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EDITORIAL

New Frontiers in Retinal Imaging

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ABSTRACT

Ophthalmology is one of the most technology-driven medical specialties with numerous recent advances due to improvements in imaging technology. Advances in retinal imaging have allowed for better understanding of the eye in health and disease, retinal pathophysiology, documenting of disease progression,and assessing therapeutic response. During the last 50 years, both the hardware such as lasers in addition to software image analysis have significantly evolved. These improvements have included facilitating the progress of the ocular examination, reducing the discomfort of patients, and obtaining high quality non-invasive images. This article will review the classic retinal imaging modalities, state-of-the-art retinal imaging technologies, and an introduction of emerging imaging technologies.

Key words: Retina; Imaging; Fluorescein angiography; Optical Coherence Tomography; Photoacoustic Imaging; Molecular Imaging

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INTRODUCTION

Retinal imaging plays a very important role in screening the status of the human eye and diagnosing many ocular diseases. In addition to ocular diseases, retinal imaging also allows for early detection, diagnosis, and management of systematic diseases of the brain, endocrine, and cardiovascular systems. The eye is mostly optically transparent, allowing a window into both the central nervous system along with the systemic vasculature.

From the first photograph of human retina in 19th century until today, there have been huge advances in ophthalmic imaging. In the 1950s, modern fundus photography was born with the advent of the electronic flash and 35-mm camera. Later, in the 1960s and 1970s, the introduction of fluorescein[1] and indocyanine green angiography[2] revolutionized our ability to assess the integrity of the choroidal and retinal vasculature. The scanning laser ophthalmoscope (SLO) imaging arrived in 1981[3], followed 10 years later by optical coherence tomography (OCT) imaging[4]. Since then, a golden age has been heralded in ophthalmic imaging. OCT has released its third generation equipment within 20 years, and the recent emerging OCT angiography (OCTA) has gained clinical popularity.

Other appealing technologies such as adaptive optics and ultra-wide field imaging have been integrated into available retinal imaging modalities, which can significantly improve our image quality and field of view, respectively. These remarkable advances in ophthalmic imaging have transformed the simple photographic documentation into a powerful investigative method enabling clinicians to make objective measurements and assessments of retinal structures in detail. This review introduces the main retinal imaging instruments, their basic principles and current clinical applications, and recent advances in imaging technology.

FUNDUS CAMERA

Since the anterior eye is in the normal state optically transparent, the retina is visible with proper illumination. Somewhat paradoxically, the optical properties of the anterior portions of the eye (such as the cornea and lens) that allow outside image formation make direct inspection of the retina more challenging. Thus, special techniques are needed to obtain a focused image of the retina. Fundus imaging is complicated because illumination and imaging beams cannot overlap,

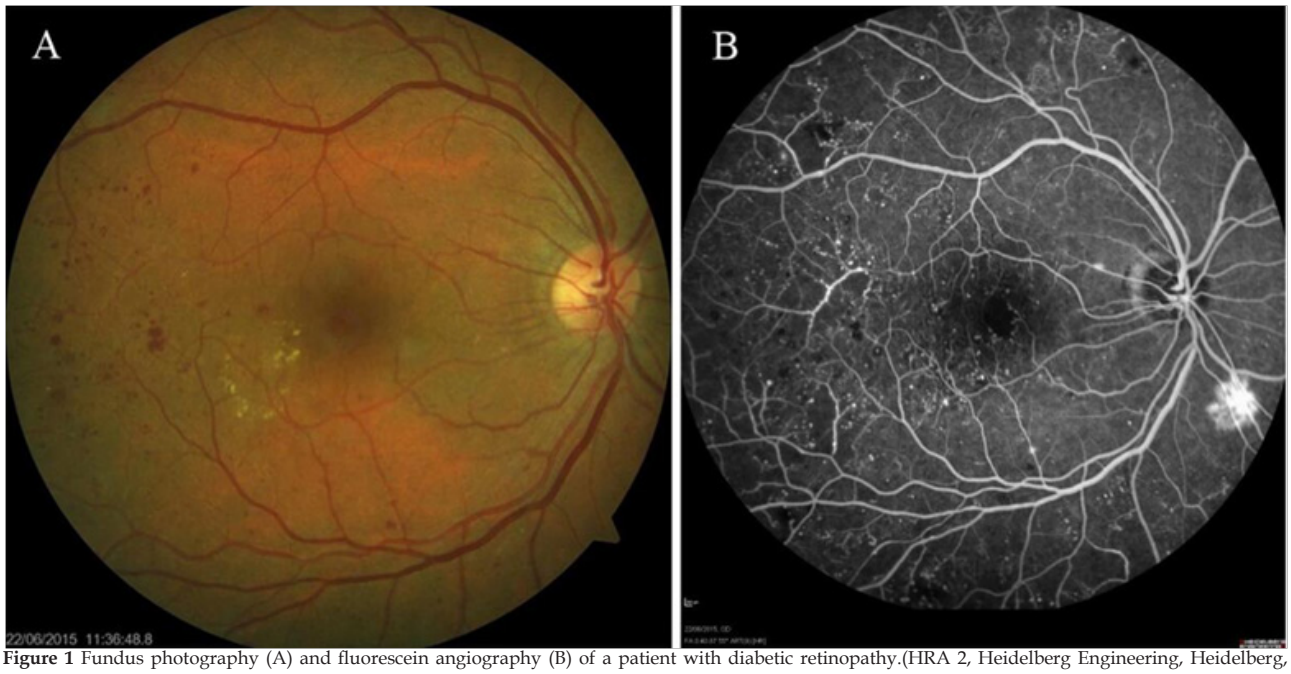

Figure 1 Fundus photography (A) and fluorescein angiography (B) of a patient with diabetic retinopathy.(HRA 2, Heidelberg Engineering, Heidelberg, Germany).

in which case, corneal and lenticular reflections will diminish image quality and contrast.

