Ken Langley

A REVIEW OF BIOLOGICAL, CHEMICAL AND OTHER APPLICATIONS

K. H. Langley
Department of Physics and Astronomy
University of Massachusetts
Amherst, MA 01003

SUMMARY

An indication of the wide variety of applications of photon correlation spectroscopy (PCS) is given by reviewing selected topics in chemistry and biology, and briefly mentioning some applications in medicine and physics. Recent progress in instrumentation and in the understanding of diffusion in polydisperse and interacting systems of particles is emphasized. The period covered by this article is roughly from mid 1978.

INTRODUCTION

The task of the would-be reviewer of photon correlation spectroscopy applications in chemistry, biology, and other non-aerodynamic fluid flow and combustion fields has expanded well beyond the capacity of a single article or review paper. So many different physical systems have been fruitfully examined using this technique that their mere enumeration has become a formidable task. PCS has been used to measure the molecular weight, size, shape, flexibility, electric charge, and interaction of particles in solution as well as to study the flow, vibration, swimming, beating, swelling, electrophoretic movement, and aggregation of supramolecular structures, organelles or cells. The range of application is rich indeed.

A number of books concerning PCS have appeared in recent years emphasizing theoretical aspects [1] and experimental methods [2], and reporting the results of NATO Advanced Study Institutes [3,4] and a light scattering workshop [5]. Reviews of applications to hydrodynamics in biophysical chemistry [6], biopolymers and bicolloids [7], molecular biology and macromolecular chemistry [8], and dynamics of macromolecular solutions [9] contain hundreds of references to the literature up to 1977 and 1978. Here I shall attempt to give some idea of the progress of the past two years by commenting on the instrumentation, discussing experiments in selected areas, and speculating on where the field (if indeed it may be referred to as a single field) might be headed.

EXPERIMENTAL TECHNIQUES

We begin by recalling the basic configuration of a light scattering experiment. A typical apparatus for measuring translational diffusion coefficients is illustrated in Fig. 1. All other experiments discussed in this article require essentially similar equipment with, of course, variations or additions to the optical arrangement and the containment or mounting of the sample.

Light from a laser (useful primarily because of its highly directional beam) is focused in a small region of the sample (in order to increase the number of scattered photons per coherence area) usually held in a round or 1 to 10 mm square cuvette. A lens images the scattering volume in the plane of aperture 2, typically 100 μm in diameter. The size of the scattering volume is defined by the magnification of the detector lens, and the diameter of aperture 2 or the imaged laser beam diameter, whichever is smaller. Aperture 1 limits the collection of light (scattered at angle θ) to a few coherence areas at most. A microscope focused on aperture 2 together with a flip-up mirror is important as it enables a visual examination of the scattering volume for adequate sample clarity and uniformity. The scattered light viewed in the microscope is identical to the light that reaches the photomultiplier when the mirror is moved out of the way. X-Y motion of the detector lens is useful for aligning the beam image on aperture 2 and for picking a scattering region free from flare, dust, or other artifacts. Obvious possible refinements are a constant temperature and/or refractive index matched fluid bath surrounding the sample, filters to block non-laser light, etc.

A pulse amplifier-discriminator (PAD) presents detected photon pulses to the correlator. The processed correlation function is typically displayed on an oscilloscope and analyzed by a computer. We note that the correlator, display, and computer analysis are now combined in a single instrument in the Langley Ford Model 1096.

Correlator Developments

Table 1 summarizes the characteristics of correlators available for photon correlation spectroscopy. For biological and chemical applications, by far the most efficient and exact are the full (multibit), digital, real time correlators (numbers 2-4). Commercially available instruments are numbers 1-4 and 6 in Table 1. It is interesting that data analysis is now built-in or available on correlators 2a, 3a and 4.

Number 5 is representative of a class of computer-based correlators which have been reviewed previously [9]. By adding a fast hardware multiplier and associated counters, it is possible to realize considerable gain in speed over a computer that is simply programmed to accept as input the number of pulses each sample time and then compute the correlation function. The efficiency of the hybrid systems is still typically 2 or 3 orders of magnitude less than the full correlators at modest sample times (2 μs, for example) and is much worse at short sample times.

