Dynamic Aperture Imaging with an Adaptive Optics Scanning Laser Ophthalmoscope as an Approach to Studying Light Scatter in the Retina

THESIS

Presented in Partial Fulfillment of the Requirements for the Degree Master of Science in the Graduate School of The Ohio State University

By

Danielle Marie Mayne

Graduate Program in Vision Science

The Ohio State University

2017

Master's Examination Committee:

Dean VanNasdale, OD, PhD, FAAO, Advisor

Bradley Dougherty, OD, PhD, FAAO

Nicky Lai, OD, MS, FAAO
Abstract

Advances in high resolution retinal imaging reveal features in normal and diseased eyes that were not previously observable, due to resolution limits of imaging systems or the optically transparent nature of many cellular structures in the human retina. Novel imaging techniques can help increase our understanding of disease processes in the eye as well as insight into more generalized systemic changes occurring in neuronal structures and vasculature.

Although there are advantages of the resolution in a highly confocal imaging system, some retinal features are more easily distinguished using more multiply scattered light.\(^1\) Multiply scattered light can be collected by offsetting a confocal aperture by varying degrees.\(^1\) It is a challenge to find the best imaging modality for the features of interest. Even though a highly confocal system usually offers the best axial resolution for optical sectioning, in this study we looked at the optical sectioning abilities using a relatively large aperture in a confocal and offset orientation in an adaptive optics scanning laser ophthalmoscope imaging system. We found that using the relatively large confocal aperture, that the system was capable of optically sectioning different retinal layers. In the offset orientation, it was also possible for different layers of the retina to be sectioned even though these were imaged with primarily multiply scattered light. This was accomplished using the retinal capillary networks to recognize axial position in the retina.
This study also explores the use of a novel imaging technique, dynamic aperture imaging, where the aperture position is varied during image acquisition. Starting in an offset orientation, the aperture is then moved towards the confocal position, to the confocal position, then past it, while the subject fixates on the same target. With this technique, both directly backscattered light as well as multiply scattered light are able to be collected and different retinal features are able to be compared with both modalities.

Dynamic aperture imaging introduces a novel approach to quantify and analyze the light scattering properties in different retinal layers. By using an intensity profile comparing the average intensity per pixel with varying offset displacement of the aperture, the scattering of a retinal layer can be quantified. Our results show differences in the scattering of the different retinal layers analyzed.
Acknowledgements

I would like to express my sincere gratitude to my advisor Dr. Dean VanNasdale for his continuous support of my study, for his guidance, patience and immense knowledge without which this paper would not have been possible.

I would like to thank the rest of my thesis committee, Dr. Bradley Dougherty and Dr. Nicky Lai for their comments and feedback. I would also like to thank Gary Huang, Keith Ramsey and Marisa Ciamacca for their assistance with the project.

Finally, I would like to thank my husband Kevin Mayne for all of his support.
Vita

June 2003 ...................................................Westerville North High School
2007.................................................................B.S. Biology, University of Michigan
2013 to present..............................................O.D./M.S candidate, Ohio State University

Fields of Study

Major Field: Vision Science
# Table of Contents

Abstract .......................................................................................................................... ii

Acknowledgements ....................................................................................................... iv

Vita .................................................................................................................................. v

Fields of Study ............................................................................................................... v

List of Figures ............................................................................................................... vii

Chapter 1: Introduction ................................................................................................. 1

Chapter 2: Methods ...................................................................................................... 23

Chapter 3: Results ....................................................................................................... 29

Chapter 4: Discussion ................................................................................................. 41

References .................................................................................................................... 50
List of Figures

Figure 1. Photoreceptors in the confocal and offset orientation. ........................................... 31
Figure 2. Superficial capillary network in the confocal and offset configuration. ........ 33
Figure 3. Dynamic aperture imaging series of blood vessels. .............................................. 35
Figure 4. Dynamic aperture imaging series of retinal cysts. ................................................. 36
Figure 5. Intensity profile for the nerve fiber layer using dynamic aperture imaging. ..... 37
Figure 6. Intensity profile for the photoreceptor layer using dynamic aperture imaging. 38
Figure 7. Intensity plot from the photoreceptor layer ............................................................. 39
Figure 8. Intensity plot from the nerve fiber layer ................................................................. 40
Chapter 1: Introduction

The human eye offers a unique opportunity to study neural and vasculature structures of the body as well as the ocular structures responsible for vision \textit{in vivo}. Novel imaging techniques can help increase our understanding of disease processes in the eye as well as insight into more generalized systemic changes occurring in neuronal structures and vasculature. A primary challenge in imaging the human retina \textit{in vivo} is the ability to match the optical properties of the tissue and structures of interest to a specific imaging modality necessary to visualize as much of the complex structure as possible. Adapting and optimizing high resolution imaging techniques can be used to match features of interest, thereby providing additional insight into normal aging and disease processes, with the goal of improving our understanding of ocular structures and their corresponding function.

The ability to resolve small features in the retina is important for our understanding of the retina’s role in the visual pathway. Resolution describes the ability for two closely spaced neighboring points to be distinguished from one another. Optimizing both lateral, or transverse resolution in the XY direction and axial resolution in the Z direction are important for this understanding.\textsuperscript{2,3} To take advantage of the accessibility of the retinal structures, imaging techniques have to overcome the innate optical challenges that the human eye presents. Pupil size as well as aberrations of the eye
can limit the resolution of an imaging system. There are also limits on the wavelength
and the amount of light that is safe to use in the human eye without inflicting damage.\textsuperscript{2}

The eye itself is a dynamic system and movement, accommodation and tear film
integrity also affect imaging. Tear film irregularities that can present only seconds after
blinking and can worsen after prolonged periods of not blinking can degrade image
quality.\textsuperscript{4} Accommodation can unpredictably shift the focal depth during imaging with
certain modalities.\textsuperscript{5} Small eye movements including tremors, drifts and microsaccades
that are normal during fixation can cause distortion errors during image acquisition and
between acquisitions of subsequent images.\textsuperscript{6} With consideration for the subject being
imaged, non-invasive as well as fast imaging techniques are the most advantageous.

The retina is a multi-layered structure that lies between the choroid and the
vitreous. It consists of ten layers that include a single layer of pigmented epithelium and
three distinct layers of neuronal cell bodies with their associated synapses and cell
processes. The outermost layer is the retinal pigment epithelium (RPE) which is
composed of a single layer of hexagonal shaped cells and is continuous with the
pigmented epithelium of the ciliary body and the ora serrata. It separates the neural retina
from the choroid. The basement membrane of the RPE is adherent Bruch’s membrane,
which forms part of the blood retinal barrier between the retina and the choriocapillaris.
The RPE serves to support the neural retina by transporting nutrients and waste through
the blood retinal barrier. It contains melanosomes and other pigment granules which
reduce light scatter and protect against reactive oxygen species. Moving internal towards
the vitreous the layer adjacent to the RPE is the photoreceptor layer. This is composed of
rods and cones, the specialized sensory cells that contain the photopigment that absorbs photons of light. It is further subdivided into the outer segment, closer to the RPE, which contains the photopigment necessary for phototransduction stacked in membranous discs and the inner segment, closer to the vitreous that contains the mitochondria and other organelles responsible for cellular metabolism. The external limiting membrane, which is not a true membrane is the zonula adherens junctions between the photoreceptor inner segments and the apical portion of the Müller cells. The outer nuclear layer is composed of the cell bodies of the rods and cones. The outer plexiform layer is made up of the inner fibers of the photoreceptors and the synapses between the bipolar and horizontal cells. The cell bodies of the horizontal, bipolar, amacrine cells, and interplexiform neurons and a few displaced ganglion cell bodies make up the inner nuclear layer. The inner plexiform layer is made up of the synapses between the axons of the bipolar cells and the dendrites of the ganglion cells as well as synapses between the amacrine and other amacrine, ganglion cells and interplexiform neurons. The ganglion cell layer is primarily made up of ganglion cell bodies with a few displaced amacrine cells. The ganglion cells layer is thickest near the macula. The axons of the ganglion cells form the nerve fiber layer which runs parallel to the retina to the optic disc, forming the optic nerve as it exits through the lamina cribrosa. The innermost retinal layer is the internal limiting membrane which is made up of astrocytes and Müller cell footplates and covered in basement membrane. It is continuous with the internal limiting membrane of the ciliary body and separates the retina from the vitreous.
Blood is supplied to the retina from the ophthalmic artery which is a branch of the internal carotid artery. The central retinal artery, the first branch of the ophthalmic artery, enters the optic nerve approximately one centimeter behind the globe. It then divides into two major branches and gives rise to the retinal arterioles and capillaries that supply the inner two thirds of the retina.\(^8\)

