Aust. J. Phys., 1981, **34**, 575–84

Photon Correlation Spectroscopy using Optical Heterodyne Detection

Guy C. Fletcher and Julienne I. Harnett

School of Mathematics and Physics, Macquarie University, Sydney, N.S.W. 2113.

Abstract

The detection by optical heterodyning of laser light scattered from solutions of macromolecules offers significant improvement in statistical accuracy over the more usual self-beating method. Nevertheless the heterodyne technique is not commonly used because of the supposed difficulties associated with stable and efficient mixing of the scattered light with a local oscillator beam. We have carried out an experimental comparison of several methods of mixing the two beams, using very dilute solutions of polystyrene latex spheres as scatterers. Experimental data are also presented for the apparent particle radius as a function of the local oscillator level, and are compared with theoretical predictions.

1. Introduction

Photon correlation spectroscopy of scattered laser light is now a well-established technique for the rapid measurement of diffusion coefficients of macromolecules and other particles, especially in the size range 10–1000 nm. The earliest experiments by Cummins *et al.* (1964) used a method of heterodyne detection in which the scattered light, spectrally broadened by the Brownian motion of the scatterers, was made to beat with a local oscillator derived from the original laser beam but shifted in frequency by 12 MHz from the frequency of the scattered beam. Later experimenters have used a local oscillator beam of the same frequency as the scattered light, either deliberately introduced or through stray scattering from optical surfaces. The homodyne or self-beat method of detection was first used by Ford and Benedek (1965), and since then most workers have preferred this method because of its greater experimental simplicity.

Heterodyne detection offers a number of advantages to be weighed against the less simple optical arrangement. The statistical accuracy in the determination of diffusion coefficients (for a given duration of experiment) is improved. The field autocorrelation function of the scattered light is determined directly, and is independent of the nature of the statistics of the scattered light; by contrast the interpretation of the intensity autocorrelation function from a self-beating experiment requires the assumption of gaussian statistics in the scattered field. In addition the fitting of more than one exponential to data from polydisperse solutions is often simpler in the heterodyne case if the scattering solution is contaminated by *small* amounts of dust. This is because the dominant effect of such dust is to increase the baseline of the autocorrelation function slightly; this can be accommodated by leaving the baseline as a free parameter to be fitted, but the form of the function is then considerably simpler in the heterodyne case.

For the preceding reasons we have carried out an experimental comparison of several practical methods of achieving optical heterodyning, both to verify the improvement in statistical accuracy and to determine the most convenient experimental arrangement. We have also made systematic measurements of the apparent particle size at various levels of local oscillator injection, and compared these with theoretical predictions.

2. Theory of Heterodyne Detection

The theory of photon correlation spectroscopy in general, and heterodyne detection in particular, has been given in a number of books (Cummins and Pike 1974; Chu 1974; Berne and Pecora 1976) and review articles (e.g. Pusey and Vaughan 1975; Oliver 1978). The normalized field autocorrelation function of the scattered light for delay time t is related to the diffusion coefficient D of the scattering particles by

$$g^{(1)}(t) = \exp(-q^2 Dt) = \exp(-\Gamma t), \tag{1}$$

where $\boldsymbol{q}$ is the scattering vector. The diffusion coefficient is in turn related to the radius r of a spherical scatterer by the Stokes–Einstein equation

$$D = kT/6\pi\eta r, \tag{2}$$

where η is the solvent viscosity and k Boltzmann's constant. This equation may be used to define a hydrodynamic radius from the measured diffusion coefficient for a particle of any shape.

The normalized intensity autocorrelation function $g^{(2)}(t)$ measured in an experiment is related to $g^{(1)}(t)$ by

$$g^{(2)}(t) = 1 + \left(\frac{2\bar{n}_s \bar{n}_o}{(\bar{n}_s + \bar{n}_o)^2}\right) g^{(1)}(t) + \left(\frac{\bar{n}_s}{\bar{n}_s + \bar{n}_o}\right)^2 |g^{(1)}(t)|^2, \tag{3}$$

where $\bar{n}_s$ and $\bar{n}_o$ are the mean numbers of photon counts per sample interval due to the scattered light and local oscillator beam respectively. The second term is independent of the statistics of the scattered light, whereas the third term requires the assumption of gaussian statistics. In a full heterodyne experiment the third (self-beating) term is made negligibly small by using a sufficiently large local oscillator signal ($\bar{n}_o \gg \bar{n}_s$). However, when clipping is used Jakeman (1972) has shown that the third term can only be minimized in this way for $\bar{n}_s \ll 1$, corresponding to very weak scattering.

