Recent Advances in Retinal Biomarkers for Diagnosis of Early Alzheimer's Disease

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Abstract

The burden of Alzheimer's disease (AD) is rapidly increasing worldwide. Developments towards early diagnosis and prevention of AD have been impeded by challenges in detection of the disease in its prodromal preclinical phase, when patients are clinically asymptomatic or exhibit mild cognitive impairments using specialized neuropsychological testing. Further, the current diagnostic processes are invasive, costly, and challenging for access. In addition, clinical testing is often initiated once the disease has progressed to late-stage, when irreversible pathological changes to the brain have occurred. As an extension of the central nervous system (CNS), the retina shares similar morphological and physiological properties. However, unlike the rest of the CNS, retinal tissue can be directly and non-invasively visualized using common ophthalmologic techniques, making this an attractive biomarker for AD. Technological advances in ocular imaging instrumentation have enabled the exploration of retinal biomarkers for detection of early AD with mild cognitive impairment (MCI). With cerebral amyloid aggregation likely occurring as early as 20 years prior to the onset of clinical dementia, it is imperative to develop a feasible, large-scale screening protocol for at-risk populations that is affordable, accessible, and non-invasive. In this review, we highlight novel ophthalmologic instrumentation used to identify and characterize retinal biomarkers in AD, such as optical coherence tomography (OCT), fundus photography, OCT angiography, and confocal scanning laser ophthalmoscopy (cSLO).

1.0 Introduction

Alzheimer's disease (AD) is the most common cause of senile dementia worldwide. AD is a prominent source of morbidity and mortality in the elderly population, ranked as the 6th leading cause of death in the United States. [1] Currently, approximately 5.7 million Americans are living with AD, and these numbers are expected to triple by 2050.[1] AD surpasses cardiovascular disease and cancer in annual health care costs, making it the most expensive disease in the United States, without taking into consideration informal care which additionally adds to this burden.[1] Despite the substantial economic and public health burden of AD, there are no effective treatments available for this disease.[2] Current therapies target cognitive deficits, which develop in the advanced stages of AD when changes appear to be permanent.

AD is a progressive neurodegenerative condition characterized by a gradual, irreversible loss of memory and cognitive function. Hallmark neuropathologic changes of AD include aggregation and propagation of misfolded amyloid beta-protein (Aβ) and hyperphosphorylated tau protein (pTau) present in neurofibrillary tangles (NFTs); these cerebral changes can precede the manifestation of clinical dementia by up to 20 years, during a phase defined as prodromal AD.[3] This emphasizes the need for early diagnosis, permitting early intervention in an effort to attain efficient therapeutic responses. Significant funding and research have been dedicated to the development of disease-modifying therapies, which can target the disease during the prodromal phase, when minimal damage to synapses and neuronal tissue have occurred. Despite this, many challenges impede the detection of early stage AD.

Current diagnosis of AD requires a medical examination, lab work, neurocognitive testing, and magnetic resonance imaging (MRI) of the brain, which is cost-and time-consuming. Amyloid positron emission tomography (PET) scans and cerebrospinal fluid (CSF) amyloid confirmatory biomarkers have received FDA approval; however, these modalities are very costly, invasive, and pose considerable access barriers.[4] Studies have demonstrated that measurable changes in PET, MRI, and CSF biomarkers occur prior to the onset of clinical symptoms.[5] However, due to the aforementioned limitations, these diagnostic procedures are not suitable for screening at a population level. In attempt to address these issues, serum biomarkers have been identified for AD.[4, 6, 7] These serum biomarkers are in the process of being validated in larger AD cohorts as well as determining whether the biomarkers can detect prodromal or early AD. In addition, the limitations of the current expensive and invasive "gold standards" have served as an impetus for further research of AD biomarkers in the retina, an extension of the central nervous system (CNS). In contrast to the rest of the CNS, the retina is easily accessible for direct, high-resolution, non-invasive imaging at a low cost. The discovery of retinal biomarkers is promising, as it has potential to effectively screen and

identify at-risk populations for subsequent testing, as well as identify preclinical AD. Retinal biomarkers may also serve as indicators of efficacy in future AD therapeutic trials.