Histological data from macaque retinas, who have similar retinal vasculature to that of humans have further demonstrated four distinct capillary networks.\(^9\) A deeper capillary network has two separate capillary planes that bracket the inner nuclear layer.\(^9\) A more superficial capillary network brackets the ganglion cell layer in the foveal region and is displaced closer to the vitreous in the extrafoveal regions.\(^9\) The area of densest vasculature is found in the peripapillary region.\(^9\) Human retinal vascular anatomy has been found to agree with the histological primate models with \textit{in vivo} studies using optical coherence tomography angiography.\(^10\) Four distinct vascular plexuses have been described in the central retina.\(^10\) A deeper network is divided into a plexus below the inner nuclear layer, the deep capillary plexus and a plexus above the inner nuclear layer, the intermediate capillary plexus.\(^10\) These networks are composed of capillaries and are all relatively uniform in size. More superficially, the superficial vascular plexus in the ganglion cell layer contains the larger arteries, arterioles, veins and venules.\(^10\) The radial peripapillary plexus in the nerve fiber layer runs parallel to the nerve fiber layer axons and is found only in the posterior pole with the area of highest density in the peripapillary region.\(^10\)
The center fovea is free from any vasculature and is known as the foveal avascular zone. The venous system has a similar arrangement to the arteriolar system and venous blood is ultimately drained through the central retinal vein. The anterior and posterior ciliary arteries, also branches from the ophthalmic artery, form the choroidal vascular system which supplies the posterior third of the retina and the fovea.\textsuperscript{8}

Different portions of the retina are composed of material that absorbs, reflects and scatters light differently at various wavelengths. For example, macular pigment, blood and melanin is very absorptive while the nerve fiber layer as well as pathology like drusen or chorioretinal scarring is highly reflective.\textsuperscript{11}

\textit{Direct Imaging}

There are several different types of systems that have been developed to image the retina, utilizing different optical properties in order to obtain the image. One of the simplest and earliest developed systems is the fundus camera. This type of system is considered a flood illuminated system, illuminating the entire field of view in the retina and collects all of the reflected light returning back from it. Light that is directly reflected from the area of interest is referred to as back-scattered light. The retinal tissue also can scatter light laterally or deeper. Light that is redirected from multiple interfaces before returning to the imaging system’s detector is referred to as multiply scattered light. In a system like a fundus camera, the collected light includes both types of light, that which is directly backscattered as well as the multiply scattered light without axial segmentation capabilities.\textsuperscript{1,11,12,13}
Fundus photography has become a standard in clinical care, as it allows for a macroscopic view of the retina, including retinal blood vessels and optic nerve. The advantage to this type of a system and what has made it so useful to integrate into clinical care is that fast acquisition speed and minimal processing time allow for images to be available immediately and it is noninvasive as no contrast agents are necessary, with several systems able to acquire usable images without pupil dilation. Optically, this type of a system is fairly simple and mechanically the equipment tends to be very stable. The disadvantage of collecting both directly backscattered and multiply scattered light is the contrast of the retinal features can be reduced by the multiply scattered light from adjacent structures/tissue. Opacities in the lens or vitreous that increase scatter can further degrade contrast. This type of imaging also only allows for two dimensional images, and the retina and optic nerve are three dimensional structures that are difficult to evaluate in two dimensions. Conventional fundus photography is also typically static in nature, allowing for a snapshot of the retina at any point in time, but does not allow for dynamic imaging such as blood flow through vasculature. The injection of contrast agents such as fluorescein dye can provide some information about blood flow and vascular integrity but this technique is invasive, time intensive and is not tolerated by all patients.12, 13, 14

Other types of imaging techniques use scanning optics, like those used in scanning laser microscopy to produce retinal images. In this type of a system, the point of interest is illuminated by a laser beam, a highly-collimated source. This point is then rapidly scanned across the area of tissue in one dimension by a high speed resonant scanner and in another dimension by a relatively slower galvanometer. Information from
successive points is brought back to a detector and an image is created by building up the information from each successive point. A scanning laser ophthalmoscope (SLO) is the most common of this type of system.\textsuperscript{15}

A SLO uses a focused laser beam to scan an area of the retina to be imaged. At any point in time, only a small point of the fundus is illuminated and the light returning from this specific area determines the brightness of a corresponding point on a monitor to form a 2-D representation. As the laser scans rapidly over successive points on the fundus, the corresponding images can be built up as an array on a monitor. The laser beam is made to scan in a raster pattern across the fundus. This is a rectangular pattern of horizontal lines scanned from left to right and top to bottom. The angle through which the laser beam is scanned determines the area of fundus that is scanned and therefore the field of view. In general, the larger the scanning angle, the larger the field of view but the poorer the magnification. Conversely, the smaller the scanning angle, the smaller the field of view and the greater the sampling density.\textsuperscript{3,16}

The photodetector in the SLO detects a signal from one point at a time building up an array to form the two-dimensional image. The types of photodetectors that are typically used are a photomultiplier tube (PMT) or an avalanche photodiode (APD). This is different than an array of detectors like those typically used in fundus cameras.\textsuperscript{3}

If the photodetector of the SLO were to accept all of the light returning from the eye, the system would be more similar to the fundus camera, with poor contrast due to multiple scattering from features adjacent to structures of interest. To block the multiply scattered light, a pinhole aperture is added to the system that is conjugate to the retinal
plane and the detector. The detector receives only the light through the aperture that is directly scattered back from the area which the laser is focused on the fundus. The conjugate points in the system are the retina, the confocal aperture and the detector. This type of system is termed, confocal. The smaller the aperture the more confocal the imaging system. The confocal aperture allows for increased contrast compared to a flood illuminated system as well as better lateral and axial resolution. Only light from the selected plane of focus that is conjugate to the confocal aperture is returned to the detector. This increase in axial resolution also allows for optical sectioning with SLO. Adding defocus to the system can change the plane of focus in the axial position. Successive optical sections can then be built up and registered in order to create a three-dimensional image.\textsuperscript{11, 13, 14, 16}

Another type of imaging system that uses scanning optics is a line scanning laser ophthalmoscope, which projects a beam rather than a point of light onto the retina in one direction and uses a galvanometer to scan the beam in the other direction. The light from the line is then descanned and detected by a linear array sensor. Because it only has one moving part, the optics are compact and simplified. The optics and utility are limited though as it is only confocal in one dimension.\textsuperscript{17}

