Impact of excess light and yellow filters on accumulation of lipofuscin

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Description
Purpose. Previous reports suggest excess high energy, short wave light increase lipofuscin fluorophore accumulation in the retinal pigment epithelium layer. Oxidation of lipofuscin has been implicated in the genesis of macular degeneration. By taking advantage of the increased exposure to light by optometry students, we tested whether optometry students accumulate more lipofuscin fluorophores than similarly aged allied health students and whether yellow filters alter lipofuscin accumulation.

Method. The sample consisted of 54 non-optometry students (Mean age 27, 4.1SD; 63% Female), 62 first-year optometry students (Mean age 27, 4.9SD; 55% female), and 39 second-year students (Mean age 26, 3.8SD, 54% female). First year practice patients were exposed primarily to anterior segment biomicroscopy, while second year practice patients included posterior segment biomicroscopy with condensing lens and binocular indirect ophthalmoscopy sessions. A two distribution mixture model estimated the gray scale of the fluorescent lipofuscin ring around the macula.

Results. There was significantly less luminance intensity in optometry students (Mean 70.8 grayscale) relative to non-optometry students (Mean 76.2 grayscale, F = 5.3, p=.024) which was opposite from our prediction. Covariates included ge (b=.9, p=.002) and baseline lipofuscin (b=1.1, p

Conclusion. Our results were more consistent with oxidation of lipofuscin fluorophores than accumulation following the excess exposure to light as practice patients. The study revealed intriguing trends in a challenging environment that suggested the topic is worth further investigation in a more rigorous experimental environment. The bottom line however is that we do not trust our results over time due to the systematic effect of multiple camera flashes that were not controlled across patients. We sincerely believe this study needs replicated with an autofluorescence camera that has a reference point to adjust for the physical conditions.

Keywords
Lipofuscin; blue light; yellow filters; fundus autofluorescence; A2E

Disciplines
Optometry

Comments
This original research manuscript has not undergone peer review.

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Impact of excess light and yellow filters on accumulation of lipofuscin

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Contact lenses were supplied by CooperVision
Lipofuscin is a complex aggregate of indigestible lysosomal material which accumulates in postmitotic cells over the lifetime of an individual. While lipofuscin has been considered a biological marker for the aging of cells, abnormally high levels of lipofuscin within the retinal pigment epithelium (RPE) are associated with retinal disease and RPE dysfunction, as in Stargardt’s and age-related macular degeneration. Laboratory evidence suggests that RPE lipofuscin pigments are unique from that of other cells as they are derived from molecular components of the visual cycle and primarily form during periods of excess light exposure with significant rhodopsin bleaching and elevated levels of all-trans-retinal in the rod outer segment (ROS) disks. Under these circumstances, the rate of generation of all-trans-retinal by photo-activation of rhodopsin exceeds the rate at which all-trans-retinal is reduced to all-trans-retinol in the ROS, providing a substrate for random side reactions to the visual cycle. Various fluorescent byproducts of the visual cycle are major components of RPE lipofuscin, including the di-retinal conjugate A2E (N-retinylidene-N-retinylethanolamine), which can induce DNA damage and RPE cell apoptosis through photooxidative processes upon exposure to short-wavelength (blue) light. These lipofuscin fluorophores have been shown to sensitize the RPE to blue light damage and are thought to play a role in the pathogenesis of certain macular diseases. It has been proposed that A2E and other lipofuscin fluorophores may be quantified via in vivo fundus autofluorescence imaging. Quantification of these molecules and methods of altering their accumulation may be of clinical interest in assessing and altering risk of various maculopathies.

