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A microinterferometric method for analysis of rotation-symmetric refractive-index gradients in intact objects

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SUMMARY

A new method for measuring refractive-index gradients in rotation-symmetric objects is described. Interference micrographs of intact objects, with the symmetry axis perpendicular to the optical axis of the microscope, are used to obtain a phase-shift profile, which is converted into a refractive-index profile by a computer program. The conversion calculations are based on iterative approximations of the gradient slope in a number of presumed shells in the object. The method is fast, convenient and yields highly accurate results even when only a small number of phase shift values can be obtained. The new method is especially advantageous for analysing many invertebrate eyes but it can also be used for measuring manufactured graded-index optical fibres.

INTRODUCTION

For many years, interference microscopy has been employed to determine the refractive indices of small objects (Hale, 1958; Kornder, 1959). The phase shift of the object is revealed by the interference fringes. If the path distance through the object is also known, the refractive index can be calculated. Although the procedure is a trivial one for optically homogeneous objects, nonhomogeneous (graded-index) objects do not permit a straightforward measurement.

The present paper deals with the analysis of a special type of graded-index object which is characterized by rotational symmetry. Such objects are found in the optical systems of many arthropod eyes (Kunze, 1979), but manufactured graded-index optical fibres also have these characteristics.

The index gradient in a rotationally symmetric object can be described by refractive-index profiles, i.e. the refractive index as a function of the distance from the central axis. To reveal this index profile, the object can be cut into cross-sections of known thickness and examined in an interference microscope. The phase-shift profile, given by the interference fringes, can then be converted into a refractive-index profile by the same equation as for homogeneous objects:

$$n_R = n_0 + (\Gamma_R/d) \tag{1}$$

where n_R is the refractive index at the radius R, n_0 the refractive index of the homogeneous surrounding medium, Γ_R the phase shift at the radius R, and d the path length through the object.

This procedure has been used for both biological objects (Hausen, 1973) and manufactured graded-index fibres (Martin, 1974; Stone & Burrus, 1975). However, measurements on sections entail a number of disadvantages, viz: (i) precision cutting of the object is time consuming and difficult, sometimes impossible for biological objects; (ii) the object is destroyed and thus cannot be used afterwards, which might be unfortunate when dealing with manufactured optical fibres; (iii) the measurement beam in the interference microscope will be bent by the index gradient in the object and this will reduce the accuracy of the measured index values (Stone & Burrus, 1975); (iv) it is difficult to reconstruct the gradient in objects where the index profile varies along the central axis (e.g. the crystalline cone of arthropod compound eyes).

To avoid the above disadvantages and to expedite analysis, we have applied interferometry to an intact object with its symmetry axis perpendicular to the optical axis of the interference microscope (transverse interferometry) (Fig. 1). Thus the object beam in the interference

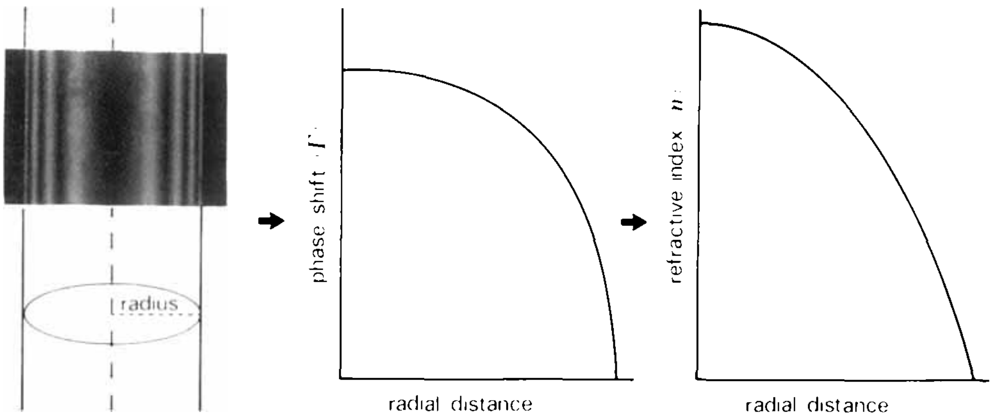

Fig. 1. The measurements of phase-shift profiles from transverse interferometry. The intact object is viewed from the side in the interference microscope. Each black interference fringe corresponds to a phase shift of one wavelength of light (inset). The position of the interference fringes is measured and used to plot a phase-shift profile. The present paper desctibes a method for the conversion of such phase-shift profiles into refractive-index profiles.

microscope will travel through a continuous gradient of refractive index necessitating computerized calculations to determine the index profile.