2.0 Retinal Biomarkers for AD

The retina is a projection of the central nervous system via the optic nerve (Figure 1). Thus, it shares many features with the cerebral cortex, including embryologic origin and blood barriers.[8] In addition, the retina contains neurons, microglia, astroglia, and microvasculature with similar morphological and physiological properties.[3] Axons of the optic nerve form the connection between the retina and the vision centers of the brain and enable vesicular transportation of amyloid precursor protein (APP), which is produced in the retinal ganglion cells (RGCs).[2] Moreover, retinal neurons and glia express proteins that are involved in the amyloid cascade (e.g. BACE1, γ-secretase, APOE).[9-11]

Animal studies of AD discovered beta-amyloid (Aβ) plaques via curcumin labeling in the retina prior to manifestation of plaques *in* the brain. There was a correlation between retinal plaque burden and progression of brain pathology.[12] Further, pathological Aβ plaques were revealed in postmortem retinas of confirmed AD patients as well as early-stage cases.[10] Upon additional neuropathological examination in these patients, a qualitative association was found between retinal Aβ plaque burden and cerebral amyloid burden, unlike the age-matched healthy controls whom exhibited minimal Aβ plaques.[3, 12] Additional pathological changes in the retina, such as dropout of retinal ganglion cells and their axons, have also been validated in animal models and post mortem studies of human AD eyes. [13-16]

Recent advances in ophthalmologic imaging technology have considerably improved the ability to visualize detailed structure and function of the retina in vivo. High-resolution imaging modalities have equipped researchers with tools to investigate the potential of the retina as an extension of the CNS in neurodegenerative conditions such as Alzheimer's disease. Techniques such as spectral-domain optical coherence tomography (SD-OCT), confocal scanning laser ophthalmoscopy (cSLO), retinal fundus photography, and optical coherence tomography angiography (OCTA) have provided insights into the discovery of retinal biomarkers in Alzheimer's disease.

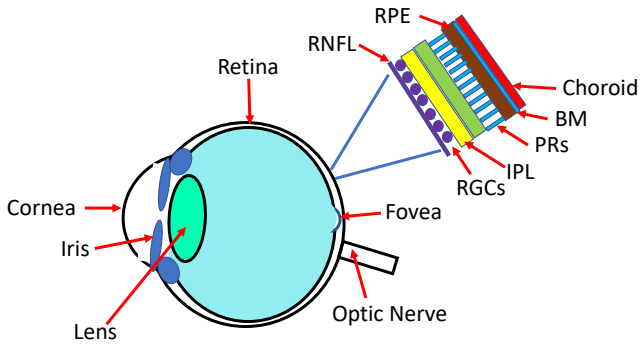

Figure 1. Illustration of the eye with key retinal anatomical structures labeled. Key: RNFL = retinal nerve fiber layer; RGCs = retinal ganglion cells; IPL = inner plexiform layer; PRs = photoreceptors; BM = Bruch's membrane; RPE = retinal pigment epithelium.

3.0 Retinal Imaging Technologies in Alzheimer's Disease

3.1 Optical Coherence Tomography

Recently, OCT has advanced to SD-OCT: a fast, noninvasive imaging device that produces 3-dimensional cross-sectional images of the retina with improved axial resolution and lower measurement variability.[17] This allows retinal neuronal layer segmentation, providing precise and accurate measurement of the thickness of intra-retinal layers.[15] OCT is widely used to identify and measure structural axonal damage in several optic nerve and neurological diseases, such as glaucoma.[18] Given that the retina is characterized as a peripheral extension of the CNS, SD-OCT has become an integral tool in the evaluation of retinal biomarkers for AD.

Several studies have demonstrated an overall reduction in mean peripapillary retinal nerve fiber layer (pRNFL) thickness in patients with both mild cognitive impairment (MCI) and AD compared to age-matched healthy controls.[19-21] In AD patients, global reduction of pRNFL was demonstrated in all four retinal quadrants, indicating that axonal loss seems to be a consequence of a diffuse retinal ganglion cell and nerve fiber layer degeneration.[18] This differentiates AD from other optic neuropathies, such as glaucoma, where RNFL reduction does not occur in all fields, but rather predominates in the superior and inferior quadrants.[22] These findings, demonstrating axonal loss with RNFL thinning, have been consistent between various commercially available OCT devices.[18]