Optometry students have unusual exposure to high levels of light as they serve as practice patients for their colleagues in learning various ophthalmoscopic examination procedures over a four-year period of professional education. Our first study objective was to determine if excess light exposure in optometry students relative to non-optometry health profession students leads to greater accumulation of lipofuscin fluorophores. Many ophthalmic instruments utilize light sources weighted toward shorter wavelengths more likely to result in photo-oxidation of lipofuscin fluorophores such as A2E. The resulting products of lipofuscin fluorophores may show increased fluorescence with early photo-oxidation; further oxidation and photodegradation, however, may cause a decrease in autofluorescence above 540 nm. Our second study objective was to determine if blue light-blocking yellow filters would alter lipofuscin fluorophore accumulation (as measured by fundus autofluorescence imaging) by having optometry students wear a yellow contact lens on one randomly assigned eye during procedure practice sessions.
**Method.** This was a prospective cohort study for the comparison of fundus autofluorescence measures of lipofuscin fluorophores in optometry and non-optometry students and a randomized controlled trial exploring the effect of yellow filters on lipofuscin fluorophore accumulation within optometry students. The research followed the tenets of the Declaration of Helsinki and informed consent was obtained from subjects after explanation of the nature and possible consequences of the study. The study was approved by the Pacific University Institutional Review Board.

**Subjects.** We recruited 61 first-year non-optometry students from the Pacific University College of Health Professions who are in a three-year program, 62 first-year optometry students from the class of 2013 and 38 second-year students from the class of 2012. Participation was voluntary. Optometry students were paid $10 per session and non-optometry students were paid $30 per session. The pay differential was due to the requirement that non-optometry students had to drive from a different campus (7 miles). Optometry students were also paid $90 for maintaining a practice log. The primary outcome variable was digital luminance levels of serial fundus autofluorescence photographs. The study was powered to detect a 0.5SD mean difference in luminance between optometry and non-optometry students with a power of .9 at an alpha = .05 assuming a correlation between the baseline luminance covariate and the follow-up of r = 0.7 with a 20% dropout.

Subjects had to have two healthy, normally functioning eyes. Those who reported any history of eye disease or hereditary eye conditions were excluded. Individuals with visual correction were allowed but must otherwise have had healthy functioning eyes. Optometry subjects had to be able to tolerate contact lens wear, but were not required to be previous contact lens wearers. Biomicroscopy was performed prior to administering mydriatic eye drops as typically done in routine eye examination to assess risk of acute angle closure. Non-optometry subjects must have had von Herrick Grade 2 or larger anterior chamber angles to ensure safety with dilation. Pregnant subjects were excluded (two non-optometry students became pregnant during the study and were excluded from further participation due to possible side effects of dilation).

**Equipment and materials.** A Topcon TRC-50DX retinal camera (Topcon, Tokyo) was used to collect data from control and optometry subjects. The camera employs an excitation filter that produces a green flash from 535nm to 595nm and a barrier filter for collection of fluorescent light from 600nm to 730nm. The camera was located in a completely dark room, and lights on the control panel were covered when taking photographs to exclude any light other than the excitation flash.

CooperVision (Pleasanton, CA) supplied Edge III ProActive contact lenses (62% polymacon and 36% water) which were tinted yellow by SpecialtyTint (Escondido, CA). Subjects used the same lens
throughout the trial and were supplied with identical contact lens cleaner and cases. The contact lenses exhibited the spectral profile of a longpass filter with cutoff at 470nm (above the 430 nm point of oxidation for A2E). Contact lenses were removed prior to fundus imaging.

Optometry subjects completed a log sheet each time they served as a patient for practicing ophthalmic procedures. Logs included the duration of light exposure to each eye and type of ophthalmic equipment used (e.g. biomicroscope with or without condensing lens, binocular indirect ophthalmoscopy). The file was monitored weekly for adherence to completion.

**Procedure.** Baseline fundus autofluorescence photographs were collected in January 2010 and follow-up data in September 2010, yielding a nine month period of accumulation. Very little practice occurred over the summer from June through August. Subjects were recruited by a general email sent to first and second-year optometry students and first-year professional students in all Pacific University’s College of Health Profession programs. Subjects completed a brief questionnaire that included gender, age, ethnicity, refractive correction, and nutritional supplementation.

Baseline photographs were taken by two photographers (optometry student research assistants). Photographers alternated taking the first baseline photograph. Immediately following that photograph the other photographer took a second photograph. For each photograph the camera was refocused. Two photographs were also taken at follow-up but by the same photographer. The initial baseline photographs were taken at flash intensity 50 watt seconds, but follow-up photographs were at flash intensity 100 watt seconds. We chose the initial intensity for subject comfort, but concluded that a higher intensity facilitated autofluorescence detection. The maximum for the camera was 350 watt seconds Changing intensity prohibited direct measures of change from baseline; instead, baseline measures were used as covariates to adjust for individual differences. Subsequent analysis demonstrated that our method of computing luminance was accurate at either level of flash intensity.

