# TRANSPORT OF FLUORESCENCE THROUGH HIGHLY SCATTERING MEDIA

## Corrections to the Determination of Quantum Yields

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ABSTRACT The equations for transport of fluorescence, generated within a highly scattering medium, are solved within the boundary conditions of the Kubelka and Munk treatment. Expressions are derived in closed form for fluorescence fluxes emanating from the front and back surfaces of a highly scattering infinite slab, whether or not fluorescence is absorbed within the sample. An "apparent" quantum yield, calculated from observed intensities of fluorescence and of back-scattered light from the front surface of the slab, can be corrected by these expressions to return the true quantum yield of fluorescence. Allowance for re-emission of reabsorbed fluorescence can be made, but may not be applicable in some experimental arrangements. Calculations performed on the fluorescence of rhodamine 101 suggest that in typical practical situations the correction factor may not be far from unity.

### INTRODUCTION

A problem of some importance to biology and other disciplines is the accurate estimation of fluorescence yields of substances excited in highly scattering media. An obvious example is the fluorescence of chlorophyll excited in leaves. The need that gave rise to the present work was that of determining fluorescence yields of chlorophyll in a model system of photosynthesis in which the pigment was adsorbed to small, but not submicroscopic, particles of swollen polyethylene, and suspended in a highly scattering but non-absorbing medium (1, 2).

There have been several solutions of the problem of light transport in highly scattering systems, the most familiar and successful of which is that bearing the names of Kubelka and Munk (K & M) (3), and more fully developed by Kubelka (4). Their equations have been applied successfully to the estimation of reflectance and absorbance of scattering materials such as coatings, and their application to biological systems has been expounded by Butler (5, 6). An alternative treatment has been presented by Hemenger (7), based on linear transport theory and with boundary conditions (e.g., collimated incidence rather than diffuse incidence) more appropriate to most experimental set-ups than those of K & M, but the form of the solution is not immediately adaptable to the problem of fluorescence generation as is that of K & M. Fluorescence generation in highly scattering layers has been examined by Allen (8), under K & M conditions, with particular reference to intensification of brightness by incorporation of fluorescent whiteners. In this paper, a perhaps more fundamental approach is taken in deriving, in closed form, equations for fluorescence excited in a highly scattering slab and radiated from front and back faces, subject to the boundary conditions of K & M, and in calculating the factor that enables one to convert certain measured light ratios to true fluorescence quantum yields.

### THEORY

The geometry and the nomenclature of Kubelka's paper (4) are followed as closely as is convenient (Fig. 1). Diffuse incidence of exciting light of intensity $I_0$ enters the surface $x = L$ of an infinite, highly scattering and absorbing slab of thickness $L$. Light traveling to the right, summed over all angles in the right hemisphere, is designated $i$; to the left, $j$. Some of the exciting light is absorbed with coefficient $K$ and reappears as fluorescence fluxes $i_f$, $j_f$ with a quantum yield of $\phi$. The fluorescence is radiated equally in both directions ($\pm x$) at point of origin. To recapitulate the results of K & M, the differential equations of light transport are

$$\frac{\mathrm{d}i}{\mathrm{d}x} = (K + S)\, i - Sj \tag{1}$$

$$\frac{\mathrm{d}j}{\mathrm{d}x} = -(K + S)\, j + Si, \tag{2}$$

where $S$ is the coefficient for reversal of direction of incident light by scattering, i.e., conversion of $i$ to $j$, and $j$ to $i$.

The solutions of these equations are

$$i = \frac{I_0\,[a \sinh(bSx) + b \cosh(bSx)]}{\Lambda}, \tag{3}$$

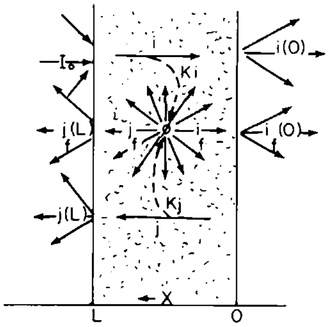

FIGURE 1 Diagram of geometry of optics for generation and propagation of fluorescence through a highly scattering and absorbing medium. Adapted from Kubelka (4).

