• Review •

Adaptive optics scanning laser ophthalmoscopy in fundus imaging, a review and update

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Abstract

• Adaptive optics scanning laser ophthalmoscopy (AO-SLO) has been a promising technique in funds imaging with growing popularity. This review firstly gives a brief history of adaptive optics (AO) and AO-SLO. Then it compares AO-SLO with conventional imaging methods (fundus fluorescein angiography, fundus autofluorescence, indocyanine green angiography and optical coherence tomography) and other AO techniques (adaptive optics flood-illumination ophthalmoscopy and adaptive optics optical coherence tomography). Furthermore, an update of current research situation in AO-SLO is made based on different fundus structures as photoreceptors (cones and rods), fundus vessels, retinal pigment epithelium layer, retinal nerve fiber layer, ganglion cell layer and lamina cribrosa. Finally, this review indicates possible research directions of AO-SLO in future.

• **KEYWORDS:** adaptive optics; scanning laser ophthalmoscopy; retina; fundus imaging

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INTRODUCTION

Adaptive Optics, From Starry Sky to Eye Adaptive optics (AO) is defined as a discipline to improve the performance of an optical system by reducing the effect of wavefront distortions[1]. Wavefront of light is a imaginary surface representing its propagating direction[2]; just like water wave, distortion of wavefront can indicate environment information. The modern AO originated from astronomy in 1950s, when Babcock[3] put forward wavefront information could be used to adjust atmosphere turbulence. This hypothesis was realized in 1977[4] and within another two decades, 10-meter class AO telescopes achieved a precision near diffraction limit[5].

The human eye is also an optical system, in which the counterpart of atmosphere turbulence is wavefront aberration. Aberration is the difference between reference wavefront and the actual wavefront for every point over the pupil[2]. In 19th century, Helmholtz described the human eye as an imperfect optic system, the main reason was the existence of aberration[6]. Aberration of the eye can be described by Zernike polynomials[7], the 2nd order components of which are defocus and astigmatism[8]; the higher order components can also pose an influence on the eye. The ocular aberration limits the resolution of non-AO equipment, such as the scanning laser ophthalmoscopy (SLO) without AO technique.

After its success in astronomy, researchers had tried to apply AO technique in ophthalmology. In 1989, wavefront corrector (an active optical focusing unit at that time) was firstly introduced into fundus imaging, with a considerable improvement on the resolution of SLO[9]. In 1990s, after the great successful introduction of Hartman-Shack wavefront sensor[10] and the deformable mirror in ophthalmology, it was available to perform single-cellular imaging noninvasively *in vivo* of cone cells[11] with AO flood-illumination ophthalmoscopy (AO-FIO). In 2002, the first AO-SLO was available by introducing AO technique into SLO, which had a higher axial and transverse resolution and better contrast comparing with AO-FIO[12].

Now, many published literatures on AO fundus imaging are done with AO-SLO. This review and update will focus on AO-SLO with the last part to indicate some future research directions.

A COMPARISON OF AO-SLO WITH OTHER FUNDUS IMAGING METHODS

AO-SLO Versus Conventional Fundus Imaging Methods Fundus fluorescein angiography (FFA), indocyanine green angiography (ICGA), optical coherence tomography (OCT) and fundus autofluorescence (FAF) are now widely used methods in fundus imaging. A comparison of these methods with AO-SLO is shown in Table 1[13-17]. The main advantage of AO-SLO over the conventional ones is a higher