In addition to pRNFL thinning, reduction of total macular volume and macular retinal nerve fiber layer (mRNFL) thickness has been discovered in patients with MCI and AD.[20, 22] Moreover, there was a significant correlation between the total macular volume and severity of AD based on the Mini-Mental State Exam (MMSE).[20] Because approximately one-third of the retinal thickness in the macular region is comprised of RGCs and their fibers, measurements of macular thickness can provide useful insight in assessing neuronal

loss in AD patients.[18, 23] With the advancements in OCT technology, layer segmentation has enabled further examination of retinal and RGC sublayers (Figure 2). Some researchers propose that the macular region is preferentially affected in early AD.[18, 24-26] Salobrar-Garcia et al. (2015) demonstrated this in patients with mild-AD; the patients exhibited significant reduction in macular parameters, and although pRNFL thinning was observed, the difference did not reach significance compared to age-matched controls.[26] Moreover, a significant, circumferential reduction in macular ganglion cell-inner plexiform layer (GC-IPL) thickness was observed when comparing AD patients with cognitively normal controls.[27] Since the macular region encompasses the greatest density of cone photoreceptor cells and RGCs, variations in the macular layers, including the GC-IPL, macular ganglion cell complex, and photoreceptor layer are anticipated to be more sensitive to neurodegenerative disease than the peripapillary RNFL, making it a promising biomarker for detecting early changes associated with AD.[28, 29]

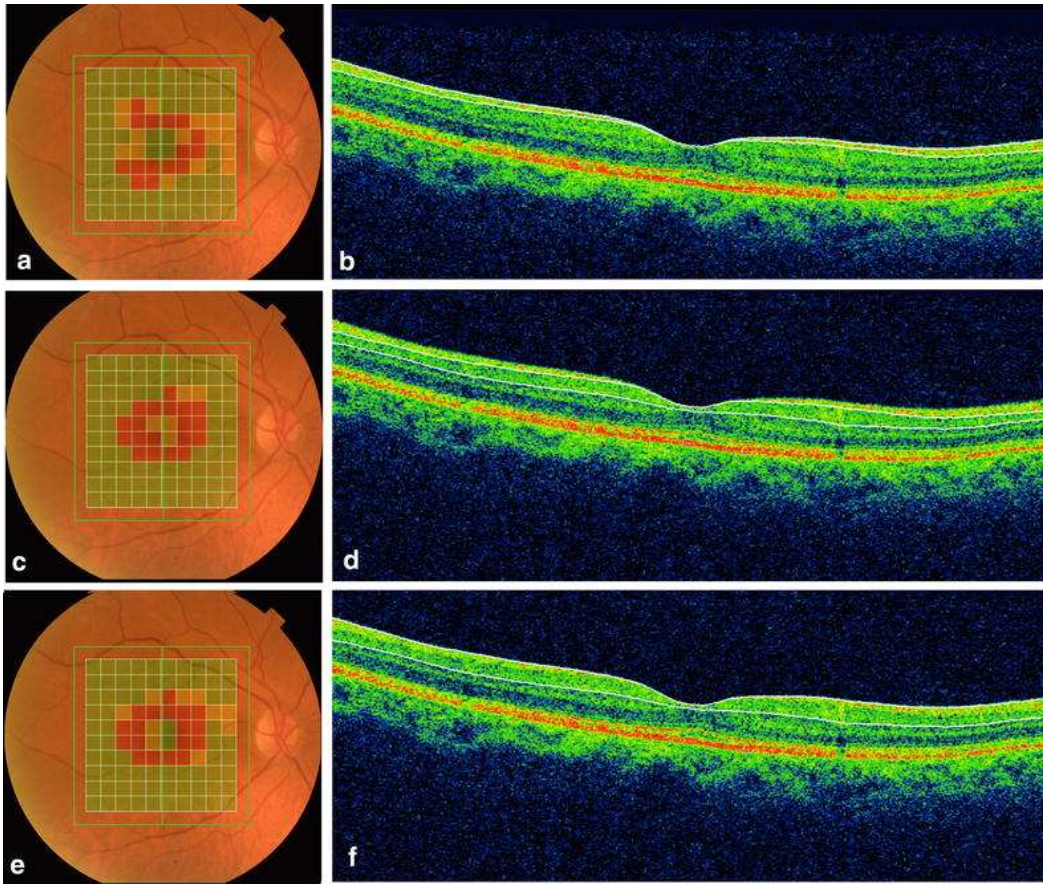

Figure 2. Depiction of inner macular thickness measurements with SD-OCT of AD patient. Pictures on the left (a,c,e) indicate the retinal areas scanned, and pictures on the right (b,d,f) show the corresponding vertical OCT scans. White lines demonstrate the limits of the areas measured for that scan. (Cunha et al., 2016)