Consequently, in the pupil plane, separate paths are applied, including an outer illumination beam and an inner imaging beam. The concept of the ophthalmoscope dates back to 1823 by Czech scientist Jan Evangelista Purkyně. It was reinvented by Charles Babbage in 1845[5,6] and by Hermann von Helmholtz in 1851[7]. The first attempt to obtain the retinal photography was reported by the Dutch ophthalmologist van Trigt in 1853[8]. Since then, fundus photography has been a routine ophthalmic examination, with many enhancements, including the transition from film-based to digital image capture, optimization for nonmydriatic image acquisition, and stereoscopic image acquisition[9,10].

Over the past two decades, efforts have been underway to make the fundus photography more accessible and more robust, with less dependence on experience and expertise. The technology of Picture Archiving and Communication Systems (PACS) allows the fundus camera to move from film-based to digital imaging. More straightforward operation of fundus cameras was further improved due to the development of nonydriatic imaging and near-infrared focusing, which increased reproducibility. For example, the Nidek Model AFC-230 (Nidek Inc., Fremont, CA), a nonmydriatic fundus camera with a 45-degree field view, has been demonstrated to be an efficient screening tool for diabetic retinopathy in a clinical trial[11].

In addition, portable, handheld smartphone-based retinal cameras have begun to play an important role as an ocular diagnostic tool and take advantages of the portability, data storage capacity, and wireless telecommunication system built into modern smart phones[12,13]. Other fundus cameras have been developed which incorporate adaptive optics or ultra-wide field imaging (see below). Combinations of these technologies make fundus photography more appealing and beneficial for the general ophthalmic examination, especially facilitating eye examination in pediatric practice and by nonophthalmic-trained physicians.

FUNDUS ANGIOGRAPHY

As an important extension of the fundus camera, fluorescein angiography (FA)[1] and indocyanine green (ICG) angiography[2] have remained integral parts of the clinical evaluation of retinal and choroidal circulations. For FA, blue light with an excitation wavelength of 465 to 490 nm is used. Barrier filters block the light between 465 and 490 nm to block reflected light and allow only the emitted light with a longer wavelength in the yellow-green spectrum at 520-530 nm. As a smaller molecule, fluorescein is not suitable for detailed choroidal imaging due to the profound leakage of dye from fenestrated blood vessels of the choroid (including the choriocapillaris).

Indocyanine green, as a larger molecule, does not leave the choriocapillaris. ICG has been used as a contrast agent for enhanced visualization of the choroidal circulation. FA is a good baseline tool for the diagnosis of neovascular macular degeneration[14] and diabetic retinopathy[15] (Figure 1), whereas ICGA complements FA and is mainly used in the diagnosis of polypoidal choroidal vasculopathy (PCV)[16], choroidal tumors, and choroidal vascular hyperpermeability associated with central serous chorioretinopathy[17]. Vessel diameter, density, tortuosity, and branching patterns may offer potential value in the future if FA or ICGA can be combined with noninvasive motion contrast image-processing and ultra-wide-field techniques (see below).

SCANNING LASER OPHTHALMOSCOPE (SLO) AND ADAPTIVE OPTICS (AO)

First described in the early 1980s[3,18], the SLO uses a single, monochromatic laser with low power and a confocal raster scanning technique to collect an image of the retina and optic nerve head. Unlike the fundus camera which produces an image using a film or a charge-coupled device (CCD), SLO systems only illuminate a small area at any one time and the intensity of each pixel is recorded using

