Derivation of Lenticular Transmittance from Fluorophotometry

Thomas J. T. P. van den Berg,1 Joris E. Coppens,1 and Jaap A. van Best2,5

PURPOSE. To derive transmittance spectra for the human lens using the ratio between posterior and anterior autofluorescence of the lens as measured by fluorophotometry.

METHODS. Transmittance spectra of the lens can be described with a one-parameter model to a high degree of accuracy. The parameter m of this model defines the differences between lens transmittance spectra of individuals. In fluorophotometry literature another parameter related to lens transmittance, T, has been defined as the square root of the ratio between posterior and anterior lenticular autofluorescence. T can be predicted from parameter m, given the spectra of the excitation light, of the fluorescence emitted by the lens and of the detecting device are known, and assuming that the anterior and posterior fluorescence efficiencies of the lens are equal. When this relation is inverted, parameter m can be derived from T, giving the complete transmittance spectrum on the basis of T.

RESULTS. A transformation curve was calculated to determine T from m and vice versa. The light transmittance spectrum of the lens was calculated as a function of T. The validity of this approach was evaluated using an independent method for assessment of lenticular transmittance. This method consisted of making color slitlamp slides, grading the observed color of these slides with the LOCS III NC grading system, and transforming these grades into the model parameter m using published transformation curves.

CONCLUSIONS. The total transmittance spectrum can be calculated reliably from a fluorophotometric scan of the human lens. (Invest Ophthalmol Vis Sci. 2002;43:3003–3007)

Light entering the human eye is partially absorbed in a wavelength-specific manner by pigments located in the crystalline lens. Absorption increases with decreasing wavelength, resulting in a typical spectral lens transmittance: a high light transmission at the extreme red end of the visual spectrum and low transmission at the blue end. The strength of this absorption influences perception, including color vision and visual field sensitivity, manifest especially in blue-on-yellow perimetry. Although eye media transmission is only fully described if the value for all wavelengths is given, there is good evidence that a single figure can suffice to specify the eye media transmission for any individual with adequate accuracy.1,2 In many publications the color of the lens is specified with one figure. Clinically important examples of one figure color specification are subjective scoring systems such as the LOCS (Lens Opacity Classification System) from Chylack et al.1–4 It has been found by direct transmittance measurements that, when the lens ages or cataract develops, the transmittance spectrum changes in a characteristic one-dimensional way. Accordingly a one-parameter model has been proposed to describe the full transmittance spectra of different human lenses.5,6 This conclusion from direct transmittance measurements underlines the appropriateness of clinical one-dimensional scoring systems. Another technique for determining lens transmittance has been developed, based on objective scanning fluorophotometry. This technique also gives a one-dimensional parameter characterizing the light transmission of the human eye lens for blue-green light.7,8 The technique is easy to perform by making an axial autofluorescence scan of the human eye media and yields reliable data. The purpose of the present study is to show how this parameter can be used to derive the complete transmittance spectrum of the lens. Because this method is intended for use in vivo, the result was checked against an in vivo reference method. It must be noted here that the study is descriptive in nature, not interpretative. The underlying distribution of chromophores and its change with aging and cataract formation is a very complex behavior whose mere shadows are visualized with a study like this.

