CHAPTER II

GENERAL THEORY OF PHOTON CORRELATION
In contrast to the simplicity of carrying out an experiment on Photon correlation spectroscopy, the theory is quite involved and comprises a number of different topics the understanding of which requires an acquaintance with the theories of light scattering, the statistics of light fields, principles and practice of light detection, the method of data evaluation and some knowledge of the fast electronics circuitry. The method is based on the property of particles suspended in liquid to scatter light from an incident beam in all directions of space. Since the molecules perform Brownian motion, the total scattered field at any point in space resulting from superposition of all wavelets scattered from individual particles fluctuate with time. The analysis of these fluctuations yields the lateral diffusion coefficient of the particles and by means of the Stokes Einstein relation, the hydrodynamic radius for the case of the spherical particles. This novel technique requires very small amounts of the often precious samples. However, the relationship between the measured quantities and the quantities to be obtained is complicated by the fact that the intensity rather than the field is measured with a common light detector and that light is measured by quantum process whose statistics have to be considered. The intensity fluctuation spectroscopy covers the general class of experiments where intensity fluctuations are analyzed regardless of the signal processing techniques actually used. Photon correlation spectroscopy is used when intensity fluctuations are processed by a digital correlation technique (Jakeman, 1968; Cummins and Swinney, 1970; Chen et al., 1972). Usually the frequency shift produced by scatterers is very small in comparison to the
frequency of light. The resulting spectral linewidth can be as narrow as few KHz. and the resolution required to decipher it is $10^{12}$ which calls for high resolution technique while a conventional optical spectroscopy fails above a resolution of $10^7$.

2.1 BASIC ASPECTS OF PHOTON CORRELATION SPECTROSCOPY:

It is well known that the small frequency broadening in the light scattered from dilute solutions of macromolecules contains information about diffusion of macromolecules and in addition DLS is being used to study the critical phenomena (Pipe and Stanley, 1981). The experimental realization of these ideas depends not only on directly measuring the small frequency changes involved by use of monochromators but the use of "optical beating" technique (Bertolotti et al., 1967; Jakeman and Pipe, 1968).

It is convenient to think of the scattered light fields from each beam as suffering a constant frequency shift due to constant rate of phase change of the scattered light seen by the detector while the particle moves through the scattering volume. Since the scattering volume is finite the number of Doppler beat cycles measured is limited which gives rise to a broadening of the spectrum that is usually referred to as the Doppler ambiguity. Alternatively, the particles give rise to a small fraction of a Doppler cycle before they change direction by collision.
and therefore concept of a frequency shift is not directly applicable in such cases. In this case, as in the case of Rayleigh scattering, it is perhaps useful to think of the scattered light as a random diffraction pattern varying on a time scale governed by the internal changes occurring in the scattering volume. Such a pattern is known as a speckle. In all cases, therefore, the detector measures the changes in light intensity, either as regular Doppler beats or as randomly varying diffraction lobes, and one can relate these fluctuations either implicitly or explicitly to the spectrum of the scattered light arising from the temporal behaviour of the sample. The technique is known as light beating or intensity fluctuation spectroscopy or, when the scattered light flux is measured by processing individual photons, photon correlation spectroscopy (PCS). PCS is characterized by a 'digital' technique for measuring intensity fluctuations in which the number of photons arriving at the detector at the set time interval is repeatedly counted and its time autocorrelation function (ACF) computed. Digital correlation techniques greatly improve the signal to noise ratio. The time scales in photon correlation studies as compared to molecular scale, correspond to long time phenomena. Thus PCS is specially well suited for measuring dynamic constants associated with macromolecular and particulate systems.