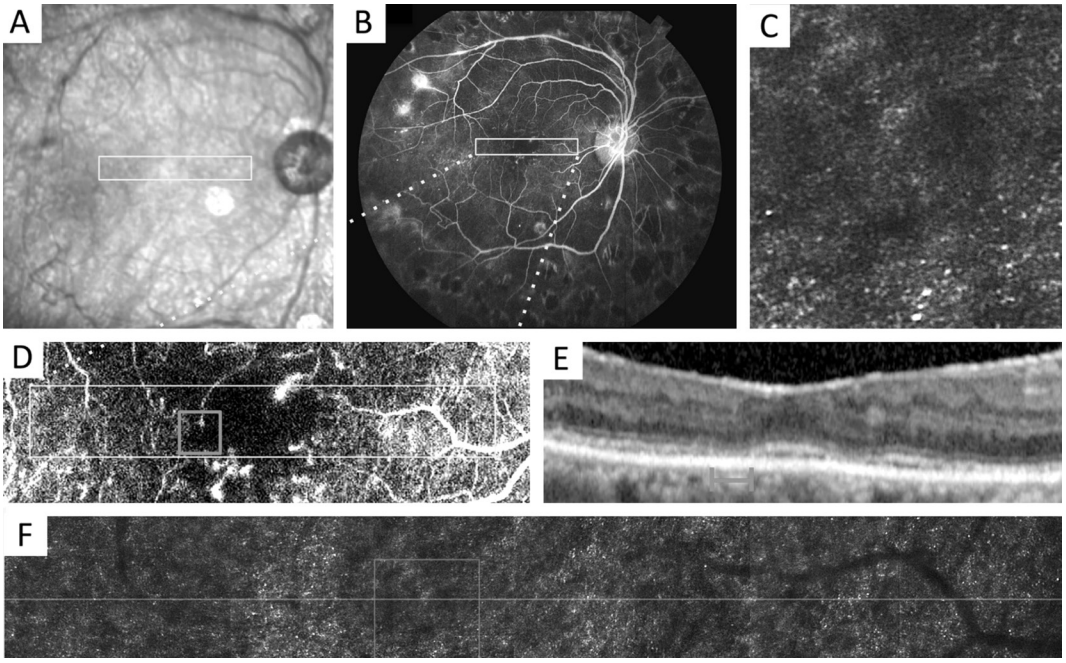

Figure 1 Multimodal imaging of a patient's right eye with diabetic retinopathy A: Fundus image with location of AO-SLO montage (box); B: Fundus fluorescein angiography (FFA); C: Enlarged 1˚×1˚ AO-SLO image from F; D: Enlarged FFA from B; E: Spectral-domain OCT (SD-OCT) registered to A; F: AO-SLO montage stitched from 2˚×2˚ images with location of SD-OCT (line) and AO-SLO inset (box). This figure is from reference[20] under the permission of cc BY 4.0.

Table 1 A comparison of AO-SLO with FAF, FFA, ICGA and OCT

Methods	Invasive	[a]Transverse resolution	[1,a]Field angle	First time available	Applications
AO-SLO	[2]N	2.5 μm	1.5°	2002[12]	Observing cones, rods, vessel and capillary, nerve fiber layer *etc* (Table 2).
FAF	N	20 μm	50°	[3]1970s[13]	Retinal pseudodrusen, macular edema, choroidal neovascularis[14] choroquine and hydroxychloroquine retinopathy[15] *etc*.
FFA	Y	20 μm	50°	1960[16]	Fundus neovascularization, aneurysms, tumor, telangiectasis, edema, vitreous inflammation[14] *etc*.
ICGA	Y	20 μm	50°	1970 in eye[17]	Choroidal vasculopathy, exudative AMD, inflammation and tumors, central serous chorioretinopathy[15] *etc*.
OCT	N	[4]20 μm	45°	1991[18]	[5]Vitreoretinal interface disorders, central serous chorioretinopathy, AMD, diabetic retinopathy[15] *etc*.

[1]Pupil diameter at about 6 mm; [2]AO-SLO fluorescein angiography not included; [3]Fundus autofluorescence was reported around 1870s[19], but it was not explored for clinical use until 1970s; [4]Axial resolution is about 5 μm; [5]AO-OCT and angio-OCT not included; [a]The approximate data.

transverse resolution without invasion, which has furthered our observation of retina. An example of multimodal fundus imaging of the same eye with diabetic retinopathy (DR) by FFA, OCT and AO-SLO is given in Figure 1[20].

Characteristics of Different Adaptive Optics Equipment

Now three AO fundus imaging methods in research are AO-FIO, AO-OCT and AO-SLO. With AO, all of them can achieve a high transverse resolution; the characteristics of them are as following:

AO-FIO AO-FIO was invented earlier than AO-SLO and AO-OCT, and is also the only equipment available on market now (rtx1, Imagine Eyes)[21]. AO-FIO is able to observe structures like capillaries and the outer segment of cones[21]; the imaging time of one frame is short with good quality. Moreover, the cost to build an AO-FIO system is usually lower than AO-SLO and AO-OCT as the structure is less complex. However, the axial resolution of AO-FIO is low (about 300 μm) and the observation in axial direction is usually limited.