MATERIALS AND METHODS

Fluorophotometry

In scanning fluorophotometry, fluorescent intensity is recorded from the anterior to the posterior pole of the lens, while the lens is illuminated with a narrow excitation beam of blue light (half-height wavelengths, 430 to 490 nm). Because during passage through the lens, part of the excitation light is absorbed in the yellow pigments of the lens, it must be assumed that the blue excitation beam is weaker as it reaches the posterior pole compared with the anterior pole. As a result, the excitation of the fluorescent materials at the posterior pole is weaker. Indeed, the recorded fluorescent intensity at the posterior pole Fp is always lower compared with that at the anterior pole Fa. The so-called transmittance for blue-green light is calculated as T = (Fa/Fp). Where Fp is recorded posterior and Fa is recorded anterior lens autofluorescence intensity.7,8 The square root is used because light resulting in Fa passes twice through the lens, first as (blue) fluorescence excitation stimulus and second as (green) emitted fluorescence light. In this formula it is assumed that the fluorescence quantum efficiency of all material at the posterior side of the lens is equal to that at the anterior side. This has indeed been found to be the case, using donor lenses.9 So, a difference measured between Fa and Fp must be attributed exclusively to light losses between both sides.7,8 The value of T gives a kind of mean value of the transmittances at the forward and backward passages. It must be noted that for this analysis, the yellow materials causing transmittance losses in the lens are not necessarily the same as those causing fluorescence.
Optical Density Spectrum

As a model for lenticular optical density spectra the weighted sum of two fixed spectra was used, based on the following evidence: Pokorny et al. observed differences in visual sensitivity as a function of wavelength for individuals of different ages. They used large data sets from literature on accurate psychophysical techniques such as color matching. They found that all differences between age groups could be explained with only one weighted density spectrum, called TL1(\(\lambda\)), expressed as optical density (\(OD = -\log_{10}\text{transmittance})\). With aging, absorption increases according to an increase in weight factor of this TL1 spectrum. This resulted in the following model for the optical density spectrum of an individual lens:

\[
TL(\lambda) = m \cdot TL1(\lambda) + TL2(\lambda) \quad \text{(unit OD),}
\]

with \(\lambda\) = wavelength and \(m\) = individual multiplier (dimensionless weight factor, \(m > 0\)). The parameter \(m\) signifies how densely colored the lens is, because of aging or cataract formation; e.g., for \(m = 0\) the lens would be very lightly colored, typical for 20 to 30 years of age, whereas for \(m = 5\) the lens would be clearly yellow, typical for 70 to 80 years of age. TL2 is a fixed spectrum for every individual. In an independent study, this model was compared to direct in vitro transmittance measurements on lenses from donors aging between 14 and 86 years. It proved to comply very well, with an average residual error of only 0.05 log unit. For TL2 the compilation of lens transmittance data of Van Norren et al. proved to be best suited. TL2 is the spectrum for the least dense lenses allowed by the model (1) when for \(m = 0\) TL1 vanishes. In order for the model to be applicable to very clear lenses also (with TL1 vanished), the weight of TL2 was allowed however to vary below 1 in the in vitro study. The extended formula for the lens spectral density model reads

\[
TL(\lambda) = \max(0, m) \cdot TL1(\lambda) + \min(1, 1 + m) \cdot TL2(\lambda) \quad \text{(unit OD),}
\]

with \(m > -1\). \(\max(0, m)\) means zero or the current value of \(m\), whichever is largest. \(\min(1, 1 + m)\) means 1 or 1 + \(m\), whichever is smallest. For \(m > 0\) formula (2) reduces to formula (1), because in that case \(\max(0, m) = m\) and \(\min(1, 1 + m) = 1\). For \(-1 < m < 0\) formula (2) reduces to \(TL(\lambda) = (1 + m) \cdot TL2(\lambda)\), applicable to very clear lenses. On the other extreme side, it must be noted that the validity of this model for very strongly colored or cataractous lenses may be limited to the highest values of \(m\) in the in vitro study. Although it must be realized that this model is an approximation, it is worthwhile as a means to specify the complete lens transmittance spectrum on the basis of only one parameter, the individual multiplier \(m\).

Link between Parameter \(T\) and Density Spectrum

With some simplifying assumptions, all the information needed for exact interpretation of the fluorophotometrically determined transmittance \(T\) for blue-green light can be derived from (a) the spectral distribution of the excitation light, (b) the lens optical density spectrum, (c) the spectral excitation efficiency of lens fluorescence, (d) the spectral distribution of the emitted fluorescent light, and (e) the sensitivity spectrum of the detecting device. The spectra (a) and (c) can be obtained from the instrumental data of the fluorophotometer, and the lens optical density spectrum (b) from Equation 2, provided parameter \(m\) is known. The spectra (c) and (d) are obtained from Figure 1 of Yu et al., assuming that their shapes are stable enough over the limited wavelength ranges selected by the excitation and emission filters of the fluorophotometer. Similarly, it is possible to calculate a relationship between the values of parameters \(m\) and \(T\). The link between \(T\) and the complete transmittance spectrum of the lens can thus be obtained from this relationship by using Equation 2.