Non-optometry students had their photographs taken on weekends and in the evening. Optometry students had photographs scheduled at times during which they would be dilated for practice or lab sessions, with images being acquired prior to light exposure as a practice patient. Eyes were not bleached prior to the test photograph. Subject pupils were dilated at least 8 mm in diameter before image acquisition as measured by a ruler prior to the photograph.

For optometry students, the yellow contact lens was randomized to either the left or right eye at study entry and the same eye was filtered for the entire study. Optometry students wore the yellow
contact lens when exposed to light as a practice patient except for limited procedures requiring direct contact of ophthalmic equipment to the cornea. During direct contact procedures, students utilized yellow filters built into biomicroscopes when the eye randomized to the yellow lens was examined. If biomicroscope filters were not available, then the student proceeded without filtering light.

Luminance calculation. The primary outcome measure was luminance intensity of the Topcon autofluorescence photograph. The grayscale varied from black (0) to white (255). Figure 1a is a sample autofluorescence photograph. A mixture model was developed using R (GNU, Free Software Foundation). Figure 1b is the frequency distribution of the pixels along the grayscale and the gamma distribution model of the distributions. The R function reading the TIFF files had automatic brightness adjustment, so a small white square (10x10 pixel grayscale 255) was added to a black corner (grayscale 0) of each photograph in order to calibrate all pictures to the same brightness scale. The top one percent of the brightness pixels and pixels less than a grayscale of four were eliminated from the distribution to remove the black corners of the photograph and the extreme upper tail of brightness. Our goal was to estimate the overall luminance of the brighter perifoveal ring of autofluorescence. The smooth lines in Figure 1b show two modal points. The left distribution represents the distribution of points primarily making up the pixels from the optic disc, macula, and main vessels. The right distribution is the bulk of the lighter vessels and the gray of the entire photograph. We had two research assistants independently use the open source graphics program GIMP to identify pixels that best represented the “hyperfluorescent ring” around the macula. They used the Threshold function within GIMP which sets all points black below a particular threshold and white above. Figure 1c is the threshold view of Figure 1a. The small inset histogram identifies the threshold at grayscale 58 which was approximately the mean of the higher distribution in Figure 1b. The researchers consistently chose a point near the mean of the upper mixture distribution. This point best defined the bright ring around the macula. Figure 1d shows the consistency in judgment (reliability) between the two research assistants in estimating the mean autofluorescence luminance. In this figure, data are also shown on judgments for major vessels. Two consecutive photographs were taken on each subject at baseline and follow-up. Photograph 1 and 2 were significantly associated for both research assistant judge 1 (b=1.4, R² = .92, p<.001) and judge 2 (b=1.01, R² = .90, p<.001) further supporting the reliability of the measure. Using the subjective estimates as a guide for defining a rule for the computer, we averaged the pixel grayscale above the mixture model mean of the second distribution as the estimate of the luminance of autofluorescence.
The validity of our measure was calculated in several ways. There was a significant association between baseline and follow-up measurements ($R^2 = 0.76$, $p<.001$) demonstrating baseline measures could predict luminance 9 months later. Age was significantly associated with baseline mean luminance ($R^2 = 0.16$, $p<.001$) as would be expected since lipofuscin accumulates over time. In testing the Topcon camera on a blank piece of paper with the words “Can you see me now?”, we noted that the measured luminance linearly increased with serial pictures every 10 seconds over a period of 40 trials (slope = .42 gray scale, $R^2 = .95$, $p<.001$). We could see the text because the copy paper we used had added fluorescence to make it brighter. The increase in luminance over time was likely due to heating of the camera flash bulb. Considering the consecutive photographs taken during the study, the overall baseline mean luminance was 45.50 gray scale for the first photograph and 45.91 for the second photograph, a difference of .41. This compared favorably to the mean 10 second difference for plain copy paper of .42 (copy paper autofluoresces due to fluorescent material added to the paper during manufacturing).