where $\Lambda = a\sinh(bSL) + b\cosh(bSL)$, and

$$j = \frac{I_0\sinh(bSx)}{\Lambda}, \tag{4}$$

subject to the second K & M boundary condition that $j(0) = 0$, i.e., that the backing of the slab is perfectly black so that no light returns to the slab after leaving the face $X = 0$, a condition normally approximated in practice. Specular reflection at the surfaces is not expressly considered in the derivation that follows. The parameter $a$ of K & M is $1 + K/S$, and $b = (a^2 - 1)^{1/2}$. At the back face ($x = 0$),

$$i(0) = \frac{I_0 b}{\Lambda} \tag{5}$$

and at the front face,

$$j(L) = \frac{I_0\sinh(bSL)}{\Lambda}. \tag{6}$$

When there is no absorption of incident light ($K = 0$, $a = 1$, $b = 0$), these quantities reduce to

$$i_0(0) = \frac{I_0}{(1 + SL)} \tag{7}$$

$$j_0(L) = \frac{I_0 SL}{(1 + \mathrm{SL})}. \tag{8}$$

In general, the exciting and fluorescence wavelengths are different and that of fluorescence is longer. Consequently its scattering coefficient $S_f$ is a little different from $S$, and its reabsorption coefficient $K_f$ may be zero. In the following, reemission of absorbed fluorescence is ignored, but later it will be shown how it can be allowed for, and of course it is assumed that excitation of fluorescence is an irreversible process, i.e., absorption of fluorescence does not regenerate exciting light.

The equations for propagation of fluorescence fluxes $i_f$ and $j_f$ from within the sample are similar to Eqs. 1 and 2 but append a source term depending on $i$ and $j$. With due regard for sign conventions they read

$$\frac{\mathrm{d}i_f}{\mathrm{d}x} = (S_f + K_f)i_f - S_f j_f - \frac{\phi K(i + j)}{2} \tag{9}$$

$$\frac{\mathrm{d}j_f}{\mathrm{d}x} = -(S_f + K_f)j_f + S_f i_f + \frac{\phi K(i + j)}{2}. \tag{10}$$

A solution is most readily effected through the sum and difference fluxes, $\Sigma = i_f + j_f$ and $\Delta = i_f - j_f$, the equations for which are

$$\begin{aligned}\frac{\mathrm{d}\Sigma}{\mathrm{d}x} &= (S_f + K_f)(i_f - j_f) + S_f(i_f - j_f)\\ &= (2S_f + K_f)\Delta \end{aligned} \tag{11}$$

and

$$\frac{\mathrm{d}\Delta}{\mathrm{d}x} = K_f\Sigma - \phi K(i + j). \tag{12}$$

Elimination of $\Sigma$ gives Eq. 13 for $\Delta$,

$$\frac{\mathrm{d}^2\Delta}{\mathrm{d}x^2} = K_f(2S_f + K_f)\Delta - \phi K\frac{d(i + j)}{\mathrm{d}x}. \tag{13}$$

First, a general solution is stated, then two special cases of importance and interest. The general solution for $\Delta$ is

$$\Delta = C_1\sinh(b_f S_f x) + C_2\cosh(b_f S_f x) + \frac{I_0\phi b^2 S^2[(a - 1)\sinh(bSx) + b\cosh(bSx)]}{\Lambda(b_f^2 S_f^2 - b^2S^2)}, \tag{14}$$

in which $a_f = 1 + K_f/S_f$, and $b_f = (a_f^2 - 1)^{1/2}$. Differentiation and insertion into Eq. 12 gives the corresponding expression for $\Sigma$.

$$\Sigma = \frac{b_f}{(a_f - 1)}[C_1\cosh(b_f S_f x) + C_2\sinh(b_f S_f x)] + \frac{I_0\phi bS(a_f + 1)S_f[b\sinh(bSx) + (a - 1)\cosh(bSx)]}{\Lambda(b_f^2 S_f^2 - b^2S^2)}. \tag{15}$$

One boundary condition is the same as for exciting light, viz. $j_f(0) = 0$, which leads to the relation

$$C_2 = \frac{b_f C_1}{(a_f - 1)} + \frac{I_0\phi bS[(a - 1)(a_f + 1)S_f - b^2S]}{\Lambda(b_f^2 S_f^2 - b^2S^2)}. \tag{16}$$

The other is either $i_f(L) = 0$, or a statement to the effect that the total fluorescence escaping the sample or absorbed within it equals $\phi$ times the exciting light absorbed:

$$\phi[I_0 - i(0) - j(L)] = i_f(0) + j_f(L) + \int_0^L K_f(i_f + j_f)\mathrm{d}x. \tag{17}$$