This restriction to dilute sample solutions is realistic in practice. For stronger scattered fields having gaussian statistics, the simpler self-beating method of detection gives good accuracy within quite short experiment times. Only when the statistics are not gaussian is it then necessary to use heterodyne detection. Further, most digital correlators should not be operated at count rates above about 1 MHz to avoid problems from pulse pile-up. With a local oscillator level some 30 times as intense as the scattered light (see next paragraph), this restricts practical heterodyne experiments to a maximum count rate from the scattered light alone of about 30 kHz. In the case of spherical particles of radius 44 nm in water at room temperature, and scattering at an angle of 90°, a suitable sample time is 20 μs for self-beating, corresponding to $\bar{n}_s \approx 1$ at this maximum count rate.

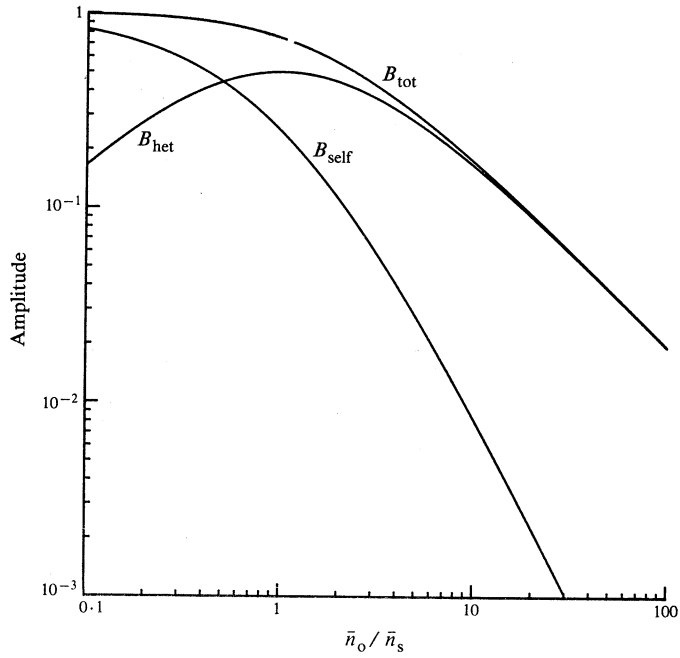

Fig. 1. Amplitudes of the heterodyne (B_{het}) and self-beating (B_{self}) components of the intensity autocorrelation function, calculated from equation (3), as functions of the relative strength $\bar{n}_o/\bar{n}_s$ of the local oscillator beam.

In a real experiment the coefficients of the two time-dependent terms in equation (3) are modified by constant (and slightly different) factors that depend on the finite size of the detector area (Jakeman 1974). For small apertures these factors are somewhat less than unity. The coefficients B_{het} and B_{self} of $g^{(1)}(t)$ and $|g^{(1)}(t)|^2$ respectively in equation (3) are shown in Fig. 1 as functions of the relative local oscillator level $\bar{n}_o/\bar{n}_s$. The coefficient of the *total* time-dependent part of $g^{(2)}(t)$, namely $B_{tot} = B_{het} + B_{self}$, is also shown. It is apparent that B_{het} goes through a maximum value at $\bar{n}_o = \bar{n}_s$; this maximum is a result of normalizing the autocorrelation function, and is not present in the unnormalized function $G^{(2)}(t)$. The total time-dependent amplitude falls steadily with increasing local oscillator level. The ratio B_{self}/B_{het} also falls, reaching a value of 1/60 at $\bar{n}_o = 30\,\bar{n}_s$; this level may be taken to correspond to effectively pure heterodyne detection.