**Figure 1.** Illustration to the derivation of lens transmittance \(T\) for blue-green light from fluorophotometry. **Thick line:** absolute transmittance spectrum of the lens of an exemplary 70 years old individual. (□ and ○), the anterior side, normalized to unity; (■ and ●), the posterior side, showing the absorption losses with respect to the anterior side. The two figures at the left side (□ and ■) show the loss of fluorescence excitation spectrum going from the anterior side of the lens (□) to the posterior side (■). The two figures at the right side (○ and ●) show the effective loss of fluorescence emission spectrum going from the anterior side of the lens (○) to the posterior side (●).

Verification of Transmittance Parameter \(T\)

A verification of the abovementioned calculations including the in vivo situation was wanted. For this, a second assessment of lens transmittance for blue-green light was needed. A method based on an established clinical scoring system was used. First, the color of the crystalline lens was scored subjectively by comparing the color of the lens as it appears in slit lamp photographs, with a series of six standard photographs from the LOC3 III system. The photographic standards in this system correspond to nuclear color scores NC1 to NC6. By visual comparison with these six standards and further inter- or extrapolation, the photograph of an individual is scored between NC0.0 and NC6.9. Measured transmittance spectra of donor lenses had been found to compare well with the spectral lens density model (Eq. 2), and a close relationship between the parameter \(m\) and the LOC3 NC score was established. This relationship can be used conversely to derive the lenticular transmittance spectrum once the lens color is documented with the NC score. This scheme was implemented for 200 ASA photographs, and also for 1600 ASA photographs to include noncataractous lenses as in the present study. From the \(m\) value, the transmittance spectrum for the lens was derived, and the value of \(T\) was calculated for the Fluorotron Master as summarized in the above paragraph and exemplified in Results.

Additionally, a fluorophotometer scan was made using a commercial scanning fluorophotometer (Fluorotron Master; Ocumetrics Inc., Mountain View, CA) and the \(T\) value was calculated from this scan. Both methods were applied to 15 healthy volunteers recruited by advertisements in local newspapers. All subjects gave their consent after explanation of the nature and possible consequences of the procedure. The study was conducted according to the principles established in the Declaration of Helsinki and was approved by the local medical ethics committee.

**Results**

Example

Figure 1 illustrates the derivation of the lens transmittance \(T\) for blue-green light of a typical 70-year-old individual as obtained with the scanning fluorophotometer. The area between the anterior (open squares) and posterior (closed squares) excitation spectra represents the change in fluorophore excitation...
between the anterior pole (no light losses in the lens) and posterior pole (taking light losses in the lens into account). These spectra were obtained as follows. The anterior excitation values were calculated as the product of (1) the halogen light spectral distribution, (2) the measured transmittance of the blue excitation filter (transmittance interval 410 to 490 nm) used in combination with a halogen lamp in the instrument, and (3) the excitation spectrum of human lens autofluorescence. These anterior excitation values were normalized (i.e., maximum = 1), and the posterior excitation values were derived by multiplication with the corresponding lens transmittance values (the bold line in Fig. 1, calculated with Eq. 2 using \( m = 2.6 \), the value for a typical 70-year-old individual\(^6\)). Note that this transmittance spectrum is the weighted sum of two experimentally derived curves, as explained in Methods.

The ratio between the integrals of these two spectral excitation distributions corresponds to the overall lens transmittance for excitation and amounted in this example to a factor of 0.44. It must be noted that the lens transmittance used here must be the total lens transmittance as usually measured, being the result of absorption light losses in both fluorescent and non-fluorescent chromophores. This spectrum is different from the excitation spectrum.