In some systems, the wavelength of light used in an SLO can also be controlled and varied. In general, the longer the wavelength of light, the deeper the penetration into the tissue. Near infrared wavelengths, 700-900nm, tend to work well for imaging using SLO. Near-infrared can image retinal and sub-retinal features better than shorter wavelengths. There is also a higher reflectance \textit{in vivo} with near infrared (830 nm)
compared to shorter wavelengths (450nm). Also, with shorter wavelengths, reflectance of
the fundus varies in a non-monotonic fashion depending on the degree of pigmentation of
the fundus. This is due to the different distribution of absorbing substances such as
melanin, macular pigment, oxygenated and deoxygenated blood in the retina and their
associated absorption spectrum. Reflectance is much more equal in the near infrared
range, which results in less inter-subject variability when imaging. Macular pigment
includes the carotenoids lutein, zeaxanthin and mesozeaxanthin which are found in the
highest concentrations within the Henle fiber layer of the macula and the inner nuclear
layer in the parafoveal area. Macular pigment has its peak absorption in the short
wavelength range, 460nm which can cause macular features in deeper retinal layers to be
blocked when imaging using shorter wavelengths, this effect is minimized with near
infrared. At much higher wavelengths, 950-1050nm, there is a large decrease in retinal
reflectance due to the high absorption of water at that wavelength. This again makes
near-infrared a good choice for imaging many retinal features. The increased scatter and
reflectance of near infrared does make it more useful in an SLO imaging system as
opposed to a fundus camera, as the SLO limits the multiply scattered light. Media opacity
such as cataract also affect imaging less using near infrared compared to white light.
Near infrared is also more comfortable to use for the imaging subjects as the visual
system is less sensitive to these wavelengths compared to white light.\textsuperscript{11,18}

SLO also allows for real time, dynamic, imaging using video images rather than
just a snapshot as flood illuminated systems. Because it is a more complicated optical
system, there is less light return from the eye resulting in the need for more sensitive light
detectors. It also has a relatively slower acquisition time compared to a flood illuminated system which provides nearly an instant image.\textsuperscript{11}

\textit{High Resolution Retinal Imaging}

While, SLO images have improved contrast and resolution compared to flood illuminated systems, the technique is still limited by the optical aberrations of the eye. The optics of the eye as it relates to imaging can be described with a point spread function, PSF. The PSF refers to the distribution of light on the retina produced by a single point source of light at infinity. In an eye where diffraction is the only thing limiting the system, the PSF diameter is inversely proportional to the size of the pupil, with the largest pupil producing the smallest PSF. In real eyes, higher order aberrations increase with pupil size. In this instance, the ideal pupil size to optimize the PSF in eyes with aberrations would be 2-3mm which is not ideal for maximum resolution in retinal imaging.\textsuperscript{19}

Adaptive optics (AO), a technique originally used in astronomy to correct for aberrations caused by the atmosphere in telescopes, has been applied to retinal imaging systems. Adaptive optics measures and compensates for higher order optical aberrations in real time allowing for essentially microscopic imaging of cellular and subcellular ocular structures \textit{in vivo} by creating nearly a diffraction limited system. In a diffraction limited system, the resolution is inversely related to the numerical aperture of the system $R=\frac{\lambda}{2\cdot NA}$, where $R$=resolution $NA=$numerical aperture. The numerical aperture is directly proportional to the entrance pupil diameter of the system $NA=n\cdot\sin(\arctan(D/2\cdot f)$
where $n=$ the index of refraction which the light is focused. $D =$ entrance pupil diameter and $f =$ focal length. The larger the pupil diameter, the better the resolution. AO allows for the pupil size to be maximized for imaging.\textsuperscript{13}

In order to correct for the higher order aberrations, they first must be measured. In order to accomplish this, a wavefront sensor, such as a Shack Hartmann wavefront sensor is used. A Shack Hartmann wavefront sensor uses a two-dimensional array of lenslets, conjugate to the pupil plane of the eye to sense the wavefront emerging from the pupil produced by a spot of light focused on the retina.\textsuperscript{20} The lenslets form spot images on a charge coupled device (CCD) detector.\textsuperscript{20} The amount that each spot is displaced at a particular point compared to the reference is proportional to the slope of the wavefront at that point.\textsuperscript{20} To correct these aberrations the wavefront sensor is paired with a deformable mirror.\textsuperscript{13} The deformable mirror is composed of many actuators that act essentially as small pistons to distort the mirror in the equal and opposite shape of the aberrations that are sensed by a wavefront sensor so the aberrations of the eye are cancelled by aberrations from the mirror.\textsuperscript{13} AO technology has been integrated into all imaging modalities including fundus cameras, scanning laser ophthalmoscopes as well as optical coherence tomography.\textsuperscript{13}

Adaptive optics is particularly well paired with an SLO system. SLO, compared to a flood illuminated system allows for real time, dynamic imaging. This gives the imager immediate feedback about the image quality and location. By correcting the optical aberrations, more light is focused through the confocal pinhole of the system, maximizing the amount of light collected and reducing photon noise, the noise due to
statistical variation in the photons detected. AOSLO also has much improved axial resolution compared to a non-AO system. Non-AOSLO systems typically only have an axial resolution of around 300µm. AO can improve the axial resolution of the system to 100µm or better. This allows for improved axial sectioning of different layers throughout the retinal tissue. Even with the earliest AOSLO system, Roorda et al were able to achieve images of the photoreceptor mosaic resolvable within 0.5 degrees of the fovea, achieving a lateral resolution of 2.5µm. They were also able to visualize blood flow though the capillaries near the foveal avascular zone as well as obtain images of nerve fiber bundles.

Because of their waveguiding properties, cone photoreceptors are amenable to visualization through AOSLO imaging. AOSLO has allowed for the photoreceptor mosaic to be studied and described in greater detail in vivo compared to what was only available from histological data previously. The cone packing density peaks at the fovea, rapidly decreases in density within the central 2mm of retina and decreases at a more gradual rate with further eccentricity. Chui et al used AOSLO to describe the cone packing density as a function of foveal eccentricity. They described a cone photoreceptor density of 27,712 cells/mm² at 0.3mm from the fovea compared to 7,070 cells/mm² at 3.4mm from the fovea in emmetropes. There was also a lower cone packing density found in myopes, as expected by the longer axial length in these eyes. While there is some discrepancy in the histological literature about the stability of things like cone packing density, Song et al found that older subjects (ages 50-65) have 75% the cone density within 0.6 degrees of the fovea as do younger subjects (ages 22-35) but
more similar packing densities with greater eccentricity. They also found that in all age
groups, a meridional difference in packing density based on retinal eccentricity. The
packing density decreases faster in the vertical meridian with greater eccentricity from
the fovea. In the horizontal meridian, the packing density does not decrease as fast.

AOSLO has also enhanced imaging of the retinal vasculature and contributed to
greater understanding of diseases affecting the vascular system. Burns et al used AOSLO
to study subjects with mild to moderate non-proliferative diabetic retinopathy. Vessel
pathology such as capillary remodeling, micro aneurysms, and intraretinal microvascular
abnormalities (IRMA) were clearly visible with this technique. Arteriole wall thickness
and arteriole lumen thickness were able to be measured and a wall to lumen ratio of 1.1
was described compared to a ratio of 0.48 in age matched non-diabetics. Blood flow
within the vessels was also examined, revealing areas of local stasis and capillary non-
profusion. Many of these pathological features are visible using standard clinical
techniques like fundoscopy or spectral domain optical coherence tomography (SD-OCT)
but AOSLO exposes much greater detail. Hard exudates less than 10µm were visible on
AOSLO images, which were not visible on SD-OCT images. Some features such as
capillary non perfusion and IRMA, which are more difficult to identify clinically, were
much more easily identified using AOSLO. Tam et al used AOSLO to look at the
vasculature of subjects with Type 2 diabetes without any retinopathy and found a
significant increase in the tortuosity of arteriorvenous channels, capillaries connecting
arteries to veins, in the parafoveal retina compared to those without diabetes.
demonstrates that AOSLO can be used to noninvasively uncover early vascular changes before the onset of more clinically evident pathology.\textsuperscript{23,24}