Jakeman (1972) has shown that, for $\bar{n}_s \ll 1$ and $\bar{n}_o \gg \bar{n}_s$, the standard deviation in Γ for heterodyning and for self-beating, obtained from a two-parameter fitting procedure and a large number of autocorrelator channels, is given by

$$\delta\Gamma/\Gamma = (2\Gamma T/\bar{n}_s^2 N)^{\frac{1}{2}} \qquad \text{(heterodyne)}, \tag{4a}$$

$$= (21\cdot 2\,\Gamma T/\bar{n}_s^2 N)^{\frac{1}{2}} \qquad \text{(self-beating)}, \tag{4b}$$

where Γ is the decay rate and N samples are taken, each of length T. This gives a theoretical advantage of 3·26 in accuracy in favour of heterodyning to determine Γ and hence the radius r of a scattering particle. However, Oliver (1974) points out that such an improvement is unlikely to be fully realized in practice.

3. Data Analysis

Equation (3) may be written in the form

$$g^{(2)}(t) = 1 + B_{\text{het}} \exp(-q^2 Dt) + B_{\text{self}} \exp(-2q^2 Dt). \tag{5}$$

In this expression $g^{(2)}(\infty)$ has been normalized to unity by dividing the experimental autocorrelation function $G^{(2)}(t)$ by $\bar{n}^2$, where $\bar{n} = \bar{n}_s + \bar{n}_o$. Thus

$$g^{(2)}(t) = G^{(2)}(t)/\bar{n}^2, \tag{6}$$

where

$$G^{(2)}(t) = N^{-1} \sum_{i=1}^{N} n(iT)\, n(iT+t), \tag{7}$$

for N consecutive sample periods, each of length T. The mean count $\bar{n}$ is accumulated in the correlator memory, along with the summations from which the autocorrelation function is calculated.

At very low sample concentrations, residual dust in preparations can become a significant problem in spite of clarification procedures using centrifugation and filtration. Such dust particles act both *in* the scattering volume, causing sudden increases in scattered intensity equivalent to an increase in local oscillator level, and *outside* the scattering volume by obscuring the incident beam and effectively reducing its intensity. Because of the very small number of dust particles in the beam at any instant, these effects are non-gaussian. Experimentally the first-order effect of *small* amounts of dust is to increase the baseline of the normalized intensity autocorrelation function $g^{(2)}(t)$ to a value slightly above unity, both for self-beating and for heterodyne detection. The initial effect on the decay rate seems to be very small, and some workers have chosen to meet this problem by leaving the baseline as an adjustable free parameter in the fit; this procedure prevents the use of cumulants analysis of the data (Koppel 1972). While the problem exists for self-beating, it is much more acute for heterodyne detection (Thomas and Fletcher 1979) because the amplitude B_{het} is quite small (at most 0·06) compared with the expected baseline value of 1·0.

It may be noted that dust is a problem not only with dilute solutions of small scatterers. Experiments with quite high concentrations of strongly scattering large particles, such as polystyrene latex spheres of diameter 1 μm, can suffer severely from the presence of dust in the original sample. This can be very difficult to remove because the dust particles and the spheres are similar in size. Both centrifugation and filtration are apt to remove the spheres as well, leaving a sample 'solution' of clean water.

Allowing for the effects of finite detector area mentioned in Section 2 above, and also for a floating baseline to accommodate small departures of this from unity, we may rewrite equation (5) in the form

$$g^{(2)}(t) = A + C_{\text{het}} \exp(-q^2 Dt) + C_{\text{self}} \exp(-2q^2 Dt). \tag{8}$$

In principle it is possible to fit experimental data with a nonlinear least squares program to equation (8) using four free parameters. In practice we have enjoyed only limited success with this approach, finding it to give consistent results only in the regime when C_{het} and C_{self} are comparable in value. To cover a wide range of

local oscillator levels we have therefore resorted to a forced single exponential fit of the form

$$g^{(2)}(t) = A_f + C_f \exp(-2q^2 D_a t), \tag{9}$$

where D_a is the apparent self-beating diffusion coefficient from which an apparent hydrodynamic radius r_a may be calculated; r_a is expected to range from the true particle radius r at $\bar{n}_o = 0$ to $2r$ for $\bar{n}_o \gtrsim 30\bar{n}_s$. To facilitate comparison between experimental data treated in this way and the predictions of equation (3), we have also force-fitted equation (9) to synthetic data calculated in accordance with equation (3). These data, which will be referred to as the 'computer data' are therefore based on the assumption of small detector area, so that the correction factors mentioned above are approximately equal and close to unity. The experimental data were collected under such conditions; the effect of finite detector area on the results is considered in Section 5*c* below.