3.1.1 BluePeak Laser Autoflourescence (BAF)

As previously discussed, both animal studies and evaluation of postmortem retinas of confirmed AD and early-stage AD patients revealed the presence of pathological retinal amyloid plaques, which qualitatively corresponded to cerebral amyloid burden in these patients.[12] Further histological studies suggest that the inclusion bodies contain fibrillar amyloid, which is characteristic of the neocortical plaque deposits seen in AD.[30, 31] Retinal inclusion bodies appeared at or near the inner plexiform layer (IPL), differentiating them from the age-related cellular drusen that typically appear between Bruch's membrane and the retinal pigment epithelium.[32] Successive research with living and postmortem AD patients has supported and expanded on these findings by identifying aggregates of Aβ plaques and neurofibrillary tangles in the retina.[3] Retinal Aβ plaques frequently exist in clusters, and, although smaller in size, are comparable representations of Aβ plaque morphology and vascular pathological alternations in the brain.[3]

Snyder et al. supplemented SD-OCT measurements by incorporating BluePeak Laser Autoflourescence (BAF) imaging, a tool which enabled identification of the number and precise location of retinal inclusion bodies (Figure 3).[32] BAF is a non-invasive, scanning laser fundus imaging modality that provides a map of the retina which can identify areas of metabolic dysfunction. Their study identified retinal inclusion bodies in individuals with significant neocortical amyloid burden, in stark contrast to the absence of retinal inclusion bodies in individuals without significant beta-amyloid aggregation. [30]

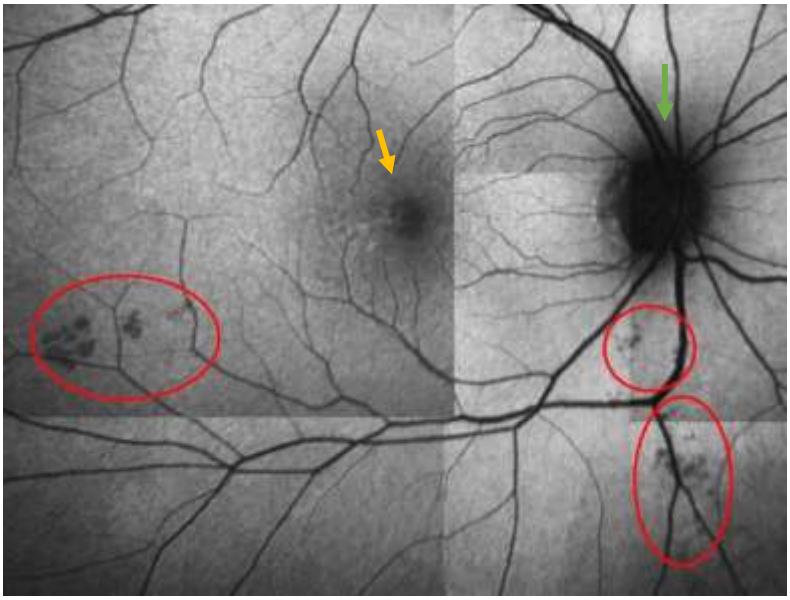

Figure 3. Retinal image with BAF of a cognitively normal adult with high neocortical A burden. Inclusion bodies are located within red circles. The green arrow is pointing at the optic nerve head and the yellow arrow is pointing at the macula. (Snyder et al., 2016)

Further, the surface area of retinal inclusion bodies increased as a function of cortical amyloid burden and was associated with an increase in volume of the IPL in amyloid positive patients [32] The IPL is a cholinergic-rich retinal layer that has been identified as an early indicator of neurochemical changes in AD. Further investigation is required to explore the potential inflammatory process occurring in the IPL, which could be a reflection of the early cholinergic changes that occur in the neocortex.