AO-OCT As a combination of AO and OCT technique, AO-OCT has a high resolution both transversely and axially, which enables 3D cellular imaging; AO-OCT may play an important role in fundus functional imaging in the future with this advantage[22]. The main limitation of AO-OCT is its inability to detect fluorescent signals, which restricts the imaging of a specific fundus structure[22]. Another limitation of AO-OCT is the slow speed of 3D imaging[23], which may reduce imaging quality with eye movement.

AO-SLO Comparing with AO-OCT, the axial resolution of AO-SLO is lower (about 5 μm versus about 100 μm[24]). AO-SLO can detect fluorescent signals, which enables imaging

Table 2 AO-SLO in fundus imaging

Fundus structures	[1]Participants
RNFL	Normal[47]; Glaucoma[49]
Ganglion cell layer	Normal[31] (multi-offset imaging)
Cones	Normal (density)[35,52-53]; RP[33b,54]; Achromatopsia[55]; Stargardt's disease[56]; APMPPE[57]; Laser maculopathy[58]; DR[36]; AMD[59]; CSC[60]; ERM[61]; AZOOR[62]; Usher syndrome[33]b; AIR[63]; NARP[64]; Pseudodrusen[65]; Type 2 Mac Tel[66]
Rods	Normal[67] (density[35] reflectance[68]); RP[33]b; Achromatopsia[55]; Stargardt's[56]; Usher syndrome[33]b
RPE	Normal[27c-28]; AMD[51]c
Lamina cribrosa	Normal[48]; Glaucoma[50]
Fundus vessel research	
Thickness and ratio	Arteriole wall thickness[40-41]; Wall to lumen ratio[69]
Blood cells	[2]Velocity of leukocyte[70-71]; Velocity[42] and aggregation[72] of RBC
Parafoveal network	Microvascular density[43]; Foveal avascular zone[44-45]; Network and vascular perfusion condition[34,43]a; Hemodynamics[73-74]; Oxygen saturation[75]
Diseases	Type 2 diabetes[76]; DR[74,77-78]; Hypertension[41,69]; MA[46]a; CRVO[79]; Choroideremia (Choroidal vessels)[80]

AIR: Autoimmune retinopathy; AMD: Age-related macular degeneration; APMPPE: Acute posterior multifocal placoid pigment epitheliopathy; AZOOR: Acute zonal occult outer retinopathy; BVMD: Best vitelliform macular dystrophy; CRVO: Central retinal vein occlusion; CSC: Central serous chorioretinopathy; DR: Diabetic retinopathy; ERM: Epiretinal membrane; MA: Microaneurysms; Mac Tel: Macular telangiectasia; NARP: Neurogenic weakness, ataxia and retinitis pigmentosa syndrome; RBC: Red blood cell; RNFL: Retinal nerve fiber layer; RP: Retinitis pigmentosa; RPE: Retinal pigment epithelium1. [1]For research compared patients with healthy controls, only the diseases listed. Special imaging methods: [a]AO-SLO fluorescein angiography (AO-SLO FA); [b]Split-detector AO-SLO; [c]AO enhanced indocyanine green ophthalmoscopy (AO-ICG). [2]Uji *et al*[71] suggested the moving transparent particles may be leukocytes or plasma gaps.

methods like AO-SLO fluorescein angiography (AO-SLO FA). Another advantage of AO-SLO is its high recording speed of single frame (about 30 frame per second; FPS)[25].

RESEARCH OF AO-SLO IN OPHTHALMOLOGY

Imaging Methods Besides the confocol photographing model, many new imaging methods spring up in AO-SLO research, which makes more structures available to be observed and more parameters to be measured.

Fluorescent imaging methods With an ability to detect fluorescence of SLO, AO-SLO fluorescent imaging methods developed. FFA is a frequently used diagnostic technique in fundus vascular diseases, however, the morphologic information from FFA is incomplete in observing structures like the capillary network[26]. With a higher resolution than FFA, AO-SLO FA can afford more detailed information. Similarly, AO-SLO autofluorescence (AO-SLO AF) and AO-SLO indocyanine green (AO-ICG), as a combination of AO-SLO with AF and ICG imaging respectively, have been applied in photographing fundus structure like retinal pigment epithelium (RPE)[27-28]. Another imaging method, two-photon fluorescence AO-SLO (TPF AO-SLO) is able to image a wide variety of structural details in animals, such as ganglion cells, Müller cell processes of the Macaca rhesus[29]. However, with an uncertain safety as using near-infrared exciting light[29-31], no research in humans has been published up to now.

