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Near Infrared Polarimetric Imaging and Changes Associated with Normative Aging

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Abstract

With aging, the human retina undergoes cell death and additional structural changes that can increase scattered light. We quantified the effect of normative aging on multiply scattered light returning from the human fundus. As expected, there was an increase of multiply scattered light associated with aging, and this is consistent with the histological changes that occur in the fundus of individuals before developing age-related macular degeneration. This increase in scattered light with aging cannot be attributed to retinal reflectivity, anterior segment scatter, or pupil diameter.

Keywords

(100.2960) Image analysis; (110.3080) Infrared imaging; (120.5410) Polarimetry; (170.4470) Ophthalmology; (290.4210) Multiple scattering; (330.4300) Vision system – noninvasive assessment

1. INTRODUCTION

There are considerable structural changes to the multi-layered retina that are associated with aging and eye disease, including a decrease of neurons and a build-up of debris in and around the retinal pigment epithelium (RPE) that provides the metabolic support for the outer retina as seen on histology [1]. In older individuals as compared with younger ones, there is less cone photopigment and macular pigment as measured with fundus reflectometry [2] and a lower density of cone photoreceptors as measured by counting the focal points of light return seen with adaptive optics scanning laser ophthalmoscopy [3]. In extreme cases of photoreceptor loss there is reduced thickness of the outer nuclear layer, comprised of the photoreceptor cell bodies and Muller cell processes, but there is a seemingly paradoxical increase of thickness in older eyes. That is, the older subjects had thicker, not thinner, outer nuclear layers, as measured by optical coherence tomography, implying that the layer had a measureable amount of additional material. This thickening is readily explained by histological findings of retinal modelling, in which neurons grow new processes, new connections are made among neurons, and structural changes occur to Muller cells, but the connections may be more like a tangle of neural components rather than subserving vision [4]. All of these results are consistent with increased light retinal scatter with aging at 1 deg from the foveal center, where we measured (Fig. 1).

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The complex light-tissue interactions of retina and RPE also include changes in polarization, with each layer or neural component having different properties. The entire eye may be considered as a series of sequential components in a double pass optical system, each with scattering and polarization altering properties [5]. Light reaching the retina has already passed through the birefringent cornea, with additional anterior segment scattering occurring particularly due to the tear film layer and the vitreous. Both of these undergo considerable change with age [6], increasing the scattered light and likely decreasing the degree of polarization (DOP) of light reaching the retina.

Two retinal layers exhibit strong form birefringence: the retinal nerve fiber layer and the Henle fiber layer [5,7–9]. These layers primarily contain neuronal axons running parallel to the surface of the retina. Each differs considerably as a function of retinal location, with very little retinal nerve fiber layer component near the fovea where there are few ganglion cells, and an increase at retinal locations farther from the fovea, with peak birefringence for the thick axon bundles arcing toward the optic nerve head. In contrast, there is a decrease of Henle fiber layer birefringence with increasing distance from the fovea, since the cone axons shorten and they become perpendicular to the retinal surface. The interaction of the phase retardation of cornea impinging onto the Henle fiber layer leads to a sinusoidal variation of the amplitude of the phase retardation signal circumscribing the fovea, resulting in the well-known bow tie pattern (Fig. 1) [7–10]. There is a decrease with aging of the amplitude of the variation between the bright and dark components of the bow tie [8]. The location of the maximum change also shifts outward from the fovea with age, consistent with the loss of cone photoreceptors.

Several computations based on measured polarization properties may be used to inform about the integrity of the structures within the retina, such as of the DOP at a specific location, the proportion of scattered light at a specific location, or the DOP uniformity averaged across a portion of the image [11,12]. The underlying assumption is that except for blood, macular pigment, and the highly pigmented and therefore scattering RPE, the healthy retina is clear and organized into multiple layers that differ in index of refraction. Injecting a systematic change of input polarization and measuring the resulting signal amplitude allows the computation of the DOP of the human retina. The values vary with optical methodology, wavelength, and target tissue. Sample values include 0.85 – 0.89 for *en face* imaging at 514 nm for the fovea [13], with more scattering for 780 nm light than for 543 or 633 nm light [14] and larger pupils [15], and varying with method of computation [16]. This high DOP is consistent with form birefringence of the axons of neurons, and little tissue disruption.

