first fundus structure to be electively detected by AO-enhanced imaging, and in the same time the first neuron to be identifiable in vivo in humans. Cone photoreceptors being larger than rods, they are more easily detected. It has to be mentioned that strict sensu only the photoreceptor outer segments are detectable, because they strongly interact with incident light. The rather regular arrangement of cones in normal eyes is described as a mosaic. The cone mosaic shows a characteristic spatial and temporal variability, that is, a striking cell-to-cell variability of reflectance, and this reflection pattern (figure 1). Cone photoreceptors modify their reflectance over milliseconds, the underlying mechanism of which is not clearly established. It is possible that reflections of photons within outer segments account for these changes. Waveguiding properties of photoreceptors may also contribute to spatial and temporal reflectance variability, since slight misalignment may disperse reflected light. Cone photoreceptors have a strong directional absorbance and reflectance selectivity, a property referred to as the Stiles-Crawford effect (SCE)[2-4]. The discovery of a reduced foveal sensitivity when illuminating the fovea off-axis revealed the functional importance of the SCE. This directional selectivity can also be documented on retinal images. Indeed, comparative analysis of photoreceptor reflectance on images taken with off-axis illumination are therefore of interest, because the visualization of the cone mosaic can be dramatically modified[5]. It is likely that these findings may help to refine our interpretations of images from diseased retinas. Automated cone counts or segmentation algorithms of OCT scans may vary in their result according to the angle of incident light, which is usually not recorded at the time of examination. Our findings indeed suggest that the decreased intensity of the reflectance of the COST line seen in a particular incidence may be due to misaligned cones. Hence, integrating images from several entry pupils may contribute to disambiguate missing from off-axis cones. Another interesting consequence of our findings is that the presence of directional reflectance may actually be helpful to identify cones within a remodeled retina. Accordingly, it has been shown that extracting the asymmetric component of photoreceptor reflectance (split-detection[6]) is a powerful mean for the identification of cones in a dystrophic retina. Normative database of cone photoreceptors are being build up. More recently, imaging of rod photoreceptors and of central foveal cones have been reported.

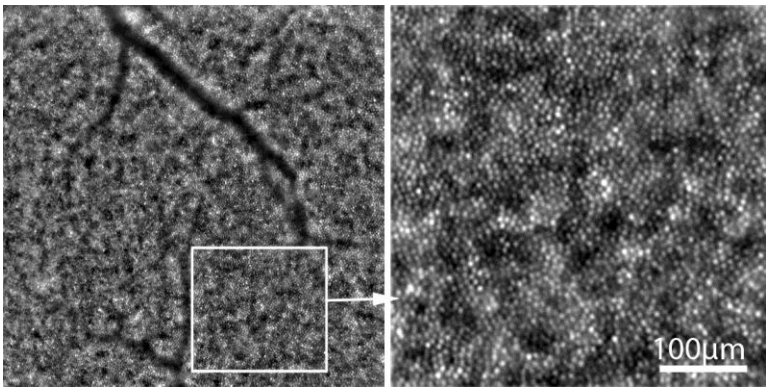

Figure 1. Imaging of a normal cone mosaic by adaptive optics (AO) flood illumination imaging.

Image processing has brought major improvements to AO imaging. Each type of AO fundus imaging (either scanning or flood-illumination) has its own constraint regarding image processing. As flood-illumination systems usually produce noisy images, registration and averaging of these images is commonly done. Myopic deconvolution of AO images[7] enables to extract cone mosaics from raw (i.e. non-averaged) images (Figure 2). Sub-pixellic registration[8b]also had a significant impact on the resulting image quality, through the suppression of peripheral rotational artifacts and hence enlarging the field over which photoreceptors can be counted. Cone counting algorithms have now reached a high degree of reliability. Recursive construction of thresholded components, when the seeds of the recursions are the regional maxima of the input image, is a reliable approach[10,11].