If the latter is chosen, it turns out that $i_f(L) \equiv 0$. This boundary condition provides a cumbersome expression for $C_1$,

$$\begin{aligned} C_1 = \frac{-I_0\phi bS(a_f - 1)}{2\Lambda\Lambda_f(b_f^2S_f^2 - b^2S^2)}\Big\{ & b[(a - 1)S + (a_f + 1)S_f]\\ & \cdot\sinh(bSL) + (a - 1)[(a + 1)S + (a_f + 1)S_f]\\ & \cdot\cosh(bSL) + (a - 1)[(a_f + 1)S_f - (a + 1)S]\\ & \cdot\left[\frac{b_f}{(a_f - 1)}\sinh(b_f S_f L) + \cosh(b_f S_f L)\right]\Big\}, \end{aligned} \tag{18}$$

in which $\Lambda_f = a_f\sinh(b_f S_f L) + b_f\cosh(b_f S_f L)$.

Fluorescence fluxes leaving the slab are

$$i_f(0) = \frac{b_f C_1}{(a_f - 1)} + \frac{I_0 \phi b S (a - 1)(a_f + 1) S_f}{\Lambda (b_f^2 S_f^2 - b^2 S^2)}, \tag{19}$$

and

$$\begin{aligned} j_f(L) = {} & \frac{1}{2}\left(\frac{b_f C_2}{(a_f - 1)} - C_1\right) \sinh(b_f S_f L) \\ & + \frac{1}{2}\left(\frac{b_f C_1}{(a_f - 1)} - C_2\right) \cosh(b_f S_f L) \\ & + \frac{I_0 \phi b S}{2\Lambda(b_f^2 S_f^2 - b^2 S^2)} \{[b(a_f + 1)S_f - b(a - 1)S] \\ & \cdot \sinh(bSL) + [(a - 1)(a_f + 1)S_f - b^2 S] \\ & \cdot \cosh(bSL)\}. \end{aligned} \tag{20}$$

This completes the solution of the general case. Eq. 20 is presumably equivalent to the equation derived by Allen (8), but is expressed in a rather different functional form.

## Special Case No. 1

If fluorescence is not reabsorbed, which is the usual situation for the longer emission wavelengths, then $K_f = 0$, $a_f = 1$, and $b_f = 0$, and Eqs. 11 and 12 simplify to

$$\frac{\mathrm{d}\Sigma}{\mathrm{d}x} = 2 S_f \Delta \tag{21}$$

$$\frac{\mathrm{d}\Delta}{\mathrm{d}x} = \phi K (i + j). \tag{22}$$

The solutions are immediate:

$$\Delta = C_5 - I_0 \phi [b \cosh\,(bSx) + (a - 1) \sinh(bSx)]/\Lambda \tag{23}$$

$$\begin{aligned} \Sigma = {} & C_6 + 2 C_5 S_f x \\ & - \frac{2 I_0 \phi S_f [b \sinh(bSx) + (a - 1) \cosh(bSx]}{bS\Lambda}. \end{aligned} \tag{24}$$

The boundary conditions give the coefficients $C_5$ and $C_6$:

$$C_6 = C_5 + \frac{I_0 \phi (a - 1)[2S_f - (a + 1)S]}{bS\Lambda} \tag{25}$$

$$\begin{aligned} C_5 = {} & \frac{I_0 \phi S_f L}{2bS(1 + S_f L)\Lambda} \{[b(a - 1)S + 2bS_f] \sinh(bSL) \\ & + [b^2 S + 2(a - 1)S_f] \cosh(bSL) \\ & - [2(a - 1)S_f - b^2 S]\}. \end{aligned} \tag{26}$$

The fluorescence fluxes are

$$i_f(0) = C_5 - \frac{I_0 \phi b}{\Lambda} \tag{27}$$

$$\begin{aligned} j_f(L) = {} & \frac{I_0 \phi}{2bS(1 + S_f L)\Lambda} \{[bS(a - 1)(2S_f L + 1) - 2bS_f] \\ & \cdot \sinh(bSL) + [b^2 S (2 S_f L + 1) - 2(a - 1) S_f] \\ & \cdot \cosh(bSL) + [2(a - 1)S_f - b^2 S]\}. \end{aligned} \tag{28}$$