\textit{Indirect Imaging}

Despite the advantages of using a highly confocal imaging system, sometimes features of interest in the structures being imaged are best viewed using multiply scattered light. To collect more of the multiply scattered light instead of light that is predominantly directly back-scattered, the aperture of the imaging system can be varied. Burns et al found that a large (10x Airy Disk diameter) confocal aperture worked better for imaging vasculature and blood flow than a small (2x Airy Disk diameter) confocal aperture because it allowed allowing for the detection of more multiply scattered light.\textsuperscript{23}

The aperture of the system can also be modified so that the directly backscattered light is blocked and only multiply scattered light is collected, permitting indirect imaging as opposed to direct imaging. Elsner et al. explored this by using a SLO with an annular aperture and a central stop.\textsuperscript{11} This technique is referred to as indirect mode imaging or dark field imaging. In this technique, light enters the eye but any that is directly reflected or back-scattered is blocked by the central stop. Only the light that is multiply scattered can return to the detector. The larger the central stop the less directly back scattered light is collected and the more laterally scattered light is collected. This was compared to confocal or direct mode images of the same eyes. Using the indirect mode made topographical features, thickness changes, more apparent. It was easier to distinguish thicker areas like drusen or neovascularization for example from areas of thinning such as
a retinal pigment epithelium window defect. Indirect mode also allowed for better imaging of features that scattered light laterally or were better visualized by retro or side illumination, like fluid accumulation. Confocal or direct mode provided better imaging for structures that were thin or were highly reflective. It also allowed for better imaging of features that were located near structures that caused a lot of light scatter such as chorioretinal scaring. Imaging tissue beneath large scars or turbid fluid accumulation was difficult using both direct and indirect mode.\textsuperscript{11}

Elsner et al. also compared images after varying the size of the aperture, in direct and indirect mode. Even using the longest wavelengths of light, 830 nm, there was a 50% decrease in the amount of light return using the large 400 um stop, compared to the 400 direct mode aperture. The amount of light return using the 100um stop was much more similar to the 100um aperture. This indicates that the quality of the image and lateral spread of light is most influenced by the central portion of the aperture and less influenced by the peripheral portion.\textsuperscript{11}

Acton et al. used what they referred to as retro-mode in order to image using multiply scattered light using an SLO system. In this technique, a confocal aperture was deviated laterally to the left or right side to block the directly backscattered light and collect the multiply scattered light from one particular direction. The aperture allows the deviated light to produce a shadow on the sub-retinal features enhancing their contrast. When compared to fundus photographs and optical coherence tomography (OCT) images, fewer drusen were detected on the fundus photographs than the subretinal deposits detected on the retro-mode SLO images. The drusen detected on the retro-mode
Images corresponded to the drusen detected on the OCT images. The retro-mode SLO images also showed some smaller subretinal deposits not visible in fundus photographs or OCT images.\textsuperscript{25}

The limitation of the works by Elsner and Acton is the resolution of the optical system because of the optics of the eye as well as the limits in the range of the detector and the video and digitizing equipment. Using this relatively low resolution system, pathological features less than 10-20 \( \mu \text{m} \) could not be resolved. It is unknown however how multiple scattering may impact axial segmenting in higher resolution systems.\textsuperscript{11, 24}

\textit{High resolution indirect imaging}

The use of adaptive optics in conjunction with a scanning laser ophthalmoscope (AOSLO) as discussed previously, allows for the resolution of very fine retinal details including individual photoreceptors, microvasculature and even the movement of red blood cells inside the vasculature. Even with the high resolution available with an AOSLO system, it is still challenging to image microvasculature in the peripapillary and papillary areas of the retina. In these areas, there is thick retinal nerve fiber layer (RNFL) or other non-neural glial tissue that causes a lot of back scattering of light, blocking the detection of the microvasculature below.\textsuperscript{1}

To overcome this, Chui et al. varied the aperture of an AOSLO system by offsetting it to block directly backscattered light and collect multiply scattered light. The size of the aperture was also varied, using a small (2x Airy Disk diameter) confocal aperture and large (10x Airy Disk diameter) confocal aperture. Chui et al studied three
different aperture manipulations. The large confocal aperture was offset -8x Airy disk diameter to +8x Airy disk diameter with a step size of 2x Airy disk diameter in a vertical, horizontal and diagonal orientation. The small diameter aperture was also tested but had such a quick drop off in intensity with offset the results with the offset aperture were not easily quantifiable. Specific areas of the retina with different amounts of backscatter were selected for imaging. This included the peripapillary region and the perifoveal region (5-10 degrees from the fovea), both areas with thick RNFL causing backscattering. They also imaged in the foveal region, an area with thin RNFL as well as in the area of the optic nerve head and optic disc crescent which is known to cause a large amount of scattering. The images using the offset aperture orientation were compared to those with the aperture in a confocal orientation. In the confocal orientation using the small and large apertures, nerve fiber bundles as well as superficial capillary beds were observed with good contrast when focused at the appropriate depth. In areas of thick RNFL, the strong backscattering signal did not allow for visualization of the deeper capillary beds. When the large aperture was offset though, the nonvascular components and specular highlights of the image were reduced and blood vessels and erythrocytes were readily visible and became the dominant features of the images. The greater the offset the more uniform the images became. This was observed in all subjects in all offset directions. Other changes in the images with the aperture offset were apparent as well. When looking at the blood vessel walls, the wall orthogonal to the direction of aperture displacement became more visible with greater offset of the aperture. They also found that with increasing offset the relative visibility of the erythrocytes in the capillaries
became greatly increased against the rest of the more uniform retina. This was compared using standard error maps, showing the standard error of each pixel over time. In the confocal configuration, the specular reflection contributed to the temporal variation of the standard error map. With increasing offset of the aperture, the change in blood flow is increasingly responsible for the temporal variation in the standard error maps. This permits perfusion maps of retinal blood flow based on motion contrast.¹

Offsetting the aperture allowed for visualization of the movement of erythrocytes in the vessels at each of the imaging locations including areas with strong backscattering like the peripapillary capillaries which provide blood to the nerve fiber bundles and over the lamina cribrosa or optic disc crescent. In the area of the fovea with minimal RNFL, offset capillary imaging was similar to the confocal configuration and the greatest improvement was noted in areas with the most scattering components. Visualization of the vessels allowed for even single erythrocytes to be imaged in very fine capillary beds.¹

Using the offset aperture also highlighted features of the arteriole walls. The three layers of the blood vessel wall were readily visible as well as some cellular structure that were consistent with previous histological images. The greater visibility of the vessel wall orthogonal to the direction of offset was apparent in all imaging locations including those in the foveal region.¹

Sulai et al. explored the use of different non-confocal detection schemes in AOSLO to image retinal vasculature and perfusion using motion contrast maps. These schemes included an annular aperture with a central stop, a circular aperture with a horizontally oriented opaque filament down the center of the aperture, a knife edge
aperture with a horizontally oriented tape over greater than half of the aperture as well as a split detection scheme containing a left and right half of a circular aperture with a vertical obscuration separating the two halves. In the split detection scheme, the signal from each half goes to a different detector. Among the different apertures tested, Sulai et al. found the highest contrast in the structural reflectance and the perfusion maps using the split detection scheme.\textsuperscript{26}

