Quantal and Visual Efficiency of Fluorescence in the Lens of the Human Eye

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Purpose. To document quantitatively the fluorescence in the human lens relevant to its interference with visual function. To explain quantitatively the experimental findings relative to loss of visual function. To study the relation between the fluorescence and the light transmission of the lens.

Methods. Three normal lenses, from 22-, 28-, and 69-year-old donors, were used. Fluorescent light was induced by a 4-mm diameter pencil beam of 380, 400, or 420 nm. It was measured as a function of the deflection angle from −10 to 150 degrees for different wavelengths.

Results. The shapes of the emission spectra were comparable to those reported in the literature. Total quantal efficiency of fluorescence was between 5% for 69 years and 380 nm excitation and 0.4% for 22 years and 420 nm excitation. The forward intensity was less than the backward intensity because of secondary absorption.

Conclusions. Fluorescence of the lens causes light with wavelengths of 420 nm and lower to be much more visually effective. A marked homogeneous veil is added to the point spread function. The total increase in luminous efficiency was a factor 3 to 6 at 400 nm, and a factor 70 to 150 at 380 nm. For other visual effects (glare) the increase can be larger. Invest Ophthalmol Vis Sci. 1993;34:3566–3573.
In a recent article, Zuclich et al. experimentally verified in monkeys that fluorescence of the lens interferes with visual function. They projected a 413 nm laser beam onto the lens at 45 degrees to the visual axis while the foveal visual evoked potential was recorded. As a result the visual evoked potential declined. Assuming 413 nm to be entoptically scattered according to Vos’s white light model, scattering could not explain the decline. Because 413 nm light is not only scattered, but also gives fluorescence, it was concluded that fluorescence was the cause.

The current study aimed to gain quantitative insight into the visual effect of lens fluorescence. Specifically, the uniform component added to the retinal PSF was sought. Intact donor eye lenses were used. We wanted to determine the intensity of fluorescent light emitted by the lens in the forward direction (the direction of the retina). But in this direction the excitation light interferes with the measurement. This is why standard fluorescence measurements are conducted at right angles. But with intact lenses, light deflected at right angles travels laterally through 6 mm of the lens toward the optically less well-defined equator. This light would not properly represent the fluorescence emitted toward the retina. The problem was solved by making a scan around the lens. From these measurements the uniform component of the PSF could be estimated. Taking the wavelength dependencies into consideration, the disturbance of visual function can be understood and quantified from the modified PSFs.

METHODS

Three lenses were used (lenses 1, 2, and 3, from donors aged 28, 69, and 22 years, respectively), forming part of a larger study on light scattering in donor lenses. Eyes with short postmortem enucleation times (8, 1, and 6 hours, respectively) and no potential damage to the lens (eg, no trauma to the head) were obtained from the Hoornvliesbank (cornea bank) in Amsterdam. The lenses were carefully extracted and mounted in a special holder with a free diameter of 8 mm, and placed between, but not in contact with, specially selected cover glasses that were renewed for each lens. As immersion medium the isotonic solution (Na⁺ 77, K⁺ 4, Ca²⁺ 2.2, Cl⁻ 85 and Glucose 139 mmol/l, index of refraction 1.336) was chosen. The mounted lenses were checked using a slit lamp. They were normal for their age, but postmortem changes (some superficial irregularity, spokelike structures) could not be excluded. The 69-year-old lens showed sclerosis including early irregularities in the nucleus. Measurements were performed during a few hours (2, 4, and 4 hours, respectively) directly after extraction of the lens. A first scattering measurement was repeated at the end of the measurements to check that no change in scattering characteristics had taken place during the experiment.