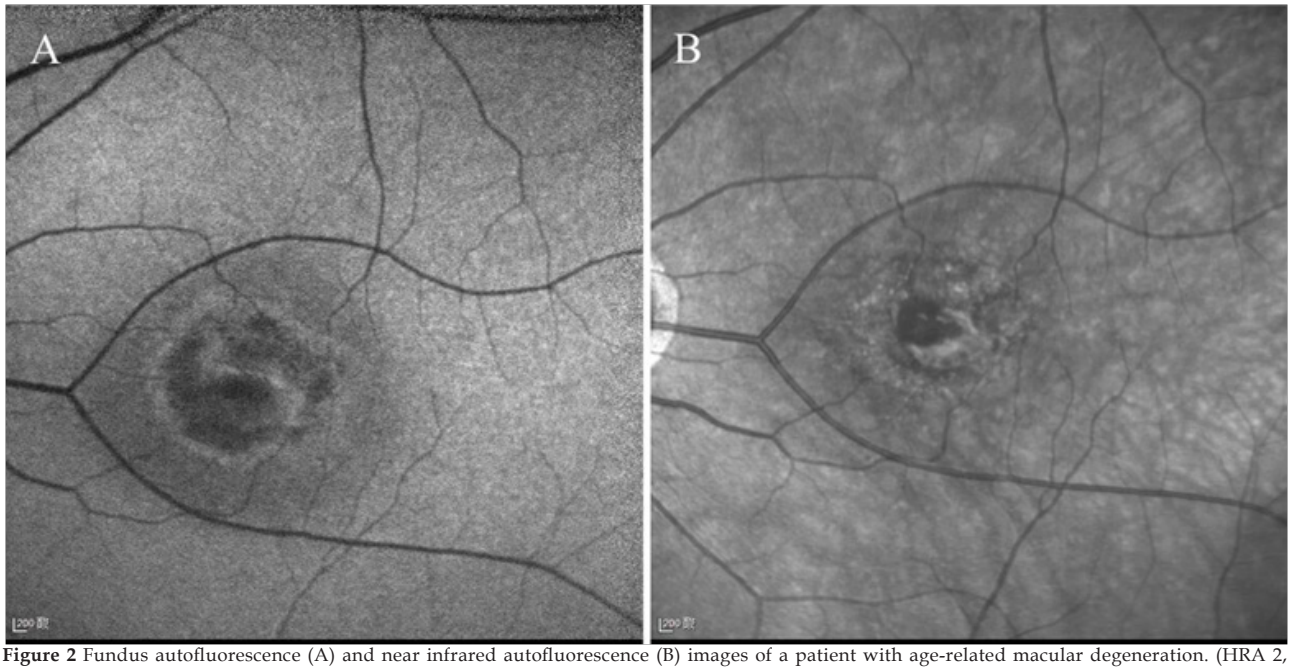

Figure 2 Fundus autofluorescence (A) and near infrared autofluorescence (B) images of a patient with age-related macular degeneration. (HRA 2, Heidelberg Engineering, Heidelberg, Germany).

a single, light sensitive detector. In this sense, the effects of light scatter are reduced so that the output images are with higher contrast than standard fundus cameras. One major advantage of SLO is its ability to perform confocal imaging[19,20].

Taking advantage of the principle of confocal microscopy, confocal SLO moves a confocal aperture between two end points to obtain a number of tomographic slices and to extract depth information[21,22]. Confocal SLO and OCT have become standard instruments for scanning the optic nerve head in glaucoma and are widely being used for imaging the RNFL[23,24]. Another important feature of SLO is the use of different wavelength lasers to enable a pseudo-color fundus image. Such devices, usually called multi-spectral SLOs, employ several laser sources of different wavelengths in the illumination. These multiple laser beams are made coaxial by a set of dichroic mirror and the images are created by a multispectral frame acquisition model. In addition to the pseudo-color fundus image, the changeable laser wavelength of multi-spectral SLOs also allows capture of near infrared reflectance images, fundus autofluorescence (FAF) images, and FA and ICG angiography. This can also be used to perform of retinal vessel oximetry, reflectometry, angioscotometry, fundus perimetry[25,26,27].

Further improvements of SLOs include time tracking and adaptive optics (AO). For patients who cannot fixate properly (i.e. diabetic retinopathy), high-speed retinal tracking is essential to improve image quality[28]. To achieve this, retinal spatial information from a fixed frame is used as a reference, and active tracking is performed by placement of a dithered beam originating from a low-power LED onto the fundus. Detection and processing of the backscattered reflectance signals by a phasesensitive detector enhances the imaging capabilities of the SLO.

SLO has been significantly improved through integration to AO, called Adaptive Optics Scanning Laser Ophthalmoscope (AOSLO). AO is used in retinal imaging systems to compensate for optical aberrations through a wave front-sensing technology and a deformable mirror. A wave front sensor, most commonly the Shack-Hartmann sensor, is employed to measure the aberration that the patient's lens, cornea, and tear film introduce in the imaging beam, and the deformable mirror, as a wave front corrector, corrects the aberration by physically changing its surface shape to compensate that of the aberration measured[29-32]. AOSLO systems have been reported to have the capability of observation of individual cone and rod photoreceptors[33,34], blood vessel and capillary imaging[35-37], and retinal pigment epithelium (RPE) (Figure 2)[38].

FUNDUS AUTOFLUORESCENCE (FAF)

Fundus autofluorescence (FAF) has grown significantly in popularity over the past decade. FAF is a non-invasive fundus imaging modality which provides information on native fluorescent molecules. The primary FAF signal is lipofuscin that has accumulated in RPE cells. Lipofuscin accumulation is due to deficient digestion of the photoreceptor outer segments by RPE cells. Elevation of lipofuscin in the RPE is indicative of cellular stress and often is a precursor of cell death[39]. Thus, autofluorescence is increased with RPE dysfunction and is decreased with the loss of photoreceptors and RPE.

Clinical applications of FAF include monitoring the progression of geographic atrophy[40], diagnosis of Best inherited macular dystrophy[41,42], intraocular tumors[43], posterior uveitis[44,45], and detecting drug toxicity such as hydroxychloroquine retinopathy[46]. Lipofuscin has a broad range of excitation from 300 to 600 nm, allowing visible light to elicit its fluorescence in the human retina[47]. Instruments for the measurement of FAF include the fundus spectrophotometer[48], fundus camera[49], and cSLO[47].