Non-confocol imaging method In split detector AO-SLO, a reflective mask is placed to reflect the confocal signal to a first detector and transmit the multi-scattered light to another two incoherent detectors, thus the confocal signals and the split-detector signals (calculated from non-confocal detectors) can be recorded simultaneously, with a perfect spatial registration[32]. The split detector AO-SLO has been applied in imaging of retinal structure like cones and rods in patients and healthy controls[33]. Using offset pinhole is another method in nonconfocal imaging, which can get structural and perfusion images noninvasively similar to those by AO-SLO FA[34]. With multi-offset pinhole, single neurons in the retinal ganglion cell layer (GCL) are able to be imaged[31], which is of significant difficulty and has only been imaged in animals with TPF AO-SLO before.

Photoreceptors In 1997, adaptive optics (an AO-FIO) was first successfully[11] applied in ophthalmology to observe cones. AO-SLO has been used in research of photoreceptors for over a decade, in a wide variety of diseases (Table 2). Several parameters of photoreceptor mosaic are studied as following:

Density and distribution Most studies are focused on cones, as imaging of rods is proved to be more difficult[35]. The density of cones is reported to decrease from fovea (about 164 000/mm^2) to periphery retina (about 6700/mm^2 NR and 5400/mm^2 TR at 30°), while density of rods reaches a peak at about 25°NR (about 124 000/mm^2) and 20°TR (about 120 000/mm^2)[35]. Alteration to photoreceptors has been observed in many different diseases (Table 2)[27-80].

Reflectance As wave-guiding cells, change to reflectance of the photoreceptors is observed in different diseases, such as the dark patches in diabetic retinopathy (DR) patients[36]. However, as the reflectance of photoreceptors in normal people can also be low, it should be careful to evaluate reflectance as an indicator of funds diseases[37-38].

Descriptive metrics of mosaic Besides density, many other metrics have been introduced to describe the photoreceptor mosaic statistically, which may be helpful in auto-analysis of mosaic pattern in the future, such as the spacing metrics like nearest neighbor distance and regularity metrics like nearest neighbor regularity[39]. The regularity metrics are proved to be more sensitive in tracking changes of mosaic like diffuse loss[39].

Fundus Vessels AO-SLO is able to image fine vessel structures like the capillary and the arteriole wall, and to detect fluoscence with a compatibility with FA, AF and ICG imaging. AO-SLO has been applied in research of different vessel parameters like arteriole thickness[40-41], velocity of blood cells in capillary[42], parafoveal capillary density[43] and area of foveal avascular zone[44-45]. AO-SLO can also reveal structural details which are difficult to be observed with conventional methods, such as the microscopic features of retinal microaneurysms[46].

Other Structures

Ganglion cell body, retinal nerve fibre layer and lamina cribrosa All these three structures may play a role in glaucoma study and have been successfully imaged in human noninvasively[31,47-48]. AO-SLO can reveal normal and abnormal retinal nerve bundles in glaucoma patients[49]. AO-SLO can also reveal pores of lamina cribrosa; comparing with normal participants, the mean area in glaucoma patients is significantly larger[50].

Retinal pigment epithelium RPE can be revealed by AO-ICG and AO-SLO AF[27]. Reflectance imaging of RPE cells with AO-SLO in normal fundus is difficult as the wave-guiding nature of overlying photoreceptors obscures signals from RPE[28]. Imaging of RPE cells with AO-ICG is based on that they can take up injected ICG dye, which can be detected by AO-SLO[27]. AO-SLO AF is able to image RPE mosaic *in vivo* because it can detect autofluorescence from lipofuscin in RPE cells[28]. In age-related macular degeneration (AMD) patients, research indicates RPE cell morphologies by AO-SLO AF are markedly similar to those seen in postmortem histological studies[51].

FUTURE RESEARCH DIRECTION

Possible research direction of AO-SLO may exist in the following fields:

Imaging Although AO-SLO has a high transverse resolution to detect single photoreceptors and capillaries, its field angle at one scan is small, or a corresponding retinal area of about 0.085 to 0.34 mm^2[81], thus it's sometimes difficult to image the whole area of interest. One method to get an image of larger area is by mosaicking multiple images, which could expand the field angle to over 20°[82]. Another efficient but difficult method is to increase field angle at one scan. Dual-conjugate adaptive optics technique may be one such method, which may increase the field angle to about 7°×7° in one scan[83].