Retinal diseases collectively termed retinal dystrophies affect electively or preferably photoreceptors and are a cause of irreversible blindness. Many of these diseases are due to gene mutation, although the role of epigenetics in the clinical presentation plays a significant role. Several vision preservation or restoration strategies are being developed, most of them based on gene therapy.AO imaging appears as a tool of choice to monitor retinal dystrophies and evaluate treatment. Loss of photoreceptors has been reported in a number of retinal dystrophies. Genotype-phenotype correlations are being actively investigated. Identifying pre-symptomatic changes, potentially allowing diseases to be detected earlier, and to monitor the treated retina non-invasively on a cellular level could be considered reasonable goals. Therefore, in patients with advanced form of retinal dystrophies, in particular showing the so-called retinitis pigmentosa in which end stage diseases are characterized by a central island of functional retina, it could have been expected to count the amount of residual photoreceptors. However, it rapidly became clear that the complexity and progressivity of tissue changes defies any straightforward interpretation of AO images. The combination of tissue remodeling, media opacities and shortening of cones indeed severely affects the contrast of cones over the surrounding milieu. Retinal dystrophies affect many aspects of the retinal structure and physiology, and there is a smooth transition from preserved to diseased retina. Other features such as photoreceptor disarray may further complicate the figure (Bottin et al, submitted). These problems are even worse when dystrophies affect the central retina such as Stargardt disease.

2.3 Age-related macular degeneration

Age-related macular degeneration (ARMD) is the leading cause of vision loss in industrialized countries. In its atrophic (dry) form, it is believed that rods dye first, followed by a synchronous loss of cones and retinal pigment epithelium (RPE) cells. Despite the identification of several genetic, molecular and environmental factors, the pathophysiology of dry ARMD remains debated and there is currently no available treatment. Two stages are distinguished during AMD, an early and a late phase. Early stages comprise fundus changes such as drusen, that often precede the onset of tissue loss.

Overall, photoreceptor counts in AMD are difficult. This has led to active investigation into the underlying reasons for poor image quality below drusen and/or at the margins of geographic atrophy. However, beyond photoreceptors a number of other features are of interest in AO images of AMD. AO imaging improves the resolution of the changes affecting the RPE[11] (figure 2). There is a high contrast of margins, and also a myriad of

melanin clumps within and outside atrophy. AO imaging, both in flood and scanning modes, therefore allows micrometric-scale monitoring of atrophy progression. Foveal sparing, that is, the persistence of an "island' of intact RPE monolayer under the fovea surrounded by RPE atrophy, can be observed. This spares central visual acuity. Infrared AO offers the possibility to monitor closely these eyes, in which a small centripetal progression may have visual consequences incommensurate with the actual dimension of progression.

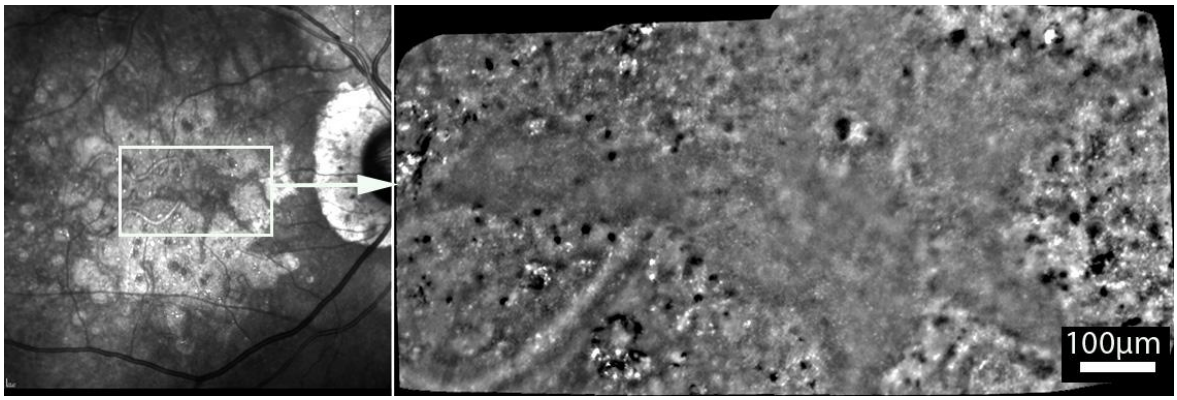

Figure 2. Age-related macular degeneration. Left, fundus infrared scanning laser ophthalmoscope image. Right, montage of AO images**.**