Figure 1 is a simplified drawing of the setup (not to scale). The hot spot of a high-pressure mercury lamp was focused on diaphragm 1. The light passed through (excitation) interference filter 1 (half band width 10 nm). The beam divergence at the site of this interference filter was approximately 0.2 degrees. This is small enough for proper functioning of the interference filter, with no need for collimation. For lens 1 excitation wavelength 400 nm was used, for lenses 2 and 3 excitation wavelengths 380, 400, and 420 nm were used. Diaphragm 1 was focused in front of the lens (small cross in Fig. 1). The divergence of the beam was adjusted by means of diaphragm 2, to obtain a

![Figure 1. Schematic drawing of the setup, not to scale. The combination of lens 3, diaphragm 3, interference filter 2, and photomultiplier could rotate, with the donor lens as the center of rotation.](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933172/)
beam width of 4 mm diameter at the site of the donor lens. Shifting lens 2 of the setup, the distance between focus (small cross) and donor lens was adjusted so that a sharp image (radius about 1 mm) was obtained at 550 mm behind the donor lens. A 9 mm radius diaphragm (diaphragm 3) was positioned at this point. This diaphragm could rotate with the donor lens as center of rotation. With the position of the image defined as 0 degrees, the diaphragm was rotated from −10 degrees to 150 degrees. Light collected by this diaphragm was fed into a calibrated photomultiplier with a calibrated interference filter in front. Analysis wavelengths of 400, 420, 440, 460, 480, 500, 520, 540, 562, 602, and 700 nm were used, all with half band width of about 10 nm. Not all these wavelengths were used for all series. The photomultiplier was used as photon counter. After each experiment the donor lens was removed from the holder and the measurement was repeated for each excitation wavelength used (analysis filter removed), with diaphragm 3 at 0.645 mm radius. In the absence of the donor lens, no imaging of the focus (small cross) in diaphragm 3 took place. The beam divergence was approximately 3 degrees radius. This scan was used to calculate (by integration over solid angle) the total number of excitation quanta/second (q/s; Table 1). For 400 nm, log(q/s) was 12.57, 12.24, and 12.54 for donor lenses 1, 2, and 3, respectively. For 380 nm these values were 1.13 log unit lower and 12.54 for donor lenses 1, 2, and 3, respectively.

Unwanted refraction, reflection (limit angle of a Ringer-air interface is about 48 degrees), and screening effects occurred in the holder. Fluorescence is basically isotropic, so the fluorescence scans should be flat. Figure 2 shows the actual shape of two fluorescence scans. They are from lens 1 at excitation wavelength 400 nm and analysis wavelengths 420 (large peak) and 480 nm (small peak). Although interference filters with very good side band suppression were used, also a small part of nonfluorescent light was transmitted to the photomultiplier. This is seen as the peak at 0 degrees. It is small for 480 nm. Thus, for 480 nm, an absolute value for the fluorescent intensity in the zero direction could be estimated by interpolation of the flat portions around −10 and 10 degrees. For wavelengths closer to the excitation wavelength (420 nm in the example of Fig. 2), the peak is much larger. To arrive at a proper estimate in the zero direction, the 420 nm curve was shifted vertically so that the part of the forward branch outside the peak coincides with that of the 480 nm curve. (The peak could have been reduced if a second set of filters would have been available to improve the side band suppression.) Because the holder was opaque, at 90 degrees the dark current from the photomultiplier was measured. Taking into account the size of the diaphragm, the spectral transmission characteristics of the respective filters, the spectral quantum efficiency curve of the photomultiplier, the vertical shift, and the number of counts/s at 480 nm, for each analysis wavelength the number of quanta/s × nm due to fluorescence emitted by the donor lens in the forward half space (in the direction of the retina) was derived. For this derivation, fluorescent emission was assumed to be independent of angle, implying that emission in the forward half space was calculated from forward (0 degrees) quanta/s × steradian by multiplication with 2π. But, at larger angles, fluorescent light travels a larger distance through the lens. This will give extra losses. Conversely, in vivo, the light travels through vitreous hu-