Let us consider the calculations of the scattered electric field when a light beam traverses a transparent medium. The incident electric field polarizes the scattering medium, creating the electric multipole all along
the medium. We will consider induced dipoles only (Crosignani et al., 1975) which oscillate with the incident light frequency. As is well known, for a non conducting medium one can simply look for the existence of the dielectric susceptibility $\chi$ relating the electric field $\vec{E}(\vec{r},t)$ and the electric polarization (dipole moment per unit volume) $P(\vec{r},t)$ as

$$P(\vec{r},t) = \chi(\vec{r},t) \ E(\vec{r},t)$$

(1)

For an equilibrium system, where coherent laser source is used as the incident electric field $\vec{E}(\vec{r},t)$, the scattered radiation which is characterized by the electric polarization $P(\vec{r},t)$ will remain devoid of any statistical fluctuations. Since it is fundamental to statistical mechanics that a system of many particles never exhibits perfect equilibrium, the thermodynamic state variables describing the system continuously fluctuate with time about their most probable values. The electric susceptibility $\chi(\vec{r},t)$ is a function of such variables, e.g., temperature, pressure, density etc. The instantaneous polarizability then can be written as.

$$\zeta(\vec{r},t) = <\zeta> + \Delta \zeta(\vec{r},t)$$

(2)

Where $\zeta$ is the most probable value of the molecular polarizability and is related to the dielectric susceptibility by the relation.

$$\chi = 1 + 4\pi\zeta$$

(3)
Accordingly instantaneous dielectric susceptibility $\chi$ can be written as

$$\chi(\mathbf{r},t) = \langle \chi \rangle + \Delta \chi(\mathbf{r},t)$$

(4)

The polarization at any point in the medium is

$$P(\mathbf{r},t) = [\langle \zeta \rangle + \Delta \zeta(\mathbf{r},t)] E(\mathbf{r},t)$$

(5)

Since the fluctuations in the dielectric polarizability is small compared to the fluctuations in the incident light field, we can neglect the time dependence of $\Delta \zeta(\mathbf{r},t)$ in the second derivative of equation (5) to get (Benedek, 1968).

$$\frac{d^2P(\mathbf{r},t)}{dt^2} = \omega_0^2 P(\mathbf{r},t)$$

(6)

The incident field is assumed to be

$$\vec{E}(\mathbf{r},t) = \vec{E}_0 \exp[i(\mathbf{k}_i \cdot \mathbf{r} - \omega_0 t)]$$

Where $\omega_0$ is the laser frequency and $|\mathbf{k}_i| = 2\pi/\lambda$, $\lambda$ is the incident laser wavelength. The total scattered electric field $\vec{E}_s(\mathbf{R},t)$ at any point $(\mathbf{R},t)$ in the space can be given as

$$\vec{E}_s(\mathbf{R},t) = \int \hat{n}_{R-r} \times \left(\hat{n}_{R-r} \times \frac{d^2P(\mathbf{r},t')}{dt^2}\right) dr$$

(7)
Where, \( t' = t - (R-r)/(c_m) \); \( c_m \) is the velocity of light in the medium and \( \hat{n}_{R-r} \) is the unit vector along the \((R-r)\) direction, Fig (2.1) where the integration is over the whole scattering volume 'V'.

Assuming that \( R >> r \) and quasi elastic light scattering, \(|K_i| \approx |K_s|\) and writing \( \hat{n}_R \) for \( \hat{n}_{R-r} \) equation (7) can be simplified as

\[
\overline{E}_s(R, t) = -\left(\frac{\omega_0}{c}\right)^2 \frac{\exp(i(\overline{k}_s \overline{R} - \omega_0 t))}{\overline{R}} \\
x \left[ \hat{n}_R \times \hat{n}_s \times \int E_0 \zeta(\overline{r}, t) \exp(i\overline{k} \overline{r}) \, d\overline{r} \right]
\]

(8)

Where \( \overline{k} = \overline{k}_i - \overline{k}_s \), \( |k| = \left(\frac{4\pi n}{\lambda}\right) \sin \left(\frac{\theta}{2}\right) \)

Where \( \overline{k}_i \) and \( \overline{k}_s \) are the incident and scattered wavevectors and \( \theta \) is the scattering angle.