Methods for analysing transverse interference patterns in graded index optical fibres have been described (see Iga & Kokubun, 1978; Chu & Whitbread, 1979) but all of these methods require a very large number of phase-shift values for each profile, and thus are not suited for objects with only a few interference fringes. The present paper describes a calculation routine for conversion of a phase-shift profile, with few values, into a refractive index profile.

PRINCIPLE OF ANALYSIS

The task is to convert phase-shift profiles, obtained from transverse interferometry, to refractive-index profiles. In order to do this, we must consider the optical geometry during the phase-shift measurement (Fig. 2). The object beam is perpendicular to the symmetry axis of the object. The object is divided into a number of shells, whose refractive index is to be determined and, as a first approximation, the ray paths are assumed to be straight. Depending on position, the four rays in Fig. 2(a) travel different distances through the object, and they travel through a number of shells of different refractive index. From the interference fringes, the phase shift for each of these rays can be found (Fig. 1). The most peripheral ray (Fig. 2a) travels through shell 1′ only, and the average refractive index in this shell can be calculated by equation (1). Ray 2 will travel through shell 2′, but also through shell 1′. Because the refractive index of shell 1′, $n_{1'}$, has already been calculated, the phase shift that ray 2 will undergo in shell 1′ can be

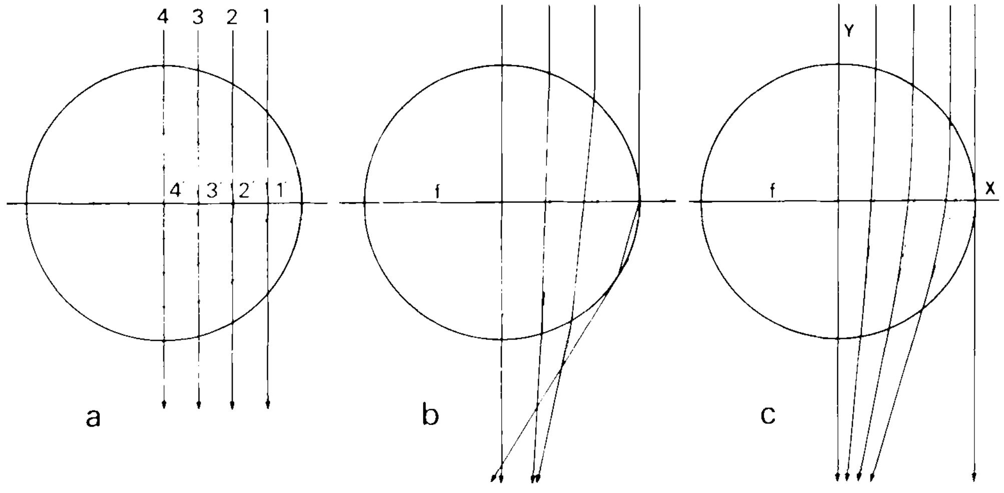

Fig. 2. The optical geometry in a given plane through the object in the interference microscope. (a) Four rays, 1–4, passing through the four corresponding shells 1′–4′. (b) The ray path in a homogeneous object with a higher refractive index than the surrounding medium; f is the focal plane of the microscope. (c) The curved ray path through a graded-index object with an increasing refractive index towards the centre.

calculated from

$$\Gamma_{2,1'} = d_{2,1'}(n_{1'} - n_0) \tag{2}$$

where $d_{2,1}$ is the path length of ray 2 in shell 1′. The phase-shift contribution from shell 1′, $\Gamma_{2,1}$, can be subtracted from the measured phase shift for ray 2, Γ_2, to obtain the phase-shift, $\Gamma_{2,2'}$, that ray 2 will experience in passing through shell 2′:

$$\Gamma_{2,2'} = \Gamma_2 - \Gamma_{2,1'} = d_{2,2'}(n_{2'} - n_0) \tag{3}$$

Thus the average refractive index of shell 2′, $n_{2'}$, can be calculated (the path distance, $d_{2,2'}$, through shell 2′ is given by the geometry in Fig. 2(a)). The calculations are continued, in a similar manner for shell 3′, for which the refractive index is derived from the equation

$$\Gamma_{3,3'} = \Gamma_3 - (\Gamma_{3,1'} + \Gamma_{3,2'}) \tag{4}$$

and equation (1). The refractive index of shell 4′ is calculated correspondingly.