3.2 Confocal Scanning Laser Ophthalmoscopy

Confocal scanning laser ophthalmoscopy (cSLO) is an imaging technique that operates by emitting a superluminescent diode light and an infrared scan to concurrently provide high resolution and high contrast images of ocular microstructures. Using a modified point cSLO, Koronyo et al. demonstrated in vivo retinal imaging using a curcumin fluorochrome to distinguish and quantify retinal amyloid deposits in humans.[3] Curcumin is a ligand that binds selectively to fibrillar Aβ, is generally safe, and has been validated in previous animal studies which showed increased fluorescence of curcumin spots, leading to specific detection of Aβ-containing deposits.[12] Ten definite AD patients and 6 healthy controls underwent a 2-day dosing regimen of oral curcumin with baseline and follow-up modified SLO scans. AD patients exhibited a 2.1-fold increase in the number of retinal amyloid plaques compared to healthy controls. Retinal plaques were associated with retinal neuronal loss, which corroborated preceding studies. Further, following curcumin administration, retinal amyloid deposits were localized above the intact retinal pigment epithelium (RPE) and Bruch's membrane, distinguishing AD retinal plaques from other ocular pathologies, such as drusen in age-related macular degeneration (AMD). Lastly, Koronyo et al. demonstrated a positive correlation between the quantity of retinal amyloid plaques and cerebral amyloid plaques. [3]

3.3 Optical Coherence Tomography Angiography

Optical coherence tomography angiography (OCTA) is a relatively novel, non-invasive retinal imaging technique that evaluates retinal and microvascular anatomy. Measurement outcomes include foveal avascular zone (FAZ) parameters, areas of abnormal flow in the outer retina and choroid, and vessel density of the superficial vascular complex, deep vascular complex, and radial peripapillary capillaries. In AD, reported vascular changes include breakdown of the blood-brain-barrier, diminished Aβ clearance, and reduced blood vessel caliber, density, and blood flow.[33] Using OCTA, retinal venous narrowing and decreased retinal blood flow have been determined in patients with AD and MCI when compared to healthy controls.[34] O'Bryhim et al. found an association between inner foveal thinning and enlargement of the FAZ (Figure 4) in CSF or PET biomarker-positive groups,

suggesting that biomarker-positive, preclinical AD may have retinal vascular and architectural changes that precede clinical cognitive symptoms.[35]

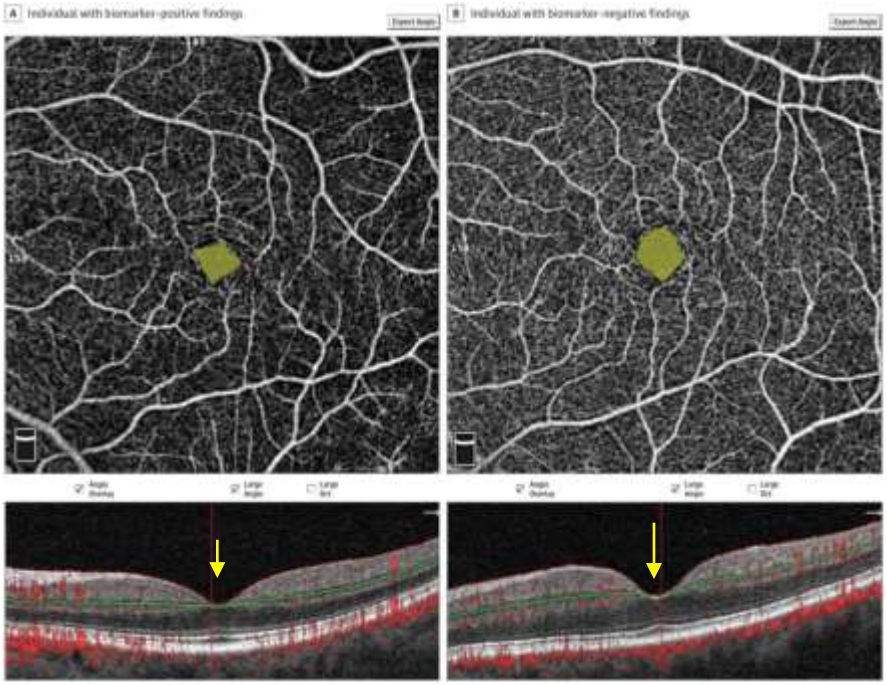

Figure 4. Example of OCTA images measuring Foveal Avascular Zones (FAZ). Top images show angiogram images with nonflow areas and bottom images depict corresponding OCT scans. Yellow arrows point at the foveal region of the OCT scans. (O'Bryhim et al., 2018)

The foveal avascular zone is a specialized, avascular region of the retina, which encompasses the highest density of cone photoreceptors and oxygen consumption.[36] A link between FAZ and visual function has been established, and changes in the FAZ have been noted in other vasculopathic conditions such as diabetes, hypertension, and retinal vascular occlusions.[34] Overall, OCTA is a non-invasive technological advancement that permits accessible visualization of retinal architecture and blood flow that has the potential to serve as another retinal biomarker for identification of early vascular changes in individuals with preclinical AD.

