J. Cell Sci. 49, 237–247 (1981)
Printed in Great Britain © Company of Biologists Limited 1981

THE INTERPRETATION OF INTERFERENCE-REFLECTION IMAGES OF SPREAD CELLS: SIGNIFICANT CONTRIBUTIONS FROM THIN PERIPHERAL CYTOPLASM

DAVID GINGELL
Department of Biology as Applied to Medicine, The Middlesex Hospital Medical School, London W1P 6DB, England

SUMMARY

In interference-reflection microscopy, used for investigating cell–substratum separation, it is commonly believed that cytoplasmic thickness can be ignored, provided a high illuminating numerical aperture (INA) is used. It is shown here that even when a maximal INA is used, cytoplasmic lamellae of 1 μm or less can be major determinants of the image. The leading lamella of spreading tissue cells and large peripheral areas of *Dictyostelium discoideum* amoebae on adhesive substrata are less than 1 μm thick and it is argued that hitherto unexplained features of the interference images of these cells may be interpreted in terms of the theory used here.

INTRODUCTION

The technique of optical interferometry using incident light illumination is becoming widely used in the study of cell adhesion and locomotion. Following the lead of Curtis (1964), Izzard & Lochner (1976), in an important and innovative study, described localized optically dense areas of close apposition between fibroblasts and a solid substratum, which they termed adhesion plaques. Widespread interest was generated by this work and it was subsequently demonstrated that cytoplasmic stress fibres terminated at adhesion plaques (Heath & Dunn, 1978). Their role as adhesive specializations of the cell surface has been carefully investigated by Rees, Lloyd & Thom (1977) who observed the interference image of cells exposed to trypsin and a chelating agent.

There is little doubt that adhesion plaques are discrete adhesive regions where the cell surface is intimately applied to the substratum, although the actual distance from substratum to the membrane bilayer is uncertain. However, other interferometric features of spread cells lend themselves less readily to interpretation. For example, the interference micrographs of Thom, Powell & Rees (1979), fig. 8 (frame D especially), and also of Haemmerli & Ploem (1979), show continuous dark fringes, which sometimes appear quite distinctly as fringes of successive order, rising from the thinnest edges of spread cells towards the thicker central region. Similarly, we have observed that amoebae of the cellular slime mould *Dictyostelium discoideum* when newly spread on a highly adhesive surface such as polylysine-treated glass or silanated glass (Vince & Gingell, 1980) produce 'fried egg' forms within a minute or so. The periphery

appears as a broad optically black region of zero order (as ascertained from colour sequences in white light); followed centripetally, by up to half a dozen higher-order fringes as the thicker nuclear region is approached. We were initially under the impression that these broad zero-order black regions represented areas where contact with the substratum was closer than elsewhere. However, scanning electron microscope studies (Vince & Gingell, unpublished) showed that the edges of such cells were spread out as very thin lamellae, and preliminary transmission electron microscopy has indicated a uniform contact between cell and substratum on these very strongly adhesive surfaces. These observations prompted calculations of the contribution of thin cytoplasm to the interference image, using the technique of Gingell & Todd (1979). This method allows us to compute the brightness of image areas seen in the interference microscope by combining all reflected wave vectors of light which illuminate the object from the entire illuminating aperture of the objective.

ANALYSIS OF A MODEL

Images of thin cytoplasm

The peripheral region of a spread cell, being essentially a wedge of small angle, is a legitimate object for the application of our analytical method. Fig. 1A shows the