The above routines can be used to calculate the refractive index in any number of shells provided that each shell corresponds to a ray for which the total phase shift is known.

However, the ray path shown in Fig. 2(a) is incorrect because the refractive-index differences will force the rays to change direction. The ray paths through a homogeneous object and through a graded-index object are presented in Fig. 2(b, c). The graded-index case is applicable to this work, and therefore we will consider it in more detail. The ray curvature has two important effects on the calculation routines presented above: (1) the path distances will be altered; (2) the location of rays will be displaced, because each ray will appear as if it had originated from a point where the emerging ray virtually projects on to the focal plane, f, through the object (see Fig. 2b). In order to calculate the refractive-index profile, these two effects have to be taken into consideration, but this cannot be done unless the refractive-index profile is already known. Because the refractive-index profile and the ray path are mutually dependent upon each other and neither of them is initially known, they have to be revealed by sequential testing. This can be done by starting with a straight ray path, as in Fig. 2(a), and using the average refractive-index obtained by the above method to construct a refractive-index profile (assumed to be linear in each shell). This profile can then be used to calculate a more correct ray path, yielding a better refractive-index profile, in turn giving an improved ray path and so on. This results in a mathematical iteration, which can be repeated until the true conditions are attained (ray path and index profile become stable). The procedure is performed until complete for one ray at a time, starting with the outermost ray. The division into shells and the successive phase-shift subtractions are done as described above.

Because this method is based on iterative approximations involving ray-tracing in a gradient, it can be realized in practice only by computerized calculations. A program application and the details of the calculations are presented below.

PROGRAM DESCRIPTION

Conversion of the phase-shift profile into a refractive-index profile was achieved by a computer program. The program was written in FORTRAN 77 and executed on a UNIVAC 1100 computer.

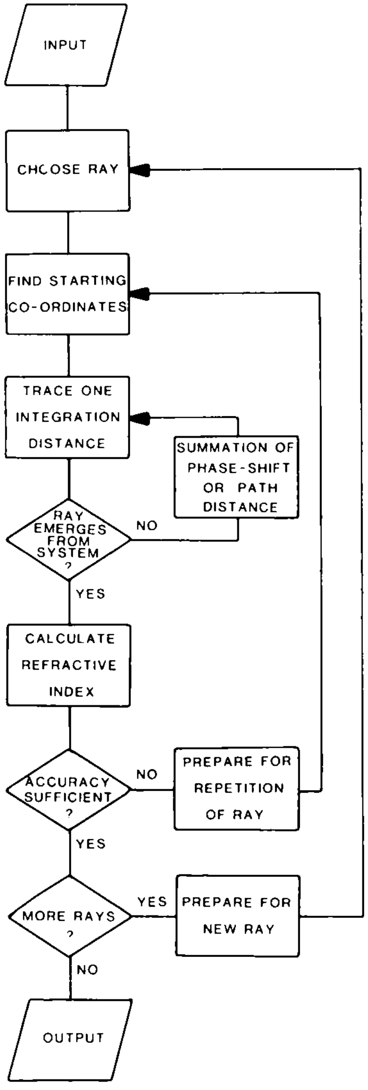

Fig. 3. Flowchart of the gradient analysis program.

The program simulates the ray path through the object in the microscope by tracing assumed rays in a coordinate system, according to the principles outlined above.

A flowchart of the program is shown in Fig. 3, and the individual processes are described in sequence below.

Input: The measured phase-shift profile and the surrounding refractive index are read. (The refractive index is initially assumed to be homogeneous throughout.)

Choose ray: One ray is traced for each phase-shift value, starting with the most peripheral one. Each ray corresponds to a shell as in Fig. 4(a).

