

Cell studies of total internal reflection fluorescence: effect of lipid membranes

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Summary

The intensity of the fluorescence generated when cell-substratum contacts are studied by total internal reflection fluorescence is calculated. In previous work, some approximations were made in respect of the effect of the lipid membranes. These approximations are removed in the present treatment.

Key words: internal reflection fluorescence, lipid membranes, adhesion.

Exact solution for fluorescent intensity

The method of frustrated total internal reflection has been shown to be especially powerful for the study of the cell-substratum contact region and indeed is the only optical method capable of giving the nanometre resolution needed for such studies. It makes use of the fact that the evanescent wave decays to a low value over a distance of the order of that of the light wavelength. The fluorescence generated by the evanescent wave falls very rapidly in the first few nanometres from the substratum surface and ceases if it meets a cell surface. Thus small variations in cell-substratum separations are converted into measurable intensity fluctuation of the emission from fluorophores contained in the immersion fluid.

In a previous paper by Gingell *et al.* (1987), the theory of this process was given. In this analysis it was assumed that the amplitude of the evanescent wave had fallen effectively to zero at the surface of the cell remote from the one in contact with the substratum. Thus the field intensities were calculated in the cell-substratum region, in the lipid layer in contact and in the cytoplasm, the latter in effect being assumed to be semi-infinite in extent. This assumption will be justified if the cell thickness is sufficiently large, but will become less valid for a flattened cell profile. This paper treats the more realistic model in which fields are calculated in all the cell regions. The exact results will thus enable cell-substratum separations to be deduced in cases where the neglect of the lipid layer remote from the contact is not justified. Calculations are made for a cell with a cytoplasmic thickness of 100 nm. For this case, the error involved in using the simplified treatment is very small.

In the previous paper, fluorescence arising beyond the

cell was estimated by assuming the lipid membrane in contact to be of zero thickness. It was clear that for cytoplasmic thicknesses much below 100 nm this effect could introduce significant errors. Simple adaptation of the procedure given here enables the intensity of fluorescence beyond the cell to be calculated without the neglect of the effects of the lipid layers. In place of the simplified model of Gingell *et al.* (1987) the more appropriate model is illustrated in Fig. 1. The incident medium, with progressive waves of amplitude, A (incident) and A' (reflected), is of refractive index n_1 . The propagation constant in this region γ_1 is given by:

$$\gamma_1^2 = n_1^2 k_0^2 - k_z^2, \quad (1)$$

where $k_z = n_1 k_0 \sin \phi$, ϕ being the angle of incidence and $k_0 = 2\pi/\lambda$.

Layers 2 and 6 represent the immersion medium, 3 and 5 the lipid membranes and 4 the cytoplasm. Waves in all but the incident medium are assumed to be evanescent, with propagation constants β_i ($i=2, 3, 4, 5, 6$), where

$$\beta_i^2 = k_z^2 - n_i^2 k_0^2. \quad (2)$$

Equating the tangential components of the electric and magnetic vectors at the five boundaries yields ten simultaneous equations, from which all the wave amplitudes $B, C, \dots, F, E', D', \dots, A'$ may be determined in terms of the incident amplitude A , and the field strength may thus be calculated at any point in the system. For internal reflection fluorescence measurements, the region of main interest is layer 2. We give below the expressions needed to determine the field-strength in this layer. The results are calculated for the model cell considered by Gingell *et al.* (1987), but with lipid membranes included so that

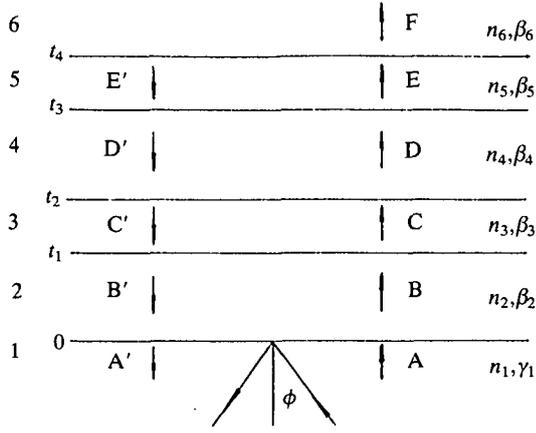


Fig. 1. Model of layered system: 1, glass; 2, water; 3, lipid membrane; 4, cytoplasm; 5, lipid membrane; 6, water.