The reabsorption of fluorescence from posterior to anterior was accounted for in the following way: The open circles in Figure 1 represent the spectral distribution of fluorescent light, emitted by the anterior portion of the lens, but after passage through the short wavelength cutoff filter in the instrument. Precisely this same spectrum would apply for the emission from the posterior side, if no reabsorption of fluorescent light would occur on the passage from posterior to anterior. However, the transmittance spectrum (heavy line) shows that reabsorption does occur in this wavelength region, having the effect of lowering this spectral distribution. In this way, reabsorption modifies the spectrum measured from the posterior side, as given by the closed circles. It is assumed that the reabsorbed light is not followed to an appreciable amount by remittance. The area between anterior emission spectrum (open circles) and posterior emission spectrum (closed circles) in Figure 1 represents the change in what is measured of the fluorescent light between the anterior pole (no light losses in the lens) and the posterior pole (light losses in the lens). Note that this only gives the change to what is measured from the posterior excitation spectrum. On top of that, the excitation itself is weaker at the posterior side. These data were obtained as follows. The anterior fluorescence emission values were calculated as the product of (1) the fluorescent emission spectrum\(^16\) and (2) the measured transmittance of the emission filter (transmittance >520 nm) used in the instrument. These anterior emission values were normalized (i.e., maximum = 1), and the posterior emission values were derived by multiplication with the corresponding lens transmittance values (bold line in the figure). The overall lens transmittance that follows for fluorescent emission light was 0.87 in this example. The combined transmittance for excitation and transmittance became \( 0.44 \cdot 0.87 = 0.38 \). Thus, \( T = 0.38 \). The transmittance \( T \) for blue-green light thus reflects the combined effects for two rather wide spectral ranges.

**Relationship between \( m \), \( T \), and the Transmittance Spectrum**

The calculated relationship between parameter \( m \) and transmittance \( T \) for blue-green light using the commercial fluorophotometer is shown in Figure 2. Parameter \( m \) can be read from \( T \) in this figure. Subsequently, from \( m \) the complete transmittance spectrum can be calculated using Equation 2. This is shown in Figure 3, where the resulting transmittances at 12 selected wavelengths are presented as a function of \( T \). Transmittances at intermediate wavelengths can be obtained by interpolation between the curves. The bold line in the figure corresponds to an equal transmission value (calculated transmission using the spectral lens density model equals transmission measured using the fluorophotometer). This illustrates the complicated character of \( T \). \( T \) does not represent the transmittance at a fixed wavelength but at various wavelengths between 430 and 470 nm, depending on the value of \( T \). So, \( T \) cannot be used directly as an accurate estimator of transmittance. Instead it could be used via transformation curves such as presented in Figures 2 or 3 to arrive at transmittance values.

**Verification of the \( T-m \) Transformation**

The transmittance \( T \) for blue-green light was determined in 15 healthy volunteers from NC scores using the spectral lens density model and from fluorophotometric scans. The NC scores as a function of the \( T \) values obtained by fluorophotometry are presented in Figure 4. The \( T \) values obtained from the NC scores as a function of the \( T \) values obtained by fluorophotometry are presented in Figure 5. A linear regression analysis of the latter data revealed a correlation coefficient of 0.81 (\( P = \)
0.00026), a SD around the line of 0.059, and a coefficient of relative deviation of 6.2%.