Statistics. A between subjects analysis of covariance with baseline luminance and age covariates assessed the first hypothesis of whether or not there was a difference between lipofuscin fluorophore accumulation in optometry and non-optometry students as measured by fundus autofluorescence. A within subjects analysis of covariance with baseline luminance and age covariates assessed the effect of yellow filters on follow-up luminance. Figures are presented with 84% confidence intervals. These confidence intervals mimic the results from unadjusted least significant difference tests at an $p<.05$. 
Results.

Sample: Demographics of the sample are in Table 1. At the outset of the study we lost 1 second year student because of dropping out of school and two non-optometry students due to pregnancy and 5 others due to an inability to locate. Photographs of sufficient quality to be processed were available for 57 (92%) first year students, 38 (97%) second year students, and 53(98%) of non-optometry. Follow-up photographs were lost due to subjects dropping out of the study. Those remaining were 41 (72%) first years, 34 (89%) second years, and 33 (62%) non-optometry students. Dropouts had significantly higher luminance at baseline for first year optometry students (45.4, 12.3SD versus 37.9, 13.8SD, t=2.93 p=.004). Second year and non-optometry dropouts tended to have higher baseline luminance means but the differences were not statistically significant (49.9, 15.6SD versus 43.3, 12.3SD and 51.2, 20.1SD versus 43.9, 16.2SD for second year and non-optometry respectively).
Table 1. Sample demographics at baseline.

<table>
<thead>
<tr>
<th></th>
<th>First Years (n=61)</th>
<th>Second Years (n=39)</th>
<th>Non-Opt (n=62)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean / % (SD)</td>
<td>Mean / % (SD)</td>
<td>Mean / % (SD)</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>27.3 (4.9)</td>
<td>26.5 (3.8)</td>
<td>26.8 (94.1)</td>
</tr>
<tr>
<td>Women (%)</td>
<td>54.8</td>
<td>53.8</td>
<td>63.0</td>
</tr>
<tr>
<td>Caucasian (%)</td>
<td>82.3</td>
<td>79.5</td>
<td>70.4</td>
</tr>
<tr>
<td>Asian (%)</td>
<td>17.7</td>
<td>15.4</td>
<td>24.1</td>
</tr>
<tr>
<td>Other (%)</td>
<td>5.1</td>
<td>5.6</td>
<td></td>
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<tr>
<td>Wore Correction (%)</td>
<td>72.6</td>
<td>64.1</td>
<td>44.4</td>
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<tr>
<td>Correct w/ contacts (%)</td>
<td>67.7</td>
<td>76.9</td>
<td>18.5</td>
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<tr>
<td>Slit-Lamp (minutes)</td>
<td>131.3 (71.6)</td>
<td>21.0 (24.1)</td>
<td></td>
</tr>
<tr>
<td>LED BIO (minutes)</td>
<td>1.4 (5.0)</td>
<td>14.9 (20.7)</td>
<td></td>
</tr>
<tr>
<td>Non-LED BIO (minutes)</td>
<td>0.7 (2.4)</td>
<td>142.1 (101.3)</td>
<td></td>
</tr>
<tr>
<td>High Plus (minutes)</td>
<td>13.5 (14.6)</td>
<td>141.6 (99.2)</td>
<td></td>
</tr>
<tr>
<td>Retinoscope (minutes)</td>
<td>34.1 (31.8)</td>
<td>2.2 (5.3)</td>
<td></td>
</tr>
<tr>
<td>Ophthalmoscope (minutes)</td>
<td>20.2 (22.3)</td>
<td>0.5 (2.0)</td>
<td></td>
</tr>
<tr>
<td>Other Exposure (minutes)</td>
<td>13.8 (4.4)</td>
<td>43.7 (11.6)</td>
<td></td>
</tr>
<tr>
<td>Total Exposure (minutes)</td>
<td>215.0 (106.9)</td>
<td>366.0 (183.8)</td>
<td></td>
</tr>
</tbody>
</table>

