Analysis of solute concentration and concentration derivative distribution by means of frameshift Fourier and other algorithms applied to Rayleigh interferometric and Fresnel fringe patterns

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ABSTRACT

The equilibrium distribution of particles dispersed in an aqueous solute situated in a centrifugal accelerative field is routinely studied by means of an optical trace recorded photographically. Rayleigh interferometric fringe patterns have been widely used to give this trace, in which the displacement of the parallel fringes is directly related to particle concentration differences. We have developed a simple but highly efficient frameshift algorithm for automatic interpretation of these patterns[1]. Results obtained from extensive use and further definition of this algorithm confirm its validity and utility.

We have also studied algorithms for the interpretation of Fresnel fringe patterns yielded by an alternative optical system. These more complex patterns involving non parallel fringes can be analysed successfully, subject to certain conditions, with a precision similar to that obtained using Rayleigh interference optics.

1.INTRODUCTION

1.1 The methodology employed

The Analytical Ultracentrifuge is used to study systems of dispersions of large particles in a fluid (usually aqueous) medium[2]. From the results yielded, important conclusions can be drawn concerning the structure, interactions and state of dispersity of the particles. A particularly powerful approach involves the balancing of radial centrifugal forces acting on the particles against the forces arising from the chemical potential gradient induced by the former. The sytem in this state is said to be at 'sedimentation equilibrium'.

The basic equation governing the distribution of particles at sedimentation equilibrium in a centrifugal field is

$$dc/dr = k c r \qquad (1)$$

where c is the concentration of particles at radial position r, and k is a reduced particle mass, given by

$$k = M_r(1 - \bar{v}\rho)\ \omega^2/RT \qquad (2)$$

where M_r is the particle mass, $\bar{v}$ the partial specific volume (≈ reciprocal density) of the particles, ρ the density of the fluid, R is the gas constant, ω the angular velocity and T the temperature (deg Kelvin).

Equation (1) is frequently used in integrated form :

$$\Delta \log c / \Delta(r^2) = k/2 \qquad (3)$$

Normally the parameter M_r will be the object of study. It may itself be a function of r, either directly as a result of polydispersity or indirectly as a result of depending upon c, which as a consequence of redistribution of the solute particles varies with r.
The basic function of any analysing optical system is thus to record a pattern capable of being interpreted to yield *either* c *or* dc/dr as a function or r. In the former case equation (3) is applicable : in the latter case equation (2) would be used.

1.2 Analysis to yield c vs r data

The classical approach has been to use Rayleigh interference optics to give a pattern in which the displacements of the fringes in a direction (z) normal to radial is a linear function of the concentration increment at the radial position in question. The fringes are of course equi spaced and parallel, and hence a scan across them in the z direction yields a sinusoidal intensity function whose phase is a measure of (the non integer part of) the fringe shift.
We have developed a simple but fast and stable algorithm for deriving the phase shift from the intensity function[1]. The latter is logged from the photographic record of the fringe pattern, using a commerical scanning densitometer, the LKB 2202 laser densitometer. Then if Q fringes are contained within the window analysed, an iterative frameshift is performed within the data set, to maximise the Fourier coefficient of order Q. The method is thus a null method, which searches for the frameshift which will set the phase term to zero[1].
Thus this algorithm, unlike earlier approaches in this area, yields estimates for the fringe increment whose precision is not a function of the latter. The precision of the recorded fringes may be gauged subjectively from Figure 1 :