The reason why melanin deposits are better detected by flood-illuminated systems than by scanning systems may be related to the contribution of backscattered light to the FIAO image, melanin hence showing a high contrast because there is much more light reflected by the choroid than by melanin pigments. As a consequence, AO imaging provides detection of very small atrophic spots, an exquisite view of the border of atrophy. At such resolution, clinicopathological correlations, that is, the comparison of histology and imaging, is important. Histology data[12] suggested that most melanin clumps seen by FIAO are intracellular. Hence the melanin deposits can be considered as intracellular tags. Accordingly, time-lapse imaging demonstrated that many of these melanin granules are migrating. We thus hypothesize that most migrating clumps are melanin-loaded cells (MLCs).Tracking of these cells is obviously of interest to explore the pathophysiology of AMD, yet such tracking is challenging. It exquisitely depends on the congruence of velocity and density of cells and sampling interval. Roughly said, few slow cells are easier to follow that rapidly, numerous cells. Also, it is conceivable that the visibility of an individual MLC depends on the intracellular arrangement of melanin granules and hence local optical density: flattened cells with show less pigment density. Also, two MLCs close to each other may not be distinguishable. These results show that diseases showing slow progression at a macroscopic scale can appear to be significantly more dynamic at a smaller scale.

Further explorations of AMD by AO imaging may address to earlier stages of AMD, to ascertain the role of MLCs in the onset and/or progression of atrophy. Histological comparisons will remains crucialto progress in the understanding of these images. MLCs are not dying cells, and as such may be considered suspects of damaging the retina until proven innocent. Further in depth immonohistological characterization of these MLCs is therefore of high interest to better understand AMD.

2.2. Arterial hypertension

As an easily accessible part of the microcirculation, the retina provides a convenient mean for *in vivo* evaluation of the consequences of arterial hypertension. Several epidemiological studies have shown that the severity of hypertensive retinopathy as observed by classical, low resolution imaging (fundus photographs) is correlated with the incidence of end-organ damage such as coronary diseases, age-related cognitive decline and stroke. Until the advent of AO imaging, vessel walls were a true frontier of fundus imaging. In addition to photoreceptors, AO imaging broke this frontier. By AO imaging, the arterial wall is visible as a linear structure along both sides of the

blood column[12]. Although it is still uncertain what are exactly the vascular structures that are observable, it can be assumed that the inner limit of the wall corresponds to the plasma-endothelial limit, and that the outer limit represent the adventicia-extravascular limit. In some subjects, especially showing a higher backscattered light (such as lightly pigmented fundus) striation within the wall can be observed. This is probably related to dispersion of light from the media layer, that is, from the smooth muscle cells. The observation of pericytes has also been reported using AOSLO[14].