Hypothesis 1: Lipofuscin autofluorescence increases in optometry students as a function of increased light exposure over non-optometry student controls (Figure 2). A between subject analysis of covariance revealed significantly less luminance intensity (implied less lipofuscin) in optometry students (Mean 70.8 grayscale) relative to non-optometry students (Mean 76.2 grayscale, F = 5.3, p=.024) which was the opposite from our hypothesis. The effect size for this difference was -.52SD (Difference_{Opt-NonOpt}/SD = -5.4/10.5). The covariate influence on follow-up luminance in the model were age (b= .9 grayscale/age year, t=3.2, p=.002), baseline luminance (b=1.1 grayscale, t=15.1, p<.001), and replicate photograph (b=1.7 grayscale for second photograph, t=2.2, p=.03). Figure 2 illustrates the adjusted grayscale means for optometry and non-optometry students at nine month follow-up. Visual inspection
revealed no observable evidence of RPE damage in the follow-up autofluorescence photographs of either group of students.

Table 2. Hypothesis 1: Model mean comparisons at follow-up luminance as a function of starting baseline. Least significant t-test of optometry versus non-optometry students (Means square error = 99.6, 107df). Effect size is the difference between means divided by the root mean square error.

<table>
<thead>
<tr>
<th>Group</th>
<th>Quartile</th>
<th>Baseline</th>
<th>Follow-up</th>
<th>Difference from Non-Opt</th>
<th>t</th>
<th>p</th>
<th>Effect Size (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>First Year</td>
<td>Minimum</td>
<td>9.8</td>
<td>25.4</td>
<td>-7.1</td>
<td>-3.55</td>
<td>0.001</td>
<td>-0.71</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>32.1</td>
<td>58.3</td>
<td>-5.1</td>
<td>-2.55</td>
<td>0.012</td>
<td>-0.51</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>42.9</td>
<td>74.3</td>
<td>-4.1</td>
<td>-2.06</td>
<td>0.041</td>
<td>-0.41</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>51.3</td>
<td>86.7</td>
<td>-3.4</td>
<td>-1.69</td>
<td>0.095</td>
<td>-0.34</td>
</tr>
<tr>
<td></td>
<td>Maximum</td>
<td>91.2</td>
<td>145.6</td>
<td>0.2</td>
<td>0.11</td>
<td>0.914</td>
<td>0.02</td>
</tr>
<tr>
<td>Second Year</td>
<td>Minimum</td>
<td>9.8</td>
<td>14.9</td>
<td>-17.6</td>
<td>-8.78</td>
<td>0.000</td>
<td>-1.76</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>32.1</td>
<td>55.3</td>
<td>-8.1</td>
<td>-4.06</td>
<td>0.000</td>
<td>-0.82</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>42.9</td>
<td>74.8</td>
<td>-3.6</td>
<td>-1.78</td>
<td>0.077</td>
<td>-0.36</td>
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<tr>
<td></td>
<td>75</td>
<td>51.3</td>
<td>90.0</td>
<td>0.0</td>
<td>-0.01</td>
<td>0.996</td>
<td>0.00</td>
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<tr>
<td></td>
<td>Maximum</td>
<td>91.2</td>
<td>162.2</td>
<td>16.9</td>
<td>8.42</td>
<td>0.000</td>
<td>1.69</td>
</tr>
<tr>
<td>Non-Optometry</td>
<td>Minimum</td>
<td>9.8</td>
<td>32.5</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>25</td>
<td>32.1</td>
<td>63.4</td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>50</td>
<td>42.9</td>
<td>78.4</td>
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<td>75</td>
<td>51.3</td>
<td>90.1</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Maximum</td>
<td>91.2</td>
<td>145.3</td>
<td></td>
<td></td>
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</tbody>
</table>

General linear model: Follow-up = 18.95 – 8.0(1st year) – 21.7 (2nd year) + 1.39 (Baseline) + 0.09(1st yr * Baseline) + 0.42 (2nd year * Baseline)
Hypothesis 2: **Yellow filters will alter the accumulation of lipofuscin fluorophores (Figure 3).** There was no effect of yellow filter for the first-year class of optometry students (mean 69.4 unfiltered and 68.5 filtered, $t=.938/1.5$, $p=.532$). The effect size for the second-year optometry students was $0.26SD (-1.97/7.58)$, which was not statistically significant (mean = 72.0 unfiltered and 74.0 filtered, $t = 1.5$, $p= .14$). Age ($b=.99$, $F=11.6$, $p=.001$) and baseline luminance ($b=1.34$, $F=309.5$, $p<.001$) were significant covariates, but replicate photograph (1.5 gray scale units greater on the second photo, $F = 2.6$, $p=.11$) did not reach statistical significance.

