

ADAPTIVE OPTICS IMAGING OF RETINAL MICROSTRUCTURES: IMAGE PROCESSING FOR MEDICAL APPLICATIONS

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ABSTRACT

Adaptive optics (AO) fundus imaging is an optoelectronic technique allowing an improvement of an order of magnitude of lateral resolution of retinal images. Currently, its main applications in ophthalmology span from photoreceptor to retinal pigment epithelial cells and vessels, each of them being affected by specific diseases. Technological and image processing improvements are expanding the scope of its medical applications. Here we will review some of the current and envisioned applications of AO in clinical practice.

Index Terms— adaptive optics retinal imaging, age-related macular degeneration, photoreceptors, arterial hypertension.

1. INTRODUCTION

Adaptive optics (AO) is an optoelectronic technique that compensates for optical aberrations by the mean of a deformable mirror. AO imaging was first developed in astronomy and later applied to fundus images. AO improves the lateral resolution of fundus images, which can reach the micrometric scale. AO-based fundus cameras allow in vivo visualization of microstructures of the retina, making the retina the only human tissue in which subcellular structures may be routinely observed *in vivo*. High resolution imaging of photoreceptors therefore holds promises for cellular-scale diagnosis and guidance of therapy. An increasing number of clinical applications are identified for AO imaging, hence it is progressively translating from the lab to the clinics and will undoubtedly become a routine procedure for many retinal diseases in the next years.

The main applications of AO in ophthalmology span from photoreceptor to retinal pigment epithelial cells and vessels, each of them being affected by specific diseases. The first photoreceptor images using AO were obtained more than 15 years ago [1]. However, there are still some obstacles that prevent the widespread use of AO in clinical routine. One

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. Other issues are related to the fundus image technology: scanning laser and flood illumination show some differences in term of resulting imaging which are not yet fully understood. A crucial factor is also that, in order to optimize the management of these complex images by ophthalmologists, carefully designed structure-specific softwares for image processing and analysis are necessary.

We will show here that there are some clinical applications of AO in public health that already offers promising opportunities for clinicians. Most of this paper relates to flood illumination-based AO.

2. MEDICAL APPLICATIONS OF ADAPTIVE OPTICS IMAGING

2.1. Photoreceptors

Historically, photoreceptors were the first microstructures specifically identified by AO imaging. The diameter of the largest cones reaches $\sim 3\mu\text{m}$, making them visible under the aspect of a mosaic of hyperreflective dots (Figure 1). Rods and the smaller foveal cones became more recently accessible with the increasing resolution of AO imaging systems. This allowed documenting the loss of photoreceptors in diseases such as retinitis pigmentosa. A precise counting of residual photoreceptors may be an essential biomarker of the effect of biotherapies such as gene or cell therapy.

Image processing has brought major improvements to AO imaging. Cone counting algorithms have now reached a

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high degree of reliability. One of the most reliable and accurate approach is based on a recursive construction of thresholded components, when the seeds of the recursions are the regional maxima of the input image [2,3].

These automatic analysis algorithms benefits from advanced preprocessing methods. As flood-illumination systems usually produce noisy images making microscopic structures hardly visible, a commonly used solution is to register these images and average them to increase the signal-to-noise ratio. Myopic deconvolution of AO images has improved the quality of flood-illumination AO images [4] since it enables to extract cone mosaics from raw (i.e. non-averaged) images (Figure 2), hence avoiding artifacts related to photoreceptor scintillation and/or subsequent image processing. Sub-pixellic registration, developed by one of us [5] also had a significant impact on the resulting image quality, through the suppression of peripheral rotational artifacts (Figure 3) and hence enlarging the field over which photoreceptors can be counted.

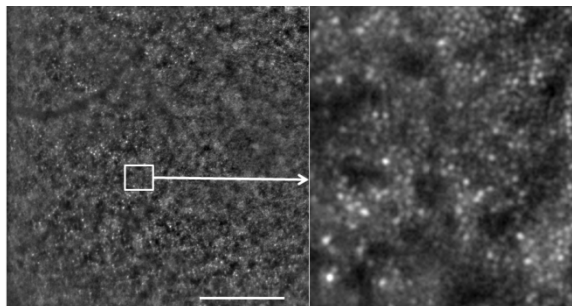


Figure 1. Imaging of the normal cone mosaic by adaptive optics (AO) flood illumination imaging (bar, 300 μ m).