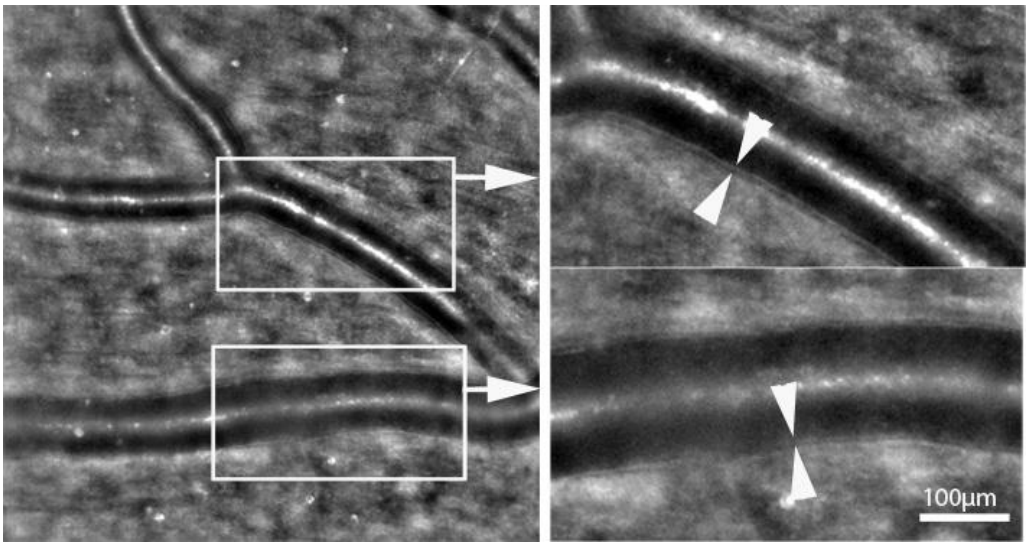

Figure 3: AO image of a normal artery and vein. Arrowheads bracket an artery (upper right image) and a vein wall.

As in photoreceptor imaging, progress in image processing are crucial in vascular imaging. Advanced image processing methods were proposed by our group, allowing automatical or semi-automatical segmentation of parietal structures of arterioles[15,16]. Figure 4 shows an example of segmentation along with several measurements: diameters and wall thickness and wall-to-lumen ratio. The wall-to-lumen ratio (WLR) represents the ratio between the wall thickness and the vessel internal diameter.

Arterial hypertension and retinal vessels

An increased WLR is a hallmark of microvascular remodeling due to arterial hypertension. The prevalent physiopathological concept of such parietal thickening postulates that a rise in blood pressure stimulates myogenic vasoconstriction, which tends to normalize parietal tension, without significant modification of the parietal structure (eutrophic remodelling). In a cohort of healthy and hypertensive subjects, using these tools we found that the thickness of the wall of arterioles was positively correlated with systolic and diastolic blood pressure. In multivariate analysis taking into account age, lumen diameter, and systolic and diastolic blood pressure, only diastolic blood pressure and lumen diameter were found independently correlated to the WLR [13].

Hypertensive retinopathy also shows diffuse narrowing of arterioles and focal lesions such as focal arteriolar narrowing (FAN) and arteriovenous nicking (AVN). We could also observe and measure changes of vascular morphometry in focal lesions, which provided insights into their pathophysiology. AVNs in the retina are the cause of retinal vein occlusions, a common cause of visual loss. They are also surrogates of cerebrovascular aging. The prevalent mechanistic model of AVNs stating that arteries crush veins remains somewhat unchallenged despite the lack of evidence other than fundus photographs. However, despite their clinical importance, the pathophysiology of AVNs received limited attention in the scientific literature. There has been a longstanding yet unsubstantiated consensus among clinicians about the compressive nature of the arteriovenous conflict underlying AVNs. By AO imaging we observed that venous nicking may be observed in the absence of any physical contact

between a venule and an arteriole[17]. Therefore, AVNs do not necessarily involve arteriovenous compression. Instead, the topology of venous changes suggests a retractile process originating in the intervascular space. These findings have important implications for the understanding of retinal vein occlusions and of cerebrovascular aging. We therefore hypothesize that a key mechanism underlying venous changes is retraction of the intervascular space; this is the simplest explanation for the apparent attraction of veins toward the artery.