One common cSLO is the Heidelberg retinal angiographs (HRA Classic, HRA 2, and Spectralis HRA; Heidelberg Engineering, Heidelberg, Germany). In this system, excitation occurs with a 488 nm illumination and the emission is detected above 500 nm (Figure 2)[50]. Image averaging occurs automatically and in real-time. In addition, normalization of pixel intensity occurs, which facilitates quantification and comparison between different FAF images[47]. In addition, near infrared AF (NIR-AF), with an excitation wavelength of 787 nm and an emission of > 800 nm, is capable of imaging the melanin in RPE and choroid layers (Figure 2)[51,52]. Further, NIR-AF in patients with AMD has been described, using the ICG mode (790 nm as excitation wavelength). The recorded signal is likely melanin-dependent[52].

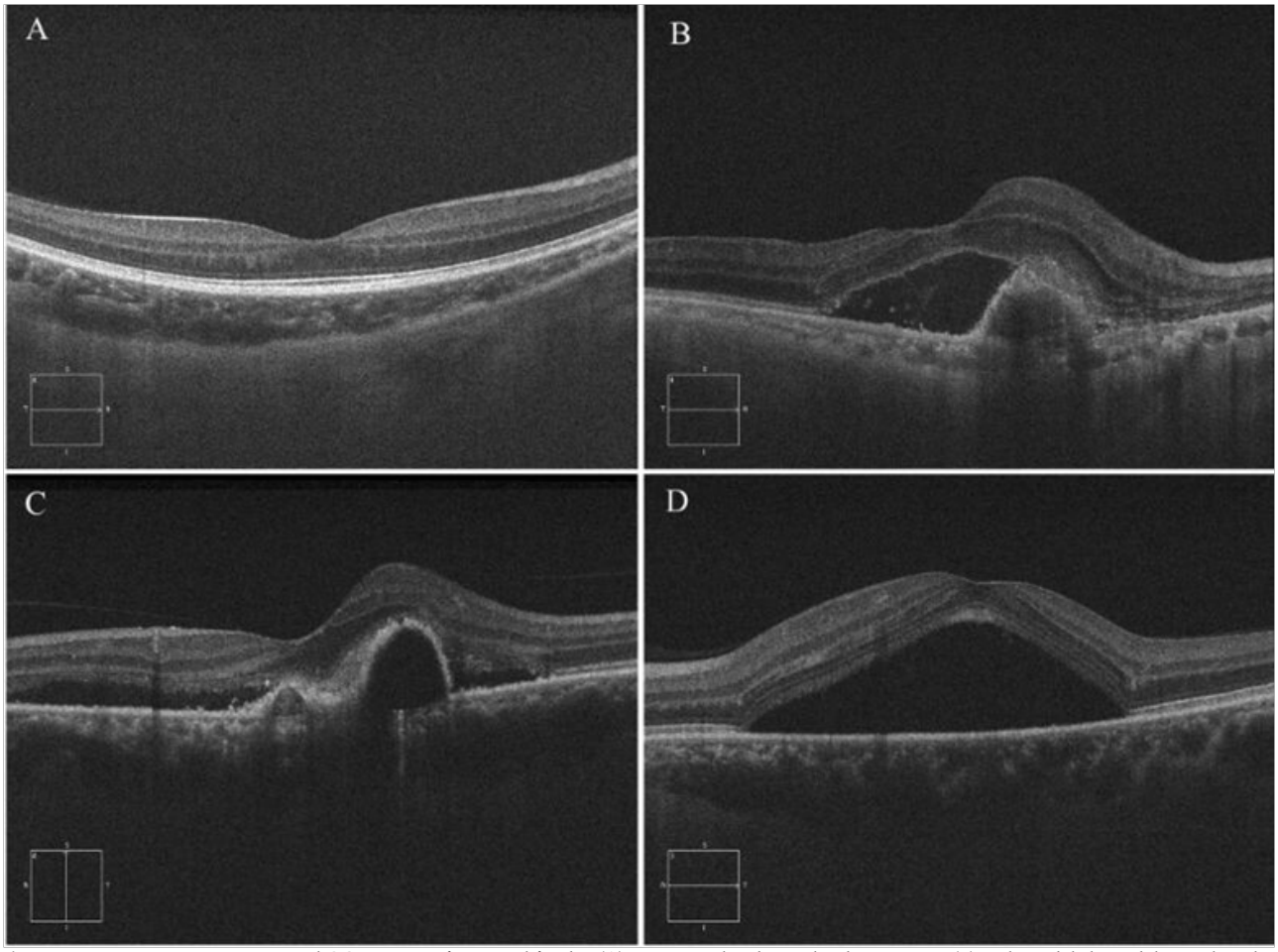

Figure 3 Representative cross-sectional OCT images of a normal fundus (A), wet age-related macular degeneration (B), polypoidal choroidal vasculopathy (C), and central serous chorioretinopathy (D). (Cirrus OCT, Carl Zeiss Meditec, Dublin, CA, USA).