When imaging the retina with scanning laser polarimetry, the light not following the polarization variation, i.e. the minimum light detected across all conditions, has been used to compute multiply scattered light images [12]. In these images, the contrast of specific retinal features is increased with tissue disruption or with normal light-tissue interactions, e.g. the strong scattering signal of the RPE serving as a contrast generator. Thus, the RPE can retro-illuminate blood vessels [17]. There is enhancement of the visibility of the structural disruption due to drusen [18,19], as well as demarcation of the protein-filled fluid border of neovascular membranes, in age-related macular degeneration [5]. Scattered light images help detect and localize structures, such as choroidal neovascularization and pigment

clumping in pigment epithelial detachments. The retina is amenable to be studied with both *en face* imaging techniques and polarization sensitive optical coherence tomography (PS-OCT). The results compare well with the size and location as seen PS-OCT [20,21]. For computations across an image, the DOP uniformity is used to segment layers to improve thickness measurements and enhance the visibility of features [20–22]. There are two important differences between PS-OCT and scanning laser polarimetry. First, PS-OCT can visualize structures in depth, whereas scanning laser polarimetry provides a weighted average of several retinal layers and the RPE. Second, several versions of PS-OCT maximize the detected signal as the difference between two detectors having orthogonal polarization properties, therefore providing no method of using multiply scattered light at discrete locations [23]. In contrast scanning laser polarimetry can detect small drusen and subtle leakage, with the scattered light images being more sensitive than confocal ones [5,12,18,19].

PS-OCT can demonstrate increased phase retardation of retinal structures in the deepest layers in older eyes, indicating structural changes [23]. The DOP uniformity across an image is altered in age-related macular degeneration, pinpointing damage to the RPE [21,22]. Taken together, these results indicate the potential for using multiply scattered light in images, or quantitative outcome measures, as an early marker for retinal disease. Such a measure should be able to distinguish between normal aging changes and onset of disease. To this end, we quantify the scattered light in the retinal images acquired with scanning laser polarimetry in a sample varying with age, and estimate the influence of anterior segment light scatter on our measurements.

2. METHODS

A. Subjects

Subjects with normal eye exams were recruited from the Indiana University School of Optometry eye clinic. We tested 120 subjects, with 20 subjects per decade, ranging from 20 to 80 years. There were equal numbers of males and females in each group. Subjects were selected from the database of normal subjects used previously and demonstrated a significant decrease with aging of foveal phase retardation [8]. Additionally, five people were selected who had dry age-related macular degeneration (AMD) for comparison. These subjects were each paired with the control subject who was of the same sex and nearest in age. One foveal centered scan was analyzed for each subject. Before any testing was performed, all subjects received a detailed explanation of the study procedure, and informed consent was obtained from all subjects. Our testing conformed to the principles expressed in the Declaration of Helsinki.

B. Instrumentation and Computations

Images were collected with a confocal scanning laser polarimeter (GDx, Carl Zeiss Meditec, Dublin, CA) that uses linearly polarized 780nm light as previously described [5,7,8,12,17–19,24,25]. Subjects were tested without pupil dilation, but the input and exit pupil on the instrument was small, approximately 2.5 mm, so that there was little difference of signal strength due to decrease in pupil size in older subjects, which is tested in computations from

measurements of reflections of larger retinal blood vessels. Also, with this small pupil size, there is little variation across the pupil. This scanning laser polarimeter has a small confocal aperture, minimizing scattered light from out of focus planes, particularly from the anterior segment. This machine lets the operator control the gain settings, and the operator was trained to not oversaturate the images. To maximize the measureable polarization signal, the corneal compensation lens was removed [8]. The input polarization angle was varied over a series of sequentially collected images. Light returning from the eye entered a polarizing beam splitter and was collected on two detectors, one that was parallel to the input polarization and one that was perpendicular to the input polarization (crossed). Images were collected pairwise on each detector, with each image covering 15 deg by 15 deg visual angle, and are saved as 256×256 pixels in 8 bit grayscale.

Custom software in Matlab (Mathworks, Natick, MA) was used to create 18 image types based on polarization content, with five used in this analysis. How these images were computed has been extensively described [5,12,17,18,26,27]. The five images types that were used in this analysis are (Figs. 2–6):

$$\text{Minimum (crossed detector)}. \quad (1)$$

$$\frac{\text{Mean (crossed detector)} + \text{Mean (parallel detector)}}{2} \quad (2)$$

$$\frac{2 * \text{Minimum (crossed detector)}}{\text{Mean (parallel detector)} + \text{Mean (crossed detector)}} * 255 \quad (3)$$

$$\frac{\text{Mean (crossed detector)}}{\text{Mean (parallel detector)}} * 100 \quad (4)$$

$$\text{Maximum (crossed detector)} - \text{Minimum (crossed detector)} \quad (5)$$