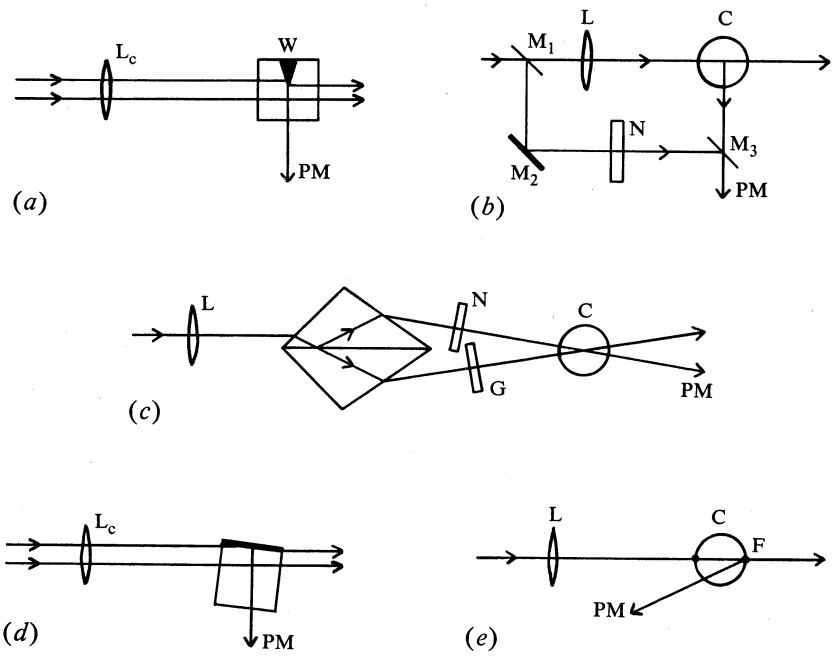

Fig. 2. Five different arrangements for optical heterodyning. The water bath surrounding the cell in each case is not shown. (*a*) Teflon wedge W inside a rectangular scattering cell; the whole cell can be translated horizontally at right angles to the beam (L_c, cylindrical lens; PM, photomultiplier tube); (*b*) mirror system to recombine the beams (M_1, M_3, glass microscope slides; M_2, aluminized mirror; L, spherical lens; C, cylindrical cell; N, neutral density filter); (*c*) beam-splitting prism with glass compensating plate G; (*d*) rectangular cell with outside of one face frosted; (*e*) plastic centrifuge tube used as scattering cell to give a strong flare spot F as local oscillator.

4. Experimental Details

(*a*) *Sample Solutions*

Polystyrene latex spheres from the Dow Chemical Company, having a nominal radius of 44 nm, were used for all the experiments. The original preparation was diluted with filtered distilled water. Samples were finally syringe-filtered (0·22 μm

Millipore filters) into the cleaned scattering cell. The dilution factor was chosen to give a final (self-beating) count rate of about 5 kHz. The addition of a local oscillator signal up to 30 times this level thus gave a maximum count rate under 200 kHz.

(*b*) *Optics and Signal Processing* (*Self-beating*)

The arrangement for self-beating is fairly standard. The focused beam from a 50 mW helium–neon laser (Spectra Physics, Model 125A) illuminates the scattering cell. An image of the scattering volume is formed on a pinhole (0·3 mm) by a converging lens. A second pinhole in front of the photomultiplier tube (E.M.I. type 9863B/100) defines the number of coherence areas seen by the detector. A permanently connected ratemeter with analogue display facilitates fine adjustment of the optics. The correlator has 64 channels (Langley–Ford) and is interfaced to a Hewlett–Packard desk-top computer (Model 9835A) with associated printer (Model 9876A). Data can also be transferred to the Macquarie University's Univac 1106 computer for analysis by more elaborate programs.