Different anatomical and functional retinal features can be obtained from images using back-scattered as well as multiply scattered light. Ideally, the most information could be obtained by optimizing the collection of both types of light in a single imaging technique. Scoles et al. accomplished this by using a system that simultaneously collects direct backscattered and multiply scattered light using what they termed a non-confocal split detector. In this adaptive optics scanning laser ophthalmoscope system Scoles et al. used a reflective mask with a transparent annulus was used in place of the confocal pinhole aperture. The mask reflects the confocal signal into a first detector and is recorded directly. Multiply scattered light is transmitted to two incoherent detectors that collect the light from the right and left semi-annuli. This split detector signal is then calculated as the difference between the signals from the nonconfocal detectors divided by their sum. The confocal and nonconfocal signals are gathered simultaneously and are perfectly registered. Scoles et al. specifically used this technique to study photoreceptors. Standard AOSLO systems that only use confocal apertures used to image photoreceptors are able to obtain high resolution images as the AO corrects for monochromatic aberrations induced by the cornea and the lens. Using this imaging technique produces
images of photoreceptors that appear as bright round spots. This is the result of the strong directional coupling or waveguiding of light by the photoreceptor inner segments into the outer segments and the higher refractive index relative to the surrounding. This visualization through, depends on the intact photoreceptor outer segment to produce this waveguiding effect which are present in normal healthy photoreceptors. This is problematic when trying to image diseased or degenerating photoreceptors that are common in conditions such as age related macular degeneration, retinitis pigmentosa or choroideremia. The collection of multiply scattered light in imaging of photoreceptors can reveal more information about the photoreceptor inner segment which may still be intact in cases where the outer segment is not intact. Scoles et al., using their non-confocal split detection technique were able to image the intact inner segments of patients with achromatopsia whose outer segments were disrupted. This may have future implications in the evaluation of the success of future gene therapy in patients with diseases that affect the photoreceptor mosaic.27

Rossi et al. expanded upon offset aperture techniques by exploring further variation in the offset distance, offset direction and aperture size imaging both humans and monkeys. Using an AOSLO system they imaged using a “multioffset” detection scheme with two different detection patterns. The first was a radial multioffset detection pattern, where the aperture was positioned a fixed distance away from the point spread function, either 6, 11 or 16 Airy disk diameters at 45 degree angles. The second was a triangular multioffset detection pattern where the apertures were arranged in a triangular grid. Both detection patterns obtain simultaneous offset and confocal AOSLO images.
When imaging the photoreceptor layer, individual human cones were visible with all offset configurations used (8-21 ADD). They described an intensity gradient across the individual cells with the gradient orthogonal to the offset direction. In a subject with a disrupted photoreceptor mosaic due to dry macular degeneration, confocal imaging revealed areas of gaps in the cone mosaic, while the multioffest revealed cones in those corresponding areas. These multioffset images could be combined and differences to enhance the contrast and features of the photoreceptors.28

When imaging blood vessels using the multioffset detection pattern, the vessel features were either enhanced or minimized depending on the directionality of the offset configuration. Offsetting the aperture in the direction perpendicular to the course of the blood vessel enhanced the image of the vessel, but is reduced when offset orthogonal to the course of the vessel.28

Rossi et al. also used the multi-offset detection technique to image cells in the inner retina in monkeys. These images were compared to images using intrinsic two photon excitation fluorescence (TPEF) which is an established technique to image Muller cells and retinal ganglion cell layers in monkeys but the high light levels required for its use limit its use to animals only. Multioffset imaging demonstrated similar cellular features in the RGC layer in register with those seen with TPEF. RGC somas were much more visible with increased offset, 13.7-21.1 ADD. Better visualization was achieved by averaging multioffset images together. These features were not at all visible with confocal imaging in the same area. A similar cellular pattern of somas in the RGC layer
was also detected when using multioffset imaging in healthy human retinas. In the corresponding confocal images of these areas, axon bundles obstructed the cell somas.\textsuperscript{28}

It is clear that it is beneficial to use both directly backscattered as well as multiply scattered light to allow for the best visualization of retinal structures. The rationale for dynamic aperture imaging is to use an AOSLO system with a single detector with an aperture that could be varied over a single imaging session. A single detector system allows for more streamlined optics, but the variable aperture allows information to be obtained from multiple offset directions in a single episode of image acquisition.
Chapter 2: Methods

Subjects

Five subjects were recruited from the Ohio State University College of Optometry. Each subject had no known history of ocular disease and had visual acuity correctable to 20/20. The study was approved by the Institutional Review Board at the Ohio State University and adheres to the tenets set forth in the Declaration of Helsinki and the Health Insurance Portability and Accountability Act regulations. Written informed consent was obtained from all subjects. Subjects were informed that they could withdraw without consequence at any time.

AO system description

All images were acquired using a custom built high resolution adaptive optics scanning laser ophthalmoscope (AOSLO). The AOSLO uses an 830nm sensing beacon for wavefront sensing and a 780nm imaging source. This is a low coherence light source in order to reduce noise due to speckle. The beam then is separated with a dichroic beamsplitter.1,29

The signal is optically relayed between the different components of the system by using spherical mirror pairs. These are slightly off-axis by a few degrees to minimize the aberrations caused by off-axis astigmatism. These angles are calculated using Zemax.
The deformable mirror as well as the scanner are both conjugate with the plane of the pupil. From the deformable mirror, light is transmitted to the combination resonant scanner galvanometer scanner. A faster resonant scanner scans the beam horizontally and is coupled to a vertical galvanometric scanner resulting in a sinusoidal raster scan of the area of interest on the retina. Images are acquired at a frame rate of 28 Hz.

Light coming back from the retina passes though the confocal aperture which is optically conjugate to the retinal plane. The system has two apertures, a 50 µm and a 150 µm. Only the 150µm aperture was used for the imaging included in this study in order to collect more multiply scattered light. The 150µm confocal aperture is approximately 3x the Airy disk diameter, with a maximum pupil size of 8 mm. This aperture can be translated in the x, y and z directions with accuracy better than 1µm. The aperture was varied between -8x the Airy Disk diameter and +8x the Airy Disk diameter away from the centered, confocal position in both the horizontal and vertical position with a step size of 2x the Airy Disk diameter. This displacement was controlled by an operator at the computer and can be varied before or during image acquisition. The computer recorded the new aperture position. Prior to each imaging session, the aperture was reset to the confocal configuration. The aperture is always focused in the same plane as the imaging beam. Defocus of the system is controlled by the operator using the computer and could be varied to select the desired depth of focus. The high-resolution field size is 1.2 degrees by 1.2 degrees on the retina.
Light that comes through the aperture is detected using an Avalanche photodiode, a semiconductor device that converts photons to electrons. This has the advantage of increased efficiency in the conversion of photons to electrons over a photomultiplier tube when using near infrared wavelengths. The signal is then input into an imaging board to create a video image. Images were captured as short sections of sequential video frames. This number of frames ranged from 50-250 frames per acquisition and is selected by the operator. The intensity of the voltage could be varied and was optimized depending on the tissue being imaged in order not to saturate the detector. Images were captured with an average total power of 260µW and an average imaging power of 200µW.

A Shack-Hartman wavefront sensor performs the wavefront sensing for the imaging system. The 12 bit 1024 x 1024 CCD detector works with the sensor that has a lenslet array, 300µm aperture and a 7mm focal length. The sensor controls the shape of the deformable mirror for wavefront correction. The shape of the mirror could be observed on the computer using the AO Control GUI and could be paused or reset to avoid too much distortion.

*Retinal Imaging locations*

Different areas of the retina could be imaged by moving the raster in the X or Y direction. The fixation point for the subject could also be varied to obtain images from the desired retinal location. The axial depth of imaging was controlled by the operator and could be varied depending on the amount of defocus selected.
Subject Imaging

Subject’s eyes were dilated with 1% tropicamide and aligned in the AOSLO system with a 3 axis adjustable head and chin rest. Alignment was aided by the use of an external camera of the pupil. Alignment was adjusted throughout the imaging sessions to maintain centration of the pupil. For all subject imaging, the 150 µm aperture was used in the imaging system. All light levels were safe according to the American National Standards Institute ANSI Z136. Prior to each imaging session, a model eye was aligned in the imaging plane and the system was tested.