Equation (8) is an interference integral and describes the superposition of phases of scattered waves from each point in the medium, i.e. field \( \overline{E}_s(R, t) \) fluctuates due to the fluctuations in polarizability \( \zeta(r, t) \). In the absence of fluctuations in polarizability the quantity \( <\zeta> E_0 \) would be constant at position \( r \). For \( V >> \lambda^3 \), except in the forward direction \( (k = 0) \), this integral is zero. Hence there would be no scattering of light from an equilibrum system except in the forward direction. For \( \theta \approx 0 \) the total scattered electric field \( E_s(R, t) \) can be written as

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$E_0 \exp[i(K_i \cdot r - W_0 t)]$

**Figure 2.1: SCATTERING GEOMETRY**
This equation indicates that the scattered field $E_s(R,t)$ fluctuates due to fluctuations in the molecular polarizability and reflects the time dependent fluctuations present in it.

Whenever a sample contains a large number of scatterers, the scattered field $E_s(R,t)$ will be Gaussian; due to the restrictions imposed by central limit theorem.

2.2 CORRELATION FUNCTION FOR CONCENTRATION FLUCTUATION

Any system can be characterized by the dielectric constant or molecular polarizability and can be described as a function of a number of thermodynamic state variables, e.g. pressure, volume, density, temperature and entropy etc. Any two independent variables are required to investigate the system and the other variables would be determined with the help of these two state variables indirectly through the equations of state.

In a two component system (a solution or a binary mixture) the local thermal fluctuations due to finite heat content of the system produce concentration fluctuations. The time dependent fluctuations of these systems can be described by the Fick's second law of diffusion.
\[
\frac{d}{dt} \Delta C(\bar{r}, t) = D \Delta^2 \Delta C(\bar{r}, t) \tag{10}
\]

Where \( D \) is the translational diffusion coefficient, \( \Delta C(\bar{r}, t) \) is the instantaneous deviation of concentration \( C(\bar{r}, t) \) from its most probable value \( <C> \), i.e.

\[
C(\bar{r}, t) = <C> + \Delta C(\bar{r}, t) \tag{11}
\]

This equation can be solved for

\[
\Delta C(\bar{r}, t) = \Delta C(\bar{r}, 0) \exp(-\gamma t) \tag{12}
\]

Where \( \gamma \) represents the reciprocal of relaxation time \( \tau \) Autocorrelation function of \( \Delta C(\bar{r}, t) \) is

\[
<\Delta C(\bar{r}, t) \Delta C^*(\bar{r}, t + \tau)> = \langle \Delta C(\bar{r}, 0) \rangle^2 \exp(-\gamma \tau) \tag{13}
\]

The dielectric constant \( \varepsilon(r,t) \) of the system can be defined as a function of concentration 'C' and entropy 'S' such that

\[
\varepsilon = \varepsilon(C,S) \tag{14}
\]

and
\[ \varepsilon (r, t) = (d \varepsilon / dc)_{<s>} \Delta C (r, t) + (d \varepsilon / ds)_{<c>} \Delta S (r, t) \quad (15) \]

the corresponding correlation function for \( \Delta \varepsilon (r, t) \) will be

\[ \Delta \varepsilon (r, t) \Delta \varepsilon^* (r, t + \tau) = (d \varepsilon / dc)_{<s>}^2 <\Delta C (r, t) \Delta C^* (r, t + \tau)> \\
+ (d \varepsilon / ds)_{<c>}^2 <\Delta S (r, t) \Delta S^* (r, t + \tau)> \quad (16) \]

Substituting the autocorrelation function (ACF) of \( S(r,t) \)
from \( \Delta S (r,t) = \Delta S(r,0) \exp (-\gamma_s \tau) \) and ACF of \( \Delta C (r,t) \) from equation (13)
we get

\[ <\Delta \varepsilon (r, t) \Delta \varepsilon^* (r, t + \tau)> = (d \varepsilon / dC)_{<s>}^2 <|\Delta C (r, 0)|^2 > \exp (-\gamma_C \tau) \\
+ (d \varepsilon / ds)_{<c>}^2 <|S (r, 0)|^2 > \exp (-\gamma_S \tau) \quad (17) \]

where \( \gamma_C \) and \( \gamma_S \) are relaxation constants for concentration and entropy fluctuations respectively. Since we are interested in the correlation studies of the time dependent fluctuations of the scattered field which fluctuates with molecular polarizability we consider normalized correlation function defined as

\[ g_1 (\tau) \propto \frac{<E (R, t) E^* (R, t + \tau)>}{<E>^2} \quad (18) \]