3.4 Retinal Photography

Retinal photography is a routinely utilized imaging technique in ophthalmic practice; it entails a retinal camera which captures a non-invasive, ultra-wide-field, high-resolution digital image of the retina in each eye. Retinal photography equips investigators with a still image to carefully evaluate for retinal vascular signs associated with AD. Cheung et al. implemented a vessel segmentation software in conjunction with retinal image analyses and determined that individuals with narrower retinal vessel caliber, changes in global vessel geographic patterns, such as reduced vessel fractal dimension, and increased vascular tortuosity

were more likely to have AD.[37] Frost et al. examined the relationship between retinal vasculature and neocortical brain amyloid plaque burden and AD; they found higher venular branching asymmetry and arteriolar length-to-diameter ratios in healthy individuals with high plaque burden, measured by PET imaging.[38]

Fundus autofluorescence (FAF) is an imaging modality that detects fluorophores, predominately lipofuscin, in the retinal pigment epithelium. Kabayasi et al. used FAF imaging to visualize changes in the retina following oral curcumin administration and noted areas of hyperintense dot-like alterations on FAF in patients with MCI. Additionally, patchy areas of hypofluorescence were revealed in the FAF images, which was likely attributable to choroidal thinning and RPE degeneration.[39]

Furthermore, retinal imaging provides a comprehensive overview of retinal structure. It has allowed for determination of cofounding variables, such as retinopathy and glaucoma, as well as identification of peripheral biomarkers, such as peripheral drusen formation after clinical progression of AD.[40] It is notable that retinal photography does pose limitations, such as physiological variation of retinal vasculature, photographic quality, and detection of retinal lesions common in several diseases. Although retinal photography may be limited as a single screening tool for detection of AD, it does provide valuable information and further studies are needed to validate the association between these retinal changes and AD.

4.0 Conclusion

A number of novel technological advancements have allowed the discovery of retinal biomarkers in mild cognitive impairment and early Alzheimer's disease (Table 1). If validated, retinal biomarkers can serve as part of a multi-tiered diagnostic process. Retinal imaging is a non-invasive, low cost, and easily accessible approach to effectively screen individuals who require subsequent, confirmatory diagnostic testing. Future studies need to determine if these biomarkers can serve as a potential screening tool in primary care. As novel therapies and clinical trials progress, it will become vastly significant to ascertain point-of-care clinical markers of early disease burden, to initiate therapy and to follow the therapeutic efficacy on AD progression.

Table 1. Imaging techniques to identify retinal AD biomarkers

Imaging Technique	Description	Advantages	Disadvantages
OCT	• Cross-sectional imaging of retinal layers	• Allows evaluation of individual retinal layers • Retinal layer thickness quantification • High resolution • Non-invasive	• Media opacities obscure image quality* • Measurement variability between available instruments • Susceptible to confounding pathology; non-specific for AD
BAF	• Scanning laser fundus imaging • Identifies areas of metabolic dysfunction	• Detection of retinal inclusion bodies specific for AD • Non-invasive	• Media opacities obscure image quality* • Additional studies required to validate method
cSLO	• Imaging of ocular microstructures	• High resolution and high contrast • Retinal layer thickness quantification	• Media opacities obscure image quality* • Poor axial resolution
OCTA	• Imaging of the microvasculature of the retina and choroid	• Enables measurement of foveal avascular zone, volumetric blood flow, and vessel density • Non-invasive; does not require an injectable dye like conventional angiography techniques • Quick • High axial resolution	• Media opacities obscure image quality* • Sensitive to motion artifact – requires patient cooperation
Retinal Photography (Fundus Imaging)	• Static, wide-field image of the retina	• Multiple imaging modalities available, such as fundus autofluorescence • Allows for evaluation of retinal vessel integrity and presence of pathology, including retinopathies	• Current findings are ambiguous and non-specific for AD • Subjective interpretation of images leads to variable outcome measures

*Cornea, lens and vitreous need to be transparent for best resolution; for example, an advanced cataract (lens opacification) is a confounding factor in these imaging techniques.

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