<table>
<thead>
<tr>
<th>Table 1. The Data Set Derived from the Scans</th>
<th>Forward Fluorescent Emission log(q/s × nm)</th>
<th>Forward/Backward Ratio in Fluorescent Emission, relative to 480 nm (log units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lens 1</td>
<td>Lens 2</td>
<td>Lens 2</td>
</tr>
<tr>
<td>Excitation wavelength</td>
<td>400</td>
<td>400</td>
</tr>
<tr>
<td>Wavelength (nm)</td>
<td>400</td>
<td>420</td>
</tr>
<tr>
<td>400</td>
<td>7.59</td>
<td>7.08</td>
</tr>
<tr>
<td>420</td>
<td>7.59</td>
<td>7.08</td>
</tr>
<tr>
<td>440</td>
<td>8.08</td>
<td>7.81</td>
</tr>
<tr>
<td>460</td>
<td>8.12</td>
<td>7.96</td>
</tr>
<tr>
<td>480</td>
<td>8.25</td>
<td>8.20</td>
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<td>500</td>
<td>8.15</td>
<td>8.25</td>
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<td>520</td>
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<td>540</td>
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<td>562</td>
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<td>602</td>
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<td>7.68</td>
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<td>700</td>
<td>6.31</td>
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</tr>
</tbody>
</table>

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FIGURE 2. Fluorescent intensity from an isolated human lens, measured as a function of deflection angle. Excitation wavelength 400 nm. Emission wavelengths 480 nm (lower curve) and 420 nm (upper curve). The 420 nm curve was shifted vertically to coincide around 50 degrees with the 480 nm curve. The peak at zero degrees is an artefact (see text). The backward branch is relatively higher for 420 nm because (re)absorption is higher for 420 as compared to 480 nm (see text).

RESULTS

In Table 1 the data set derived from the scans is shown. An estimate for total fluorescent light that in vivo would reach the retina is given as explained in the Methods section. The log ratio between forward and backward fluorescent emission (relative to 480 nm) is given. Negative values indicate backward emission to be relatively stronger. In Figure 3 absolute results are shown (not relative to 480 nm) for the ratio of forward fluorescent emission to backward fluorescent emission, as a function of wavelength. As explained in Methods these data can be interpreted as $T_{1-2f} = \frac{T_{1}(wavelength)}{T_{1-2f}(480 \text{ nm})}$. (Throughout this article Briggsian (base 10) logarithms are used.)

3570

3570


FIGURE 3. Differences in intensity (due to reabsorption) of fluorescent light emitted by a human lens in the forward and the backward direction. Excitation wavelengths as indicated. Ages: above, 28 years; middle, 69 years; below, 22 years. Each data point followed from a comparison between backward branches as depicted in Figure 2.

This formula was fitted to the data of Table 1 (+ a constant representing the 480 nm result) using the least-squares criterion. The constant was simultaneously fitted. The result is shown in Figure 3. For lens 1 (400 nm excitation only) \((1-2f) \times 0.93 \times \log(T_s)\) (upper continuous line) was fitted to the data + a constant (symbols). The fit gave \(f = 0.36\) (so, the continuous line is \(0.27 \times \log(T_s)\)), and \(-0.02\) for the constant. Compare this value \(f = 0.36\) to the \(1/e\) penetration depth for 400 nm, that follows from \(\log(T/2) = -1.35\): \(f_{1/e} = 0.32\).

With lenses 2 and 3, in addition to 400 nm, measurements were performed with 380 and 420 nm. It should be realized that the 380 nm excitation light penetrates less far, whereas the 420 nm excitation light penetrates farther into the lens. If the analysis presented above is correct then the log forward/backward ratios for 380 and 420 nm must have the same wavelength dependence as for 400 nm, apart from a multiplication (and an addition) constant. To check this, the 380 and 420 nm data were fitted to the 400 nm data, estimating these constants. For 380 nm the multiplication constant was 1.23 and 1.30 respectively. For 420 nm it was 0.52 for lens 2. The absorption in lens 3 was too weak for the estimation of a 420 nm multiplication constant (set to 1). In Figure 3 the 380 and 420 nm data are multiplied by these constants (and shifted vertically). The correspondence with the 400 nm data is rather precise. The continuous curves resulted from a fit as above. For lens 2 the continuous curve is \(0.99 \times \log(T_s)\) \((f = 0.24, f_{1/e} = 0.16)\), for lens 3 the continuous curve is \(0.20 \times \log(T_s)\) \((f = 0.39, f_{1/e} = 0.34)\).