ULTRA-WIDEFIELD IMAGING

The "standard" field of view of the traditional, commercially available fundus camera is 30 to 50 degrees, which correspond to approximately 5% to 15% of the retinal surface area, respectively. With traditional cameras, it is difficult to photograph the peripheral retina. Even with montaging techniques, the standard 7 fields cover around 75 degrees. Moreover, certain constraints exist in FA or ICGA in particular, as fluorescence in transit is time-sensitive and different parts of the retina cannot be imaged simultaneously. Imaging angles larger than 50 degrees have been referred to as "wide-field". More recently, the term "ultra-wide field" (UWF) fundus imaging has gained popularity, although the exact definition and area of the retina imaged remains ambiguous[53].

Several camera designs were released with different properties. Optos (Optos, PLC, Scotland) increases the field of view up to 200º covering approximately 82% of the retina in a single image. Heidelberg Spectralis (Heidelberg Engineering, Germany) is another UWL camera with a 102º field of view with noncontact lens and 150º with Staurenghi contact lens. The Optos captures an appreciably wider view of the retina temporally and nasally, despite of peripheral distortion, while the ultra-wide field Heidelberg Spectralis is able to image the superior and inferior retinal vasculature more peripherally[53]. Of note, the Heidelberg Spectralis provides a higher resolution image than that of Optos[23].

Recent advances in UWF imaging software, such as stereographic projection software, now have been available in commercial UWF devices. This allows images obtained at different gaze angles to be montaged and corrected for peripheral distortion[54]. Not limited to the fundus camera, UWF has also been employed in other retinal imaging modalities like fundus fluorescein angiography[55], OCT[56], autofluorescence[57], and scanning laser ophthalmoscopy[58].

OPTICAL COHERENCE TOMOGRAPHY (OCT)

OCT is a powerful imaging system for acquiring cross-sectional images of tissue non-invasively (Figure 3). OCT creates an image based on low-coherence interferometry and analyzes the difference in back-scattered light comparing the tissue to a reference arm[59]. A broad band light source is first split into two beams: a reference and sample beam. The back-scattered beam from the retina generates an interference pattern with the reference beam. From this, a reflective depth and intensity profile of the retina can be constructed[4]. Initially, the interference patterns generated in the early OCT systems varied as a function of time using a moving mirror in the reference pathway. Such devices were termed time-domain OCT(TD-OCT)[60]. TD-OCT systems like Stratus OCT (Carl Zeiss Meditec, Inc., Dublin, CA, USA) acquire image at 400 A-scans per second scan with an axial resolution of approximately 10 μm[61].

The second generation is spectral-domain OCT (SD-OCT), which employs an interferometer and spectrometer to analyze back-scattered light interference pattern simultaneously through the Fourier transform algorithm[62,63]. The SD-OCT systems such as the Cirrus

OCT (Carl Zeiss Meditec, Dublin, CA, USA), Spectralis (Heidelberg Engineering GmbH, Heidelberg, Germany), RTVue-100 (Optovue Inc., Fremont, CA, USA), and Topcon 3D-OCT 2000 (Topcon Corporation, Tokyo, Japan) allow much faster scan rates at 20,000 to 52,000 A-scans per second, better signal-to-noise ratio compared with TD-OCT, and a higher an axial resolution of 5 to 7 μm[62,63].

The third generation OCT, so called swept-source OCT (SS-OCT), alternatively employs a tunable frequency-swept laser light source and a photodetectors to assesses interference patterns[64]. Such devices as DRI OCT-1, Atlantis (Topcon Corporation, Tokyo, Japan) has an image acquisition speed of 100,000 A-scans per second and ultra-fast SS-OCT with an acquisition speed as high as 6,700,000 A-scans per second[65]. Another advantage of SS-OCT is the improvement of the signal quality in deep tissue by elimination of the sensitivity of a spectrometer to higher frequency modulation as with SD-OCT, thereby improving the visualization of the choroid[66]. With the application of longer-wavelength light source—approximately 1,000 nm, the SS-OCT systems are expected to improve the visualization of choroid–sclera interface[67]. This is very important for the diagnosis of diseases such as central serous chorioretinopathy (CSCR), and pathological myopia, where the thickness of choroid is abnormal. This also improves choroidal volumetric analysis and visualization of various pathological features such as choroidal neovascularization and subretinal/intraretinal fluid.

In addition to the commercially available desktop systems, prototype OCT systems have greatly contributed to an ever-growing body of OCT research field. These include, but are not limited to, the ultra-high resolution OCT (UHR-OCT), handheld OCT, and intraoperative OCT (iOCT). Ultra-high resolution OCT typically employs a broader bandwidth laser of over 100 nm at the wavelength of 830 to 870 nm to provide axial resolutions of 2-3 μm, which reveals retinal morphology in high detail. However, because it requires femtosecond laser and expensive light sources, UHR-OCT has not become widely used in the clinical setting at this time[68-70].

Handheld OCT, or mobile OCT, may further expand the application spectrum of OCT to help the analysis of subjects who cannot be ambulatory or cooperative, including adults and pediatric patients. Ashwin *et al* in 2015 reported that with a handheld SD-OCT Envisu 2300 (Bioptigen Inc., Research Triangle Park, NC, USA), they studied 975 infants and children[71]. The handheld SD-OCT can assist with the monitoring and evaluation of hereditary maculopathies[72,73]. The upgraded handheld OCT is capable of imaging both the anterior and posterior segments of the eye in rapid succession[74].