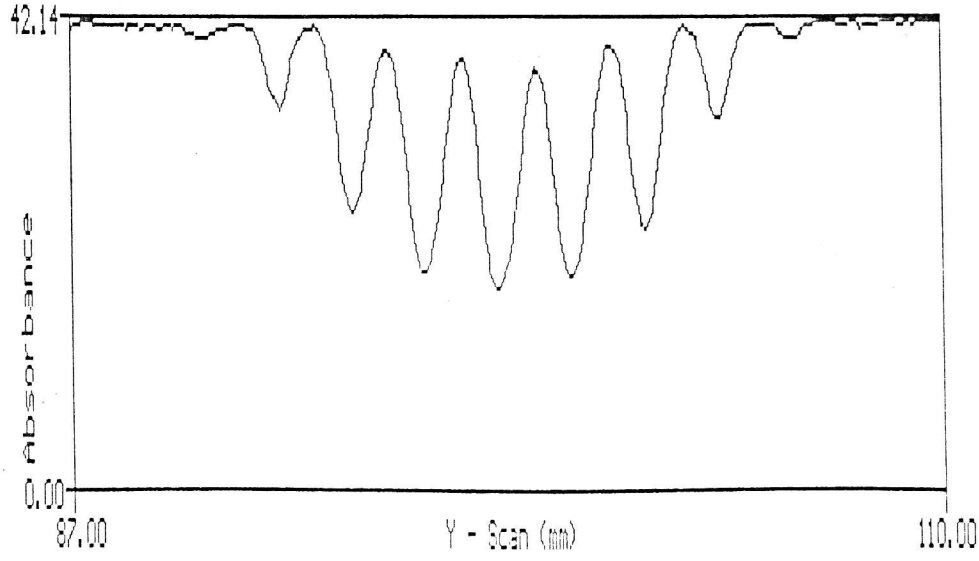

Figure 1 Digitised optical density values outputted from an LKB Ultroscan XL 2222 (two dimensional scanner) scanning at a single radial position. 575 values were logged in this case at each of 175 radial positions in the cell, and these form the data set for subsequent analysis. Data for sedimentation equilibrium experiment on lipase, using Rayleigh interference optics.

Initial results using our algorithm suggested a precision of f/500 (f is a single fringe increment) as being attainable. Further development and applications of the algorithm have followed, and are now presented and discussed.

1.3 Analysis to yield dc/dr vs r data

The earliest optical method used to analyse distributions within the ultracentrifuge cell was the 'Schlieren' optical system, in which an analysing diaphragm is inserted into the back focal plane of the camera lens employed to image the cell. Shadows or other traces are produced, whose displacement, again in the z direction, is proportional to the first derivative of solute concentration with respect to radial distance. Other than in the earliest work, a 180^{o} phase plate has been used as analysing diaphragm. The resulting single trace is rather broad as compared to an interference fringe (Figure 2).

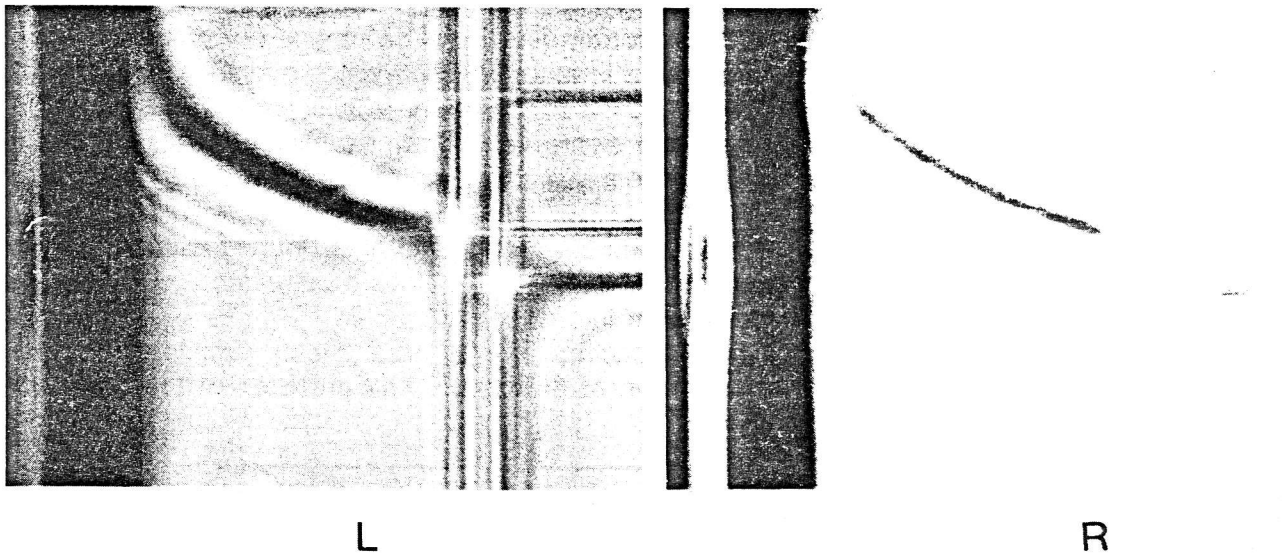

Figure 2 Phase plate Schlieren records of a solution at sedimentation equilibrium in an ultracentrifuge cell (MSE Mk II Analytical Ultracentrifuge). The solution column is some 2 mm long in real space. From original negatives conventionally (L) and correctly (R) exposed.