(*c*) *Optical Heterodyne Arrangements*

Five different methods of achieving efficient optical heterodyning were considered, and four of these were investigated experimentally. The methods are illustrated in Fig. 2.

The first method (Fig. 2*a*), proposed by Cummins *et al.* (1969) and subsequently modified by Wada *et al.* (1971), uses a cylindrical lens L_c to produce a horizontal ribbon of light at the focus in the scattering cell. A Teflon wedge is moved into this beam to produce a local oscillator signal of variable intensity. The photomultiplier sees only the vertical profile of the ribbon, and thus good coherence is achieved at the detector.

A mirror arrangement (Fig. 2*b*) can also be used to superpose a portion of the incident laser beam at the detector (Uzgiris 1972). Neutral density filters are then used to control the level of this local oscillator signal. Careful alignment is needed to ensure proper superposition of the two beams in both position and direction.

Pike (1977) has proposed a method (Fig. 2*c*) in which the laser beam is split into two by a beam-splitting prism. The emerging beams converge on the scattering cell directly without the use of mirrors, thus ensuring greater freedom from problems due to vibration. The local oscillator level is again controlled by a neutral density filter N, with a compensating glass plate in the other beam. A disadvantage is the low scattering angle, making this system more susceptible to dust in the sample (already a problem in very weakly scattering solutions). A suitable prism was not available, so this method could not be tested experimentally.

In the fourth method (Fig. 2*d*) we propose the use of a rectangular scattering cell of which one outside face is frosted using fine carborundum. The cell is used with a cylindrical lens to focus the beam as with the Teflon wedge, and is slightly rotated on its base so that the frosted rear face intercepts a portion of the ribbon beam. Limited (but sufficient) control of local oscillator level is achieved by varying the angle of rotation. The method uses no mirrors and is simple to adjust.

We have frequently used cylindrical centrifuge tubes as scattering cells, being careful to ensure that the flare spots, where the laser beam passes through each wall, are invisible to the detector. Our final heterodyning arrangement (Fig. 2*e*) makes

deliberate use of one of these flare spots F in a plastic tube to provide a local oscillator signal. It is usually convenient to work at a scattering angle different from 90°.

5. Results and Discussion

(*a*) *Heterodyning Methods*

With the exception of the flare-spot method (Fig. 2*e*) all experiments were carried out at a scattering angle of 90°. For each method of mixing, 25 experimental runs were completed with and without local oscillator injection. All runs were of duration 60 s. The sample interval was 20 μs for self-beating and 50 μs for heterodyne experiments. The count rate in self-beating experiments simulated weakly scattering samples, being about 5 kHz. In heterodyne experiments the local oscillator increased the total count rate to 150 kHz. At such a count rate full (four-bit) correlation could be carried out on all data without the use of clipping or scaling. Data were fitted to equation (9) using a simple nonlinear regression program; the reason for this (as opposed to the more usual cumulants method) is further discussed below.

The Teflon-wedge method (Fig. 2*a*) was easy to adjust but very susceptible to vibration. Our normal system uses a 3 m path from the laser under the 3 t optical table to the scattering cell via four mirrors. The stability of this system proved inadequate, resulting in significant 'noise' on the local oscillator due to movement of the beam on the Teflon wedge. The use of a much shorter path (0·2 m) without mirrors cured this problem completely. A fairly large scattering cell was required to accommodate the Teflon wedge, and because the wedge is in the solution some difficulties were experienced in obtaining clean samples.

The mirror method (Fig. 2*b*) was somewhat difficult to align but performed well after this. Perhaps because of the compact layout, no problems were experienced from vibration of the reflectors, and there was no evidence of poor wavefront matching in the amplitude of the correlation function.

The frosted cell method (Fig. 2*d*) stood out for its ease of setting up and, because the inside of the cell is unchanged, no problems of cleaning were encountered as with the rather similar Teflon-wedge method. The chief limitation was in the range of local oscillator levels which could be achieved for a reasonable rotation of the cell; in practice this placed an upper limit of 5 kHz on the scattering from the sample alone if effectively pure heterodyning was to be achieved. Since heterodyning is only likely to be used with weakly scattering samples this was not a serious restriction in practice.