The entropy fluctuations decay to equilibrium much faster \((10^3 \text{ times})\) than the concentration fluctuations, therefore contributions of the
entropy fluctuations can be neglected and the field correlation function can be expressed as

\[ g_1(\tau) \propto (d\epsilon/dc)^2_{\text{CS}} \langle |\Delta C(r,0)|^2 \rangle \exp(-\gamma_c \tau) \] (19)

In the Gaussian signal limit, the scattered intensity correlation function \( <I_s(t)I_s(t+\tau)> \) can be expressed in terms of the scattered field correlation function \( <E_s(t)E^*_s(t+\tau)> \) as

\[ <I_s(t)I_s(t+\tau)> = 1 + \left\{ \frac{<E_s(t)E^*_s(t+\tau)>}{<I_s>^2} \right\}^2 \] (20)

Therefore intensity autocorrelation function (ACF) can be written as

\[ g_2(\tau) \propto \exp\left(\frac{d\epsilon}{dc}\right)^2_{\text{CS}} \langle |\Delta C(r,0)|^2 \rangle^2 \exp(-2\gamma_c \tau) \] (21)

and the normalized intensity ACF \( g_2(\tau) \) can be expressed as

\[ g_2(\tau) \propto \exp(-2\gamma_c \tau) \] (22)

Or simply as

\[ g_2(\tau) \propto \exp(-2\gamma \tau) \] (23)
This equation shows that the decay of intensity autocorrelation function $g_2(\tau)$ is exponential. The plot of $g_2(\tau)$ vs $\tau$ gives the correlogram with half width equal to $1/2 \gamma$. Where

$$\gamma = DK^2 \quad (24)$$

Here $D$ is the translational diffusion coefficient of the molecules and $K$ is the scattering wave vector given by

$$K = \frac{4\pi n}{\lambda}, \sin \theta/2 \quad (25)$$

Where $n$, $\lambda$ and $\theta$ are refractive index, wavelength and scattering angle respectively.

2.3 SIZE DETERMINATION

These equations (24,25) have been used to experimentally investigate the diffusion coefficient of the proteins and polymers in aqueous solutions. The other parameters such as hydrodynamic radius $R_h$, molecular weight $M_w$, and aggregation number $A$ can be obtained through the diffusion coefficient.

The $R_h$ is obtained from the Stoke's Einstein relation

$$D = \frac{K_B T}{6\pi \eta R_h} \quad (26)$$
Where $K_B$ is the Boltzmann's constant, $T$ is the absolute temperature, $\eta$ is the viscosity of the medium.

2.4 POLYDISPERSY ANALYSIS

In monodispense case the normalised modulus of the first order correlation function of the scattered field, can be expressed (Bertero M, et al; 1987), Using (20), as

$$|g_1(\tau)| = \exp(-\gamma \tau)$$

(28)

The data reduction problem in this monodisperse case, namely fitting a single exponential curve poses no problem. When particles of different sizes, however, are present - the polydisperse case - each size fraction gives rise to its own exponential curve and hence the total scattering produces an autocorrelation function, which is the sum of exponentials, given by

$$|g_1(\tau)| = \int_\gamma e^{-\gamma \gamma} G(\gamma) d\gamma$$

(29)

where $G(\gamma)$ is the normalized linewidth distribution function. The problem is the determination of $G(\gamma)$ from the measured values of $|g_1(\tau)|$. As follows from (24) and (26), the determination of $G(\gamma)$ is equivalent to the determination of the distribution function of the particle sizes.
A number of methods (Mcwhirter, J.G.; Pike, E.R. 1978; Koppel, 1972; Gulari et al., 1979; Provencher, 1982; Cha and Min, 1983; Vaidya and Hester, 1984) have been proposed for the determination of $G(\gamma)$ which can be divided into two main types: - The empirical methods - the methods facing the ill conditioned nature of the problem implies that the equation can not be solved analytically. Numerical solution is difficult because the existence or uniqueness of a computed solution is not guaranteed and convergence to a solution is nonuniform. Any proposed method for determining particle size distributions from the equation (29) must converge to the correct solution and must do so rapidly. Convergence to the correct solution is difficult because several local solutions can exist apart from the global solution. This problem is most serious with broad unimodal and multimodal distributions.