It has been universally considered that the precision with which this trace can be interpreted falls well short of what can be achieved using Rayleigh intereference optics. Subjectively this is understandable. The Schlieren trace appears relatively broad, and only a single trace is yielded, thus making unavailable the reduction in noise/signal normally achieved from multiple records. Yet the principal optical components of the two optical systems are identical, and are used at the same working aperture. Insofar as distinctive components are introduced in either method, there is no reason to suppose that these limit the information transfer function, which one would expect to be very similar in both cases, given adequate interpretative algorithms.
We have therefore researched the possibility of developing the interpretation of Schlieren records to a much higher level than heretofore. There are a number of practical reasons for doing this. As detailed below, we find that with suitable developments of the methodology, and subject to certain relatively minor reservations, results from the Schlieren optical method can indeed be

interpreted with a precision approaching those obtained by the Rayleigh interference method. The basis of this is the recording and interpretation of the more complex *Fresnel* fringe patterns generated by the Schlieren optical diaphragms. Several approaches to the interpretation of Fresnel fringe patterns can be defined. It seems likely that an optimal approach has yet to be delineated, but results to date are more than adequate to demonstrate the potential of work in this area.

2. FRINGE SHIFTS IN RAYLEIGH INTERFEROMETRIC PATTERNS

We have completed the construction of a 2 dimensional data acquisition system and the writing of a package of user friendly interpretative software, built around the frameshift Fourier algorithm described earlier[1]. Sophisticated search procedures have been incorporated to ensure that the system reproducibly and stably finds the correct fringe intensity maximum in what is now a full 2 dimensional record (cf our earlier version[1] which was a series of individual one dimensional scans) . As it is now possible to analyse data at up to 200 radial positions from a single experiment, rigorous tests can be performed to assess such factors as sample homogeneity and interactions (Figure 3) :

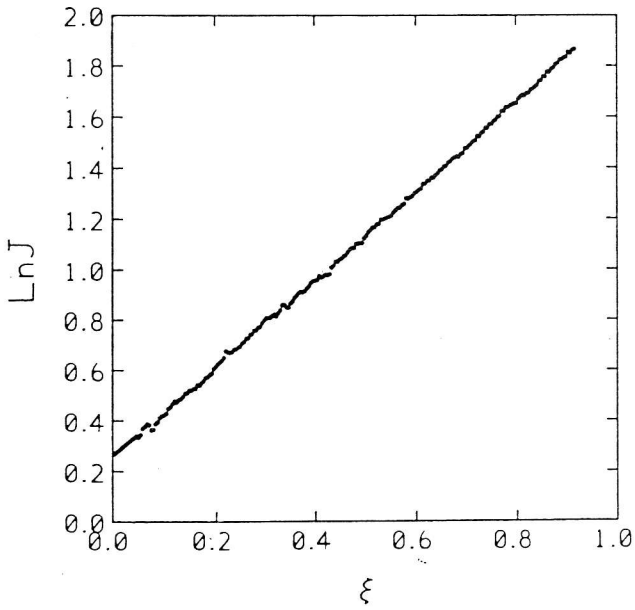

Figure 3 Plot of the logarithm of the solute concentration (expressed in absolute fringe numbers, J) versus the radial displacement (squared) parameter ξ . Data from a low speed sedimentation equilibrium experiment on recombinant Hirudin, loading concentration 0.8 mg/ml. From the slope a weight averaged molecular mass of 7080 ± 100 is computed (from sequence = 6964).

The completed system is now in intensive use, and results on many systems have fully justified our initial estimates[1] of the precision attainable.