Another exciting application of OCT is the potential use of intraoperative OCT (iOCT) during surgery, especially for vitreoretinal surgery. The great advantage of iOCT is to assist with the prompt decision-making process and to allow additional deliberate surgical maneuvers aimed at improving surgical outcomes. Traditional table-top designed OCT systems have been playing an essential role in the diagnosis, treatment, and evaluation of macular diseases[75]. However, accurate image acquisition with these modalities needs a compliant patient who can sit in an upright position, which is paradoxical considering the intraoperative posturing of patients. With the development of integrative OCT technology, surgical instrumentation, and software algorithms, intraoperative OCT (iOCT) is becoming increasingly utilized.

Three kinds of iOCT have been employed during surgery, which include hand-held SD-OCT devices (for example, iVue, Optovue Inc., Fremont, CA, USA)[76], microscope-mounted OCT system (Bioptigen Envisu SDOIS; Bioptigen, Research Triangle Park, NC, USA)[77], and intra-ocular side-scanning OCT endoprobe (C7 System; Light Lab Imaging, Inc/St Jude Medical, St. Paul, MN, USA)[78]. Since the current commercially available handheld OCT systems must move the OCT system in and out of the surgical field, the microscope-mounted OCT system and intra-ocular OCT endo probe are more readily incorporated into the surgical field.

DOPPLER OCT

OCT is performed using intrinsic contrast alone, not requiring the use of dyes or extrinsic contrast agents. This non-contrast capability is attractive for longitudinal studies where cumulative dye toxicity is a concern. Since OCT can measure well-defined volumes using predominantly single scattered light, quantitative assessment of hemodynamic parameters such as the diameter of blood vessel and blood flow using Doppler algorithms are possible. Doppler OCT is based on the traditional OCT system with the phase-resolved axial scan information.

Fourier-domain OCT has proven to enable Doppler OCT through simpler and more robust measurements of human retinal blood flow in real time using depth-resolved information on vascular structure and Doppler velocity[79]. However, the flow measurement was highly sensitive to errors in the Doppler angle, especially when the vessel is nearly vertical to the OCT beam around the optic disc margin. Eye-motion artifacts as well as varied vessel geometries can make the angle measurements challenging and require additional data analysis.

Another method, bidirectional Doppler OCT measures Doppler velocity in two probe beam directions and uses trigonometric relations to calculate the absolute flow velocity[80] and blood flow[81]. In addition, the improvement of OCT scan speed enabled another Doppler OCT approach, which analyzes blood flow in the en face plane and therefore, avoids the need to measure the Doppler angle[79]. In ophthalmology, *en face* Doppler OCT does not require the measurement of Doppler angle because the detected flow velocity component is always perpendicular to the *en face* plane. Lee *et al*[82] suggested that precise measurement of total retinal blood flow using *en face* Doppler OCT at commercially available scan speeds should be feasible while further validation in diseased subjects is required.

OPTICAL COHERENCE TOMOGRAPHY ANGIOGRAPHY (OCTA)

Optical coherence tomography (OCT) angiography (OCTA) is an emerging and promising approach that uses SD-OCT for non-invasive visualizing of retinal and choroidal vessels based on flow rather than simple reflectance intensity. An important advantage of OCTA over traditional fluorescein angiography (FA) is that it provides three-dimensional, depth-resolved functional information of bloodflow in vessels (Figure 4). OCTA works by evaluating the difference between sequential OCT B-scans in the retina. Since the tissue structure does not change in this short interval, change is attributed to movement of erythrocytes within the blood vessel lumen. By applying the key technology of the split-spectrum amplitude decorrelation algorithm (SSADA)[83], OCT signal is first split into several spectral bands, each of which forms a different speckle pattern. The next step is the sum of amplitude decorrelation images derived from these spectral bands, which increases the flow signal and reduces the image noise.

As OCTA is a depth-resolved technique, precise axial segmentation of retinal layers is needed as to acquire important data on perfused structures and simultaneously. OCTA thus avoids the risk of generating superimposed images, which are typical of FA or ICGA. To achieve this, several methods have been employed. An