Two methods, namely, the method of cumulants and constrained regularisation method which fall into each of the above mentioned categories, are described below.

1. **The Method of Cumulants:**

   This is an empirical method and one of the very first methods used to analyze $G(\gamma)$. It was first described by Koppel 1972.

   Using the taylor series expansion of $\exp(-\gamma r)$ around $\bar{\gamma} = \int \gamma G(\gamma) \, d\gamma$ we can rewrite (29) as
\[ |g_1(\tau)| = \exp(-\overline{\gamma}\tau) \left[ 1 + \sum_{n=2}^{\infty} \mu_n \frac{t^n}{n!} \right] \] (30)

Where the \( \mu_n \) are the nth moment of the distribution \( G(\gamma) \) around, \( \overline{\gamma} \):

\[ \mu_n = \int (\gamma - \overline{\gamma})^n G(\gamma) \, d\gamma \] (31)

The fitting of the experimental data to the theoretical form (30) is generally done on a semi-logarithmic scale, i.e.

\[ \ln(|g_1(\tau)|) = -\overline{\gamma}\tau + \frac{1}{2} \mu_2 \tau^2 - \frac{1}{3!} \mu_3 \tau^3 + \frac{1}{4!} (n_4 - 3\mu_2^2) \tau^4 \] (32)

In most experiments, this expansion is limited to the quadratic term, occasionally to the cubic one. It is therefore only valid for small polydispersity, typically for \( V = \mu_2/\gamma^2 < 0.1 \). \( V \) is often called "polydispersity index". Based on the above method, Malvern (Malvern Instruments, U.K.) has developed a computer code for use with Malvern correlators (7032 CN).

2. Constrained Regularisation Method

The constrained regularisation technique has been developed into a computer package (CONTIN) by Provencher (1982) and has been used for size distribution. For a better estimate of \( G(\gamma) \) it combines two strategies: (i) incorporation of a priori knowledge and (ii) Parsimony. Absolute a priori knowlege (e.g. nonnegativity of \( e^{\tau} \) eliminates
members of $G(\gamma)$ that violate basic truths such as the nonnegativity of mass or number distribution functions. Statistical a priori information (e.g. on the expectation values and variances of the $e^\gamma$) is incorporated into the regularizer, whose strength depends on the regularization parameter $\alpha$, using a Bayesian approach (Provencher, 1981). Usually this statistical a priori information is insufficient or missing and the regularizer is specified by parsimony. The principle of parsimony says, of all solutions in $G(\gamma)$ that have not been eliminated by the constraints, choose the simplest one, i.e., the solution that reveals the least amount of detail or information that was not already known or expected. This is almost certainly not the true solution, but it does tend to protect from artifacts; i.e., while it probably does not have all the details of the true solution, the detail it does have is necessary to fit the data and therefore more likely to be real and not artifact. The most common definition of "simplest" is smoothest and CONTIN automatically does this by setting $\alpha$ and other parameters in the regularizer to penalize deviations from smoothness.

Each time that CONTIN IS RUN, the best fit solution is determined using a range of values for $\alpha$, so that each run produces a series of answers which are more or less smooth. Sophisticated statistics are used to pick one of these answers as the "chosen solution". As well as penalizing non-smooth solutions, CONTIN also requires input of the size range over which the analysis should be carried out, this is a powerful constraint, even when used conservatively. Over the size range
chosen, a number of particular sizes are chosen to model the distribution. There are many other options that can be set in CONTIN. The important choices that have been made are:

i) A weighted analysis has been used. Fit errors at points early in the correlation function are penalized relative to those at long delay times.

ii) The sizes used to fit the distribution form a geometric series. Ostrowsky (1981) showed that this was the best way to extract information from a correlation function, using arguments based on information theory.