3. REFRACTOMETRIC OPTICS AND FRESNEL FRINGE PATTERNS

3.1 Refractometric optics

The presence of an analysing diaphragm in the back focal plane of the camera lens imaging a cell, in this case located in an ultracentrifuge rotor, leads to the formation of a 'Schlieren' pattern in the image plane. The presence of a cylindrical lens results in a pattern in the image plane in which the z deflection of the trace at any radial position is linearly related to the refractive index change (dc/dr) in the conjugate locus in the cell plane (Lloyd[3]). Although any physical form of diaphragm can in principle be employed, it has long been customary to use a 180° phase plate (Wolter[4]), which causes no loss of transmitted light and has been considered on general principles (rigorous analysis appears not to have been performed) to maximise the information transfer function[3]. As a pure phase plate records no signal as dc/dr > 0, a thin line of metal evaporated onto the half wave step is normally added[3]. The latter *only* produces a trace for zero or very low dc/dr values. The optics of the transition region of dc/dr have yet to be been defined.
The resulting trace shows a well defined but rather broad line (Figure 1). This represent the zeroth order fringe of an often poorly resolved Fresnel pattern. We have addressed ourselves to the definition at high resolution of the co ordinates of this pattern, both by location of the zeroth order fringe, and by an alternative approach in which we derive and apply a relationship between Fresnel fringe *spacing* at defined r and the corresponding dc/dr and Δc values.

3.2 Definition of the zeroth order fringe in a Fresnel pattern

As noted above, the conventional approach here has been to record the Schlieren pattern using a modified phase plate diaphragm. The pattern is in principle symmetric with respect to the zeroth order fringe, which is located in the centre of the line trace. However, as was noted by Rowe and Khan[5], a simple knife edge diaphragm has a better established optical theory, and can yield results of a precision equal to that given by a phase plate. We have therefore explored the use of a simple knife edge diaphragm to generate the Fresnel pattern.
We have in all cases adopted certain modified procedures for setting up of the ultracentrifuge cells and optics designed to maximise the information transfer function. These will be described elsewhere. They are not relevant to the interpretative algorithms described below, but are indispensible if results of the precision described are to be obtained in practice.

3.2.1 The zeroth order in a phase plate generated Fresnel pattern

The zeroth order in this case is simply the maximum density (minimal intensity) in the z scan across the line pattern (Trautman and Burns[6]). Unfortunately as normally recorded the line is rather broad and the precision in the estimation of the position of the maximum not high. This is the conventional reason behind the general opinion that Schlieren optics are inherently of low precision.
Several experimental procedures can be adopted which largely circumvent the problem of the width and lack of definition of the optical trace. The experimental procedure most relevant to the present discussion is the use of long photographic exposures to bring the optical record *near to the centre of the line trace* into the linear part of the gamma curve of the recording material. It seems not to have been generally appreciated (though Lloyd[3] commented briefly on the matter) that giving a 'normal' exposure as gauged for the whole photograph seriously degrades the transfer

function in the critical region.
Locating the zeroth order in a suitably exposed photographic image (i.e. highly *over* exposed with respect to 'non information') by analysis of successive radial z scans results in a very smooth data set. Figure 3abelow illustrates typical final results computed from data measured in this way.