C. Data Analysis

To quantify scattered light and other light-tissue interactions of retinal tissue, four measurements of 5×5 pixels were taken using Photoshop (Adobe, San Jose, CA) 1 deg from the fovea. At this location, there is not a thick retinal nerve fiber layer, and therefore the deeper (outer) retinal layers and RPE contribute more significantly than inner retina. The RPE is a contrast generator and leads to considerable variation in light return for certain image types, particularly by the ones generated by equations 1 and 3. By characterizing

scattered light values for focal locations in control eyes, we provide confidence limits against which pathological features can be compared in diseased eyes.

The boxes were large enough to produce a standard deviation to build confidence limits, but small enough to sample from predominantly small drusen or focal hyperpigmentation. The region was selected to avoid the central reflection artifact of the instrument, but near enough to the fovea to be representative of potential damage to visual acuity.

Foveal location was determined by finding the center of the macular bowtie (Fig. 1) [24,28], and saving that location so that the same 4 regions were sampled for each image type. Most of the computed images, and the raw data assessed for quality control, were transformed in intensity using histogram equalization or linear scaling, since otherwise the images would be too dark to be inspected.

The average pixel intensity was computed. Single factor analysis of variance and planned comparisons were performed on each of the image types from equations 1–5 with the value from equations 1–5 as the dependent variable and age in years as the independent variable using SPSS (IBM, Armonk, NY). Furthermore, linear regressions were performed for each image type for average pixel intensity as a function of age.

D. Anterior Segment and Superficial Retinal Measurements

Additional measurements were taken to estimate the effect of the anterior segment and most superficial retinal layers. Using the first image collected from the parallel detector, five points were selected along a reflective portion of a large blood vessel, and the average intensity was computed. Using the same coordinates, the average intensity was computed for each image collected on both detectors. The Matlab curve fitting toolbox was then used to fit a sine wave of the form

$$y = a + b * \sin(c * x + d) \quad (6)$$

to the average intensities from each detector, separately (Fig. 7). The fixed variable values and the computed R-square for the fits were analyzed.

3. RESULTS AND CONCLUSIONS

A. Normal Subjects

There was a significant difference between age groups ($p < 0.05$) for the image types represented by the first four equations using a one-way ANOVA, however, this was not true for the fifth ($p = 0.351$). The image generated by equation 1 provides a metric of the scattered light at the retina. Nearly every age group had a mean intensity that was larger than the previous age group, with the one exception being that the people in their 60s had a slightly smaller mean than people in their 50s (Fig. 2). There was a significant difference between the age groups for this image ($p = 6.33 \times 10^{-15}$), and these results can be seen in Table 1. Most age groups were not significantly different from the immediately preceding and proceeding age groups, with the one exception being between the group in their 60s and the

group in their 70s, which were significantly different. ($p=0.002$). Generally, there were statistically significant differences for gaps that were greater than 20 years. A linear regression followed $y=0.4122x+12.369$ and had an R-squared value of 0.4445, which was significant ($p=9.36 \times 10^{-17}$) and had an adjusted z-score of 0.8050. This potentially indicates that as people age, there is more scattered light at the retina. Below we described the extent to which this scattered light could be attributed to experimental method or artifacts not removed by the confocal aperture, such as anterior segment scatter.

The images generated by equation 2 demonstrate the average amount of light over both detectors that is detected, and is a typical confocal image that does not analyze the polarization content. There was a significant difference between age groups ($p=0.003$). The main differences that appeared in the planned comparisons were that the subjects in their 30s had less light on average in both detectors compared to people in their 50s and 70s, and that people in their 50s had more light on average in either detector than people in their 60s (Fig. 3). A linear regression followed $y=0.1811x+64.481$, which was significant ($p=0.0110$) and had a z-score of 0.2400.

Our results imply that there was an increase with increasing age in the average light reaching the detectors. This could be due to experimental method or less absorption by the older fundi. However, the amount of increase with age had a slope of only 0.181 as compared with the slope for the increase in scattered light of 0.412. Thus, whether the increase in signal for the confocal images is due to experimental method or decreased absorption near the fovea, scattered light *per se* increased more with aging.