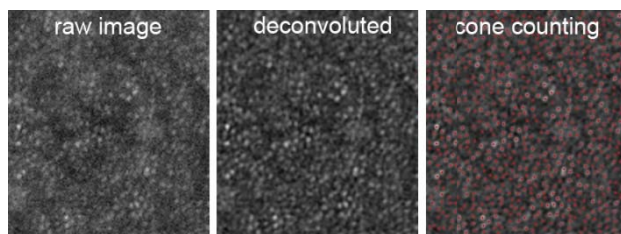


Figure 2. increased contrast of the cone mosaic on raw image resulting from myopic deconvolution, which enables cone counting to be performed.

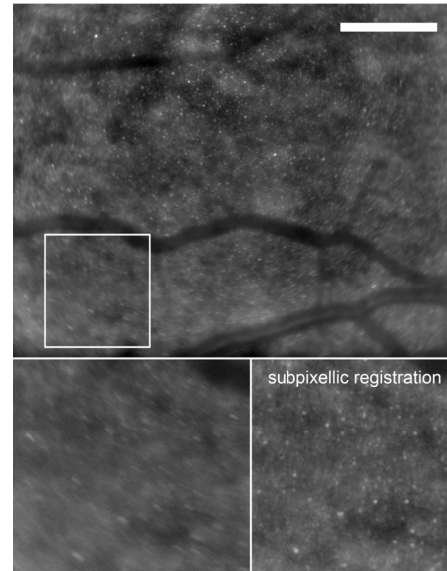


Figure 3. Improvement of AO images by subpixellic registration (bar, 300 μ m).

Cone photoreceptors have a strong directional absorbance and reflectance selectivity, a property referred to as the Stiles-Crawford effect (SCE) [6-8]. This may be observed on AO image by the strong variations of the cone mosaic under different illumination incidence. Preliminary work from our group suggests that the analysis of the SCE, which requires the fusion of images taken at various illumination angles, may provide a novel biomarker related to the healing process of photoreceptors.

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 on fundus photographs is indeed correlated with the incidence of arterial hypertension or of end-organ damage such as coronary diseases, age-related cognitive decline and lacunar stroke. By AO imaging, the blood column of vessels appears as dark stripes 50-100 μ m wide with an axial light reflection (Figure 3). A linear structure along both sides of the blood column of arteries is visible, which most likely corresponds to parietal structures [9].

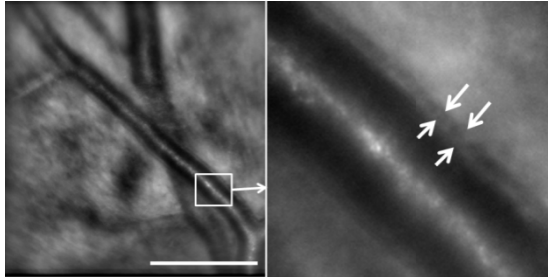


Figure 3. Imaging of the wall of an arteriole (between arrows in the magnification) by AO flood illumination imaging (bar, 300 μ m).

Advanced image processing methods were proposed by our group, allowing automatic or semi-automatic segmentation of parietal structures of arterioles [10,11]. Our approach relies on three main steps: (i) detection and accurate localization of the vessel axial reflection, (ii) pre-segmentation through a tracking procedure relying mostly on gradient information, (iii) accurate delineation of the arterial walls, based on a new active contour model integrating parallelism information [10]. The proposed energy functional, that defines the active contour model, makes evolve independently four curves towards the artery wall contours assuming that they are almost parallel to the axial reflection. In [11], other structural features were also modelled and integrated, in order to improve the robustness against low contrasted walls and morphological deformations that occur along vessels in case of pathologies. For this, new coupled energy terms were added in the energy functional, modeling symmetry properties on either side of the axial reflection. In this new model, the four curves do not evolve independently any more but jointly. The strength of parallelism and symmetry constraints is controlled through weighting parameters that have been optimized experimentally so as to cover the largest variety of images and pathologies. However, in case of failure, the clinician can adjust them manually (semi-automatic mode). From the segmentation result, graphic representations of morphometric parameters of a given vessel segment can be generated, which facilitates medical analysis of vascular morphometry. Figure 4 shows an example of segmentation (a), along with several measurements: diameters and wall thickness (b) and wall-to-lumen ratio (c). The latest morphometric biomarker (wall-to-lumen ratio, WLR) represents the ratio between the wall thickness and the vessel internal diameter (lumen). It is calculated all along the artery segment, from measurements averaged on a sliding window (50 μ m wide in this case).