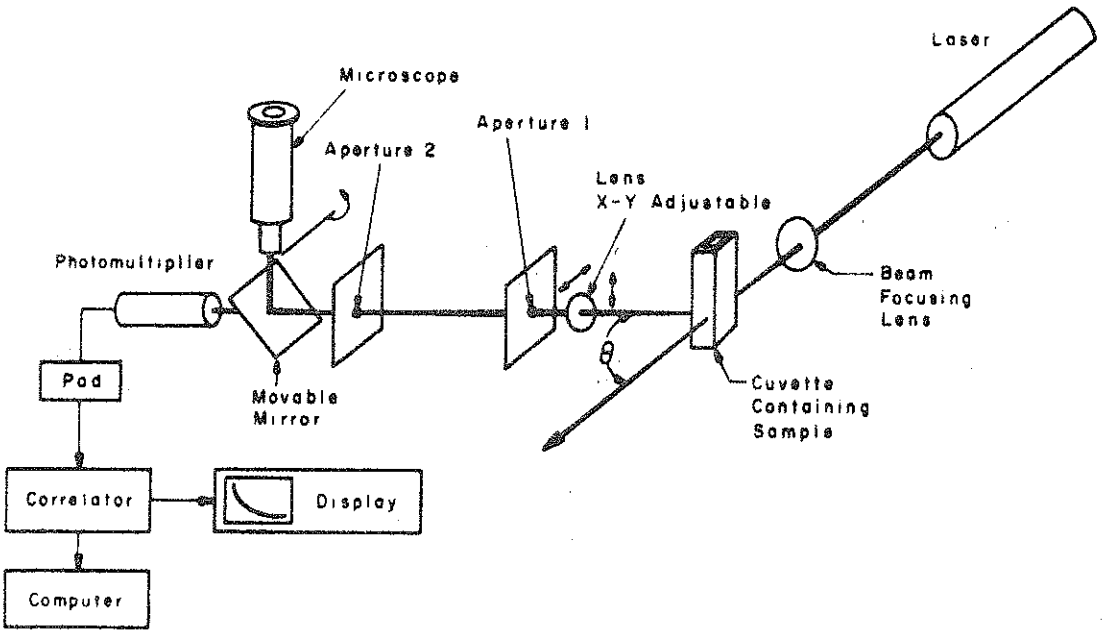

Figure 1. Typical experimental arrangement for diffusion measurements by photon correlation spectroscopy.

Table 1. Summary of Correlator Characteristics

	Model	Type	Number of Channels	Minimum Sample Time	Comment
1. Honeywell	SAI-48	Hard-ware	400	100 ns*	Batch or modified processing at sample times < 200 μs (inefficient) analog input (digital optional)
2. Langley-Ford Instruments	(a) 1096	Hard-ware	72-1096+	100 ns	μ processor controlled, graphic and alphanumeric display, analysis capability digital/analog input
	(b) DC-64	Hardware	64	100 ns	digital/analog input, low cost
	DC-128	Hardware	128**	100 ns	digital/analog input, low cost
3. Malvern Instruments	(a) 7025	Hardware	64**	100 ns	μ processor, analysis capability
	(b) 7023	Hardware	24-96	50 ns	single clipped correlator
4. Nicomp	6864	Hardware	64	100 ns	μ processor controlled, performs 2nd and 3rd order cumulants fit
5. Lempert and Wang (Ref. 73)	--	Software	50	100 ns	uses PDP 10/11 computer with added multiplier, counter, memory
6. Coulter Electronics	Nano-sizer	Software			PCS system dedicated to simple measurement of particle size

* "usable" at 50 ns, according to the manufacturer
\+ time shared
** expandable to 256 channels

Efficiency, and therefore the rate at which adequate signal to noise can be achieved, may be absolutely vital in studying biological samples which deteriorate, react, or otherwise change with time. The Coulter instrument (Table 1, No. 6) is a system dedicated to simple particle size determination by correlation of 90° scattered light from a 2 mW HeNe laser. The built-in microprocessor performs a second order cumulants fit to the measured correlation function and displays the results on two digital meters, one reading particle diameter in nanometers (400 to 30,000) and the other a relative polydispersity index derived from the second cumulant. The correlation function is not output or displayed.

We have purposely omitted high speed correlators (10 to 20 nsec sample time) from Table 1, as extremely fast detectors and electronics are expensive and are not usually required in biological or chemical research, with the possible exception of the measurement of rotational diffusion coefficients.

Other Developments

The range of PCS applications continues to broaden as improvements in light scattering technique are developed: Thomas and Schurr [10] report PCS in the near ultraviolet, extending studies of internal motions in DNA to shorter wavelength. Sanders and Cannell [11] used a cylindrical beam focusing lens to spread out the region of laser power absorption in a solution of hemoglobin without reducing the scattered light coherence. Pusey [12] has demonstrated a new "anticorrelation" method of measuring the rotational diffusion coefficient of anisotropic molecules (discussed in more detail below). The Chromatix low angle scattering instrument has been adapted for diffusion measurements using light scattered at $\theta=6°$.

TRANSLATIONAL DIFFUSION

Translational diffusion studies of macromolecules, particles, or colloids in solution are by far the most numerous of all the applications of photon correlation spectroscopy. From its very inception, the advantages of rapidity and ease of measurement (typically a few seconds to a few minutes), small sample quantity requirement (typically 1 cm^3, but as little as 10 μ liter), accuracy (1-2% in a clean monodisperse sample), and lack of perturbing influences such as concentration gradients, excess pressure, and nonequilibrium conditions have been well recognized. It seems reasonable to say that in chemical and biochemical applications to relatively monodisperse samples, photon correlation spectroscopy has replaced the classical technique of analytical ultracentrifugation as the method of choice.