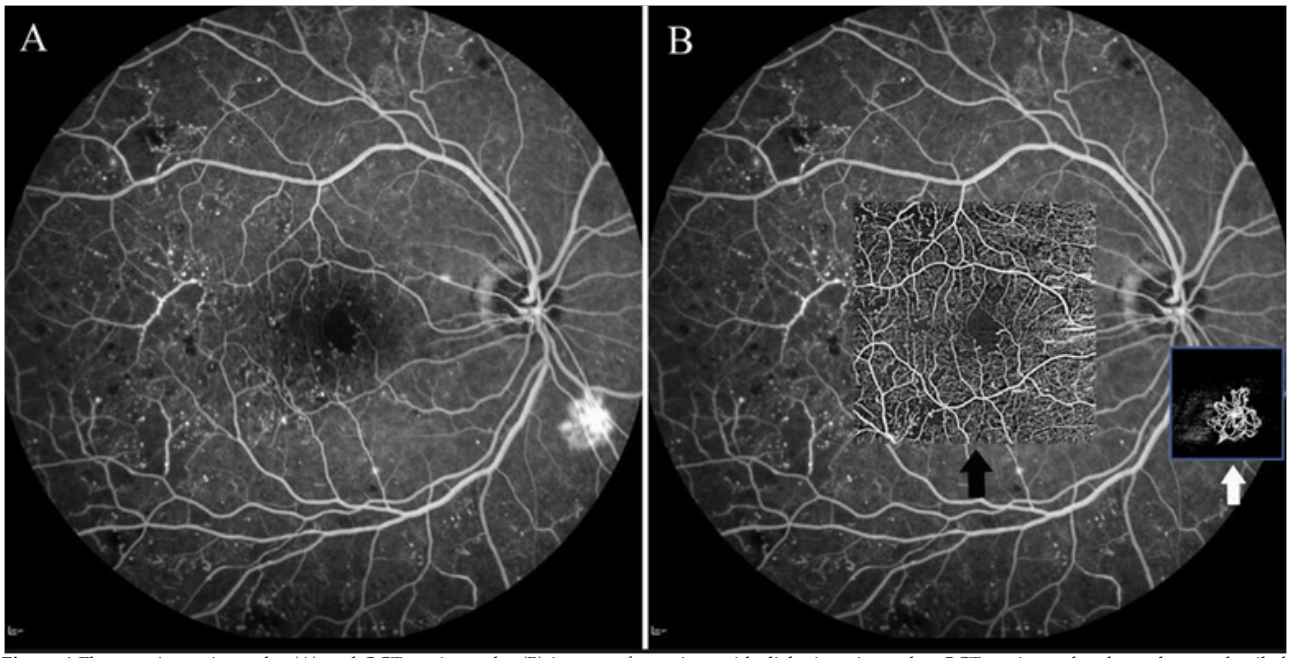

Figure 4 Fluorescein angiography (A) and OCT angiography (B) images of a patient with diabetic retinopathy. OCT angiography showed more detailed information of the macular microvascular structure (white arrow) and indicated the neovascularization (segmentation at the level of vitreous-retinal interface) near the optic head where FA only showed dye leakage.(HRA 2, Heidelberg Engineering, Heidelberg, Germany; ZEISS Angioplex, Carl Zeiss Meditec Inc., Dublin, CA, USA).

automated segmentation algorithm is provided by the majority of different OCTA devices[84]. This algorithm is capable of providing an extremely fast way to delineate the presence of a decorrelation signal and to distinguish different retinal layers, from the inner limiting membrane (ILM) to the RPE, including the external limiting membrane, ellipsoid zone, and outer segment.

Another case is *manual segmentation*[84], where the manual selection of C-scans at different depths is performed with either horizontal or variably shaped sections. The horizontal section (not aligned to any retinal layer) can reduce artifacts due to segmentation errors. Despite both methods, a major and unavoidable limitation of OCTA is projection artefacts, in which superficial retinal vessels are projected onto the deep plexuses and choriocapillaris. This can limit the accurate interpretation of vascular pathology in the deeper layers. Other challenges of OCTA include inability to view leakage from vessels like FA or ICGA, limited imaging of slow flow situations such as microaneurysms or fibrotic CNV, a limited field of view (3 × 3 or 6 × 6 mm)[85], and eye motion artifacts[86].

The currently commercially available OCTA systems used in clinic include the Angio Vue Imaging System (Optovue, Inc., Freemont, CA)[87], ZEISS Angioplex (Carl Zeiss Meditec Inc., Dublin, CA, USA), Swept-Source Optical Coherence Tomography Angio (Topcon Corporation, Tokyo, Japan), and Heidelberg Spectralis (Heidelberg engineering, Heidelberg, Germany). OCTA has been playing an active role in the evaluation of common ophthalmologic diseases such diabetic retinopathy (DR), age related macular degeneration (AMD), artery and vein occlusions, and glaucoma. OCTA has been demonstrated to visualize areas of retinal non-perfusion[88,89] and the foveal avascular zone (FAZ) of the retinal capillaries[90], which are essential to describe the DR progression[91].

OCTA has also been reported to be sensitive in detecting Type I[92], Type II[93], and Type III[94] choroidal neovascularization (CNV) in AMD patients. Furthermore, OCTA has been suggested to visualize alterations in the choriocapillaris of patients from dry AMD[95,96]. For retinal vein occlusions(RVO), Kashani *et al* reported findings in OCTA of 26 eyes with RVO that were consistent with clinical, anatomic, and fluorescein angiographic findings including areas of impaired vascular perfusion, retinal atrophy, vascular dilation, shunt vessels, and some forms of intraretinal edema[97]. Researchers also demonstrated the usefulness of OCTA in evaluating the response to anti-VEGF treatment[98,99]. OCTA has been demonstrated to be useful to identify the retinal vascular pathology in diseases such as macular telangiectasia Type 2[100], sickle cell retinopathy[101], central serous chorioretinopathy (CSCR)[102], and glaucoma[103].