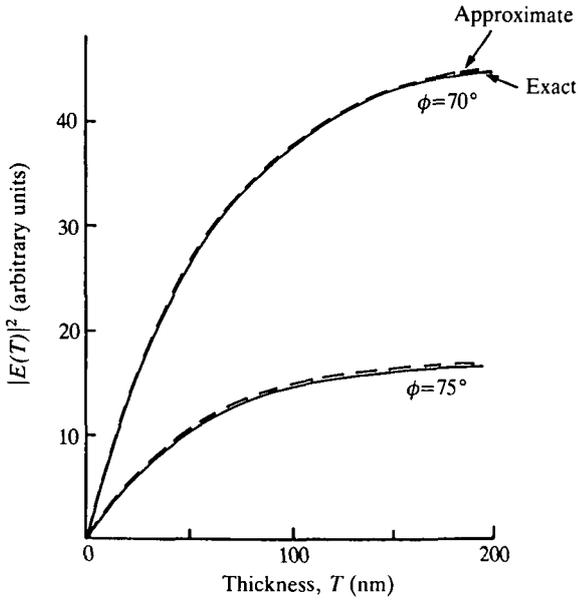


Fig. 2. Calculated values of $|E(T)|^2$: broken curve, $t_3 \rightarrow \infty$; continuous curve, exact theory, for parameters given in text.

the approximate and more-exact predictions can be compared.

We set:

$$\phi_i = \beta_i (t_{i-1} - t_{i-2}) \quad (i = 3, 4, 5) \quad (3)$$

$$a_{ij} = \exp(\beta_i t_j); \quad a_{ij}^* = \exp(-\beta_i t_j) \quad (4)$$

$$\left. \begin{aligned} a &= (\beta_4 + \beta_3) a_{31}^* a_{32} + (\beta_4 - \beta_3) a_{31} a_{32}^* \\ b &= (\beta_4 - \beta_3) a_{31}^* a_{32} + (\beta_4 + \beta_3) a_{31} a_{32}^* \\ e &= \cosh \phi_4 (a \beta_4 \beta_5 \cosh \phi_3 + b \beta_3 \beta_4 \sinh \phi_3) \\ &\quad + \sinh \phi_4 (b \beta_4^2 \cosh \phi_3 + a \beta_3 \beta_5 \sinh \phi_3) \\ f &= \cosh \phi_4 (a \beta_4 \beta_5 \sinh \phi_3 + b \beta_3 \beta_4 \cosh \phi_3) \\ &\quad + \sinh \phi_4 (b \beta_4^2 \sinh \phi_3 + a \beta_3 \beta_5 \cosh \phi_3) \end{aligned} \right\} \quad (5)$$

$$\left. \begin{aligned} p_1 &= \beta_2 \beta_6 a_{64}^* (e \beta_3 \cosh \phi_2 + f \beta_2 \sinh \phi_2) \\ q_1 &= \gamma_1 \beta_6 a_{64}^* (f \beta_2 \cosh \phi_2 - e \beta_3 \sinh \phi_2) \end{aligned} \right\} \quad (6)$$