The simplest system in which one can think of making a diffusion measurement is that of a dilute solution (but yet concentrated enough so that the number of scatterers within the scattering volume is much greater than one) of small (compared to the wavelength of light), identical, noninteracting particles. In this case the scattered intensity autocorrelation function has the well known exponential form

$$g^{(2)}(\tau) = 1 + \alpha e^{-2\Gamma\tau}$$

where the decay rate $\Gamma = D_T q^2$; D_T and q are the translational diffusion coefficient and the scattering wavevector respectively. Γ is often referred to as the linewidth, since it is also the half width (in rad/sec) of the Lorentzian shaped Rayleigh spectral line. Recent theoretical developments have been directed to removing the restrictions mentioned above. A theory for the correlation function (at least in the Rayleigh-Debye approximation) now exists for large, rigid, cylindrically symmetric, optically anisotropic particles of arbitrary shape [13]. It is cast in a form that explicitly displays the dependence on all scattering geometry parameters. In addition, considerable effort has been focussed on two problems which are central to the broader application of PCS: polydispersity and particle interaction.

Polydispersity

In systems containing independent diffusing particles of different sizes or shapes, each species of particle present will contribute an exponential decay term to the field autocorrelation function. Assuming that the specific polarizability is independent of molecular weight, the amplitude of each term $G(\Gamma_i)$ is proportional to the weight fraction, molecular weight, and structure factor for that species i. The decay rate is proportional to the diffusion coefficient for that species, and the normalized field autocorrelation function becomes

$$g^{(1)}(\tau) = \sum_i G(\Gamma_i) e^{-\Gamma_i \tau} / \sum_i G(\Gamma_i) \quad .$$

Here $\Gamma_i = D_i q^2$ is the decay rate for species i. In the case of a continuous distribution of diffusion coefficients.

$$g^{(1)}(\tau) = \int_0^\infty G(\Gamma) e^{-\Gamma\tau} \, d\Gamma / \int_0^\infty G(\Gamma) d\Gamma \quad .$$

PCS would indeed be an even more powerful experimental technique if it were practical to measure the correlation function of light scattered from a polydisperse collection of macromolecules, perform the inversion implied in the above equations, and obtain directly the distribution of diffusion coefficients, or better still, obtain the distribution of particle sizes or molecular weights. Unfortunately this goal remains attainable only in certain specific situations. The principal difficulty lies in the fact that the data required to accurately perform the inverse Laplace transform of the above equation and obtain the decay rate distribution function, $G(\Gamma)$, must be of extremely high precision over a very wide range of values of τ.

Many approaches have been made to using the autocorrelation function to characterize polydisperse systems. These attempts basically fall into two categories, those which require some assumption or knowledge about the form of the decay rate distribution function and those which do not. One attraction of the earliest analysis schemes, Koppel's popular method of cumulants [14] (and later simplifications) [15], is that the decay rate distribution is characterized by two or (in favorable cases) three parameters, representing the average decay rate $\bar{\Gamma}$, its variance $\mu_2/\bar{\Gamma}^2$, and the skewness μ_3 of the distribution. These

parameters are obtained independent of the details of the shape of the distribution. The cumulants method therefore cannot distinguish between unimodal and bimodal distributions or between discrete and continuous distributions.

Recent reports involving polydispersity have included both kinds of analysis, those assuming some knowledge of $G(\Gamma)$ and those treating $G(\Gamma)$ as entirely unknown. Schmidt et al. [16] have extended the work of Brehm and Bloomfield [17] to particles of larger size (polystyrene latex spheres approximately 110 and 480 μm diameter) and explored the nonlinear q^2 dependence of the apparent diffusion coefficient of the two component mixture caused by the polydispersity and by the change in scattering factor of the particles. Their unsuccessful attempts to fit the observed correlation functions to two exponentials with freely varying amplitudes and decay rates, serves to illustrate the advantage of some knowledge of the distribution function. Fits performed using either known scattering factors or known diffusion coefficients of the two species were quite satisfactory, although known values of D for the individual species could not be reproduced to better than about 10 to 30%.

Photon correlation spectroscopy has been applied to the measurement of continuous molecular weight distributions in a number of situations where there is an a priori knowledge of the particular form of the distribution function such as Pearson, Schulz, gamma or log-normal. The approach is essentially to perform a least squares fit to the correlation, varying the parameters of the distribution function (or variables related to these parameters) to realize the best fit [18-20]. Morris et al. [21] have presented a method for analyzing polydisperse suspensions of spheres, thin rods and discs (all of the same minor dimension), and ellipsoids of revolution (of constant axial ratio) in the case of rigid particles small enough to obey the Rayleigh-Debye approximation. Parameter values for two-parameter distribution functions (log normal, for example) are derived from Z average values of the squared radius of gyration $\langle R_G^2 \rangle$ and the Z average diffusion coefficient, $D_z = \bar{\Gamma}/q^2$. $\langle R_G^2 \rangle$ is obtained from a Zimm-type plot of inverse scattered intensity vs. q^2, and D_z is obtained from the initial slope of the correlation function. The authors do not present experimental data or assess the errors inherent in the procedure.