OCTA represents a promising emerging retinal imaging modality that can potentially be applied in the screening, monitoring and treatment of retinal diseases. Most of the current literature describing OCTA has been small cross sectional studies. Thus, larger, prospective, randomized clinical trials are required. Establishing a normative and pathologic database and evaluating the sensitivity and specificity of OCTA is important for an emerging technology. It is also important to evaluate the clinical applications of OCTA in assessing therapeutic effects of various treatment strategies in retinal and choroidal diseases. Additionally, considering its limitations, future improvements in OCTA may focus on new algorithms that maximize signal-to-noise-ratio, new OCT devices with faster scanning speed to expand the field of view, and eye-tracking system to decrease eye motion artifacts.

PHOTOACOUSTIC IMAGING

As a hybrid biomedical imaging method, photoacoustic (PA) imaging exploits both optical and acoustical properties and provides functional and structural information of bio-tissues. A nanosecond pulse-duration laser beam is employed to irradiate the target tissue of interest, causing photons to propagate inside the tissue. Absorption of these photons leads to a slight localized transient temperature rise of the tissue, followed by a transient thermoelastic expansion. Ultrasonographic pressure waves, called PA waves, can then be induced by the transient thermoelastic expansion, detected by

broadband ultrasonic transducers, and imaged[104].

In the eye, the major light-absorbing materials are hemoglobin inside the blood vessels and melanin inside the RPE and choroid. PA offers the first possibility for direct measurement of light absorption by retinal tissues. PA can be a potential tool for noninvasive and sensitive imaging of retinal and choroidal structures[105]. Apart from the photoacoustic tomography (PAT) used to visualize the entire eye[106,107], photoacoustic ophthalmoscopy (PAOM) is emerging as a promising tool. With the guidance of OCT, PAOM has been reported to be capable of image the retinal vessels[108,109,110], RPE cells[109,110], and choroidal vessels[108,110] in rodent animals with a axial resolution of around 20 μm[111,112] and a higher resolution that can reach 4 μm[112,113].

In addition, photoacoustic imaging provides a new method to accurately sense oxygen saturation through direct measurement of light absorption within blood with multiple laser wavelengths[114]. In future research, PAOM can be improved in terms of its resolution, use of contrast agents for molecular imaging, and harmonious integration with existing fundus imaging modalities.

MOLECULAR IMAGING

Although ophthalmic imaging modalities are capable of imaging retinal morphology with unprecedented resolution, challenges still remain to detect subclinical molecular changes. Subclinical changes are subtle cellular and molecular changes which occur before retinal disease can be detected by current anatomy-driven ophthalmic imaging instrumentations. Molecular imaging includes strategies capable of detecting disease biomarkers in the retina. Two important components of retinal molecular imaging are the potential biomarkers to be targeted and the molecular profiling techniques to be employed. A number of studies have reported feasibility of molecular imaging in detecting retinal ganglion cells (RGCs), RPE cells, endothelial cells, and leukocytes.

Cordeiro *et al*[115,116] used molecular imaging technology named detection of apoptosing retinal cells (DARC) for the single-cell detection of RGC apoptosis, where Annexin V was intravitreally injected to specifically bind the apoptosis biomarker phosphatidylserine (PS) and then was detected by ophthalmic fluorescence imaging instrumentation. Barnett *et al*[117] utilized a peptide-based fluorescent probe (TcapQ) which is sensitive to active caspases such as caspase 3 involved in RGC apoptosis to *in vivo* quantify apoptotic RGCs. Another promising opportunity for molecular imaging of RGCs lies in the imaging of RGC dysfunction before cell death. Several imaging probe such as reactive oxygen (ROS)[118], mitochondria selective JC-1[119], and E glutamate[120] hold great promise in improving the molecular imaging of RGCs.

Beyond autofluorescence imaging, several RPE-related molecular targets may also warrant consideration, such as ROS[121], β-amyloid, esterified cholesterol and carbohydrate moieties in drusen[122]. As for endothelial cells, the surface biomarkers of inflammation or angiogenesis are potential candidates for development of targeted contrast agents for ophthalmic imaging of retinal or choroidal neovascularization. The C-C chemokine receptor 3 (CCR3) is a promising biomarker of choroidal neovascularization (CNV), as demonstrated by CCR3 expression on choroidal neovascular endothelial cells in human CNV specimens[123].

Other promising biomarkers include targeting proliferating endothelial, endoglin[124], and integrin $\alpha_v\beta_3$[125,126]. These have been successfully imaged in patient specimens *ex vivo* and in cardiovascular diseases and cancer. While currently in its infancy, molecular imaging of the retina will likely play a critical role in disease diagnosis and monitoring in the future.

CONCLUSIONS

Ophthalmic imaging has rapidly developed and advanced during the last 30 years. Traditional fundus camera and FA/ICGA still play pivotal roles in disease diagnosis and monitoring. Integrated technologies with previously developed retinal imaging instruments such as hand-held, ultra-wide field, and adaptive optics have improved and transformed our imaging capabilities. The non-invasive OCT and OCTA also have further profound benefits in providing improved resolution anatomic imaging, intraoperative surgical planning, and expanding scan area. Finally, photoacoustic imaging and molecular imaging are novel fields in their infancy, which will likely play a larger and more critical role in the future through providing a window on dynamic disease processes, such as inflammation, apoptosis, and neovascularization. Ophthalmic imaging will continue to rapidly advance and result in improved patient care for years to come.

CONFLICT OF INTERESTS

The authors declare that they do not have conflict of interests.

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