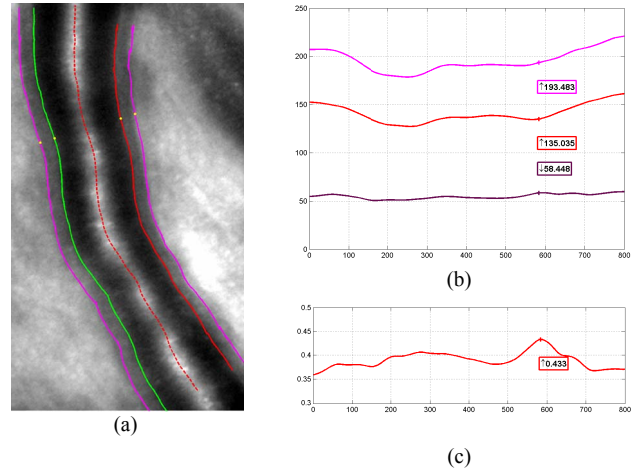


Figure 4. Segmentation of a retinal artery segment in AO imaging (a) and extracted measurements (μ m): internal diameter (lumen, in red), external diameter (magenta) and wall thickness (brown) (b); WLR calculated from the previous measures (c).

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 [12]. We could also observe and measure changes of vascular morphometry in focal lesions, which provided insights into their pathophysiology.

AO imaging is currently the only technique allowing a direct view of vascular parietal structures. 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. Longitudinal explorations of hypertensive patients are underway, addressing questions such as the predictive value of such measures on the incidence of end-organ damage, and the effect of treatment on microvascular parameters. 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.

2.3 Age-related macular degeneration

Geographic atrophy (GA), the atrophic form of age-related macular degeneration, is one of the most frequent causes of vision loss in industrialized countries. The disease is characterized by the primary alteration of a single layer of cells, the retinal pigment epithelium (RPE). Accurate characterization of the progression of atrophic lesions

during GA is important for the estimation of its long-term prognosis and consequently for the evaluation of therapeutic results. It is likely that improved imaging of small GA lesions may allow evaluating the effect of treatment at earlier stages of the disease, where such treatment may be more susceptible to show efficacy.

AO imaging improves the resolution of the changes affecting the RPE [13] (figure 5). Time-lapse imaging revealed for the first time the migration of these cells, presumably inflammatory cells. Such dynamic changes can be observed within as well as outside atrophic areas. Time-lapse AO imaging also allowed detection of very small spots of atrophy and follow-up of their progression.

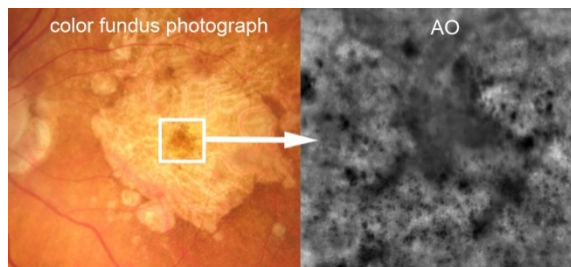


Figure 5. Illustration of pigment redistribution in a case of geographic atrophy. Note the multiple black dots in the AO image, presumably representing inflammatory cells.

AO imaging is therefore of interest to detect the emergence of atrophic spots, to document the progression of GA at a small temporal and spatial scale, and also to investigate the dynamic process underlying the redistribution of (presumed) inflammatory cells. A complex, dynamic process of redistribution of melanin-loaded cells indeed precedes and accompanies the emergence and progression of GA. Further studies correlating AO to conventional en face imaging and OCT would help to better define the place of AO imaging in the management of GA patients.

2.4. Vascular inflammation

Recently, we observed that AO may reveal paravascular cellular infiltrates in eyes with vascular inflammation [14], which are not otherwise detectable. This offers the opportunity of diagnosing vascular inflammation and also monitoring the therapy of such patients in a noninvasive way. Since vascular inflammation occurs in many general diseases, including multiple sclerosis, detection of paravascular infiltrates may of interest in a number of neurological and/or inflammatory diseases.

3. 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.

With a simple procedure applicable in a routine setting, AO imaging can document microscopic features of common ophthalmological and general diseases and will possibly become a reference tool for their monitoring. Such medical applications will rely heavily on image processing and analysis, for which specific applications are needed for each structure of interest, and possibly of each disease.

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