In contrast, Provencher [22] has presented a method which yields a determination of molecular weight distributions of polymers directly from an analysis of the autocorrelation function. In earlier papers, Provencher had explored an eigenfunction expansion method which fit the correlation function by a discrete series of exponentials using only the correlation function as input data. The number of exponentials required was determined by the procedure as well as the amplitude and decay rate of each term. In the newer method the problems of instability and nonuniqueness of the decay rate distribution which usually result from inversion of the integral equations are overcome by constraining the distribution to be the smoothest non-negative one that is consistent with the data. If the distribution is known to be bimodal or known to be unimodal, constraining the distribution accordingly can further improve the stability and accuracy. The method was used

[23] to determine the molecular weight distributions of two well characterized polystyrene samples in cyclohexane at 35°C (the relation between D_T and molecular weight, $D_T = K_T M^{-b}$, is known so that the distribution in Γ can be related to the distribution in molecular weight M). One distribution was quite narrow (NBS SRM 705, M_z: M_w: M_n = 1.12 : 1.07 : 1) and the other quite broad (NBS SRM 706 M_z: M_w: M_n = 2.9 : 2.1 : 1.0). In both cases, the light scattering results reproduced the values of M_z, M_w, and M_n known from osmotic pressure, sedimentation equilibrium and fractionation to within a few percent. It appears that this method can be consistent and reliable as long as dust, stray light and other systematic errors are reduced to the level of the statistical errors in the correlation function.

A histogram method of molecular weight distribution analysis has been developed by Chu and co-workers [24,25], compared to the methods of cumulants, Pearson distribution, and Laplace transform, and applied to a test of the Yamakawa, Imai, and Pyun and Fixman theoretical descriptions of a polymer in a dilute theta solution [13]. The method consists of approximating the continuous distribution of decay rates, $G(\Gamma)$ by a histogram such that the correlation function may be written as

$$g^{(1)}(\tau) = \sum_{i=o}^{m} G(\Gamma_i) \int_{\Gamma_i-\Delta\Gamma/2}^{\Gamma_i+\Delta\Gamma/2} \exp(-\Gamma\tau)d\Gamma$$

where $G(\Gamma_i)$ is the total integrated intensity scattered by all molecules having decay rates in the range $\pm \Delta\Gamma/2$ about Γ_i. The computed correlation function is least squares fit to the measured correlation function by varying the values of $G(\Gamma_i)$ to minimize the χ^2. Gulari et al. [26] were able to obtain the molecular weight distribution of polystyrene in cyclohexane (a theta solvent) accurately enough from a single PCS experiment to determine the constant K_T in the relation between diffusion coefficient and molecular weight $D_o^{\theta} = K_T M^{-\frac{1}{2}}$. They were then able to test the Yamakawa, Imai, and Pyun and Fixman theories by determining which one yielded an identical molecular weight distribution when the experiment was repeated at a second concentration. Chu and Gulari [27] combined light scattering intensity determination of molecular weight, second virial coefficient, and radius of gyration, with the concentration dependence of D_T and the molecular weight dependence of the radius of gyration to transform a measured histogram of decay rate distribution to a distribution of molecular weight for the polymer poly[bis (m-chlorophenoxy) phosphazene].

The histogram method provides a general approach to the polydispersity problem, and in cases where the relation between molecular weight and diffusion coefficient is known, provides considerable detail on the molecular weight distribution, in contrast to the momements analysis. The method has been verified successfully for bimodal as well as unimodal distributions [28].

Interacting Particles

The simple exponential behavior of the correlation function and its straightforward interpretation in terms of single particle diffusion

is expected only in the limiting case of infinitely dilute, and therefore noninteracting particles. At finite concentration, the motion or mere presence of one molecule influences the motion of the next and the entire diffusive process begins to take on certain collective or odered properties. The particle interactions are of two types: Coulombic interactions due to charges on the molecule and its surrounding cloud of ions, and hydrodynamic interactions resulting from the fluid flow field around a moving particle. The appropriate experimental response to this situation has usually been to measure some average diffusion coefficient at several solute concentrations, and extrapolate to zero concentration to obtain D_o. In many cases this procedure is impractical, if not impossible, as the charge interaction may extend to distances greater than the interparticle spacing even at concentrations yielding impractically weak scattered intensity. In a flexible polymer there is an obvious mutual influence of neighboring sections of the chain.