$$r_1^2 = p_1^2 + q_1^2 \quad (7)$$

$$\left. \begin{aligned} s &= \cosh \phi_3 \cosh \phi_5 [\beta_3 \beta_4 \beta_5 (\beta_2 + \beta_6) \cosh \phi_4 \\ &\quad + \beta_3 \beta_5 (\beta_2 \beta_6 + \beta_4^2) \sinh \phi_4] \\ t &= \cosh \phi_3 \sinh \phi_5 [\beta_3 \beta_4 (\beta_2 \beta_6 + \beta_5^2) \cosh \phi_4 \\ &\quad + \beta_3 (\beta_2 \beta_5^2 + \beta_4^2 \beta_6) \sinh \phi_4] \\ u &= \sinh \phi_3 \cosh \phi_5 [\beta_4 \beta_5 (\beta_2 \beta_6 + \beta_3^2) \cosh \phi_4 \\ &\quad + \beta_5 (\beta_2 \beta_4^2 + \beta_3^2 \beta_6) \sinh \phi_4] \\ v &= \sinh \phi_3 \sinh \phi_5 [\beta_4 (\beta_2 \beta_5^2 + \beta_3^2 \beta_6) \cosh \phi_4 \\ &\quad + (\beta_2 \beta_4^2 \beta_6 + \beta_3^2 \beta_5^2) \sinh \phi_4] \end{aligned} \right\} \quad (8)$$

$$q_2 = \gamma_1 a_{64}^* a_{21} (s + t + u + v) \quad (9)$$

$$\left. \begin{aligned} w &= \cosh \phi_3 \cosh \phi_5 [\beta_3 \beta_4 \beta_5 (\beta_6 - \beta_2) \cosh \phi_4 \\ &\quad + \beta_3 \beta_5 (\beta_4^2 - \beta_2 \beta_6) \sinh \phi_4] \\ x &= \cosh \phi_3 \sinh \phi_5 [\beta_3 \beta_4 (\beta_5^2 - \beta_2 \beta_6) \cosh \phi_4 \\ &\quad + \beta_3 (\beta_4^2 \beta_6 - \beta_2 \beta_5^2) \sinh \phi_4] \\ y &= \sinh \phi_3 \cosh \phi_5 [\beta_4 \beta_5 (\beta_3^2 - \beta_2 \beta_6) \cosh \phi_4 \\ &\quad + \beta_5 (\beta_3^2 \beta_6 - \beta_2 \beta_4^2) \sinh \phi_4] \\ z &= \sinh \phi_3 \sinh \phi_5 [\beta_4 (\beta_3^2 \beta_6 - \beta_2 \beta_5^2) \cosh \phi_4 \\ &\quad + (\beta_3^2 \beta_5^2 - \beta_2 \beta_4^2 \beta_6) \sinh \phi_4] \end{aligned} \right\} \quad (10)$$

$$q_3 = \gamma_1 a_{64}^* a_{21} (w + x + y + z) \quad (11)$$

The field strength E at a depth T in medium n_2 , where the main fluorescence is generated, is given by:

$$E(T) = \mathcal{Y}(T) + iK(T), \quad (12)$$

where

$$\left. \begin{aligned} \mathcal{Y}(T) &= -\frac{q_1 q_2}{r_1^2} \exp(-\beta_2 T) + \frac{q_1 q_3}{r_1^2} \exp(\beta_2 T) \\ K(T) &= -\frac{p_1 q_2}{r_1^2} \exp(-\beta_2 T) + \frac{p_1 q_3}{r_1^2} \exp(\beta_2 T) \end{aligned} \right\} \quad (13)$$

The fluorescent flux generated is proportional to $|E(T)|^2$, where $|E(T)|^2 = [\mathcal{Y}(T)]^2 + [K(T)]^2$. (14)

Curves of $|E(T)|^2$ versus T are shown in Fig. 2 for the following parameters: $n_1=1.539$ (glass), $n_2=n_6=1.337$ (aqueous medium), $n_3=n_5=1.45$ (lipid membrane), $n_4=1.37$ (cytoplasm), $t_2-t_1=t_4-t_3=4$ nm, $t_3-t_2=100$ nm, $\lambda=488$ nm. The broken curves are obtained by putting t_2-t_1 and t_4-t_3 equal to zero and $n_6=1.337$, corresponding to the treatment described by Gingell *et al.* (1987). The largest discrepancy, for $T=200$ nm and angle of incidence 70° , amounts to less than 0.5%, so justifying the use of the approximate model for the parameters used.

References

- GINGELL, D., HEAVENS, O. S. AND MELLOR, S. J. (1987). General electromagnetic theory of total internal reflection fluorescence: the quantitative basis for mapping cell-substratum topography. *J. Cell Sci.* **87**, 677-693.

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