The images generated by equation 3 are a linear transformation of the results of equations 1 and 2, therefore showing the change with age of the scattered light when normalized by the overall light return. There was a significant difference between age groups ($p=1.48 \times 10^{-11}$), and the planned comparisons from Tukey's HSD were similar to those for the images generated by equation 1 (Table 2). The mean intensity increased with age for every age group (Fig. 4), and a linear regression followed $y=0.2968x+14.298$. This was significant ($p=3.66 \times 10^{-14}$) and had a z-score of 0.7271. These computations agree with the results from images 1 and 2, in which the scattered light increased with aging at a higher rate than the increase of the overall light return.

As a related measure, the DOP was derived from this image and computed (Fig. 8). For increasing age, there was a decrease in the DOP for a double pass, with a total range of 0.8059 – 0.9492. These numbers are similar to those reported by other groups in the past [13,16]. The decrease is slight, however, with a linear regression yielding $y=-0.0012x+0.9439$. The linear regression was significant, with $p=3.66 \times 10^{-14}$, and had a z-score of 0.7206.

Images generated by equation 4 report on both light that is rotated into the crossed detector and increased multiply scattered light that is divided between detectors, i.e. when there is less light retaining polarization and remaining in the pathway of the parallel detector. There was a significant difference between ages groups, with the crossed detector having a larger proportion of the light as age increased ($p=2.61 \times 10^{-7}$). The main differences were that

people in their 20s and 30s had a significantly lower mean intensity than people in their 60s and 70s. A linear regression followed the form $y=0.1378x+12.005$, with an R-squared value of 0.2414 (Fig. 5). The linear regression was significant ($p=1.21 \times 10^{-8}$) and had a z-score of 0.5373.

The images generated from equation 5 provide the amount of phase retardation, as measured at the crossed detector. There was no significant difference between age groups for the images generated according to equation 5 ($p=0.351$). The trend is generally decreasing with increasing age, as indicated by the negative slope of the linear regression $y=-0.0504x+14.57$, but there are large individual differences. The linear regression was also not significant ($p=0.1710$) and had a z-score of 0.1271.

To understand the influence on the measurements of retinal light scatter of the anterior segment and superficial retina, we analyzed the light reflected off the top of retinal blood vessels. These lie in relatively superficial retina. For the curve fitting analysis, the average R-squared value for the parallel detector curves was 0.3984, whereas for the crossed detector the average R-squared value was 0.9314. For analysis, only the crossed detector fits were used since they were more reliable. A minimum threshold for being used in analysis was set for having an R-squared value of 0.8. This criterion led to 111 of the 120 subjects remaining in this group, and the average R-squared value for the curve fits for these subjects was 0.9499.

A one-way ANOVA on the average intensity of the crossed detector indicated a significant difference between age groups ($p<0.0003$). Tukey's HSD test revealed that the groups in their 20s, 30s, and 40s were significantly different at the 0.05 level from the group in their 70s, and that the group in their 70s had a higher average intensity. However, there was not a significant difference between the groups for the maximum amplitude of their fitted sine waves ($p=0.559$) (Fig. 9). This indicates that the signal remains roughly the same for people as they age, but it is associated with an increase in multiply scattered light.

To provide an estimate of how the anterior segment and superficial retina contributed to the multiply scattered light estimates from the images generated by equation 1, we normalized the images in Fig. 2 by the average amplitude computed from the crossed detector curve fittings. Taking this into account implies that the increase in multiply scattered light that was associated with aging is only partially due to changes in the anterior segment or superficial retina (Fig. 10). A linear regression followed $y=0.0097x$, and was significant ($p=0.0170$) and had a z-score of 0.2227.

In addition to the estimate of the anterior segment's effect on multiply scattered light, the DOP of the anterior segment and superficial retina was also estimated (Fig. 11). Similar to the DOP for the eye as a whole, which decreased with increasing age, the estimate for DOP for the anterior segment also slightly decreased with age. A linear regression followed $y=-0.002x+0.9601$ and had an R-squared value of 0.4849. A linear regression was significant ($p=1.04 \times 10^{-18}$) and had a z-score of 0.8603.

These data indicate that retinal scatter light increases with increasing age. There are many factors that could contribute to this. One is decreased absorption due to loss of

photoreceptors, as well as the potential decrease of other absorbers, such as melanin, although none having strong absorption in the near infrared regime [29]. This is consistent with the results of slightly increased amplitude of confocal images as shown in Fig. 3. However, whether the increase in signal amplitude in confocal images is due to decreased absorption, methodological considerations, or both, the increase in scattered light with aging is too great to be due solely to an increase in the average amount of light returning from the retina. The ratio measurements in Fig. 4 emphasize the greater increase of scatter as compared to the overall light return that was measured as a function of aging.