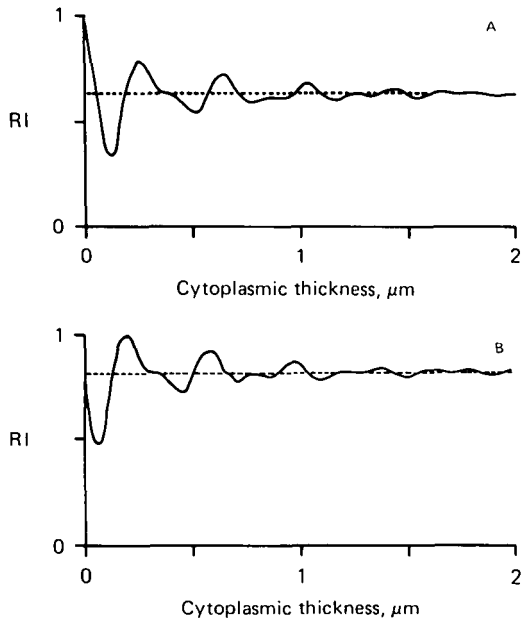

Fig. 1. A. Relative irradiance (RI) *versus* cytoplasmic thickness (μm) for a zero water gap between cell and glass. INA = 1·18. The asymptote for limiting thick cytoplasm is shown as a dotted line. Background brightness, 1·0. B. As 1A but water gap = 50 nm.

relative brightness (or relative irradiance, RI, defined as photon flux reflected from object/flux from background) of the edge of a cell spread on glass with no aqueous separation. The illuminating numerical aperture (INA) is 1·18, corresponding to a

cone of light of solid angle ~ 100°. It is apparent that fringes will be seen as the cytoplasm thins from around 1 μm. Only strongly damped fringes are seen for cytoplasmic thicknesses greater than 1·0 μm (the graph extends to 2 μm but computations have been carried out to several μm). If the aperture of illumination is now cut down, giving INA = 0·69 (Fig. 2A), a series of strongly oscillating fringes appears, extending out to 2 μm cytoplasm. We therefore see that despite the uniform cell–substratum distance (zero μm in this example) *thin cytoplasm alone is sufficient to generate interference fringes*. Their amplitude increases as the cytoplasm gets thinner, and their number increases as the illuminating aperture is reduced. For zero cell–substratum

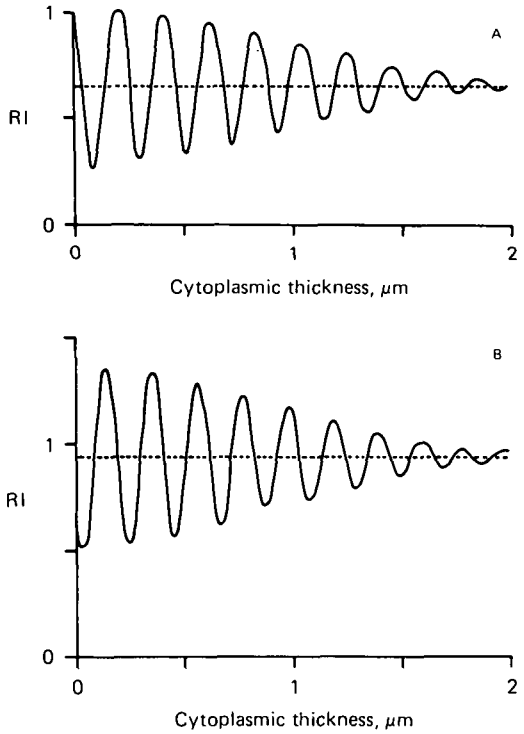

Fig. 2. A. As 1A except that INA = 0·69. B. As 1B except that INA = 0·69.

separation the relative irradiance shows damped oscillations, which converge to a constant value independent of INA as the cytoplasmic thickness increases to 1 or 2 μm. When the cell–substratum separation is zero, the RI tends to a value near 1·0 at the limit of zero cytoplasmic thickness, regardless of INA. If the aperture is then increased most of the fringes are damped out, but the zero-order black region remains practically constant (RI ~ 0·3), where the cytoplasm is about 0·1 μm thick.