After controlling for age and baseline luminance, there was no dose effect of total light exposure on follow-up luminance ($p=.95$). There was significantly more practice light exposure in the second year class (Mean 366 minutes) than in the first year (Mean 215 minutes, $p<.001$). However there was no exposure difference between filter and no filter ($p = .574$) or the group by filter interaction ($p=.365$).
Discussion.

Prior research has demonstrated that light exposure results in the accumulation of RPE lipofuscin fluorophores. We hypothesized that optometry students’ exposure to excess light while serving as practice patients would lead to more lipofuscin autofluorescence. After adjusting for age and baseline autofluorescence luminance (lipofuscin proxy), optometry students had significantly less accumulation at nine months follow-up. The effect was a scientifically meaningful .5 standard deviation difference.

While most previous research has supported the concept of increased lipofuscin with increased light exposure, Morgan et al have shown a reduction in autofluorescence in macaques after light exposure at 568 nm. They found transient decreases in autofluorescence at low levels of light exposure. Subsequent analysis suggests that the processes of photoisomerization, photooxidation and photodegradation may explain this phenomenon. In Morgan's study, higher levels of light exposure led to permanent RPE cell dysfunction as viewed with an adaptive optics scanning laser ophthalmoscope (AOSLO). We noted a decline in autofluorescence in our optometry students but did not detect damage to RPE cells, although we lacked an AOSLO.

The second hypothesis tested whether yellow filters could alter lipofuscin accumulation. We found no scientifically relevant trend of filter for first years, effect size equal to .12SD. However, we
noted a small trend for second year students (.26SD) which was not significant. We could not rule out chance as a reasonable alternative to the differences found.

One possible explanation for our findings is that excess light exposure led to increased oxidation and photodegradation of lipofuscin fluorophores such as A2E within eyes of optometry students relative to non-optometry students. Oxidation of lipofuscin fluorophores decreases fluorescence above 540nm (our study camera’s barrier filter collects fluorescent light from 615-715nm). Oxidation of lipofuscin occurs more readily at short wavelengths, and thus may have been reduced by the presence of a yellow filter, as has previously been demonstrated to occur in vitro. Light exposure in second-year optometry students was greater as they were exposed to procedures involving more direct illumination of the fundus (high plus and biomicroscopy (BIO)), while first years were exposed primarily to anterior segment BIO. This may explain why the protective effect of yellow filters was present only in second-year optometry students. The filter effects were trends only and were not significantly different.

We acknowledge a number of limitations to our study which should provoke a cautious interpretation of results. While we were confident in our ability to quantify autofluorescence with the Topcon camera, the device was not designed for this purpose. The quantification method of analysis was by computer program and there was no subjective component. The difference between consecutive photographs was detectable and similar to the difference between consecutive photographs of plain copy paper with added fluorescence caused by the heating of the instrument’s flash bulb.

We lost access to our Topcon camera after nine months, at which point a Heidelberg Spectralis with BluePeak SLO/OCT (Heidelberg, Carlsbad, CA) was acquired and utilized in following the subjects for another year. The Spectralis was also not designed to quantify lipofuscin. The Spectralis proved more difficult for our photographers to operate, and blue light laser exposure time was variable for the subject as the instrument required multiple photographs of a certain quality. The Spectralis could not be run in a dark room, allowing uncontrolled ambient light during photography. We were not able to demonstrate reliability or validity for quantifying luminance with the Spectralis in any of the measurements described above for the Topcon camera. Subsequent analysis showed no correlation between the Topcon baseline and Spectralis follow-up, or even an appreciable correlation between first and second consecutive photographs within a Spectralis imaging session. There was no correlation with age. A reliable and valid method of quantifying lipofuscin has been more recently designed for the Spectralis, but the new modification of the camera was unavailable to us at the time of our study.
The study suffered from attrition. For many of the first year optometry students, managing a practice log and using the contact lens was too much of a distraction to continue in the study. The non-optometry students were from a different campus in a different town and the inconvenience of the trip may have contributed to a number of subjects choosing not to participate in the follow-up visit. Future studies will need to consider ways to reduce these barriers to study completion.