**DISCUSSION**

In the present article we describe a method to derive full transmittance spectra for human lenses from the parameter \( T \), obtained by fluorophotometry. With these as well as with most other transmittance spectra, it must be kept in mind that the transmittance may not only depend on light absorption processes, but also on light scattering. Both processes contribute to removal of light from the direct beam. This holds equally well for the measurement of light transmittance as for the situations to which the results may be applied, such as psychophysics. A problem might be, however, that the scattering angles involved are different, so that the measurement is not precisely valid for the application. However, compared with absorptive light losses, light losses by scattering are in the lens as a rule much smaller\(^{18,19}\) so that this problem will not be very significant. This may seem odd, because it is well known that functionally, for its detrimental visual effects such as glare, scattering in the lens can be very significant. However, the quantitative figures involved in glare versus transmittance are much different. In normal lenses, <1% of the light is scattered over \( >10^\circ \), giving rise to significant glare. A figure of 1% light loss, however, would correspond to a transmittance of 0.99 or \(-0.004\) log units. And even this 1% may not fully be lost for transmittance, dependent over what angular acceptance range transmittance is defined. With aging and cataract formation scattering may increase 10-fold, which causes a serious handicap to the patient, but still remains to be not very important from the point of view of transmittance. In the cornea, the situation is quite different because absorptive light losses are much weaker anyhow.\(^{20,21}\) So, the results on lens transmittance of the present measurements can be used in, e.g., psychophysics, even if the scattering angles involved would be different.

It must be noted that, given a proper model, other measured parameters could serve as well, with appropriate changes to the transformation curves (see the relation between the parameters \( m, T \), and the NC scores in Figs. 2 and 4). The assumptions to the model used may be a matter of concern. In the present study, one could, e.g., wonder whether the assumption of the original authors\(^7\) about equality of anterior and posterior autofluorescence was sufficiently justified. An argument in favor is the fact that with lightly colored lenses the fluorescence readings are the same anteriorly and posteriorly. However, one cannot exclude that with aging, the anterior and posterior sides change at a different pace. This question was addressed with a direct comparison of anterior and posterior fluorescence using donor lenses.\(^9\) Almost identical values were found irrespective of age\(^9\) in correspondence with the assumption of the present study. Also it was assumed that the shapes of fluorescent excitation and emission spectra within the limits of the excitation and emission filters do not differ too much between individuals. In this respect it was reassuring that Figure 5 shows good absolute correspondence between predicted values of \( T \) (using NC scores) and observed values (using fluorophotometer). The scatter around the regression line (SD = 0.059) is acceptable in view of the uncertainties in the fluorophotometric measurement of \( T \) (SD = 0.02)\(^6\) and the NC scores (SD = 0.3).\(^6\)

With the present data, especially Figure 3, it is possible to interpret the parameter \( T \), defined as lens transmittance for blue-green light in the original studies.\(^7-9\) The value of \( T \) corresponds with the transmittance at 430 nm for the clearest lenses and with the transmittance at 470 nm for the darkest lenses. However, from Figure 1 it can be seen that a wide distribution of wavelengths contributes to give \( T \) its value.

A basic problem is the attempt to reduce a complex behavior of a lens to a single numerical parameter. Not only must the validity regions for proper description of the lens transmittance be noted, as mentioned in the methods section. The method and model works as a mathematical approximation, and it works within limits. No claim can be derived from it about the underlying processes. As mathematical description its validity is limited by two things: its accuracy and the range of applicability. Within the normal population its accuracy is about as good as the measurement precision. This makes it a good approach for normals, with the restrictions mentioned in mind. How far can it be extrapolated to cataracts? It may have limited validity there, although its applicability for donor lenses up to the age of 87 years is promising. It may very well be that the good accuracy for the model mentioned of 0.05 log units, deteriorates with cataract. Also, in cataract the suggestion that the underlying process may be relatively simple as in normals, cannot be maintained. Another uncertainty with the one parameter approximation to lens transmittance might be its applicability to lens populations from geographical areas with high insulation where different chromophores and fluorophores may be significant.

It is concluded that the proposed analysis is suitable to derive lens transmittance spectra from fluorophotometric data. The literature data on the shapes of the autofluorescence spectra for the human eye lens and the literature model for lenticular transmittance spectra can fruitfully be used to inter-
pret the drop in recorded autofluorescence from the anterior to the posterior pole of the lens.

References