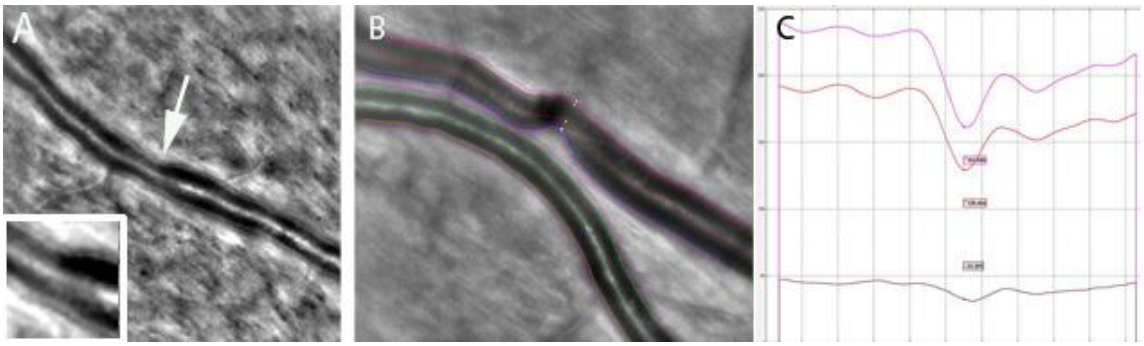

Figure 4:A ; focal arteriolar narrowing (note the parallelism of vessel walls); B, narrowing of a vein close to an artery suggesting indirect interact; C, segmentation of vascular lumen and wall from B.

AO imaging of retinal arterioles therefore offers a unique opportunity to explore microvascular changes related to age, diabetes or arterial hypertension in vivo in humans at a near-histology level. Phenotyping of retinal vessels by AO imaging may contribute to a better management and understanding of end-organ damage, especially in the brain given the functional and anatomical similarities of the retinal and cerebral circulations. Stratification of end-organ damage risk may be improved by biomarkers issued from AO imaging.

3. CHALLENGES AND PERSPECTIVES

There are still some obstacles that prevent the widespread use of AO in clinical routine. Some are related to the technology: scanning laser and flood illumination show some differences in term of resulting imaging which are not yet fully understood, and present a certain complexity, especially scanning systems. Another challenge relates to the integration of information from AO into medical decisions. Although AO fundus imaging technology and image processing is reaching technological maturity, clinical interpretation of the complex features of diseased retina indeed remains challenging because of a number of factors. Among these factors are the level of pigmentation of the fundus, the transparency of the retina, the presence of other sources of light reflection in diseased retina, the spatial and temporal variability of photoreceptor reflectance, and the variable orientation of photoreceptor outer segments. In order to optimize the management of these complex images by ophthalmologists, carefully designed specific softwares for image processing and analysis are necessary. Approaches such as artificial intelligence may prove of interest.

Among the current limitations of AO imaging are the costs. This is intimately linked to the absence of mass production of these systems. Mass production of AO systems will rely on the demand from clinicians and from reimbursement. This will come from the demonstration of clinical utility, that is, diagnosis or treatment monitoring. This in turn requires accurate metrics. Normative database are still scarce. Other challenges include the time to acquire data, the tradeoffs between dense spatial sampling, field of view, and data storage, the expertise and software required for image processing, interpretation and quantification. Finally, the clinical experience of AO imaging is still lacking, hence there are no consensual interpretation schemes of AO images.

All these are surmountable, and in fine the integration of AO imaging in clinical routine will emerge from the fact that OCT cannot provide the exact same information, and also because there is no definitive technological obstacle. In the near future, implementation of novel approaches and multimodal imaging, including in particular combination with OCT, will undoubtedly expand our imaging capabilities. The considerable interest of the pharma industry for treatment of AMD and for gene therapy will probably be crucial for the development of AO imaging investigations.

4. CONCLUSION

Up to now, the main application of AO fundus imaging has been photoreceptor detection and counting; this somewhat overshadowed the exploration of other retinal structures. Small scale observation of macroscopically slow biological processes reveals a high level of apparent activity. This transforms slow into rapidly evolving diseases which may prove important as predictive biomarkers. The clinical applications of AO-enhanced imaging are still hampered by the combination of technical complexity, costs and the paucity of interpretative scheme. In the near future, implementation of novel approaches and multimodal imaging, including in particular OCT, will undoubtedly expand our imaging capabilities. Tackling the technical, scientific and medical challenges offered by high resolution imaging are likely to contribute to our rethinking of many retinal diseases, and, most importantly, may find applications in other areas of medicine.

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