Next, suppose the cell to be separated from the glass substratum by a uniform gap of, say 50 nm (Figs. 1B, 2B). The same general tendencies will be apparent, but the main difference is that as the cytoplasm becomes 'thick' (1 or 2 μm) the RI will approach distinctly different mean values which depend upon the INA chosen; as the aperture is decreased from 1·18 to 0·69 the image of the thick cytoplasm changes in

RI from 0·8 to 0·95. At the other extreme, for INA = 0·69, as the cytoplasm thins from 2 μm we see fringes ranging from RI = 0·5 (darkest) to 1·3 (brightest); on increasing the INA from 0·69 to 1·18, the number of fringes again decreases, and the darkest regions again are almost invariant with aperture (though brighter at 50 nm separation than zero water gap). Comparison of Figs. 1B and 2B brings out a further point of distinction: there is a lateral shift of the fringes with changing INA. Thus, a 0·1 μm cytoplasmic lamella separated from the substratum by a uniform water gap of 50 nm would appear darker than background (RI = 0·6) at high aperture, INA = 1·18, but brighter than background (RI = 1·1) at INA = 0·69.

Now imagine a cell making adhesion plaques in intimate contact with the substratum (zero separation assumed) where the cytoplasm is 1 μm thick, and also extending a lamellipodium tapering down to 0·05 μm, separated by 50 nm from the substratum. We see (Fig. 1B) that at INA = 1·18 the lamellipodium has an RI of 0·48, whereas the adhesion plaque gives a larger value of 0·68. The essential point is that the cytoplasmic lamellae can appear in the interference microscope as dark or darker than adhesion plaques, even when the latter are considered to be in molecular association with the substratum and the lamellae are separated by several tens of nanometres. Furthermore, the 'grey' regions (monochromatic light), which can appear where the cytoplasmic thickness is less than 1 μm, arise from thin cytoplasm and do not primarily give information about cell–substratum contact distances.

How can useful information be obtained from interference images?

An unambiguous measurement of cell–substratum separation requires an independent measurement of cytoplasmic thickness, by either transmission interferometry or electron microscopy. This requirement can, however, be waived if it is known that the cytoplasm exceeds a certain minimal thickness, related to the illuminating numerical aperture; for INA = 1·18 this is about 1 μm. The critical thickness increases as the INA decreases, until at INA = 0·69 it is about 2 μm. It is nevertheless possible to get some idea of separations by reflection measurements alone when thickness is unknown. For example, suppose opening the aperture from 0·69 to 1·18 produces no effect on RI, which remains at about 0·6, the most likely interpretation is that the cytoplasmic thickness exceeds 2 μm and there is no water gap. If RI $\sim$ 0·3 then the cytoplasm is probably 0·1 μm thick and the water gap is absent. If there is a gap between cell and substratum the 0·69 and 1·18 images can differ; the values of RI will be constant in regions where the cytoplasm is thicker than 1 or 2 μm but will vary where it is less.

Analysis of the computed results

It is far easier to generate fringe diagrams from the computer than it is to understand how they arise. Physical intuition can be elusive in cases where multiple films are illuminated by wide cones of light. However, there are several features of the results presented which can be appreciated on a semi-quantitative footing.

Case 1. Asymptote of image brightness (RI $\sim$ 0·6) as cytoplasmic thickness increases and cell–substratum separation remains zero. The mathematical analysis given by Gingell & Todd (1979) shows that for a symmetrical glass/water/glass film a common

asymptote of 2·0 is reached as film thickness increases, regardless of INA. If we neglect the lipid membranes, which contribute little in the present case, we see that the cell model reduces to an asymmetric 3-layer sandwich, glass/cytoplasm/water, which also approaches a constant value as cytoplasmic thickness increases, regardless of INA. Much more extensive analysis would be needed to show why the particular value approached is ~0·6.