A great deal of effort in both theory and experiment has been made in an attempt to understand and interpret the results of PCS experiments in charged colloidal and macromolecular solutions. A number of theories have emerged using different starting points and treating systems of different physical structure [29-36]. In principle, a theory should take into account both charge and hydrodynamic effects, but since the charge effects are often much stronger, hydrodynamic interactions are frequently neglected. Hess [37] has briefly reviewed a number of the theories and clarified their relationship to the solution of the generalized Smoluchowski equation by Ackerson [38]. In the same volume, Pusey [39] has reviewed the results of several experiments on small highly charged polystyrene latex spheres in aqueous solutions reduced to very low ionic strength by the presence of ion-exchange resins. The experimental situation and its physical interpretation are roughly as follows: As ionic strength is lowered, the range of repulsive Coulomb interactions increases, becoming comparable to the interparticle spacing and the wavelength of light. The spatial arrangement of the solute becomes liquid-like i.e. ordered at short range but not over macroscopic distances. Each particle is held in a "cage" of repulsive forces due to its neighbors. Neglecting hydrodynamic interactions (valid for dilute solutions) a given particle experiences the usual rapidly fluctuating Brownian forces due to solvent molecules plus small but long-lived interaction forces from its charged neighbors. We must distinguish two time regimes. (1) For times less than the fluctuation time of the interaction force, essentially free diffusion obtains, but with an effective diffusion coefficient $D = D_o/S(q)$ where $S(q)$ is the static structure factor. The diffusion coefficient (measured from the initial decay rate of the correlation function) plotted as a function of q has peaks and valleys corresponding to the minima and maxima, respectively in $S(q)$. (2) For times long compared to the fluctuation time of the interaction force the theory is more complicated. The result is that for large q (such that $S(q) \approx 1$) a slow diffusive motion is expected; the correlation function decays exponentially, but at a rate much slower than for the uncharged free particles. At small q (at least in monodisperse systems), the effective diffusion coefficient is expected theoretically to approach the uncharged free particle value, but experimentally it does not. Until recently it had been thought that the effect of polydispersity on this "small q, slow mode" diffusion would be small. However, exploiting a

simplification suggested by Weissman [36], it now appears that self (single particle) diffusion contributes strongly to the slow mode at small as well as large values of q, and the observed slow diffusion is due to the long time required to relax a fluctuation of a specific species of particle in a polydisperse system.

In addition to the studies of highly charged colloidal particles there has been recent progress in both theoretical understanding and experimental evaluation of the diffusion of charged macromolecules in solution. Here the situation is somewhat different. The molecular charge is much lower, reducing the range of interaction; the scattering wavevector q is much too small to probe features of the structure factor S(q), and hydrodynamic interaction is certain to play a more significant role. Schor and Serrallach [40] have compared the bovine serum albumin (BSA) experimental data of Doherty and Benedek with two modifications of the Phillies theory: (1) A hard sphere approximation to the radial distribution function of molecules yields a good approximation to the earlier Doherty-Benedek-Stephen theory. (2) Addition of a dilute gas radial distribution function results in significantly better agreement with the data. Weissman and Ware [41] have applied fluctuation transport theory to dynamic light scattering and electrophoretic mobility measurements in protein-counterion solutions, and considered the effects of coupling between charge and concentration fluctuations. They found reasonable agreement with the diffusion measurements of Doherty and Benedek, but note that there does not seem to be experimental evidence for the distinction between fluctuation and single particle transport one would expect in an electrophoretic light scattering experiment. Weissman et al. [42] did careful simultaneous intensity and diffusion measurements on BSA at low ionic strength, and showed that the decrease in S(q) and corresponding increase in D are not by the same factor, and that solute interactions contribute strongly to the solution viscosity. The interrelated questions of ionic contributions to the fluid friction, the influence of hydrodynamic interactions, and the coupling of single particle diffusion to concentration fluctuation modes continue to be actively pursued [43-46].

Schurr [46] has considered the effect of electrolyte friction on flexible polymer polyelectrolyles and Böheim [47] has shown that at least in the double scattering approximation, charged particle interactions also affect the correlation function of the depolarized scattered light. A review article on interacting particle systems by Pusey and Tough is planned for a forthcoming book [48].

Applications of Translational Diffusion

Photon correlation is now routine in studying the hydrodynamic properties of macromolecules. The translational diffusion coefficient may be used with the sedimentation coefficient and specific volume to yield molecular weight through the Svedberg equation, $M = sRT/D_T(1 - v\rho)$, or D_T may be of interest in its own right as a probe of molecular conformation. Many detailed studies of the diffusion coefficient dependence on solution conditions would have been unthinkably time consuming if the rapid and convenient technique of PCS were not available. In the next few paragraphs we mention some studies of the past two years. The list is by no means exhaustive.

0, 1, 2, 3 ... 6 tail fibers attached were separated by an applied electric field into 7 bands according to their electrophoretic mobilities. The mobility and diffusion coefficient were then measured simultaneously in the same experiment. Identical principles of simultaneous electrophoresis and laser light scattering were used to determine the size and charge distributions of 2 radiocolloids commonly used in nuclear medicine [65].

Diffusion studies of polystyrene in trans-decalin (a theta solvent) have been reported in a series of papers by Chu and Nose [66-68,28]. In the course of testing the renormalization group scaling theories for the power law behavior of dynamical properties of polymer solutions, they found a fast gellike relaxation mode which was responsible for an anomalous increase in the variance, $\mu_2/\bar{\Gamma}^2$, of the linewidth distribution, $G(\Gamma)$, as the solute concentration was increased. Their data imply a different behavior of the temperature-composition diagram than initiall predicted, and show that power law relations are valid only for limited ranges of concentration.

Micelles of gangliosides [69], bile salt-lecithin mixtures [70], and sodium dodecyl sulfate (SDS) [71,72] have been examined by photon correlation spectroscopy. For the bile salt-lecithin solutions [70] a new "mixed bilayer" model in which bile salts are included in a fixed stoichiometry within the bilayer interior is proposed. A thermodynamic theory of SDS micelle formation in high salt, based on the two chemical potentials associated with adding a molecule to either the cylindrical or hemispherical end region, is presented by Missel et al. [71], while Corti et al. [72] raise the possibility that the observed large aggregates are a hydrated crystalline phase formed by aggregation of micelles.