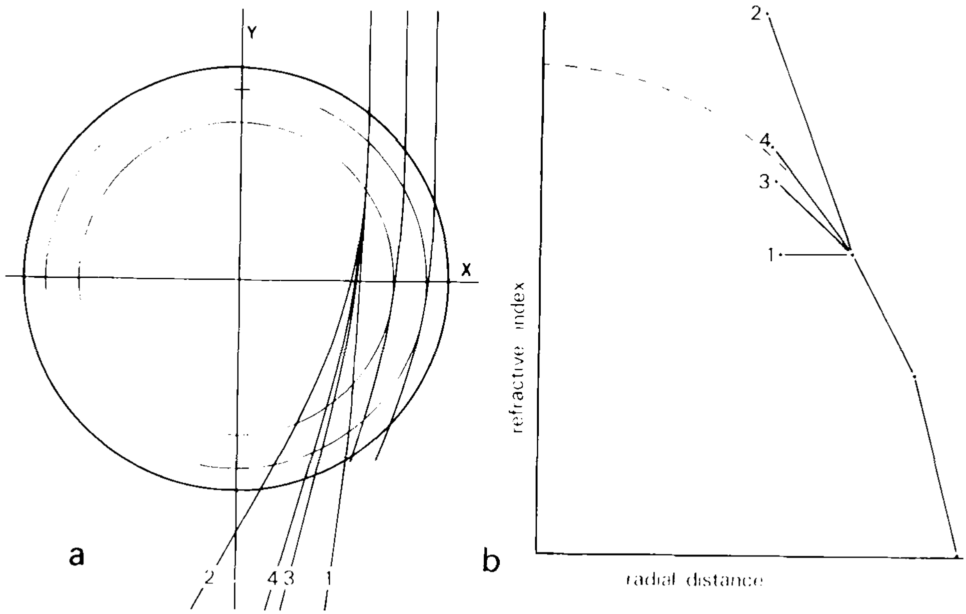

Fig. 4. Iterative approximations of the refractive-index gradient in one shell (the third). Four subsequent approximations are shown. For the initial assumption, 1, the refractive index of shell 3′ is set to be homogeneous. (a) Successive approximations to the ray path. (b) The same approximations as they will appear in the refractive-index profile.

Find starting co-ordinates: The starting co-ordinates are found on the peripheral border of the system, at the same X co-ordinate as the corresponding phase-shift value of the measured profile. The starting angle is always zero degrees, corresponding to the stopped down condenser diaphragm.

Trace one integration distance: Ray-tracing in the linear gradient of each shell is performed by employing the equation of Meggitt & Meyer-Rochow (1975):

$$r = \frac{(n_a + n_b)/2}{\sin\theta\,[(n_b - n_a)/D]} \tag{5}$$

where r is the local radius of ray curvature; n_a is the outer refractive index (at the outer edge of the given shell; for ray 1 this is the value of the surrounding medium and for subsequent rays it is the refractive index that was established by the previous ray); n_b is the refractive-index approximation being tested; D is the radial distance between n_a and n_b, and θ is the local angle between the gradient normal and the ray. The ray curvature, r, is assigned a local coordinate system (Marchand, 1978), and the change in coordinates caused by the path of one integration distance is calculated. The new coordinates in the local system are transformed into global coordinates, with the origin in the centre of the system. Because θ continuously varies through the ray path, the integration distance must be kept small. We used an integration distance less than 1% of the path distance for one ray. This means that in order to trace one ray through the system, equation (5) and the coordinate calculations have to be performed at least 100 times.

If the ray is perpendicular to the gradient (passing through the centre of the system), θ in equation (5) will be zero. To avoid this, equation (5) is not used; but the ray is instead traced uncurved, which is the consequence of θ being zero.

Ray emerges from system?: When a ray has passed the peripheral border of the system, the ray-tracing loop is terminated.

Summation of phase-shift or path distance: As the ray is traced through a shell for which the refractive index is to be established, the integration distance at each step is added to a variable, that, after the entire path, will contain the total path distance through this shell, d_Σ.

After the ray has been traced one integration distance through a shell for which the refractive index is already established, the resulting phase shift is calculated as in equation (2). This phase shift is then added to a variable, that, after the entire path, will contain the total phase shift caused by passing through shells with established gradients, Γ_Σ.

Calculate refractive index: When a ray emerges from the system, it is extrapolated backwards to the focal plane (see Fig. 2c), which it intersects at distance R from the centre of the object (the origin), where

$$R = X - Y \tan \alpha \tag{6}$$

α is the exit angle of the ray to the Y axis; X and Y are the co-ordinates of the position where the ray emerges from the system (the X-axis is the focal plane of the microscope and the Y-axis is the optical axis of the microscope).

A value for the measured total phase-shift, Γ_{tot}, corresponding to the radial position, R, is interpolated from the phase-shift profile (Fig. 1).

A refractive-index approximation for shell s is obtained by

$$n_s = n_0 + \frac{\Gamma_{tot} + \Gamma_{ref} - \Gamma_\Sigma}{d_\Sigma} \tag{7}$$

where n_s is the approximation to the average refractive index in shell s; Γ_{tot} is the total measured phase-shift; Γ_{ref} is the phase shift of the reference ray (see Kahl & Mylin, 1965); Γ_Σ is the summed phase shift for the path through established shells and d_Σ is the summed path distance through the shell s for which n_s is to be established. Equation (7) is a combination of equation (1) and the equations for subsequent subtraction, equations (3) and (4). The refractive index n_s is assigned a radius within the shell s in order to create a linear gradient that yields the same phase-shift for the ray as the average refractive-index above. The gradient is assumed continuous across shells.