We recognize that talking about non-significant trends is hazardous. This study should be replicated once a reliable and validated measure of quantifying autofluorescence is available. Our student population appeared to be a reasonable working sample for this type of study; however, the age and health of this study population may limit application of results to older patients more at risk for retinal disease. Replication of these findings has important clinical implications for understanding the role of light exposure in lipofuscin fluorophore measures and its possible relation to retinal disease.
References


ent of retina outer nuclear layer thickness. &aulast=Sparrow&pid=%3Cauthor%3ESparrow+JR%3BBlo nska+A%3BFlynn+E%3BDuncker+T%3BGreenberg+JP%3BSecondi+R%3BUeda+K%3BDelori+FC%3C%2Fau thor%3E%3CAN%3E23548623%3C%2FAN%3E%3CDT%3EJournal+Article%3C%2FDT%3E. Accessed 20130418.
Appendix. R program to calculate mean luminance from autofluorescence photos. The program used a mixture to compute the proportion of pixels, mean, and standard deviations of two distributions within the file. The luminance attributed to lipofuscin was based on the mean luminance of the brighter distribution calculating the mean luminance of the pixels of the pixels greater than the mean of the second distribution.

R code
library(pixmap)
library(rtiff)
library(mixtools)
library(mixdist)

path<-c(
  'd:/blue light/study data/Class 2012/Sept 10/Left/'
) */ For example

np<-length(path) # Number of folders to review. Each subject class has their own folder for each eye
p=1
i=1
while (p<=np)    # Go through each folder
{

  eyepic=dir(path=path[p] ,pattern="*.tif",ignore.case=TRUE)
  sort(eyepic)
  ni<-length(eyepic)
  ###
  pic_mixture<-function(i){
    filename<-paste(path[p],sep='',eyepic[i])
    #filename<-'d:/blue light/new baseline unfiltered/126 af 2.tif' # for example
    getDescription(filename)
    pic<-readTiff(filename)
    plot(pic,main=path[p],sub=eyepic[i])
    mat<-getChannels(pic)
    mat<-sort((mat))
    hist(mat)
    mat<-mat*255
    mat<-round(mat)
    q1<-quantile(mat,probs=c(.05,.5,.999),na.rm=TRUE)
    mat<-ifelse(mat>4&mat<q1[3],mat,NA) #Eliminate black corners and text on the photo
    mat<-na.omit(mat)
    q<-quantile(mat,probs=c(.05,.5,.9),na.rm=TRUE)
  }

}
pi<-c(.05,.95)
u<-c(structure(c(q[1],q[3]),names=NULL))
sigma<-c(.3*u[1],.2*u[2])
parms<-data.frame(pi,u,sigma)
matdata=mixgroup(mat,breaks=200)
matmix<-as.mixdata(matdata)
fitmat1<-mix(matmix,mixpar=parms,dist="gamma",constr=mixconstr(consigma="SFX",fixsigma=c(FALSE,FALSE),conmu="MFX",fixmu=c(FALSE,FALSE)),iterlim=150)
x<-fitmat1[[1]]
dev.next()
plot(fitmat1)
plotname<-c(paste(substr(filename,1,nchar(filename)-4),sep="",".jpg"))
dev.copy(jpeg,plotname)
dev.off()

mat2<-ifelse(mat>x[[2,2]],mat,NA)  #/select pixels > mean of the upper distribution
lipo<-mean(mat2,na.rm=TRUE)
id<-substr(eyepic[i],1,3)
eye<-1
#

line<-c(id,eye,lipo,q,x[[1]],x[[2]],x[[3]],fitmat1[[5]],fitmat1[[6]],filename)
#line<-c(id,eye,picnum,lipo,q,x[[1]],x[[2]],x[[3]],fitmat1[[5]],fitmat1[[6]],filename)
line
out<-paste(  'd:/blue light/lipo 20120510/',sep="","2012Sept10Left.txt")
write.table(t(line),out,append=TRUE,quote=FALSE,sep="",row.names=FALSE,col.names=FALSE)
print(q)
fitmat1<=""
mat<-""
pic<=""
return(TRUE)