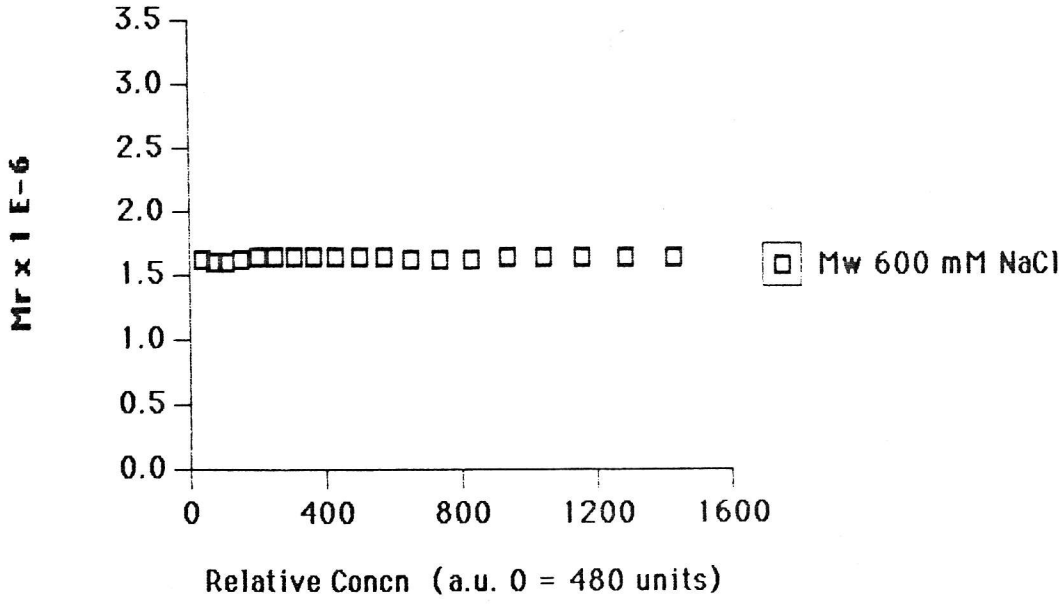

Figure 3a $M_{w,r}$ values computed for the protein dynein at a cell loading concentration of 0.5 mg/ml. These point weight average values are computed by numerical integration of the dc/dr values to give c values, the constant of integration being found by a numerical manipulation based upon the equivalence of harmonically related averages.

These results are entirely comparable to those which would be obtained using Rayleigh interferometric optics together with the Fourier algorithms described above, and would correspond to a precision of at least f/300 in the latter terms. There are however certain qualification which must be made concerning the absolute *accuracy* as distinct from the *precision* of the results. As noted above, for low values of dc/dr, the optical behaviour of a compound phase plate is far from well defined. This can be circumvented by avoidance of such conditions. More seriously, it seems not to have been appreciated that the physical properties of the phase plate and in particular its phase angle are critical.
We have computed intensity distributions in the image plane for a phase plate of various angles, using the Cornu Spiral construction[6]. The treatment given by Trautman and Burns[6] assume a phase angle of 180^{o}. We have extended this to the more general case, and as the tabulated values for the Cornu Spiral are in some cases of insufficient resolution we have computed the co-ordinates from the relation[7] :

$$F(x) = (2/\pi)^{0.5} (x + jx^3/3 + (j^2/2!)(x^5/5) + \ldots\ldots + (j^n/n!)(x^{2n+1}/2n+1))^{0.5} \quad (4)$$

for n = 0,1,2 . . . and the spiral is plotted in the complex plane $j = (1)^{0.5}$

The results are shown in Figure 4 below for phase angles of 180, 150 and 120 degrees :