Case 2. Zero cytoplasmic thickness with zero water gap gives an image equal to background brightness. For all INA values considered (0·45, 0·69, 0·91 and 1·18, though graphs are given only for 0·69 and 1·18) the RI value reached is close to 1·0. Here the situation reduces to a single ~8 nm lipid film (2 membranes without intervening cytoplasm) between glass and water. For this finite thin film there is reinforcement between waves from the N_1/N_2 (glass/membrane) and N_2/N_3 (membrane/water) interfaces; there is no phase change on reflection, because $N_1 > N_2 > N_3$.

Analysis (see Appendix) shows that for the general case the multiply reflected wave from a vanishingly thin film of refractive index N_2 is equal in amplitude to that reflected from the N_1/N_3 interface with no film present. Thus for a sufficiently thin lipid layer the RI will be close to 1, as seen in Figs. 1A and 2A. Where there is an additional thin film due to a water gap (Figs. 1B, 2B) this no longer holds true.

Case 3. Minimal image brightness. In Fig. 3 we model a cell stuck intimately to glass, without a water gap.

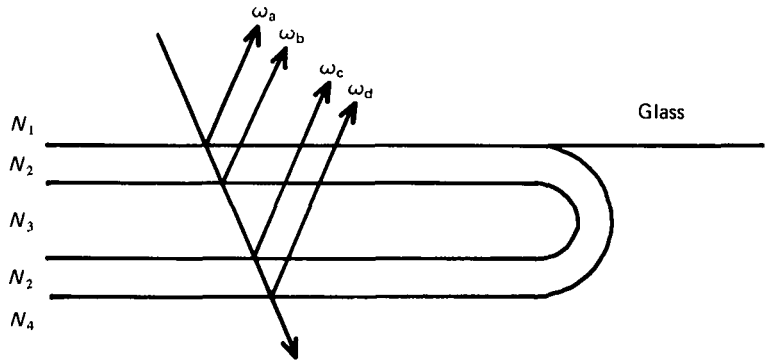

Fig. 3. Waves reflected from a multilayer representing a cell stuck to glass without a water gap. Wave ω_a is reflected from the glass–membrane interface.

There is a strong wave from the (upper) film bounded by the glass and a weaker one of the same phase from the (lower) film bounded by water on the far side of the cell. Quite useful knowledge can be gained in this case by a simple normal incidence analysis. Amplitudes and phases (+/−) are as follows, writing A for the amplitude of wave ω_a etc:

$$\text{Glass–membrane} \quad A \approx \frac{1{\cdot}52 - 1{\cdot}45}{1{\cdot}52 + 1{\cdot}45} = (+)\ 0{\cdot}024$$

$$\text{Membrane–cytoplasm} \quad B \approx \frac{1{\cdot}45 - 1{\cdot}37}{1{\cdot}45 + 1{\cdot}37} = (+)\ 0{\cdot}028$$

$$\text{Membrane–cytoplasm} \quad C \approx -B \qquad = (-)\ 0{\cdot}028$$

$$\text{Membrane–medium} \quad D \approx \frac{1{\cdot}45 - 1{\cdot}33}{1{\cdot}45 + 1{\cdot}33} = (+)\ 0{\cdot}043$$

The amplitude of the wave from the upper film is 0·024 + 0·028 = 0·052. That from the lower film is 0·043 − 0·028 = 0·015. When the cytoplasm imposes a phase difference of π between waves from the upper and lower films the resultant wave will have a minimum amplitude of 0·052 − 0·015 = 0·037. Since the background amplitude is given by the Fresnel coefficient for the glass/water reflection (0·0666) we find the RI for minimum brightness is $(0{\cdot}037/0{\cdot}0666)^2 = 0{\cdot}31$, which corresponds very closely with the values in Figs. 1 and 2.