There are two types of birefringence measurements: the overall ocular birefringence, as seen in Fig. 8, and the birefringence of the anterior segment and superficial retina, as seen in Fig. 11. As found previously [8], the birefringence at the retina that is near to the fovea decreases with increasing age. Our study did not include peripheral measurements that would be highly influenced by the thickness of the retinal nerve fiber layer [7]. In contrast, the birefringence for the anterior segment and the superficial retina, as measured from the light reflected off the top of large retinal blood vessels, there was no decrease in birefringence with aging, but there was an increase of scattered light. This more anterior segment scattered light is insufficient to explain the magnitude of the increased scattered light at the retina (Figs. 9 and 10). The DOP decreased with increasing age for retinal light scatter measurements, as well as for the anterior segment plus superficial retina measurements.

B. Comparison with AMD Subjects

Images generated by equations 1 and 3 were used to analyze retinal light scattering differences between control subjects and subjects with dry AMD. There were no significant differences in reflectivity for either image type ($p=0.0684$ and 0.4427 respectively). However, this is likely due the subjects with AMD having greater focal variability in intensity than the control subjects (Fig. 12).

The coefficient of variation was computed for each of the four sample locations for both image types, and then the average of the variabilities was computed (Fig 13). A two-tailed paired t-test was performed, and there were significant differences in the variability for both image types ($p=0.0472$ for image type 1, and $p=0.0018$ for image type 3). A 95% confidence interval for image type 1 has a range from 0.0658 to 0.0738 for average variability. Four out of the five AMD subjects fell outside of this range. The more striking results for image type 3 are expected because this is a ratio measurement, in which the overall light return normalizes the scattered light signal.

These results mirror those of aging changes and disease findings of RPE with an independent technique, fundus autofluorescence [30,31]. Aging changes to the RPE are demonstrated by increased scattered light in our measurements, and increased lipofuscin with fundus autofluorescence. However, in both techniques the older subjects can have more within group variability than do the younger ones, as can be seen in figure 2. Further, patients with AMD are well known to have strikingly different values of autofluorescence across the macula [31], which agrees well with our findings of a patchy pattern that occurs over a large range of signal strength.

Our results indicate that retinal scatter light is measureable and increases with aging. That is, not all the scattered light in older eyes, which has the potential to decrease the contrast of the visual signal, is generated by scatter in the anterior segment. Thus, while improvements of the tear film and removal of cataractous lenses may improve vision, there is nevertheless the remaining retinal light scatter that can degrade vision. Further, it may be possible to develop techniques that would quantify the visual function expected following the alleviation of anterior segment scattering sources. This method also documents RPE changes such as the patchy signal variation in figure 12 using comfortable and safe near infrared light [5,19–21,25,28], instead of the bright short wavelength light typically used for fundus autofluorescence [31].

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Fig. 1.

The image on the left was calculated using equation 1, from a 22 yr female. Brightness was adjusted to make features visible. The white squares indicate where the 5×5 pixel sample regions are located. The image on the right was calculated from equation 5 for the same subject. The macular bow-tie is present in this image, and was used to locate the fovea.

Fig. 2.

The images on the top were derived from equation 1. The image on the left is from a 22 yr female subject, and the image on the right is from a 75 yr female subject. The plot on the bottom are of the results from all 120 subjects for the images derived from equation 1. Shaded circles indicate the mean intensity for each age group, and the error bars show the range. A linear best fit, shown as the dotted line, follows $y=0.4122x+12.639$ and has an R-squared value of 0.4445. This implies that there is an increase in multiply scattered light as an effect of age.

Fig. 3.

The images on the top are derived from equation 2. The image on the left is from a 22 yr female subject, and the image on the right is from a 75 yr female subject. The plot on the bottom are of the results from all 120 subjects for the images derived from equation 2. Shaded circles indicate the mean intensity for each age group, and the error bars show the range. A linear best fit, shown as the dotted line, follows $y=0.181x+64.481$, and has an R-squared value of 0.0538.

Fig. 4.

The images on the top are derived from equation 3. The image on the left is from a 22 yr female subject, and the image on the right is from a 75 yr female subject. The plot on the bottom are of the results from all 120 subjects for the images derived from equation 3. Shaded circles indicate the mean intensity for each age group, and the error bars show the range. A linear best fit, indicated by the dotted line, follows $y=0.2968x+14.298$, and has an R-squared value of 0.3861.

Fig. 5.