Table 2 gives an overview of these and other derived parameters. Values are given in parentheses for some of the 380 and 420 nm data. This is because these data were derived using an inferred, not measured value for \(T\) at these wavelengths. The value for \(T\) was derived using the above one-component multiplicative model for lens transmission. \(\log(T_s(380 \text{ nm})) = -2.61, \log(T_s(420 \text{ nm})) = -0.59\) This gives \(\log(T_s(380 \text{ nm})) = -4.96, \log(T_s(420 \text{ nm})) = -1.12, \log(T_s(380 \text{ nm})) = -2.31, \log(T_s(420 \text{ nm})) = -0.52\).

In Figure 4 the emission spectra for the forward direction are shown (symbols). The continuous lines are replots of Figures 2 and 3 from Zuclich et al\(^{12}\) for 360 and 413 nm excitation. At the peaks \(\log(\text{emission q/s nm}) - \log(\text{excitation q/s})\) were: lens 1 (400 nm) \(-4.32\), lens 2 (380, 400 and 420 nm) \(-3.57, -4.02\) and \(-4.07\) and lens 3 (380, 400 and 420 nm) \(-3.79, -4.34\) and \(-4.51\). The two curves from Zuclich et al\(^{12}\) have more or less the same shape. The present data seem to follow the same shape also. For each excitation wavelength the 413 nm curve was shifted horizontally to coincide with the data set. These shifted curves were used to arrive at Figure 5 (see below). They were also used to estimate the quantal efficiency of the fluorescence. To arrive at the total number of fluorescence quanta generated at the site of emission, a correction had to be made for the ab-
### TABLE 2. Main Results

<table>
<thead>
<tr>
<th>Age (yr)</th>
<th>Lens 1</th>
<th>Lens 2</th>
<th>Lens 2</th>
<th>Lens 2</th>
<th>Lens 3</th>
<th>Lens 3</th>
<th>Lens 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Excitation wavelength (nm)</td>
<td>28</td>
<td>69</td>
<td>69</td>
<td>69</td>
<td>22</td>
<td>22</td>
<td>22</td>
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<tr>
<td>Log relative transmission at excitation wavelength</td>
<td>400</td>
<td>400</td>
<td>420</td>
<td>380</td>
<td>400</td>
<td>420</td>
<td>380</td>
</tr>
<tr>
<td>1/e fractional depth of excitation intensity</td>
<td>-1.35</td>
<td>-2.76</td>
<td>-1.12</td>
<td>-4.96</td>
<td>-1.28</td>
<td>-0.52</td>
<td>-2.31</td>
</tr>
<tr>
<td>Mean fractional depth of light absorption</td>
<td>0.32</td>
<td>0.16</td>
<td>0.39</td>
<td>0.10</td>
<td>0.54</td>
<td>0.84</td>
<td>0.19</td>
</tr>
<tr>
<td>Mean fractional depth of fluorescent emission (f)</td>
<td>0.28</td>
<td>0.16</td>
<td>0.31</td>
<td>0.10</td>
<td>0.29</td>
<td>0.40</td>
<td>0.19</td>
</tr>
<tr>
<td>Log quantal efficiency of fluorescence</td>
<td>0.36</td>
<td>0.24</td>
<td>0.36</td>
<td>0.18</td>
<td>0.39</td>
<td>---</td>
<td>0.35</td>
</tr>
<tr>
<td>Log visual efficiency of fluorescence</td>
<td>-2.19</td>
<td>-1.82</td>
<td>-1.95</td>
<td>-1.30</td>
<td>-2.21</td>
<td>-2.41</td>
<td>-1.61</td>
</tr>
</tbody>
</table>
| Absorption losses occurring between the site of emission and the posterior pole of the lens. For this purpose the curves were divided by \( T_j(1-f) \). The resulting curves were integrated over wavelength and multiplied by 2, to allow for emission toward the backward halfspace. Log(total number of emitted quanta thus derived) \(-\log \) (number of incident quanta) gave as result: lens 1 (400 nm) -2.19, lens 2 (380, 400 and 420 nm) -1.30, -1.82, and -1.95, lens 3 (380, 400 and 420 nm) -1.61, -2.21, and -2.41.