Macular Montage

After the subject was centered in the imaging system, the plane of focus was moved to the level of the photoreceptors. The aperture of the system was in a confocal configuration. The central 2.4 degrees of the macula were imaged at the photoreceptor level in 1.1 degree increments. In order to obtain this macular montage around the central macula, subjects were instructed to fixate at nine points on the raster pattern, specifically the top left, top center, top right, right center, bottom right, bottom center, bottom left, left center and the center of the raster. At each of these points an image of the photoreceptor mosaic was captured. The cones were easily resolvable within 0.5 degrees from the center of the macula.

Parafoveal photoreceptors

In order to image the photoreceptors outside the macular region the subjects were instructed to fixate on four specific points in each of the four meridians, one each superiorly, nasally, inferiorly and temporally. This procedure was repeated at each of the
four fixation points. Images were captured systematically at varying eccentricities as the raster scanner was moved in a stepwise fashion closer to the target. The aperture was also in a confocal configuration for this imaging.

*Through focus*

Through focus imaging was accomplished by shifting the plane of focus of the system anteriorly until none of the retinal structures were in focus. Images were captured systematically as the plane of focus was shifted posteriorly in equally spaced increments as subjects fixated on a target. This target was varied to obtain a through focus series at varying eccentricities from the macula. The through focus imaging was completed with the aperture of the system in the confocal configuration as well as the offset configuration. For the offset configuration, the aperture was displaced by 2x the Airy Disk diameter. This was completed with displacement in the horizontal direction, the vertical direction as well as in both the horizontal and vertical direction.

*Dynamic aperture imaging*

This technique was performed to obtain both images with the system in the confocal configuration as well as varying degrees and direction of offset configuration. The imaging plane was focused at the depth of interest. The aperture was then offset by a total of -4x the Airy disk diameter and as the frames were being captured the aperture was moved systematically towards the confocal position, then past the confocal position to +4x the Airy Disk diameter. The aperture was moved with a step size of 2x the Airy Disk diameter. This technique was performed in both the vertical and horizontal direction and at different axial depths. Dynamic aperture imaging was used to examine different
features such as vessel crossings and retinal cysts. It was also used to compare the
differences in scatter at different axial positions in the retina.

*Outcome measures*

In order to compare the percentage of light returning to the detector at increased
offset manipulations compared to a centered confocal configuration, the average intensity
of the image was plotted for each successive frame collected while using dynamic
aperture imaging. This plot was created using Matlab and fit to a Gaussian curve. This
was performed at different axial retinal locations in order to compare differences in the
scatter at these locations. The differences in resulting curves were then compared to help
quantify the differences in scatter from each axial location.
Chapter 3: Results

Direct Imaging of the photoreceptor mosaic

Imaging at the photoreceptor layer with the aperture in a confocal position produced clear images of the photoreceptor mosaic that are easily resolvable within approximately 0.5 degrees of the fovea. This macular montage imaged a total of the central 2.4 degree x 2.4-degree area surrounding and including the fovea in nine separate images. This was possible even using a relatively large, 150µm aperture, where photoreceptor imaging was not optimized. Quality of the unprocessed images varied depending on the fixation ability of the subject.

If the raster was moved more peripherally and the subject’s fixation point was changed to a location off center in the direction of the raster movement, the more eccentric photoreceptor mosaic was imaged. These series demonstrated the range of the system for how far peripherally the system could image. In these images, cones as well as peripheral rods were resolvable.

Offset Aperture Imaging

The structure of the blood vessel walls became much more apparent when the aperture of the AOSLO was moved to the offset configuration. A direction dependent component was observed as the vessel walls became much more distinct in the direction orthogonal to the direction of offset. These results are consistent with what was found by Chui et al. In areas where relatively larger vessels were crossing, a more robust vessel
wall was observed in the vessel on top compared to the vessel on the bottom. This is likely because the arteries usually cross over the top of veins. Arteries have a thicker vessel wall, due to the thicker layer of smooth muscle in the media layer of the wall compared to a much thinner media in veins.\(^8\)

Offsetting the aperture also increased the visibility of the capillaries, particularly the superficial capillary network in the nerve fiber layer. It also allowed for greater visibility of the blood flow within the capillaries themselves. This visibility varied depending on retinal eccentricity. In the parafoveal area, where there is thick nerve fiber layer, the superficial capillary network is completely obscured in the confocal configuration. The nerve fiber layer has a very strong backscattered signal and the individual nerve fiber bundles are visible. With the aperture offset, this same capillary network is very prominent against a more uniform background. The nerve fiber bundles are barely visible and the vasculature is the dominant feature. This was apparent to a lesser extent in the deeper capillary network. There is less direct backscatter at this retinal depth than with the confocal configuration and the capillaries are visible. With the aperture offset, the capillary walls are more apparent.

The effect of offsetting the aperture at the level of the photoreceptors did not allow for the photoreceptor mosaic to be as clearly delineated as it was with the confocal configuration, with a relatively static signal returned from this area. Figure 1 depicts in A the photoreceptors using the confocal orientation and B the photoreceptors with the offset orientation. Similar results were observed at varying foveal eccentricities. This is the
result that we would expect in subjects with healthy photoreceptors with strong waveguiding properties.

![Figure 1. A. Photoreceptors in the confocal orientation. B. Photoreceptors in the offset orientation.]

*Through Focus and Optical Sectioning*

The through focus imaging series revealed different retinal structures at different axial positions if the aperture was in the confocal versus the offset orientation but both aperture orientations allowed for imaging distinct retinal layers.

With the confocal orientation, the NFL appeared highly reflective and the striated nerve fiber bundles were apparent. Larger vessels appeared to be focus in the more superficial vascular network at the level of the nerve fiber layer. The blood cells within the larger vessels were highly reflective, but features of the vessel walls were not easily observable. Other than the intermittent observation of the motion from the highly reflective blood cells, the vascular structure of the superficial capillary networks was not
readily visible. Moving more internally, the deeper capillary beds were more apparent as was the motion of the blood cells within the vessels. On the deepest plane of focus, the photoreceptor mosaic was easily resolvable. These results, demonstrate that this AOSLO imaging system is capable of achieving a narrow focal depth which allows for optic sectioning, even though a relatively large aperture is used as distinct layers of the retina were clearly discernable with the through focus imaging series in the confocal orientation.

In the offset orientation, the nerve fiber layer appears very dim and more uniform, without obvious striations of nerve fiber bundles. As the depth of focus moves deeper in the tissue the superficial capillary network becomes very apparent. In some of the images, there appeared to be two superficial capillary networks at the level of the nerve fiber layer each with a slightly different orientation. The motion of the blood cells within the capillaries was more visible as well although the actual blood cells themselves appear less bright as they do in the confocal configuration. A clear vessel wall structure is apparent on the larger vessels which are visible in the superficial vascular network.

Moving more internal in the retinal tissue, the deeper capillary network was also apparent. The superficial capillary bed and the deeper capillary bed appear to be distinct from one another with differences in branch patterns noted. The photoreceptors were grossly visible at the deepest plane of focus.