## Special Case No. 2

It will have been noticed that if $b_f S_f = bS$, the particular integrals in Eqs. 14 and 15 are infinite. The singularity cannot be removed, at least not easily, by algebraic trickery and one must return to the original differential equations. Eq. 13 becomes

$$\begin{aligned} \frac{\mathrm{d}^2\Delta}{\mathrm{d}x^2} = {} & b^2 S^2 \Delta \\ & - \frac{I_0 \phi b^2 S^2 [(a - 1) \sinh(bSx) + b \cosh(bSx)]}{\Lambda}, \end{aligned} \tag{29}$$

the general solution of which is

$$\begin{aligned} \Delta = {} & C_3 \sinh(bSx) + C_4 \cosh(bSx) \\ & - \frac{I_0 \phi b S x [b \sinh(bSx) + (a - 1) \cosh(bSx)}{2\Lambda} \end{aligned} \tag{30}$$

and from it,

$$\begin{aligned} \Sigma = {} & \frac{b_f}{(a_f - 1)} \Big\{ C_4 \sinh(bSx) + C_3 \cosh(bSx) \\ & + \frac{I_0 \phi}{2\Lambda} [b(1 - (a - 1)Sx) \sinh(bSx) \\ & + (a - 1)(1 - (a + 1)Sx) \cosh(bSx)] \Big\}. \end{aligned} \tag{31}$$

Applying boundary conditions gives

$$C_4 = \frac{b_f}{(a_f - 1)} \left[ C_3 + \frac{I_0 \phi (a - 1)}{2\Lambda} \right] \tag{32}$$

$$\begin{aligned} C_3 = {} & \frac{I_0 \phi}{4\Lambda\Lambda_f} \{[((a_f - 1)b + (a - 1)b_f) bSL \\ & - (b_f b + (a - 1)(a_f + 1))] \sinh(bSL) \\ & + [((a - 1)(a_f - 1) + b_f b) bSL \\ & - 2(a - 1) b_f] \cosh(bSL)\}. \end{aligned} \tag{33}$$

Note that $b_f S_f = bS$ does not, in general, imply $a_f = a$, etc. These lead to the fluorescence fluxes

$$i_f(0) = \frac{1}{2}\left[ C_4 + \frac{b_f C_3}{(a_f - 1)} + \frac{I_0 \phi b_f (a - 1)}{2\Lambda (a_f - 1)} \right] \tag{34}$$

$$\begin{aligned} j_f(L) = {} & \frac{1}{2} \Big\{ \frac{b_f C_4}{(a_f - 1)} - C_3 \\ & + \frac{I_0 \phi}{2\Lambda} \left[ \frac{b b_f (1 - (a - 1)SL)}{(a_f - 1)} + b^2 SL \right] \Big\} \sinh(bSL) \\ & + \frac{I_0 \phi}{4\Lambda} \left[ b(a - 1)SL - \frac{b^2 b_f SL}{(a_f - 1)} \right] \cosh(bSL). \end{aligned} \tag{35}$$

## EXPERIMENTAL APPLICATION

In the apparatus we have assembled for fluorescence measurement in highly scattering suspensions, exciting light is focused at ~20° from the normal onto a 1-mm layer of sample in a demountable cell. Back-scattered exciting light and fluorescence from the illuminated spot are focused into a monochromator, integrated over wavelength, and corrected for instrumental sensitivity. Calibration is achieved through comparison of back-scattering from a layer of Kodak white reflectance coating ($BaSO_4$) with that from the suspension at appropriate wavelengths. Thus, the K & M boundary condition of diffuse incidence is not at all observed in our apparatus, nor is it observed in most fluorimeter arrangements, and it would be interesting to discover to what extent the equations derived above can be applied to practical apparatus for measuring quantum yields.

As an approximation, a quantity we call the apparent quantum yield ($\phi_{ap}$) is calculated from the integrated back-scattered exciting and fluorescence intensities, expressed in quantum units. It is the ratio of the fluorescence intensity to the difference between back-scattered exciting light with and without the absorber present. This quantity is easily determined experimentally, and is clearly related to the true quantum yield. The equations of the preceding section enable one to calculate the fluxes that enter into the definition of $\phi_{ap}$, $\phi_{ap} = j_f(L)/[j_0(L) - j(L)]$, in terms of the true quantum yield $\phi$ and parameters characterizing the medium. This procedure has been incorporated into a program, written in IBM PC BASIC, which calculates the ratio between the true quantum yield and the apparent quantum yield.