Accuracy sufficient?: The ray is traced through the system repeatedly (Fig. 4) until two successive refractive-index approximations are equal to three decimal places.

Prepare for repetition of ray: The summation variables for phase shift and path distance are cleared.

More rays?: The calculations are terminated when all rays have yielded stable values.

Prepare for new ray: The summation variables for phase shift and path distance are cleared.

APPLICATION

The computer program was tested on graded-index objects and on a homogeneous object. The graded-index objects were crystalline cones obtained from the compound eye of the mysid shrimp *Neomysis integer*. Intact live crystalline cones were investigated using the present method (Fig. 5a, b), whereas for comparison, sliced cones were investigated employing the conventional method. We found extremely small variation between four sets of measurements when employing the new method, which is interpreted as indicating high accuracy. The refractive-index profile revealed by the conventional method displays standard deviations about 10 times larger than the variation obtained by the new method. These large variations are thought to

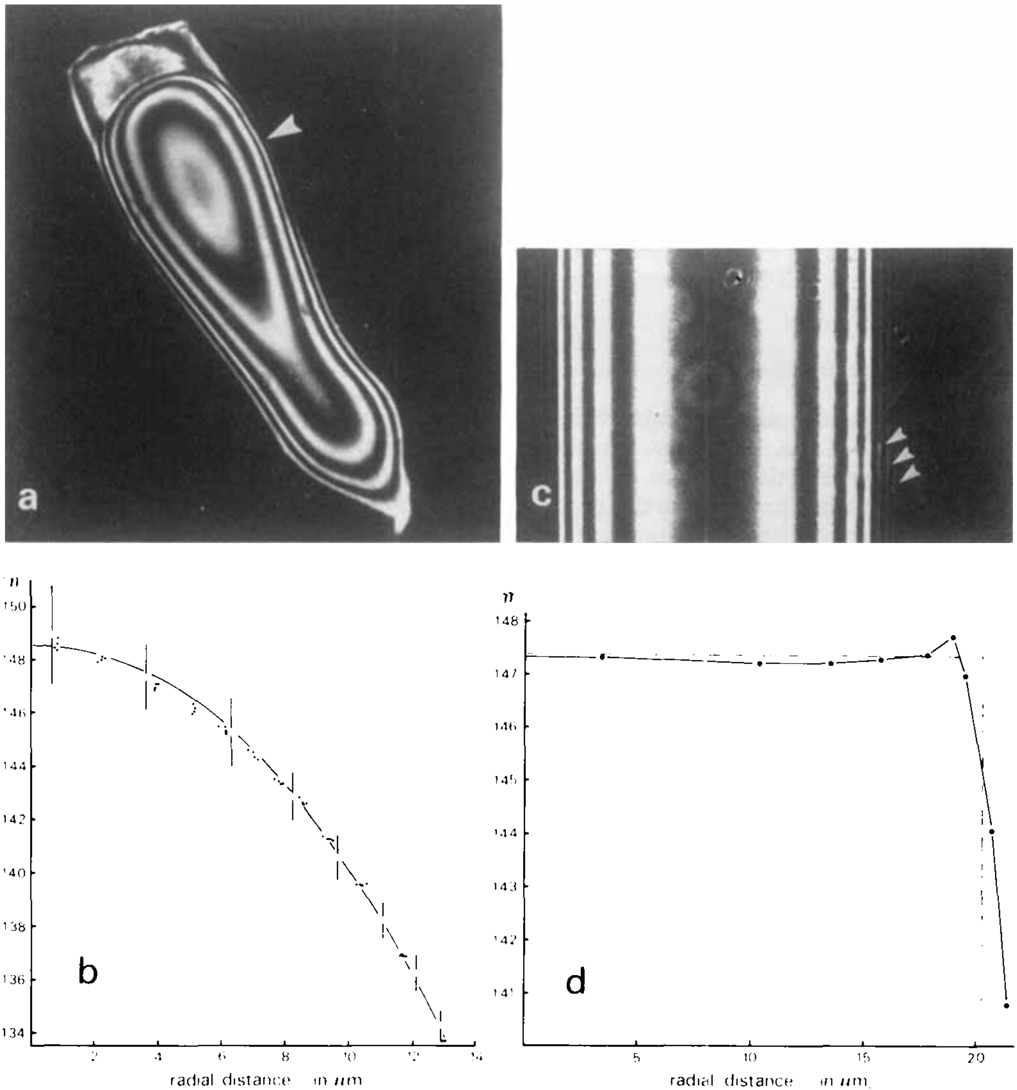

Fig. 5. Testing of the computer program on two different objects. (a) An interference micrograph of a crystalline cone from the compound eye of a mysid shrimp. The arrow indicates the position at which the phase-shift profiles are taken. (b) The refractive-index profile from the shrimp crystalline cone. Values from four sets of measurements (dots) are plotted with a fitted parabolic function (a parabola is the expected shape of the profile). For comparison, values obtained with the conventional method, involving sectioning, are included (circles with standard deviation bars based on ten measurements). (c) Interference micrograph from a homogeneous glass fibre, $n = 1{\cdot}473$, immersed in ethyleneglycol, $n = 1{\cdot}4072$. Note the diffraction (Fresnel) fringes at the edge (arrows). (d) The refractive index profile resulting from analysis of the interference pattern in (c). The true profile is also plotted (dashed line).

result from variations in section thickness, from the focusing effect described by Stone & Burrus (1975) and from damage caused by sectioning. The variations observed with our method should be attributed only to the accuracy in determining fringe positions in the microscope.

Upon testing our method on a homogeneous glass fibre, less impressive results were obtained (Fig. 5c, d). The new method, however, is designed to analyse graded-index systems rather than systems with discontinuous transitions. The sudden rise of refractive index from the surrounding medium to the edge of the fibre results in Fresnel fringes, which are interspersed with the other interference fringes. Further, the emerging rays will project outside the fibre, resulting in an apparent diameter that is too large (Fig. 2b). It is also very difficult to be certain that the microscope is correctly focused, because at the true focus (through the symmetry axis of the fibre) the edge of the fibre will appear diffuse. These reasons make a reliable