In Figure 5 a measure for the visual effectiveness of the fluorescence is given. For this purpose the intensities \((q/s)\) were transformed into radiometric units (Watts) and these were transformed into photometric units (lumens).\(^{15}\) The last step involves multiplication by the luminous efficiency function. But this function is defined for light outside the (standard) eye, whereas the emission intensity was measured behind the eye lens. A correction for the transmission \( T_j \) of the (standard) eye lens therefore had to be made. Plotted is: (emission lumens/nm) / \( T_j \) (emission wavelength) \times (excitation lumens). These curves are intended to give by integration the ratio (weighted for photopic vision) between the total amount of fluorescent light (totalized over the entire retina) vs the amount of incident light. The logarithms of these ratios are: lens 1 (400 nm) 0.44, lens 2 (380, 400 and 420 nm) 2.17, 0.82, and -0.16, lens 3 (380, 400 and 420 nm) 1.83, 0.42, and -0.66.

### DISCUSSION

In Figure 3 the (fractional) transmission spectra showed good agreement between the different excitation wavelengths. This suggests that the presented analysis is valid. The validity rests on the implicit assumption that the chromophores responsible for lens absorption above 400 nm are more or less equally (not necessarily homogeneously) distributed. The agreement with the curves derived from the literature (valid for young lenses\(^{15}\)) was good for the two young lenses, but not for the older lens. The transmission of the older lens increases more gradually. This indicates that the one-component multiplicative model used to construct log spectral transmission for different ages from the literature\(^{18}\) data was too simple. Indeed, a closer look at the literature\(^{15,19}\) shows older lenses to have the more gradual shape as found here. This change with age has already been noted\(^{20,21}\) and was used for a two-component model\(^{21}\).

In the discussion of the \( f \) values, it must be realized that emission does not take place at one site. The experimentally determined \( f \) values represent a (weighted) mean overall emission site. The \( f \) values can be interpreted as fractional depth in the lens if the chromophores were distributed homogeneously. However, this is not the case.\(^{11,14}\) More precisely, the interpretation of \( f \) should be: the fraction of the chromophores passed by the excitation light before arrival at the emission site. These fractions (0.36, 0.24, and 0.39) were found to be larger than the fractions at which the penetrating light had decreased to 1/e (0.32, 0.16, and 0.34). But it should be noted that the emission does not take place at one site. Briefly, calculation by integration of the mean sites (given as a fraction) for absorption of 400 nm light in the three lenses.
yielded 0.28, 0.16, and 0.29 (Table 2). These values are smaller than the experimental values, so it seems that the fluorescence is not distributed in the same way as the chromophores. The fluorescence seems to be concentrated more in the center.

Another indication for dissociation between chromophore and fluorophore can be found in the values given in Table 2 for quantal efficiency. The maximum and minimum of these figures −1.30 and −2.41 correspond to 5% and 0.4%, respectively. These values might be considered rather low for a fluorescent process. But it must be realized what these figures actually represent: the ratio between the total number of incident quanta (almost equal to absorbed quanta) and the total number of fluorescent quanta for a whole lens. So, it is the quantal efficiency for the complete mixture of substances present in the human lens. It might be that some of these substances are efficient fluorophores, whereas others only contribute to the absorption.