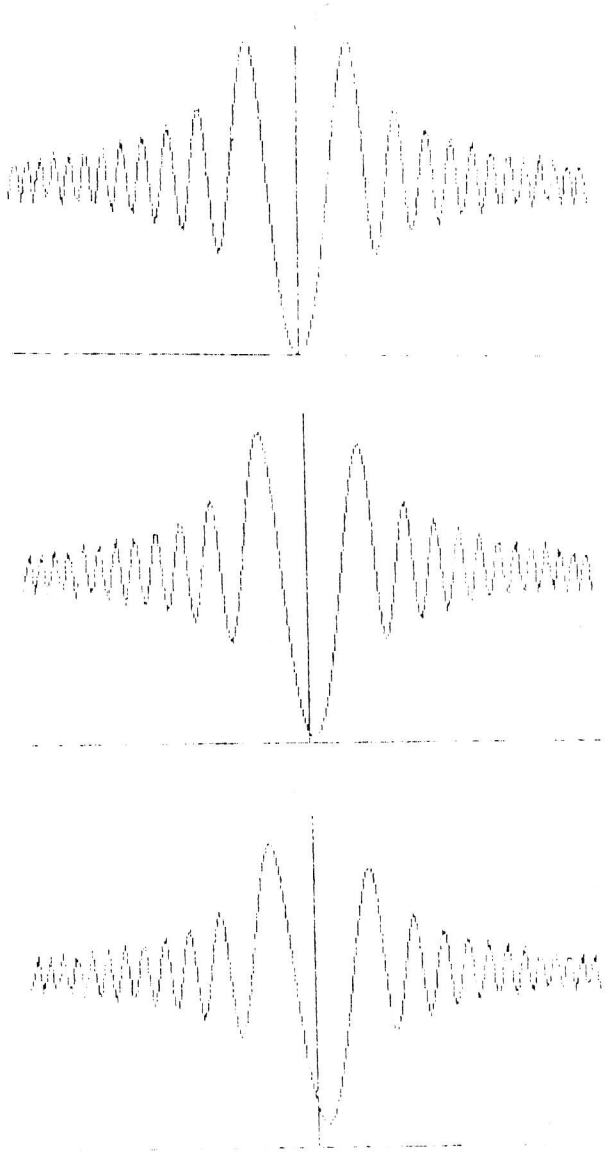

Figure 4. Computed intensity distribution of the Fresnel pattern yielded by a phase plate of phase angle 180 degrees, 150 degrees and 120 degrees. The location of the true geometrical edge is shown by the vertical line in each case.

It is clear that the location of the true geometrical edge coincides with the minimum of the intensity distribution *only* for the case of a 180 degree phase angle. Moreover the pattern is not symmetrical for other phase angles. Thus the wavelength used is critical, and must be tuned to the particular phase plate used, a precaution which has not formed part of normal practice, and can give rise to practical problems in securing adequate light intensity in monochromated light.

3.2.2. The zeroth order from a knife edge pattern

A simple knife edge used as a diaphragm results in a 'shadow' pattern, with a set of Fresnel fringes (Figure 5). Although actually of slightly smaller amplitude than those generated by a phase plate[6], th lower dynamic range of the image means they are frequently better registered.

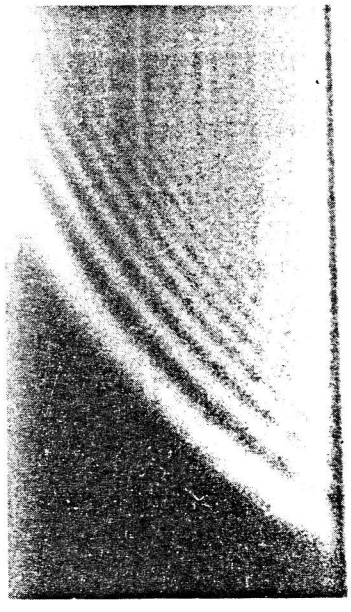

Figure 5. A part of a Schlieren pattern recorded with a knife edge diaphragm from a solution of an enzyme (chloramphenical acetyl transferase) at sedimentation equilibrium. Centrifugal direction is from left to right.

The location of the zeroth order fringe can be computed by measurement of (say) the 1st and 2nd order minima, and from the knowledge that their location (z) with respect to the zeroth order is given for order i by

$$z = (4(i + 1) - 0.5)^{0.5} \qquad (5)$$

In practice only the first two or three orders can be measured in a z scan (Figure 6) :