The method of Fig. 2*e* using a flare spot to produce the local oscillator level was predictably the least successful of the four methods actually tested. The size of the flare spot made adjustment very critical for the detector to see light scattered from both the sample and the tube wall at suitable relative levels. Nevertheless, satisfactory measurements were achieved.

The improvement factor of heterodyne detection over self-beating was calculated from the standard deviation of the particle radii taken from each set of 25 experiments. Heterodyne detection consistently reduced the standard deviation of the measurement by a factor of $1{\cdot}9 \pm 0{\cdot}1$, under otherwise identical experimental conditions. This value may be compared with Jakeman's (1972) factor of 3·26. Our experimental result falls short of the theoretical result for a number of reasons. These include the finite number of autocorrelator channels, and inefficiency in mixing the scattered

and local oscillator fields. Also, in such dilute sample solutions, residual dust, even after careful clarification, undoubtedly contributes to the intensity fluctuations and uncertainty in the measured radius.

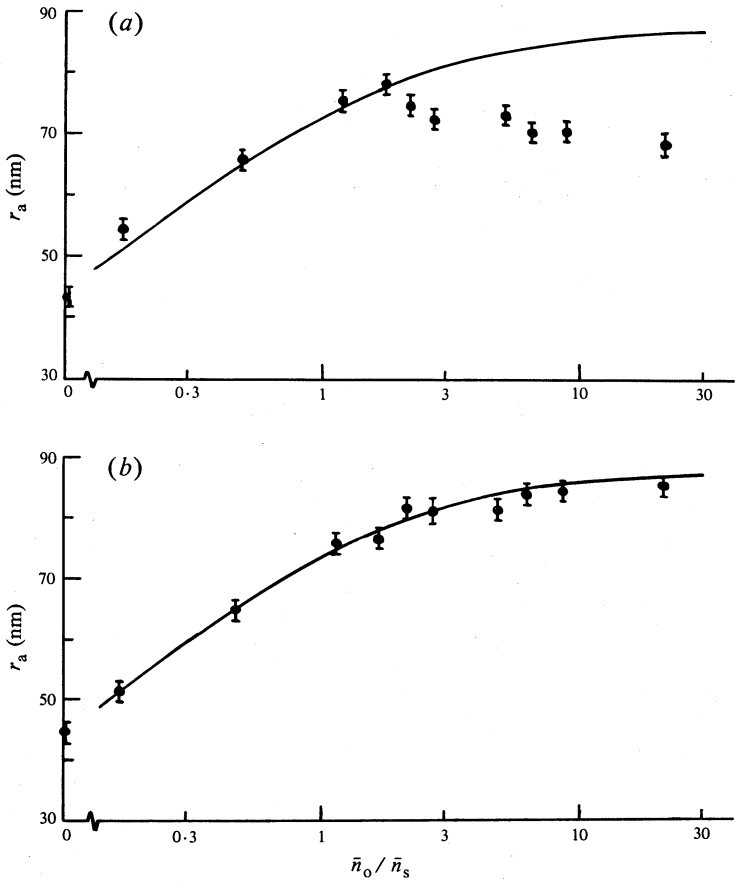

Fig. 3. Plots of apparent particle radius r_a as a function of relative strength $\bar{n}_o/\bar{n}_s$ of the local oscillator beam using equation (9). The data were obtained using the Teflon-wedge method of Fig. 2*a*. The curves show the theoretical values taken from 'computer data': (*a*) two-parameter fit with fixed baseline (1·0); (*b*) three-parameter fit (with floating baseline) to the same data.

(*b*) *Partial Heterodyning*

The level of local oscillator injection was varied systematically from zero to 30 times the intensity of the scattered light alone for each of the four heterodyning methods tested. A typical set of results (using the Teflon-wedge method of Fig. 2*a*) is shown in Fig. 3. The data were analysed first using a two-parameter fit to equation (9) with a fixed baseline ($A_f = 1 \cdot 0$) and subsequently with three parameters and a floating baseline. In each case an apparent hydrodynamic radius is plotted against local oscillator level. The theoretical value of the apparent radius is represented by the curve showing the computer data force-fitted to the same equation. The agreement between theory and experiment is very poor with a fixed baseline (Fig. 3*a*) but much

more satisfactory when a floating baseline is used (Fig. 3*b*). Heterodyne detection, because of the much smaller amplitude of the decaying exponential in the normalized correlation function, is particularly sensitive to errors in the effective baseline arising from even quite small amounts of dust, as discussed above, and to accommodate this it is important to leave the baseline as a free parameter in the fitting procedure.