The cytoplasmic thickness which gives a phase difference of π and hence minimum brightness, is readily obtained for the case of zero water gap. An optical path difference of $\lambda/2$ gives a phase difference of π, thus the corresponding thickness for normal incidence is given by $2nd = \lambda/2$, i.e. $d = \lambda/4n$, where n is the cytoplasmic refractive index. Thus, for green light, $d = 546/5{\cdot}480 \sim 0{\cdot}1\ \mu$m. This is of course an approximation, since in the computer model a finite cone of light is used; nevertheless 0·1 μm is close to the computed values (0·13 μm and 0·08 μm).

DISCUSSION

Izzard & Lochner (1976) have stated that the use of a high illuminating numerical aperture (INA > 1·0) results in negligible contributions to the final image arising from reflections generated by the far side of a cell and claim that under this condition the image gives information exclusively about the separation of the cell from the adjacent substratum. They recognize, however, that at lower INA (0·84) cytoplasmic lamellae several tenths of a micron thick can give rise to fringes. Our analysis has shown that when the cytoplasmic thickness is < 1 μm reflections from the far side of the cell can have profound effects on the image, even at INA > 1·0. Cells which flatten and spread on smooth solid substrata, such as fibroblasts or epithelia in culture, produce one or more peripheral fringes apparently generated by thin cytoplasm, which are predicted by the mathematical model. Polymorphonuclear leucocytes on glass in a salt solution (Gey's medium) can produce a broad black circumferential lamella, which is practically indistinguishable from that produced by *D. discoideum* on strongly adhesive substrata (Keller, Barandun, Kistler & Ploem, 1979). The edges of cultured cells can taper to less than 1·0 μm; a vertical section through the leading lamella of a chick heart fibroblast spread on glass shows a wedge thinning uniformly from 0·3 μm to less than 0·05 μm over a distance of 10 μm (J. P. Heath, personal communication; see also Abercrombie, 1980, fig. 9, and Heath & Dunn, 1978). This is precisely the range of thickness in which thin cytoplasm is predicted to generate strong interference fringes. One or more dark fringes are frequently seen at or very close to the leading edge, following its contour (see Thom, Powell & Rees, 1979, fig. 8D, where a fringe ~ 1 μm wide is to be seen at the upper margin of the cell). A dark fringe is predicted by the model to occur where the cytoplasm is 0·05–0·13 μm thick

and another occurs near 0·5 μm; the exact values depend on the cell–substratum separation. The separation range investigated is from 0–50 nm. These values refer to INA = 1·2, where a minimal number of fringes due to thin cytoplasm occur; at INA = 0·69 (Fig. 2A, B) many more fringes are generated by a wedge of cytoplasm up to 1 μm in thickness.

In the case of *D. discoideum* amoebae spreading on polylysine-treated glass or on methylated glass, the broad black or blackish-blue band formed at the margin can be interpreted as arising from thin cytoplasm. This is described further by Vince & Gingell (unpublished). In white (xenon arc) light, the peripheral black/blue is always darkest (and blackest) at the edge of the cell: no separation of this band into red and blue is ever seen and with increasing optical path difference, a whitish colour, typical of the zero-order fringe is evident. One should not, however, place too much reliance on colours when discussing complex films, for no treatment, even semi-quantitative, exists. The observation of an immediate loss of the dark fringe on treatment with the detergent C_{16}TAB is consistent with its arising from a thin slab of cytoplasm. Even more convincing is the fact that at λ = 546 nm the RI of this band is far too low to be attributable to a thick cell of uniform refractive index making a contact of any distance whatsoever with the substratum; only if the cytoplasm is $\sim$ 0·1 μm thick can the results be rationalized and this thickness is within the range estimated by transmission- and scanning-electron-microscope techniques (Vince & Gingell, unpublished). In monochromatic light, λ = 546 nm, the central region of *D. discoideum* amoebae gives an RI value of $\sim$ 0·8 (INA = 1·18) corresponding to a 'thick' cell separated by a gap of $\sim$ 45 nm, previously found to be an irreducible minimum on treating the cells with high concentrations of monovalent and divalent cations (Vince & Gingell, 1980); it is likely that this separation is due to a carbohydrate coat on the plasma membrane, for which independent evidence exists (Hellio & Ryter, 1980). The dark periphery gives an RI $\sim$ 0·6 and this corresponds closely with a lamella 0·05–0·15 μm thick separated by $\sim$ 45 nm from the substratum. Although we have not yet made a quantitative photometric analysis of the peripheral fringes seen in the images of other cells in culture, our analysis predicts the correct number of fringes for chick limb bud mesenchyme cells. At INA = 0·69 about 10 fringes are discernible, with slight re-focusing on moving across the pattern; at INA = 0·91 (1·5 mm) about 5 fringes are seen, and at INA = 1·18 (2·0 mm) 2 or 3 are visible. Where fringes are generated by a wedge of appreciable angle the image plane will be curved, forming a Feussner surface, which will require appropriate focusing for viewing. The method of computation adopted here assumes the image to be coplanar, so it may well be that the higher-order fringe irradiances will be found to be in only more or less qualitative agreement with the theoretical prediction.