Although many of the features seen with the confocal orientation through focus series that demonstrate the optical sectioning capabilities were not apparent with the offset orientation, new features were visible that could help identify which axial plane of
the retina was being imaged. The most identifiable features using multiply scattered light were the vascular structures including the superficial and deep capillary networks. High resolution was achieved as the fine capillary networks were resolvable. Although the striations of the nerve fiber layer are not obvious with the offset orientation and the background appears much more uniform, the capillary beds are highly visible as well as the movement of the blood cells within the vessels. By observing these capillary networks, axial position in the retina can likely be identified. A distinct separation is seen between the superficial and deep capillary networks. In some videos, the superficial network could be separated into two separate capillary beds. Figure 2 depicts examples of the capillaries from the superficial vascular network. In A, the aperture is in the confocal orientation and in B the aperture is in the offset orientation.

Figure 2. A. Superficial capillary network with the aperture in the confocal configuration. B. The superficial capillary network with the aperture in the offset configuration.
Dynamic Aperture Imaging

Dynamic aperture imaging combines both offset and confocal imaging in a single imaging acquisition. In these imaging series, the retinal features that were visible with the confocal configuration as well as the offset orientation could be observed at the same retinal depth of interest. When looking at a crossing change of two retinal vessels, the directional dependent nature of imaging with an offset aperture was apparent as the vessel wall structure orthogonal to the direction of offset was highlighted and more visible. Figure 3 depicts a series of frames taken from a video collected with dynamic aperture imaging. A through C were from one series and D through F were from another. In A and C the aperture is offset in opposite vertical directions with B representing the confocal configuration. In A and C the opposite vessel walls are highlighted depending on the orientation of the displacement of the aperture. In D and F the aperture is offset in the opposite horizontal directions while E represents the confocal orientation. In this series, the vessel walls are not as distinct as in A and C, with more uniformity in the images. Based on the appearance of this crossing, the horizontal vessel appears to be an artery and the vertical vessel appear to be a vein.
Figure 3. Series of frames from dynamic aperture imaging. In A and C the aperture is offset in opposite vertical directions. B and E are in the confocal orientation. In D and F the aperture offset in opposite horizontal directions.

Although all of the subjects included in the study were not known to have any retinal pathology, one subject had two small retinal cysts that were found incidentally. This offered the opportunity to use high resolution imaging to study a type of retinal pathology. Using dynamic aperture imaging, the cysts could be studied using directly back scattered and multiply scattered light. The cysts were visible with the confocal orientation. But when the aperture was in the offset orientation, the borders of the cyst developed more contrast. This had a directional dependent component as well, as greater contrast to the cysts were noted in the direction orthogonal to that of the offset. The offset orientation also helped better visualize a capillary running through the center of the cysts, which could have been the source of the fluid. Figure 4 depicts frames from a video of the cysts collected using dynamic aperture imaging. In A and C, the aperture is offset vertically in opposite directions and the contrast of the lateral walls of the cysts are much more apparent than with the aperture in the confocal configuration.
Figure 4. Selected frames from dynamic aperture imaging of retinal cysts. A and C represent an offset aperture configuration in opposite vertical directions and B represents the confocal configuration.

**Dynamic Aperture Imaging Intensity Profile**

Dynamic aperture imaging was used to create a model to attempt to quantify the inherent light scatter of different layers in the retina. Using the dynamic aperture technique, the amount of light intensity returning to the detector was compared to the amount of displacement of the aperture which was varied over time as the frames were being collected during an imaging session. This intensity was plotted using Matlab. The first part of the curve represents the intensity with the initial offset. As the aperture is moved closer to the confocal position, the amount of intensity increases as the frames progress. As was expected, the highest intensity resulted when the aperture was in its confocal orientation, returning the greatest amount of backscattered light which resulted in the highest peak on the curve. As the aperture is then moved away from the confocal position in the opposite orientation, the intensity again decreases as the frames progress. The average intensity plot was then fit to a Gaussian curve with the centered, confocal
position as the normalization from which the other intensities are compared. This plot is shown in Figure 5 for the nerve fiber layer and Figure 6 for the photoreceptor layer.

Figure 5. Intensity profile for the nerve fiber layer using dynamic aperture imaging.
The intensity profile plots were then calculated for different retinal layers, specifically the nerve fiber layer and the photoreceptor layer. The curve along with an associated image at each aperture position is depicted in Figure 6 for the photoreceptor layer and in Figure 7 for the nerve fiber layer. Each curve demonstrated a similar pattern, with the highest intensity at the confocal position and decreased intensity for the offset positions. When comparing the intensity profile plots between the different retinal layers, we notice that the photoreceptor layer has a much broader curve compared to the curve.
from the nerve fiber layer. This indicates that during the collection of the images, more scattered light was collected when the aperture was in a non-confocal configuration when sampling from the photoreceptor layer compared to the nerve fiber layer.

Figure 7. Intensity plot from the photoreceptor layer with associated image from each aperture position.
Figure 8. Intensity plot from the nerve fiber layer with associated image from each aperture position.
Chapter 4: Discussion

Confocal Imaging vs. Offset Aperture Imaging

As the demands to visualize smaller and smaller retinal structures have increased, so have the resolution demands for retinal imaging systems. The incorporation of adaptive optics into imaging systems has resulted in an incredible increase in imaging resolution by correcting higher order aberrations. Many highly confocal systems are able to produce detailed images of the photoreceptor mosaic, nerve fiber layer bundles as well as other small highly reflective, directly backscattered retinal features. With smaller apertures, and more sensitive aberration correcting technology the lateral resolution is greatly increased.

The disadvantage with such a highly confocal system is that the strong collection of directly backscattered light can obscure other retinal features that multiply scatter light. This was demonstrated in our results when looking at the superficial capillary beds in the NFL that are not visible in a confocal orientation as well as the vessel walls that were more visible on the offset orientation.

This is more significant when trying to image eyes with ocular pathology. It has also been reported that when imaging subjects with ocular diseases that affect the photoreceptor layer, it is difficult to achieve images of the entire photoreceptor mosaic with a highly confocal system. The direct backscatter of this tissue is highly dependent
on the wave-guiding of the photoreceptors. In ocular diseases, such as Usher Syndrome or achromatopsia, cones have poor wave guiding and appear absent in images of the photoreceptor mosaic with a confocal system.\textsuperscript{27} When the aperture is modified to collect more multiply scattered light the cones that are multiply scattering the light are detected. Early vessel changes in vascular diseases such as diabetes can also be more apparent when using a detection scheme that collects more multiply scattered light.\textsuperscript{23}

Offsetting the aperture to collect multiply scattered light does not come without disadvantages. To obtain more multiply scattered light, a larger aperture is needed, which results in less confocality. The consequence of this a decrease in the lateral and axial resolution of the optical system. This can make things like optical sectioning difficult. Also, the overall intensity of light going into the system has to be increased if primarily multiply scattered light is being collected as a portion of the light going into the system is blocked from returning from the detector. Luckily with near infrared, these light levels are very comfortable for the subject as the eye is not as sensitive to these wavelengths.

There is also a directional dependence on the aperture displacement. The features of the vessel walls for example are much more visible in the direction orthogonal to the offset of the aperture. Certain features are not equally observed depending on their orientation. Depending on the feature of interest this could be a factor in the resultant images.

\textit{Sectioning Retinal Layers with Confocal Compared to Offset}

In a scanning laser ophthalmoscope, the confocal aperture is conjugate to the particular retinal plane being imaged. This conjugacy is maintained even when the axial
position is moved. The confocality of the system creates a narrow focal depth as tissue above or below the focal depth is not conjugate to the imaging plane as is not in focus. This allows for different retinal depths to be imaged separately, creating optical sectioning of the tissue. Generally, the smaller the aperture, the better the lateral and the axial resolution. The aperture used in this AOSLO was 150µm or approximately 3x the Airy Disk diameter. This is a relatively large aperture, which results in the collection of some multiply scattered light even in the confocal orientation. Despite this larger size, the images that were obtained with the through focus series do demonstrate optical sectioning with different distinct tissues visible at different retinal depths. This indicates that the focal depth of the system is narrow enough to perform optical sectioning. Particularly visible were the nerve fiber layer, the deep capillary network and the photoreceptor layer.