Inputs to the program are five: the quantities $a$ and $a_f$ at exciting and fluorescence wavelengths (considered monochromatic), $SL$, the ratio $S_f/S$, and the true quantum yield $\phi$. The program then calculates, relative to $I_o = 1$, the quantities $i(0)$, $j(L)$, $j_o(L)$ and incident light absorbed, $i_f(0)$, $j_f(L)$, and fluorescent light reabsorbed, $i_f(L)$ (=0) as a check on the program, and the apparent quantum yield $\phi_{ap}$. If $\phi$ is set equal to 1 initially, the ratio of the apparent yield to it is obtained immediately.

One of the inputs, $SL$, must be calculated by Eq. 7 from the fraction of light transmitted by a non-absorbing suspension, measured in an apparatus that collects all transmitted light (for example, a Cary 14 spectrophotometer adapted for turbid samples). Where light is absorbed, $a$ may be calculated from the ratio of back-scattered exciting light in the absence of absorber, $j_o(L)$, to that in its presence, $j(L)$, through the implicit Eq. 36:

$$\frac{j_o(L)(1 + SL)}{j(L)\,SL} = a + b\coth(bSL). \qquad (36)$$

The ratio $S_f/S$ is calculated from the measured or extrapolated turbidities $\rho_0$ and $\rho_{f_0}$ at the two wavelengths in the absorption spectrum of the sample by Eq. 37, which is derived from Eq. 7:

$$\frac{1 + S_f L}{1 + SL} = 10^{\rho_{fo} - \rho_o} \qquad (37)$$

The parameter $a_f$ could be calculated from Eq. 36, but the appropriate wavelengths at which measurements should be made may not be known a priori. It could alternatively be calculated from $a$ and the absorbances $\rho$ and $\rho_f$ in the absorption spectrum of the sample by Eq. 38, which is derived from Eq. 5. A somewhat different procedure will however be used in the example that follows:

$$\frac{\Lambda_f}{b_f} = \frac{\Lambda 10^{\rho_f - \rho}}{b}. \qquad (38)$$

An example will illustrate the application of the correction to apparent quantum yields. The example is not intended to justify use of the procedure with an apparatus in which K & M conditions are not satisfied; this would require a much more detailed investigation. The apparatus was first calibrated with rhodamine 101 (a.k.a. rhodamine 640; Exciton Chemical Co., Dayton, OH), $3.56 \times 10^{-5}$ M in 95% ethanol in a 1-mm path length cell, for which $\phi = 0.96$ (9). The quantum yield of the dye in glycerol solution was then determined; it was corrected for refractive index difference between glycerol and ethanol, and multiplied by 5/6 to compensate for lack of rotational equilibration in glycerol (this factor overcorrects to some extent [10]). The resulting values were then $\phi = 0.91$ for 550-nm excitation and 0.90 for 530 nm. A highly scattering suspension was prepared by adding Ficoll (Pharmacia Fine Chemicals, Piscataway, NJ) and cellulose (Sigmacell, 20 $\mu$m; Sigma Chemical Co., St. Louis, MO) to the solution and the apparent quantum yield was determined as described. Estimates of $SL$ at various wavelengths were made by comparison of transmittances with those of an opal glass. The values of $a$ at 550 and 530 nm were calculated by Eq. 36 from the reduction in back-scattered light. The transmitted absorption spectrum of the dye was unexpectedly weak, by a factor of ~2, for reasons that are not presently clear; $a_f$ values calculated from it by Eq. 38 were inconsistent with parameters obtained from back-scattering. Since $a - 1$ is proportional to $K$, which is in turn proportional to absorbance, values of $a_f - 1$ at several wavelengths, relative to values of $a - 1$ obtained from back-scattering, were calculated from the absorption spectrum of the dye in glycerol solution. This procedure appears to be self-consistent and does not depend on the absorption spectrum of a highly scattering suspension.