The images on top are derived from equation 4. The image on the left is from a 22 yr female subject, and the image on the right is from a 75 yr female subject. The plot on the bottom are of the results from all 120 subjects for the images derived from equation 4. Shaded circles indicate the mean intensity for each age group, and the error bars show the range. A linear best fit, indicated by the dotted line, follows $y=0.1378x+12.005$, and has an R-squared value of 0.2414.

Fig. 6.

The images on the top are derived from equation 5. The image on the top left is from a 22 yr female subject, and the image on the top right is from a 75 yr female subject. The plot on the bottom are of the results from all 120 subjects for the images derived from equation 5.

Shaded circles indicate the mean intensity for each age group, and the error bars show the range. A linear best fit, indicated by the dotted line, follows $y = -0.0504x + 14.57$, and has an R-squared value of 0.0158. This implies a slight decrease in total ocular birefringence that can be associated with aging.

Fig. 7.

The top image is a subsection of the first raw image collected on the parallel detector for this subject. Five locations were selected from the large blood vessel in its highly reflective center. The graph on the bottom is the averaged pixel intensity from the 20 images collected with the crossed detector, at the same five coordinates selected from the top image. A sine curve was fitted to the data for all subjects, as in equation 6. This subject's curve has an R-squared value of 0.9961. The horizontal dashed line is the average amplitude of the sine wave, and the solid vertical lines are the maximum amplitude of the sine wave.

Fig. 8.

Degree of polarization, derived from the image as computed from equation 3. Shaded circles are the mean degree of polarization for each age group, and the error bars indicate the range. A linear best fit, indicated by the dotted line, follows $y = -0.0012x + 0.9439$, and has an R-squared of 0.3861.

Fig. 9.

The graph on the top is the average intensity on the crossed detector, determined from fitting a sine curve in the form of $y=a+b*\sin(c*x+d)$ to the data. These data are the variable “a”, and can be visualized as the dashed horizontal line in Fig 7. A linear best fit follows $y=0.1245x+7.56$ and has an R-squared of 0.1525. The graph in the middle is the absolute value of the variable “b” from the fitted sine equation above. This is represented by the vertical lines in Fig 7. A linear best fit follows $y=0.0009x+5.704$, and has an R-squared of

0.00002. The graph on the bottom is the ratio of the maximum amplitude to the average signal. A linear best fit follows $y = -0.0033x + 0.5682$, and has an R-squared of 0.1583.

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Fig. 10.

The ratio of the first image type to the value of the average signal to the crossed detector, as determined by fitting a sine wave to the data. Shaded circles are the mean value for each age group, and error bars indicate the range. A linear best fit, indicated by the dotted line, follows $y=0.0097x+2.1802$, and has an R-squared of 0.0477.

Fig. 11.

Degree of polarization, computed from the fitted sine wave data. Shaded circles show the mean value for each age group, and the error bars indicate the range. A linear best fit, indicated by the dotted line, follows $y = -0.002x + 0.9601$, and has an R-squared of 0.4849. A linear regression was significant ($p = 1.04 \times 10^{-18}$) and had a z-score of 0.8603.

Fig 12.

Image generated by equation 1 on the left, and equation 12 on the right, for a 70 yr female with dry AMD. There is considerable variation in signal strength across the macular region, with a patchy pattern, considerably different from the systematic change in signal seen Figs. 2 and 4.

Fig 13.

The top plot is the average variability of the intensity for each of the four sample locations, for five dry AMD subjects and five age-matched controls, for the images generated by equation 1. The two groups were statistically different ($p < 0.05$). The bottom plot is the average variability of the intensity of the four sampled locations for images generated by equation 3, for the same 10 people. The two groups were statistically different as well ($p < 0.05$).

Table 1.

Tukey's HSD test for statistical differences between age groups for the image generated by equation 1. Asterisks indicate statistical significance.

	30s	40s	50s	60s	70s
20s	0.999	0.027 *	9E-6 *	1.07E-4 *	1.2E-12 *
30s		0.075	4.4E-5 *	4.7E-4 *	0.027 *
40s			0.259	0.614	3E-6 *
50s				0.991	0.014 *
60s					0.002 *

Table 2.

Tukey's HSD test for statistical differences between age groups for the images generated by equation 3. Asterisks indicate statistical significance.

	30s	40s	50s	60s	70s
20s	0.976	0.036 *	0.003 *	3E-6 *	7.8E-10 *
30s		0.212	0.029 *	6.4E-5 *	3.13E-8 *
40s			0.963	0.12	0.001 *
50s				0.519	0.011 *
60s					0.543