Figure 5 and Table 2 indicate that fluorescence is visually important at excitation wavelengths of 420 nm and less. For the intact eye, a correction must be made because of light losses in the cornea. But this correction is small, about 0.1 log unit at 380 nm (manuscript in preparation). Above 420 nm the luminous efficiency of the excitation light itself dominates in the visual effect. Below 420 nm, eg, down to 330 nm, the fluorophore with peak emission at 360 nm (kynurenine metabolites of tryptophan) is probably responsible. Below 330 nm excitation tryptophan fluorescence may become more important, but the resulting wavelength ranges are of less visual consequence (high absorption by the eye media, low sensitivity of the visual pigment, low occurrence in ambient light).

The Results section (Table 2) indicates that for the young eye stimulated with 380 nm light, fluorescence adds a visually effective stimulus that is larger by 1.83 log unit (a factor 68) than the 380 nm light itself. At 400 nm the addition is larger by 0.43 log unit (a factor 2.7). At 420 nm the addition is smaller by 0.66 log unit (a factor 0.22). For the older eye the additions were more than twice as large: for 400 nm 0.82 log unit (a factor 6.6), for 380 nm 2.17 log unit (a factor 148), and for 420 nm −0.16 log unit (a factor 0.69). It must be noted that these figures are based on the luminous efficiency function. But this function is not valid for old eyes. The sensitivity of old eyes to short wavelengths is much lower. So, in fact for old eyes the ratio in luminous effect between the direct light, and the fluorescence it causes, is much larger. For example, at 380 nm \( \log(T_0) = -2.61 \) but \( \log(T_2) = -4.96 \). So, for this old eye the difference in visual effect between 380 nm light and its fluorescence is \( 2.17 + 4.96 - 2.61 = 4.52 \) log units (a factor 33000).

To evaluate the importance of this added light the following comparison may help. It is based on a model for the PSF, including parameters for age, pigmentation, and pupil diameter: note that the added light is homogeneously distributed over the retina, whereas the normal light follows the PSF. The PSF has a high peak and declines with angle up until 90°. For the 35-year-old, average white person with a 4-mm pupil, the PSF has a homogeneous component of its own of 0.135 (−0.88 log unit). This homogeneous component dominates the PSF for angles larger than 10°. If the PSF is compared with a homogeneously distributed light of some other origin (fluorescence), the two may cross at some point. For homogeneous light that is larger than the PSF by 2, 1, or 0 log units, crossing takes place at 0.92°, 2.2°, and 6.4°, respectively. For example, taking the above value for 420 nm (the addition is 0.66 log unit smaller), at angles larger than 10° the PSF for 420 nm light is increased by a factor of 2.7 on account of fluorescence. From the functional point of view (glare, contrast sensitivity, etc.) this is a serious deterioration of the PSF and hence of vision. The deterioration increases sharply with decreasing wavelength (and increasing age).

As mentioned in the introduction, Zuclich et al experimentally verified that the fluorescence of rhesus monkey lenses interferes with visual function. They projected a 1.5 mW 413 nm laser beam at 45 degrees to the visual axis onto the lens, while the foveal visual evoked potential was recorded using a 60 cd/m² screen. The visual evoked potential declined about 20%. The present data form a basis for the interpretation of such findings. According to Vos’s PSF model, entoptic scattering of the laser beam would correspond to a veiling equivalent luminance of only 0.5 cd/m². Fluorescence was therefore proposed as an alternative explanation for the decline. From the above figures for 400 and 420 nm, an interpolated value for 413 nm of −0.23 log unit follows. The photopic intensity of the laser beam was −2.75 log lumen. Plus −0.23 gives −2.98 log lumen that entered the eye to be homogeneously distributed over the retina. Assuming a pupillary diameter of 4 mm (surface −4.90 log m²), this gives a veiling equivalent luminance of \( 10^{-2.98 + 4.90}/2\pi = 12 \text{ cd/m}^2 \). This is 20% of the screen luminance. It should be realized that the correspondence with the 20% mentioned earlier may partly be fortuitous in view of the uncertainties present, such as that human data are used here to explain monkey results.

**Key Words**

human lens, fluorescence, aging, glare, straylight

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