analysis of refractive-index transitions difficult. Therefore, such transitions should be avoided by adjusting the refractive index of the surrounding medium. Minor transitions may be tolerated if the demand of accuracy is decreased. The presence of discontinuous transitions at the edge of an object is revealed by the presence of distinct Fresnel fringes (seen even when the reference beam is blocked) and by a discontinuity in the phase-shift profile (best seen in fringe-field interferometry).

DISCUSSION

Gradient-index optics are today being revealed in a continuously increased number of animal eyes (see Land, 1981). Also the manufacturing of graded-index fibres and the physics of non-homogeneous optical systems are under rapid development (see Marchand, 1982).

The new method presented in this paper permits high-accuracy measurements of the refractive-index profiles of rotation-symmetric objects. The method is fast and convenient in that it is based on interferometry of intact objects. Previous methods, involving sectioning of the object, were difficult and time-consuming. Sectioning of biological objects also decreases accuracy. The new method is convenient for analysing objects where the refractive-index profile varies along the symmetry axis (as in most biological objects).

However, our computer application of the analysis principle did not perform perfectly on objects having a peripheral refractive index considerably higher than the surrounding medium. This problem can be obviated either by adjusting the refractive index of the medium or by modifying the computer program. The basic principle of the analysis permits simulation of the ray path even in objects with discontinuous transitions of refractive index, for example by introduction of Snell's law but such improvements are, at present, only of secondary interest. It should be pointed out here that our computer program is only one of several possible solutions contained in the basic iterative principle.

Several other, essentially different (non-iterative), methods for analysing transverse interferograms are described (Saunders & Gardner, 1977; Iga & Kokubun, 1978; Chu & Whitbread, 1979). One of these methods (Iga & Kokubun, 1978) includes a full correction for the curved ray-path and thus should be as accurate as the present method. The unique advantage of our method is that it yields high accuracy also when only a few (five to ten) phase-shift values are available for each profile. This makes the present method especially advantageous for biological objects (such as crystalline cones of arthropod compound eyes) where the transverse interference pattern sometimes is restricted to one or a few fringes.

At present, two main fields of application can be distinguished: (1) the investigation of optics in invertebrate compound eyes, where this method opens the possibility of fast, convenient and accurate analysis (Nilsson, 1982); (2) the continuous quality control of manufactured graded-index fibres. Because the method is non-destructive, the fibres can be used after the investigation. However, for this purpose alternative methods already exist.

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