ROTATIONAL DIFFUSION

For solute particles which are anisotropic in shape, polarizability, or both, photon correlation offers the opportunity to measure rotational as well as translational diffusion. We can appreciate this possibility by realizing that the tumbling of a molecule will lead to fluctuations in the scattered light intensity provided that either the dimensions of the molecule are large enough to produce orientation dependent intramolecular interference terms in the scattered field, or that the polarizability is anisotropic. These fluctuations will give rise to a time dependence of the correlation function which is characteristic of the molecular rotational diffusion time, which in turn is related to the molecular size and shape through the Perrin equations [Ref. 1, p. 143]. In large molecules, internal motions such as stretching, flexing, or twisting may also give rise to fluctuations which influence the field autocorrelation function. Studies of these internal motions will be discussed in the next section.

The scattered field correlation function for particles undergoing simultaneous translational and rotational diffusion is quite complex, but we can gain an understanding of the general features of the problem by immediately considering the results for a simpler class of scatterers. Aragon and Pecora [74,13] have shown that if rotation and translation are uncoupled, in the Rayleigh-Debye approximation the correlation func-

tion for large cylindrically symmetric, optically anisotropic particles is an infinite series of decaying exponentials with decay rates given by $D_T q^2+\ell(\ell+1)D_R$ where D_T and D_R are the translational and rotational diffusion coefficients, and $\ell=1,2,3, \ldots$ The amplitude of each term depends on the size, shape, and polarizability of the particles and on the details of the scattering geometry.

Specializing further to optically isotropic rod shaped molecules, the result is that for small values of qL (L is the rod length) the amplitude of the rotational contribution is small and the field correlation function is identical to that for pure translational diffusion. At larger qL, the $\ell=0$ (translation) term weakens and the $\ell=2$ term contribution increases (to about 10% at qL=5) so that at larger scattering angles the correlation function is a sum of two exponentials: $\exp(-D_T q^2 \tau)$ and $\exp(-D_T q^2 \tau - 6D_R \tau)$. If the rod (or other particle)is optically anisotropic, the possibility arises to measure the correlation function of the very much weaker depolarized light. At small scattering angle, translational contributions are negligible and $g^{(1)}(\tau)$ is proportional to $\exp(-6D_R \tau)$. More recently, Schurr [75] has treated the simultaneous translational and rotational Brownian motions of an assembly of interacting polar molecules, generalizing Ackerson's [38] solution to obtain the initial slope of the depolarized autocorrelation function.

From the above we see there are at least two experimental possibilities, both of which have been exploited. The first, useful if the shape of the particle is known, is to use D_T determined in a small scattering angle experiment to aid in fitting $g^{(1)}(\tau)$ measured at larger q to a sum of exponentials, thus obtaining D_R. The second is to measure D_R directly by depolarized scattering, using the extremely weak signal resulting from the small anisotropy in the polarizability.

Probably because of the technical difficulties, relatively few experimental results have actually been reported [76]. Kochi et al. [77] used both a photon time-of-arrival technique with time resolution of a few nsec, and a Fabry-Perot interferometer to measure the correlation time due to the rotational relaxation of depolarized light scattered at 90° from transfer RNA. The measurement is negligibly affected by translational diffusion relaxation which is about 3 orders of magnitude slower. Crosby et al. [78] used forward depolarized scattering to determine the persistence length in a polydisperse sample of PBT, a stiff linear synthetic polymer. Given a knowledge of the molecular weight distribution, together with the Yamakawa [79] and Hearst [80] theories of rotational friction coefficient, the correlation functions were fit using the only adjustable parameter, the persistence length, which is the quantity of interest. Bruning and Fijnaut [81] have measured the rotational diffusion coefficient of 70S ribosomes, using depolarized light scattering.

Griffin and Pusey [12], in a novel approach, have measured the orientational relaxation of rodlike tobacco mosaic virus by cross correlating the signal from two detectors collecting 90° scattered light from opposite sides of the incident beam. The small ($\simeq 6\mu m^3$) scattering volume contains about 1 particle, on the average. The asymmetry in scattering into the two detectors leads to an anticorrelation in $g(\tau)$ at smaller values of τ.

Nucleic acids ranging in molecular weight from a few times 10^7 down to about 2.5×10^4 daltons have been investigated using dynamic light scattering. High molecular weight DNA has proven to be an excellent system for testing models of flexible polymer chains [49] as is dicussed later. Voordouw et al. have characterized ColE1-plasmid DNA [50] and studied the conformation of its complexes with histone proteins [51]. Wilson et al. [52] found a collapse of T7 DNA to a monomolecular condensed form upon the addition of spermidine or spermine to aqueous solution or addition of Mg^{2+} or putrescine to water-methanol solution. Collapse occurred when 90% of the DNA phosphate charges were neutralized by counterions. The role of proteins in stabilizing RNA chains against unfolding during ribisomal subunit assembly was studied by Bogdanov et al. [53]. The effect of solution conditions and aminoacylation or transfer RNA has been extensively investigated by Potts et al. [54-56]. The question of whether or not a conformational change accompanies deacylation of the molecule has been resolved by showing that cooperativity including the binding of Mg^{2+} ions is involved in the two state transition model [54].