Bereiter-Hahn, Fox & Thorell (1979) observed Friend erythroleukaemia cells on polylysine-treated glass by interference-reflection microscopy. The authors took no account of cell thickness and concluded that the central and peripheral regions of the cell must have different refractive indices of 1·36 and 1·38, respectively. However, their own electron micrographs show that the peripheral cytoplasm is less than 0·1 μm thick and their densitogram showing relative irradiances of $\sim$ 0·7 (central) and

$\sim$ 0·5 (peripheral) is consistent with a cell having a 0·05–0·1 μm thick periphery and a uniform refractive index of 1·360, separated from the glass by 45 nm. For this calculation the angle of illumination was modelled as a cone from 10·6 to 31° appropriate to the Leitz system and the method of computation was slightly modified to take account of the state of polarization of the measuring beam in the Leitz system. While this result does not disprove the contention of Bereiter-Hahn *et al.* (1979) that the cytoplasmic refractive index is non-uniform, it does show that another interpretation is possible.

Izzard & Lochner (1980) have discussed in some detail the interference-reflexion image of the lamellipodium of chick heart fibroblasts. Their analysis is also based on the assumption that at high INA values no significant reflection is generated from the far side of the organelle. However, since this structure is far less than 1·0 μm thick the considerations outlined above also apply. The fringes in the image of the lamellipodium seen in fig. 2 of Izzard & Lochner's paper are probably due to its inclination to the substratum, as the authors claim; indeed, it is most unlikely that they are due to the lamellipodium being sufficiently wedge-shaped to generate 5 minima as this would need a wedge about 1·0 μm thick at its base, using an INA of 0·69. Nevertheless, a reflected component from the far side of the lamellipodium will be included in the image. This should be taken into account in attempting optical measurement of the separation distance of the lamellipodium from the substratum (see Izzard & Lochner, 1976).

The technique used for deducing separation distances by Izzard & Lochner (1976) is open to serious criticism. These authors deposited MgF_2 films of measured thicknesses on glass in order to increase path length and thereby alter the colours characterizing the cell–glass separation to a more sensitive region (see Michel-Levy interference colour chart, Zeiss, W. Germany, number S.41–500.0-e). They do not, however, take account of the wave strongly reflected from the MgF_2/medium interface; this technique would be legitimate only if the deposited film had a refractive index very close to that of the medium. Calculations for monochromatic light show that the error in using MgF_2 without allowing for the additional reflection renders the method useless as a quantitative technique. Further, it is not strictly legitimate to assess separations using colours where a large INA is employed, since colour is to some extent dependent on aperture. Consequently, there is no reason to accept the values of $\sim$ 10 nm for focal contacts and $\sim$ 300 nm for close contacts, insofar as these have been derived from interference-reflection microscopy. If it turns out that these are close to the actual separations the similarity will be fortuitous. Although electron microscopy (Heath, personal communication) shows separations of such dimensions, no quantitative assessment of the interference–reflection image before and after fixation and embedding has yet been reported, and it would be surprising if these processes involving drastic changes in material dielectric properties with concomitant changes in electrostatic forces, hydrogen bonding, electrodynamic attraction, etc. were not to modify the gap dimensions significantly.