The fluorescence spectrum was divided into 10 regions of equal area (except the last). Each region was corrected for instrumental sensitivity, and the factor $\phi_{ap}/\phi$ was calculated for each area separately. Fig. 2 shows the absorption and uncorrected fluorescence spectra of rhodamine 101 in glycerol, the integrated fluorescence yield corrected for sensitivity and then multiplied by the factor

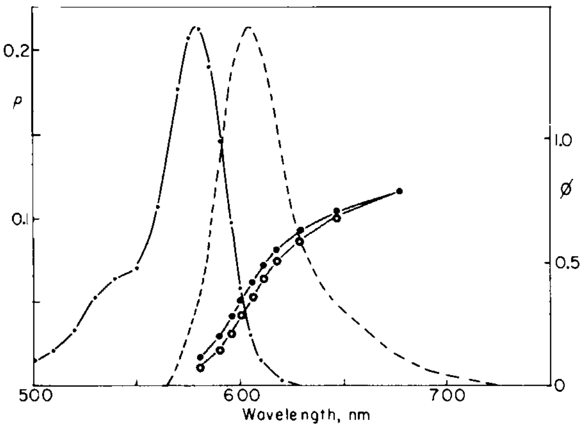

FIGURE 2 Absorption spectrum of rhodamine 101 in glycerol (*solid line*, left absorbance [$\rho$] scale), uncorrected fluorescence spectrum in glycerol-Ficoll-cellulose suspension (*dashed line*, normalized at peak to absorption spectrum), the cumulative apparent quantum yield, $\phi_{ap}$, under 550-nm excitation corrected for instrumental sensitivity (*open circles*, right scale), and the cumulative quantum yield corrected by ratio $\phi_{ap}/\phi$ as described in text (*solid circles*). The two integrals happen to coincide in this example.

$\phi/\phi_{ap}$. It is fortuitous that the integrated apparent quantum yield at 550-nm excitation is almost exactly the same as the corrected quantum yield, viz. 0.800. With 530-nm excitation, a correction of ~1% was calculated, bringing the quantum yield for that wavelength up to 0.794. These values are smaller than those for glycerol solution by ~12%. It would not be surprising if a systematic error were responsible for the difference, but it may also be that some of the rhodamine was adsorbed to the Ficoll or cellulose particles so as to fluoresce less efficiently. Since the conditions of these fluorescence measurements are fairly typical, the conclusion that may be drawn from them is that calculated correction factors are in general not likely to be large, and that the apparent quantum yield will be a good approximation to the true yield in many cases.

## Correction for Reemission

The equations derived so far take reabsorption of fluorescence into account as a matter of course but make no allowance for reemission of fluorescence. However, this can easily be done, at least within the approximation that the fluorescence can be treated as monochromatic.

Reemission is brought into Eqs. 9 and 10 simply by adding a source term dependent on $i_f$ and $j_f$.

$$\frac{\mathrm{d}i_f}{\mathrm{d}x} = (S_f + K_f)i_f - S_f j_f - \frac{1}{2}\phi K(i + j) - \frac{1}{2}\phi K_f(i_f + j_f) \qquad (39)$$

$$\frac{\mathrm{d}j_f}{\mathrm{d}x} = -(S_f + K_f)j_f + S_f i_f + \frac{1}{2}\phi K(i + j) + \frac{1}{2}\phi K_f(i_f + j_f). \qquad (40)$$

Combining terms shows that these equations are equivalent to Eqs. 9 and 10 if the substitutions

$$S_f \rightarrow S_f(1 + \phi(a_f - 1)/2) \qquad (41)$$

$$K_f \rightarrow S_f(a_f - 1)(1 - \phi) \qquad (42)$$

are made. These substitutions imply

$$a_f \rightarrow \frac{a_f - \phi(a_f - 1)/2}{1 + \phi(a_f - 1)/2}. \qquad (43)$$

Since the equations are no longer linear in $\phi$, they must be solved by an iterative procedure: first Eqs. 9 and 10 are solved as usual, then with the value of $\phi$, i.e., $[\phi/\phi_{ap}] \cdot \phi_{ap}$, in the substitutions Eqs. 41 and 43 until self-consistency is obtained.

If this correction procedure is applied to the example described above, the quantum yield for 550-nm excitation converges to 0.712. However, it is not clear that any correction for reemission should be made on yields from our apparatus, since much of the light will be reemitted from regions not observed by the detecting optics.