Other biological macromolecules and particles studied recently include hemoglobin, enzymes, collagen, fibrinogen, viruses, bacteriophages, and radiocolloids. In a very precise experiment Sanders and Cannell [57] report an 0.8% decrease in the diffusion coefficient of hemoglobin upon oxygenation at a concentration of 13 mg/ml, and extrapolate to a 1.6% decrease at infinite dilution, in disagreement with the 3% increase predicted from X-ray diffraction measurements in the crystal. Haas and Ware [58] confirmed the dissociation of tetrameric carboxyhemoglobin to dimers above pH 10 and estimated values of the subunit dissociation equilibrium constants. Simultaneous measurements of the electrophoretic mobility revealed an increase of the negative charge on the dimer upon dissociation. Conformational changes (or lack of change) in the enzyme phenyl-alanyl-tRNA synthetase upon binding to various small substrates and their involvement in functioning of the enzyme have been noted by Holler and Ford [59].

Silver et al. [60] found evidence for a two step process in the initial aggregation of collagen monomers from molecular dimensions derived from observed intensity and diffusion rate changes. The clotting mechanism of blood is ultimately determined by the structure of the protein fibrinogen, for which several quite different structures have been proposed. PCS experiments by Palmer et al. [61] were not able to determine the solution structure unambiguously, but did indicate that the molecule is rigid, does not dissociate at low concentration, low ionic strength or at 37°C, and has a diffusion coefficient in agreement with values from sedimentation. The hydrodynamic sizes of five naked icosahedral animal viruses were measured by Dobos et al. [62] and found to be separable from the family reoviridae and distinct from each other on the basis of their diameters and other biophysical properties.

Reorientation of the tail fiber of a bacteriophage from a retracted to an extended position results in a large diffusion coefficient change. The concentration and ionic strength dependence [63] and thermodynamic parameters [64] of this transition as well as the tail fiber attachment have been studied by PCS. In the later experiment, the particles with

Rotational effects alter the correlation function significantly in scattering from large particles. Only recently have these effects been accounted for in PCS of motile organisms (see below). Wilson and Bloomfield [82,83] found that an apparent difference in bacteriophage molecular weight between tail fiber retracted and extended conformations was eliminated when scattering factors and rotational diffusion were correctly accounted for by modeling the phage with an extended array of identical scattering elements.

INTERNAL MOTION

In large molecules, internal motion can alter the relative phase of light scattered by different parts of the molecule. In solution, fluctuations in this intramolecular interference appears as an additional relaxation mode in the correlation function. The review by Schurr [7] discusses applications of the Rouse-Zimm, Harris-Hearst, and mean-force models of flexible polymers to PCS observation of these modes in polymer solutions. Thomas et al. [10,49] and Lin et al. [84] have been quite successful in fitting PCS data on DNA's to the Rouse-Zimm model which pictures the polymer as a string of beads connected by springs and characterized by 3 parameters: the number of beads, the hydrodynamic friction factor of each, and the mean squared spring extension. Plots of the apparent diffusion coefficient (obtained by force fitting a single exponential to the correlation function) vs q^2 show an increase from the normal translational diffusion coefficient at low q^2 to a plateau value as q increases to $qR_G >> 1$, where R_G is the radius of gyration. The model is found to explain reasonably well the effects of pH, salt concentration, single strand breaks and molecular weight. The relaxation time of the lowest frequency internal (Langevin) mode deduced from the experiment is consistent with other measurements, and no "long time tails" on the relaxation as observed in other high molecular weight preparations were observed. The model parameters were used to detect the presence of flexible joints introduced by polycation or speridine binding [84].

MOTILE SYSTEMS

The central principle of photon correlation spectroscopy is this: if the scatterer moves, there is a possibility that the field autocorrelation function will provide some information about the nature of that motion. With this in mind, it is natural to expect that PCS may be used as a sensitive probe of biological motion at the microscopic level to obtain such information as the average speed and fraction of live and dead organisms in solutions of swimming bacteria and spermatozoa, the frequency and degree of activity of beating cilia, and the velocity distribution of flowing protoplasm inside an intact cell. In these applications, particularly in vivo, interpretation of the correlation function must be done with great care as the scatterer motion is rarely simple diffusion or uniform translation, and frequently involves such complications as large scatterers, polydispersity, combined translation and rotation, and internal motion of the scatterers.