While the available evidence certainly does not suggest that the concept of close contact is artefactual, insofar as such contacts are reported to exist at more central

regions of the cell where its thickness exceeds 1 μm, it is possible that in more peripheral thinner regions images of putative close contacts may be generated by thin cytoplasm. Even if close contacts are formed in these regions, their images will be modulated by waves reflected from the far side of the cell, even at the highest INA. The cell–substratum separation at close contacts remains to be measured photometrically.

The results presented here are not intended to form a detailed critique of work on the interference-reflection images of cells. They are meant only to inject a note of caution into the interpretation of cell images. A study of cell thickness in conjunction with quantitative interference-reflection image analysis is evidently required. Nevertheless, it seems that the peripheral few microns of spread cells fall into just the right thickness range in which interference fringes would be generated by the thin cytoplasm. It should be emphasized that this prediction could be made only after developing a mathematical model for finite INA. The perpendicular illumination approximation would predict that fringes would be generated all the way along a wedge of small angle, regardless of its thickness, whereas the finite INA model predicts that as the aperture is opened up the fringes retreat and become confined to the thinner end of the wedge. For INA = 0·69 they are significant only where the wedge is thinner than 2·0 μm, while for INA = 1·18 they are confined to the region where the wedge thickness is below 1·0 μm. Under these conditions, a cell making uniform contact over its entire lower surface with a flat substratum would be expected to have peripheral fringes but show a uniform intensity over the central region. Variations in cell–substratum separation further modulate this basic pattern.

REFERENCES

ABERCROMBIE, M. (1980). The crawling movement of metazoan cells. *Proc. R. Soc. B*, **207**, 129–147.

BEREITER-HAHN, J., FOX, C. H. & THORELL, B. (1979). Quantitative reflection contrast microscopy of living cells. *J. Cell Biol.* **82**, 767–779.

CURTIS, A. S. G. (1964). The mechanism of adhesion of cells to glass, a study of interference reflection microscopy. *J. Cell Biol.* **20**, 199–215.

GINGELL, D. & TODD, I. (1979). Interference reflection microscopy: a quantitative theory for image interpretation and its application to cell–substratum separation measurement. *Biophys. J.* **26**, 507–526.

HAEMMERLI, G. & PLOEM, J. S. (1979). Adhesion patterns of cell interactions revealed by reflection contrast microscopy. *Expl Cell Res.* **118**, 438–442.

HEATH, J. P. & DUNN, G. A. (1978). Cell to substratum contacts of chick fibroblasts and their relation to the microfilament system. A correlated interference-reflexion and high voltage electron microscope study. *J. Cell Sci.* **29**, 197–212.

HELLIO, R. & RYTER, A, (1980). Relationships between anionic sites and lectin receptors in the plasma membrane of *Dictyostelium discoideum* and their role in phagocytosis. *J. Cell Sci.* **41**, 89–104.

IZZARD, C. A. & LOCHNER, L. R. (1976). Cell to substrate contact in living fibroblasts: an interference reflexion study with an evaluation of the technique. *J. Cell Sci.* **21**, 129–159.

IZZARD, C. A. & LOCHNER, L. R. (1980). Formation of cell-to-substrate contacts during fibroblast motility: an interference-reflexion study. *J. Cell Sci.* **42**, 81–116.

KELLER, H. U., BARANDUN, S., KISTLER, P. & PLOEM, J. S. (1979). Locomotion and adhesion of neutrophil granulocytes. *Expl Cell Res.* **122**, 351–362.