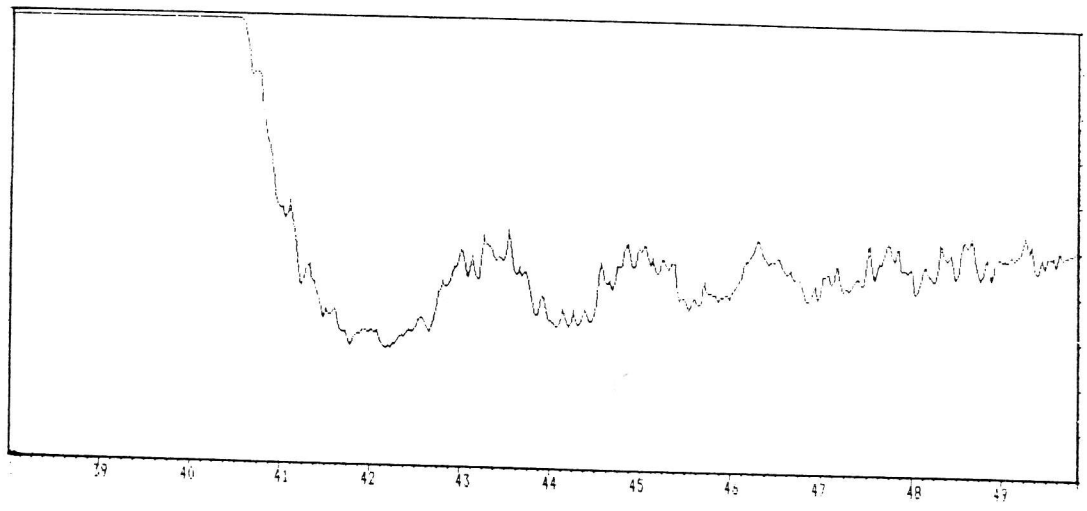

Figure 6 Vertical (z) scan across a set of fringes from a knife edge diaphragm (as in Figure 5) The location of the centre of the peaks in the scans can be determined by established procedures[1]. The resulting precision is found to be of the order of 1 to 2 % of the fringe spacing.

3.3 Zeroth order determined from the Fresnel fringe spacing

It is possible to use the values of the fringe spacings in the z direction to evaluate Δc *directly* instead of by computation of the location of the zeroth order. This is because of the defined relationship[8] between fringe spacing and the second derivative of the refraction (and hence concentration) gradient, from which it follows at once that

$$\Delta c = \int_{r_a}^{r_b} \int \{ \left(\sum_{i=1}^{i=(n-1)} (z_{i+1} - z_i) / (4(i+1) - 0.5)^{0.5} / (4i - 0.5)^{0.5} \right) / (n - 1) \} \qquad (6)$$

This equation defines the relationship between Fresnel fringe spacings and Rayleigh fringe shifts. The double integration is highly favourable with respect to error reduction in the data set, achieving at least an order of magnitude of diminution, greater if the summation can be effected by measurement of multiple fringes. A constant of integration is required for the first integration. This is in fact the zeroth order spacing, but as an independent estimate of this constant can be obtained from each radial scan, errors in its estimation are not serious.

We have evaluated several procedures for determining the z spacings in a multiple fringe pattern. Direct fitting of the Fresnel function by a least squares algorithm has been implemented, but is not totally successful. This is primarily because the intensities in a Fresnel pattern, ranging from true zero upwards, cannot possibly be recorded photographically within the linear part of the gamma curve of the emulsion. The true pattern is thus convoluted with an envelope function, which as the Fresnel amplitude/intensity function is anharmonic, cannot readily be deconvoluted as with Rayleigh patterns[1]. Furthermore, as noted above, although up to 10 or more Fresnel fringes can be discerned by eye, the intensity scans give only 2 or 3 clearly defined maxima/minima (Figures 5 & 6).

Thus this approach, whilst somewhat superior to the simple evaluation of the zeroth order, has yet to be developed to its full potential.

4. CONCLUSIONS

The evaluation of relative solute concentrations within an ultracentrifuge cell by Rayleigh interferometric fringe shifts can, using optimal procedures and interpretative algorithms, yield a data set with a precision approaching f/500, where f is a single fringe shift[1]. Our results to date using the alternative Schlieren optical system show that under identical experimental conditions (i.e. same solute concentration and optical path length) this latter system can approach the Rayleigh fringe level of precision. In terms of Rayleigh fringe shifts, the direct evalution of the zeroth order by a phase plate diaphragm (3.2.1) attains f/300, though with some danger of systematic error, and by knife edge diaphragm attains f/150 to f/200. By the use of the fringe spacings (3.2.2) and the transformation noted above (equation 6) a precision of close on f/300 is attained.

Refractometric (Schlieren) optics have a number of advantages over Rayleigh interferometric optics. Their alignment is much simpler, and window distortions are a much less serious problem. The widely held supposition that their precision is much inferior to Rayleigh optics lacks a theoretical basis and is now shown to be untrue in practice. Given more advanced two dimensional analysis of the recorded fringe patterns to enable up to 10 fringe spacings to be analysed, the precision of refraction and hence concentration increment determinations will be essentially the same by either method, and it will be possible to choose the simpler refractometric system when experimental conditons so dictate. It is possible that the transformation between the two types of fringe pattern noted above and the interpretative algorithms developed may have application to other systems.

ACKNOWLEDGEMENTS

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