We conclude by presenting plots of the ratio $\phi_{ap}/\phi$ as a function of the independent variables $a$, $a_f$, and $SL$. If there is no reabsorption of fluorescence ($a_f = 1$), the ratio is always greater than 1, but approaches values quite close to 1 as $SL$ increases (Fig. 3). This justifies use of apparent quantum yields as approximations to true yields, especially when the scattering power of the sample is large. The ratio diverges as $SL$ becomes small, apparently because $j_0(L) - j(L)$ approaches zero more rapidly than $j_f(L)$ does. Fig. 4 demonstrates the effect of reabsorption of fluorescence ($a_f > 1$) on the ratio $\phi_{ap}/\phi$. For moderate values of the parameters, the effect of reabsorption largely compensates

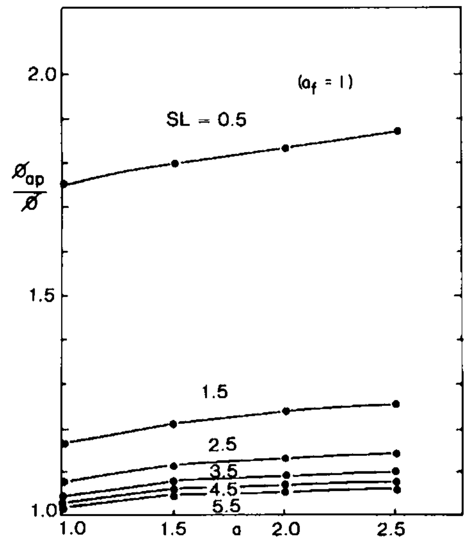

FIGURE 3 Dependence of the correction factor $\phi_{ap}/\phi$ on the parameter $a$ for selected values of the scattering power $SL$ when there is no reabsorption of fluorescence ($a_f = 1$).

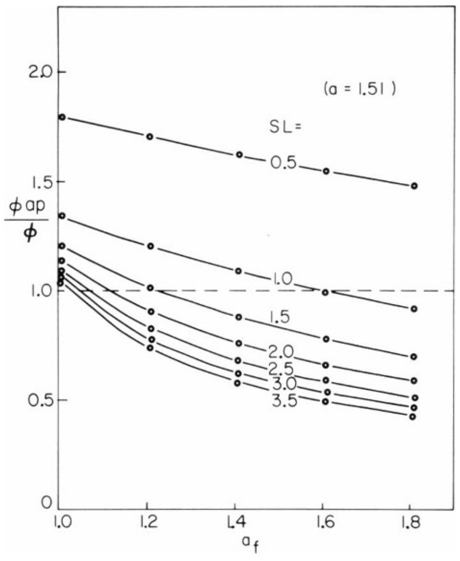

FIGURE 4 Variation of the correction factor $\phi_{ap}/\phi$ with the reabsorption parameter $a_f$, for selected values of $SL$, and $a = 1.51$.

for the effect of fluorescence dissymmetry, so that values of the ratio within 20% of unity are commonplace.

A test of the internal consistency of the derived equations is the observed convergence of values calculated for the general case (Eqs. 19 and 20) to those calculated for the special cases (Eqs. 27, 28, 34, and 35) as $a_f \rightarrow 1$ or $b_f S_f \rightarrow bS$. It is further evident from Fig. 3 that in the absence of reabsorption, the ratio $\phi_{ap}/\phi$ closely approaches 1 as $SL$ increases. This is to be expected, because at infinitely high scattering power ($SL$), light can escape only from the front face. It can be shown analytically that $\phi_{ap} \rightarrow \phi$ as $SL \rightarrow \infty$. If $SL \rightarrow \infty$ while $S$ remains finite, $\cosh(bSL) \rightarrow \sinh(bSL)$, and $j_o(L) - j(L) \rightarrow I_o(a + b - 1)/(a + b)$. Similarly, from Eq. 28, as $SL \rightarrow \infty$, $j_f(L) \rightarrow I_o\phi(a + b - 1)/(a + b)$, so that $\phi_{ap} \rightarrow \phi$.

Unfortunately, most fluorimeters are not constructed to conform to K & M boundary conditions, i.e., diffuse incidence of exciting radiation and $2\pi$ detection. Nevertheless, the equations derived here should enter into consideration in two distinct ways. When K & M conditions are closely approximated, as in coatings and biological tissues exposed to daylight, distribution of emission from a fluorophor within them could be calculated. In an apparatus such as ours with narrow cones of incident and intercepted fluorescent light, proper calibration and estimation of effective parameters should make possible consistent calculations of relative quantum yields. With our samples, the angular distribution of transmitted light is a particular concern, which may require closer examination. As the example of rhodamine shows, it may still be possible to get reasonable quantum yield estimates even in the absence of K & M conditions.

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