When the aperture in a scanning laser ophthalmoscope is manipulated away from a confocal orientation, the axial resolution of the system is decreased making optic sectioning more difficult. In this AOSLO system, even though a relatively large aperture was offset images of distinct retinal layers were still able to be obtained. This indicates that the system is still able to collect a narrow enough focal depth to separate different layers even though the system was primarily collecting multiply scattered light. With the through focus series with the aperture in the offset configuration, the dominant features of the videos collected were the capillary networks and other vasculature. The superficial vascular network is known from histology to be composed of the radial peripapillary plexus in the nerve fiber layer and the superficial vascular plexus in the ganglion cell layer. The deeper capillary network, composed of the intermediate capillary plexus and
the deep capillary plexus is above and below the inner nuclear layer. In the though focus images with the offset aperture, the superficial capillary network was clearly distinct from the deeper capillary network, in focus at different axial positions. The deeper capillary network was composed of smaller more uniform sized capillaries, whereas the superficial layer had more variation in the size of the vessels. In some videos, it appeared that there were two distinct capillary plexuses in the area of the nerve fiber layer as well, with vessels demonstrating different orientations. This demonstrates that two to three retinal layers can be sectioned using primarily multiply scattered light based on the vascular network.

*Dynamic Aperture Imaging*

Using dynamic aperture imaging also allows for the collection of both directly backscattered and multiply scattered light in a single imaging acquisition. This technique takes advantage of both a confocal and an offset system, therefore imaging can be optimized depending on the specific features being imaged as images can be easily compared. This could be particularly useful in cases where imaging with multiply scattered light and directly backscattered light is important to understanding the clinical picture. As was mentioned above, some diseases that affect the photoreceptors can selectively affect their waveguiding ability.²⁷ By including both the confocal and offset images, both types of scattered light could be included and more photoreceptors could be imaged. All of the subjects that were included in this study had no known retinal pathology of the photoreceptors, in all of the images we achieved a well visualized
photoreceptor mosaic on the confocal configuration but this could be a possible future application for dynamic aperture imaging.

The retinal cysts that were studied in our subject offered an opportunity to look at retinal pathology with this high-resolution system and dynamic aperture imaging. Cysts such as the ones imaged, would be very difficult to identify using normal clinical techniques such as fundoscopy or binocular indirect ophthalmoscopy. The retinal cysts were also difficult to visualize using a commercially available OCT, Spectralis (Heidelberg Engineering, Heidelberg Germany). With the AOSLO imaging system, the cysts were highly visible and allowed us to visualize features like a small capillary running though the center of the cystic space. This suggests that a similar imaging system would be very useful in the study of other types of retinal pathology such as vascular diseases like diabetic retinopathy. This technique could be useful in monitoring changes in vessels and blood flow within vessels over time and to monitor the effect of treatment.

Other techniques have been described that collect both directly backscattered and multiply scattered light such as split detection or multioffset.\textsuperscript{27,28} Because this information is all collected from a single acquisition, the images are already perfectly registered and are all from the exact location. This is useful for image processing and can allow for easier differencing of the images. A limitation of this technique is that using multiple detectors forces the light returning to the detectors to be divided. This results in a weaker light signal returning to the detectors which in turn requires more light going into the system to obtain enough of a signal at all of the detectors. Utilizing multiple detectors also increases the complexity and overall cost of the optical system.
Using dynamic aperture imaging also allows for the collection of directly backscattered and multiply scattered light. The advantage to this technique is that it can accomplish similar results to split detection or multioffset with only one detector. Because a single detector is used at the same voltage, the sensitivity should be equal across all detector settings. This allowed an existing AOSLO system to be used for this technique. Because only one aperture was used, this decreases the cost and reduces the overall complexity of the system.

One of the disadvantages of dynamic aperture imaging is the images are not perfectly aligned when acquired, significantly more image processing is required in order to register the images. Also, all images are not obtained from the exact location and are subject to variation that is dependent of fixation. This makes this technique more reliant on the fixation ability of the subject. It also increases the time of the image acquisition. The system collects a total of 200 frames per imaging block which lasts over 6.5 seconds, making it more dependent on the subject’s ability to hold fixation more than other imaging faster imaging modalities. Another limitation is that the aperture can only be offset in one orientation per image acquisition. Multiple acquisitions could be performed to obtain the images with the offset in multiple orientations. This would be desirable as the collection of multiply scattered light with this technique is direction dependent.

*Modeling Light Scatter Using Dynamic Aperture Imaging*

Although multiply scattered light has been used in high resolution retinal imaging to reveal features that are normally blocked by directly backscattered light, the amount of scatter inherent to the different layers of retinal tissue is difficult to model. Based on
observing differences in the confocal and offset images in different axial positions, it is likely that different retinal layers scatter light differently, but this difference has not yet been quantified. Dynamic aperture imaging introduces novel way to quantify and analyze the different light scattering properties. In this study, the average intensity plot using dynamic aperture imaging was to model this inherent scattering property. The more scattering the tissue layer, the greater the amount of light will be returned when the aperture is in offset configurations. The broader the Gaussian curve, the more the tissue is scattering light.

The results of these plots comparing the curve from the nerve fiber layer and the curve from the photoreceptor layer are not what we had expected. Intuitively, we would expect the nerve fiber layer to have much more scatter than the photoreceptor layer, but we found a broader curve in the intensity plot of the photoreceptor layer. This result could be due to several reasons. The photoreceptor layer could be inherently more scattering. This could be true, but is unlikely. When comparing the video images, the photoreceptors only maintain visibility within a small amount of offset from the confocal position. Photoreceptors, especially in the central retina, have strong waveguiding effects, which promotes direct backscatter and reduces multiply scatter light. Another option is that the scatter is not coming from the photoreceptors but the retinal pigment epithelium (RPE). Even though the system is using a relatively large offset aperture, the focal volume being sampled to obtain images is relatively small resulting in a narrow focal depth. We do not know this exact focal depth though. Although the subjective focus is on the photoreceptors we could also be collecting light from the RPE or a combination of the
RPE and the photoreceptors. On OCT imaging this area is difficult to separate out anatomically as well due to the close association of the RPE and the photoreceptors and is referred to as the cone interdigitation zone (CIZ).\(^3\) The retina does not absorb a lot of light in the near infrared spectrum. Because it is not absorbed, a large proportion of this is directly back scattered or multiply scattered. It is possible that the light is being multiply scattered by the RPE and then directly backscattered by the photoreceptors. The scattered light could be captured and funneled by the photoreceptors and redirected out of the eye. As light is collected from the CIZ during dynamic aperture imaging, it could be sampling both the directly backscattered light from the photoreceptors and the multiply scattered light from the RPE.

This model fitting curve does have a few limitations based on the constraints of the system. The aperture itself is moved in discreet steps, not in a continuous manner. The duration per aperture setting was not able to be standardized with our available software. This could result in a different amount of time spent at each aperture position. If there was longer stopping time on each aperture position for the photoreceptor layer compared to the nerve fiber layer, the resulting average intensity could be broader across the frames in the video. If the process of moving the aperture could be standardized and automated by a computer program, that would help reduce the variability in the time at each aperture position.

This study provides a qualitative mechanism to describe differences in the scattering properties of different retinal layers. Although we found differences in scattering properties in the nerve fiber layer and the photoreceptor layer or CIZ these results cannot yet be generalized to a larger population yet. Our results were from a very
small sample size at only a few different retinal locations. Other studies would need to be performed on a larger sample size at different retinal eccentricities.
References


7. Remington L. Clinical anatomy of the visual system. 2005