REES, D. A., LLOYD, C. W. & THOM, D. (1977). Control of grip and stick in cell adhesion through lateral relationships of membrane glycoproteins. *Nature, Lond.* **267**, 124–128.

THOM, D., POWELL, A. J. & REES, D. A. (1979). Mechanisms of cellular adhesion. IV. Role of serum glycoproteins in fibroblast spreading on glass. *J. Cell Sci.* **35**, 281–305.

VINCE, S. & GINGELL, D. (1980). Cationic modulation of the interaction of *Dictyostelium discoideum* amoebae with glass. Evidence from quantitative interference reflection microscopy. *Expl Cell Res.* **126**, 462–465.

(*Received* 10 *September* 1980)

APPENDIX

A vanishingly thin film

Referring to Fig. 4. we introduce the Fresnel coefficients P_1, P_2, P_3

$$P_1^2 \equiv F_1 = \left(\frac{N_1 - N_2}{N_1 + N_2}\right)^2. \tag{1}$$

$$P_2^2 \equiv F_2 = \left(\frac{N_3 - N_2}{N_3 + N_2}\right)^2. \tag{2}$$

$$P_3^2 \equiv F_3 = \left(\frac{N_3 - N_1}{N_3 + N_1}\right)^2. \tag{3}$$

$$1 - F_1 = \alpha. \tag{4}$$

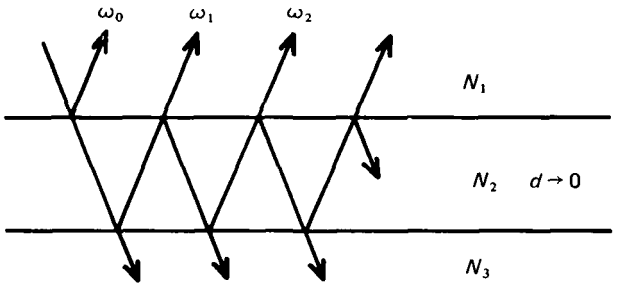

Fig. 4. Multiple reflections in a vanishingly thin film.

Individual reflected wave intensities in medium N_1 are

$$\omega_0 = F_1$$
$$\omega_1 = \alpha^2 F_2$$
$$\omega_2 = \alpha^2 F_1 F_2^2$$
$$\omega_3 = \alpha^2 F_1^2 F_2^3.$$

Thus
$$\omega_n = \alpha^2 F_1^{n-1} F_2^n. \tag{5}$$
$(n > 0)$

These waves combine to give a wave in medium N_1 of amplitude

$$A_T = F_1^{\frac{1}{2}} - \alpha \sum_{n=1}^{\infty} (F_1^{n-1} F_2^n)^{\frac{1}{2}}. \tag{6}$$

Using equations (1) and (2)

$$A_T = P_1 - (1 - P_1^2) \sum_{n=1}^{\infty} P_1^{n-1} P_2^n. \tag{7}$$

It is required to show that

$$A_T = P_3, \tag{8}$$

where P_3, the wave reflected from the N_1/N_3 interface when no film is present, is defined in terms of N_1 and N_3 in equation (3).

Expressing P_3 in terms of P_1 and P_2, using equations (1), (2) and (3), we obtain

$$P_3 = \frac{(1+P_2)(1+P_1)-(1-P_1)(1-P_2)}{(1+P_2)(1+P_1)-(1-P_1)(1-P_2)}. \tag{9}$$

Substituting for P_3 from equation (9) into (8), equating the result with equation (7) and cross-multiplying,

$$P_1 - P_2 = S(p)\,(1 - P_1 P_2),$$

$$\text{where } S(p) = P_1 - (1 - P_1^2)\,(P_2 + P_1 P_2^2 + P_1^2 P_2^3 \ldots . + P_1^n P_2^{n+1}).$$

Multiplying out we obtain the desired equivalence

$$P_1 - P_2 = P_1 - P_2.$$