In axial direction, with development of imaging techniques especially non-confocal ones (split detection, offset pinhole, *etc*), more fundus structures can be observed with AO-SLO, such as ganglion cell body[31] *etc*. In the near future, structures like choroidal capillaries may be imaged noninvasively.

Eye movement will bring intraframe distortion which affects the imaging quality of AO-SLO, especially in less cooperative patients. Even in eye fixed on a target, there is continuous eye movement[25]. A higher scanning speed or movement compensation may be helpful to achieve a better imaging quality. Lu *et al*[25] has succeeded to reduce distortion by 50.9% to 79.7% with high imaging speed at 200 FPS.

Automatic algorithms Processing images from AO-SLO can be time consuming and expert-depended, with requirement of knowledge on both image processing and medicine, which forms a barrier to promote this technique. Therefore some algorithms have been developed for AO-SLO, in automated reference frame selection[84], multiple-images montaging[81] and quantitative analysis of the photoreceptors[53,85]. However, as far as we know, there is no fully-automatic software available now for the whole procedure from imaging to outputting analytic result, which should be user-friendly and no need of coding.

Clinical practice Available firstly in 1991[18], OCT was soon applied in research of fundus diseases[86-87]. OCT got popular in hospital after entering market in 1996 and its success is dependent on the development of OCT-guided clinical practice. OCT guided diagnosis[88], therapy decision[89] and follow-up[90] has been a daily part of clinic ophthalmology.

Like OCT, AO-SLO is a non-invasive examination. Development of AO-SLO guided clinical practice will improve our understanding and management of ocular diseases and promote this technique concurrently. Future research may focus on the following aspects: 1) Diagnosis. AO-SLO can detect microstructural alterations, such as microvascular damage and morphologic changes of photoreceptors before appearing clinical symptoms, which may be applied in early diagnosis and prevention. In type 2 diabetes without sign of DR, AO-SLO can detect changes of parafoveal capillary network[76]. In patients with retinitis pigmentosa or Usher syndrome, it's reported the visual acuity could be kept in normal range with over 35% fall of cone density[33]. AO-SLO may detect some pathological alterations not seen in OCT[91]. One problem in diagnosis with AO-SLO is lack of reliable reference value, such as the density of photoreceptors, nearly all published

studies are with small sample and without a consideration of factors like age, gender and ethnicity[35,52-53]. 2) Treatment evaluation. There are not many published studies to explore AO-SLO in estimating ocular treatment. In autoimmune retinopathy patients, AO-SLO was used to evaluate the density of cones after taking rituximab, which was stable during the period of treatment (but no control group in this study)[63]. Actually, AO-SLO has great potential to assist clinical therapy and one example is gene therapy of monogenetic retinopathy. In retinal degeneration characterized by cones or rods dysfunction, AO-SLO can be applied to monitor morphologic improvements of photoreceptors. AO-SLO endpoints may play a role as structural marker in clinical trial and practice. 3) Follow-up. With a high transverse resolution, AO-SLO guided follow-up may help us understand the structural and functional alterations in ocular diseases. AO-SLO has been reported to follow the longitudinal changes of photoreceptors (AZOOR[62]) and microvessels (DR[78]). More follow-up studies of one or multiple structures may appear in the near future.

Basic medicine research Combining with fluorescent label, the distribution of a certain protein in retina could be detected with AO-SLO in animals[92], which makes it possible to study a particular fundus structure or cell type *in vivo*. AO-SLO may also be applied to research fundus physiology, especially in photoreceptors[93] and fundus vessels[94], with a transverse resolution of micron dimension and the convenience to record a video. AO-SLO may help us understand the mechanism of glaucoma better as it can image retinal microvessles, RNFL, GCL and lamina cribrosa *in vivo* noninvasively[31,47-48].

CONCLUSION

With increasing studies and publications, AO-SLO has been a promising technique. AO-SLO has played an important role in observing microstructures of living human retina with growing popularity. Development of imaging method and progress in clinical application will be made in the coming years, with innovation and cooperation of multiple disciplines from physics to medicine. However, there is still much research work to pave the road for clinical practice of AO-SLO. Thus more high-quality studies are expected on imaging, algorithms and clinical applications in future.

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