(*c*) *Effects of Finite Detector Area*

The computer data were generated from equation (3) on the basis of a very small detector area so that no coherence area effects occur. The effect of a finite area A of a detector is to multiply each of the coefficients B_{het} and B_{self} in equation (5) by factors $f_{\text{D}}(A)$ and $f(A)$ respectively. Jakeman (1974) has shown that for a finite detector area $f(A)$ is somewhat smaller than $f_{\text{D}}(A)$. The effect is to bias equation (5) in favour of the heterodyne term at the expense of the self-beating term. The apparent radius should be slightly greater than calculated in the computer data at values of local oscillator level such that both heterodyning and self-beating terms contribute significantly. Thus the curves in Fig. 3 should be shifted very slightly upwards in the middle region but not at the ends, in order to account for the finite detector area used in the experiments. The effect is quite small and no correction was made for it. The satisfactory agreement between experiment and theory in Fig. 3*b* is not affected.

6. Conclusions

We have investigated the performance of four experimental configurations suitable in optical heterodyne experiments. One of these (using a cell with a single frosted side) stands out for its effectiveness and simplicity, though all the methods tested were feasible. Experiments with partial heterodyning under conditions of inadequate local oscillator level yield an apparent hydrodynamic radius which compares well with theoretical predictions.

The use of heterodyne detection offers special advantages for scattered fields which do not conform to gaussian statistics, and for scatterers undergoing translation as well as diffusion. For systems undergoing Brownian motion and having good scattering, the method of self-beating is preferred for its greater simplicity, but for weakly scattering systems the use of heterodyne detection offers an improvement of close to 2 times in the standard deviation of the measured radius under otherwise identical experimental conditions.

References

Berne, B. J., and Pecora, R. (1976). 'Dynamic Light Scattering' (Wiley: New York).

Chu, B. (1974). 'Laser Light Scattering' (Academic : New York).

Cummins, H. Z., Carlson, F. D., Herbert, T. J., and Woods, G. (1969). *Biophys. J.* **9**, 518.

Cummins, H. Z., Knable, N., and Yeh, Y. (1964). *Phys. Rev. Lett.* **12**, 150.

Cummins, H. Z., and Pike, E. R. (Eds) (1974). 'Photon Correlation and Light Beating Spectroscopy' (Plenum: New York).

Ford, N. C., Jr, and Benedek, G. B. (1965). *Phys. Rev. Lett.* **15**, 649.

Jakeman, E. (1972). *J. Phys.* A **5**, L49.

Jakeman, E. (1974). *In* 'Photon Correlation and Light Beating Spectroscopy' (Eds H. Z. Cummins and E. R. Pike), p. 75 (Plenum: New York).

Koppel, D. E. (1972). *J. Chem. Phys.* **57**, 4814.

Oliver, C. J. (1974). *In* 'Photon Correlation and Light Beating Spectroscopy' (Eds H. Z. Cummins and E. R. Pike), p. 151 (Plenum: New York).

Oliver, C. J. (1978). *Adv. Phys.* **27**, 387.
Pike, E. R. (1977). *In* 'Photon Correlation Spectroscopy and Velocimetry' (Eds H. Z. Cummins and E. R. Pike) (Plenum: New York).
Pusey, P. N., and Vaughan, J. M. (1975). *In* 'Dielectric and Related Molecular Processes', Vol. 2, Specialist Periodical Report (Chemical Society: London).
Thomas, J. C., and Fletcher, G. C. (1979). *Biopolymers* **18**, 1333.
Uzgiris, E. E. (1972). *Rev. Sci. Instrum.* **43**, 1383.
Wada, A., Tsuda, T., and Suda, N. (1971). *Jpn J. Appl. Phys.* **11**, 266.

Manuscript received 6 April 1981, accepted 3 June 1981