Although light scattering experiments on motile systems date from early work on spermatozoa by Berge et al. [85], a full understanding of

the observed correlation functions is a very recent achievement. Two excellent examples of this recent progress are provided by the series of papers by Chen and co-workers [86-89] on the motile bacteria E. Coli, and the series by Hallett and co-workers on bull spermatozoa [90-92]. In 1971 Nossal [93] showed that if scattering organisms move in straight lines for times of order of 1/qv where v is the velocity, and if the organisms are point scatterers, the correlation function is related to the swimming speed distribution function P(v) by

$$g^{(1)}(\tau) = \int_0^\infty [\sin(qv\tau)/(qv\tau)]P(v)dv \quad .$$

It rapidly became clear that observed correlation functions for bacteria and spermatozoa could only be explained if the particle structure factor and rotational swimming motions were accounted for. It has been found for E. Coli that modeling the bacteria as a prolate ellipsoid swimming with a Maxwellian velocity distribution in straight lines along the figure axis is adequate to explain the angular dependence of the scattered intensity and the correlation function half width at scattering angles up to about 10 degrees, but for larger angles it is necessary to considerably improve the model.

Additional complications required to satisfactorily explain the more rapid decay and the amplitude and period of the oscillation in the plot of half width vs. scattering angle were: a coating of the ellipsoid by a layer of different index of refraction [86], and inclusion of (1) planar wiggling about the direction of translation, (2) rotation about axes perpendicular and parallel to the translation direction (helical motion) [87] and (3) modeling of E. Coli as a large prolate ellipsoid [88] (although it was found that because of the small difference between the 1 μm long bacteria index of refraction and that of water, the Rayleigh-Gans-Debye approximation is valid within about 10%). It appears that a model of the "twiddle" motion must still be found to remove the last discrepancies.

Craig et al. [92] found that in bull spermatozoa, at a scattering angle of 15%, the half width of the Lorentzian field autocorrelation function is dependent on the mean head rotation frequency and not on the translation speed of the moving cells, showing that PCS clinical evaluations of bull [91] or human [94] sperm viability and vigor, unless performed at very small scattering angle, must be viewed with a great deal of caution. In a related study, Lee and Blandau [95] showed that the hormone progesterone significantly reduces human spermatozoan average swimming speed, but only at concentrations 3 orders of magnitude greater than physiologic levels. The mode of action of progesterone releasing intrauterine devices may still involve motility inhibition, provided it is the hormone to spermatozoa ratio that is important.

ELECTROPHORETIC LIGHT SCATTERING

The principles of electrophoretic light scattering (ELS), (also known as laser Doppler spectroscopy) are almost self explanatory. Electrophoresis is the uniform translation of charged particles through a fluid, acted on by the counterbalanced forces of an applied electric field, E, and hydrodynamic drag. The velocity, v, is detected by laser

Doppler velocimetry, usually by detecting the audio beat frequency between frequency shifted light scattered from the moving particle and unshifted light directly from the laser. The beat frequency spectrum generated by scattering from a mixture of several particle species consists of a series of peaks, the center frequency of a peak corresponding to the velocity of that species; $f = \underline{q} \cdot \underline{v}/2\pi$. The mobility, U, is related to the velocity and electric field by $\underline{v} = U\underline{E}$, obtained from the center frequency. The peak width yields the diffusion coefficient of the species. Details of the theory [96] and apparatus [97] have been reviewed.

While the method has been useful in studies of proteins, nucleic acids, viruses, synthetic polyelectrolytes, and colloids [98,99], perhaps the most exciting applications result from the ability to measure the surface charge density which is important in the functioning of large membrane bounded biological particles. Systems investigated have included chromaffin granules [100], synaptic vesicles and synaptosomal membranes [101], rat mast cells [102], macrophages [103,104], leukocytes [105] and lymphocytes [106].

OTHER APPLICATIONS

The references cited in the above sections are representative, but are by no means complete. Diffusion and conformation studies of many other biological macromolecules and synthetic polymers have been reported. A number of interesting topics related to biology have not been touched upon, such as molecular motions in functioning neurons [107], lateral diffusion in membranes [108], blood flow [109], and fluorescence correlation spectroscopy [110]. Many PCS applications in physics, including liquid interfacial viscosity and surface tension [111,112] and studies of surface statistical roughness [113], critical phenomena [114, 115], liquid crystals [116], and atmospheric turbulence [117] are beyond the space limitations of this review.

CONCLUSIONS

The number of photon correlation applications continues to grow at a steady pace as the technique becomes more widely known and accepted. An appreciation for the subtleties of the theory and for various experimental effects is required as we seek to apply PCS to more and more complex situations. Only translational diffusion measurements of relatively monodisperse solutes can now be considered routine, but progress in the areas of polydispersity, concentration effects, and charge interaction will continue to encourage broader application of the method outside the research laboratory. Electrophoretic light scattering holds great promise, but difficulties in the consistent preparation of adequate scattering chambers continues to frustrate more general use.

With the above exceptions, instrumentation has improved to the point where sample preparation, rather than experimental apparatus, is usually the limiting factor in data quality. Progress is still needed in the areas of faster time resolution and more flexible distribution or greater number of correlator channels, but already the field has matured to the stage where photon correlation is being designed into

reasonably low cost instruments that are dedicated to the complete performance (including analysis) of specific measurements.

ACKNOWLEDGEMENTS

I would like to express my thanks to authors, too numerous to list individually, who sent copies of their papers and preprints. This work was supported in part by NSF grant PCM 78-15795.

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