

Direct measurement of a distinct correlation function by fluorescence cross correlation

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A new experimental technique, fluorescence cross correlation, is proposed to directly measure correlations in motion of pairs of interacting particles. The theoretical basis of the technique is worked out, taking into account, in addition, nonideal features of the experiment. Preliminary experimental results are obtained on latex suspensions. They demonstrate the possibility of measuring not only a distinct correlation function but also the corresponding self-contribution as well as a collective correlation function of the suspension simultaneously in one experiment.

I. INTRODUCTION

Important aspects of the microscopic dynamical behavior of a homogeneous and isotropic system of pairwise interacting particles, such as a simple liquid or a colloidal dispersion, can be conveniently described in terms of Van Hove's space-time correlation function $G(\mathbf{r}, \tau)$.^{1,2} Depending on the experimental situation one of the various representations of $G(\mathbf{r}, \tau)$ turns out to be convenient, the choice in the present paper being the intermediate scattering function (sometimes also called the dynamic structure factor)

$$S(\mathbf{q}, \tau) = \frac{1}{N} \left\langle \sum_i^N \sum_j^N e^{-i\mathbf{q}[\mathbf{r}_j(\tau) - \mathbf{r}_i(0)]} \right\rangle. \quad (1)$$

Here N is the number of particles in a macroscopically large volume V and $\mathbf{r}_j(\tau)$, $\mathbf{r}_i(0)$ are the positions of the particles j, i at times τ and 0. Angle brackets denote the thermal average. Experimentally observable with various quasielastic scattering methods is the related quantity

$$S'(\mathbf{q}, \tau) = \frac{1}{N} \left\langle \sum_i^N \sum_j^N b_i b_j e^{-i\mathbf{q}[\mathbf{r}_j(\tau) - \mathbf{r}_i(0)]} \right\rangle. \quad (2)$$

The Fourier variable \mathbf{q} assumes here the physical meaning of the scattering vector and b_i are the scattering amplitudes.

The (collective) correlation function $S(\mathbf{q}, \tau)$ can be decomposed into two contributions called the *self* and the *distinct*,

$$S_S(\mathbf{q}, \tau) = \frac{1}{N} \left\langle \sum_i^N e^{-i\mathbf{q}[\mathbf{r}_i(\tau) - \mathbf{r}_i(0)]} \right\rangle, \quad (3a)$$

$$S_D(\mathbf{q}, \tau) = \frac{1}{N} \left\langle \sum_{i \neq j}^N \sum_j^N e^{-i\mathbf{q}[\mathbf{r}_j(\tau) - \mathbf{r}_i(0)]} \right\rangle. \quad (3b)$$

Both correlation functions depend on interactions between the particles. The distinct contribution $S_D(\mathbf{q}, \tau)$ vanishes in the absence of interactions (except for a singularity at $\mathbf{q}=\mathbf{0}$) and appears thus to reflect the interactions more directly than the self-contribution $S_S(\mathbf{q}, \tau)$. It is therefore somewhat unfortunate that, in scattering experiments, S_D is accessible only indirectly.

Assuming the particles differ only in their amplitudes b_i the experimental structure factor $S'(\mathbf{q}, \tau)$ can be written as a sum of the so-called coherent and incoherent contributions²⁻⁵

$$S'_{\text{inc}} = \sigma^2 S_S(\mathbf{q}, \tau), \quad (4a)$$

$$S'_{\text{coh}} = b^2 S(\mathbf{q}, \tau). \quad (4b)$$

Here b is the average particle amplitude and σ the standard deviation of $\{b_i\}$. S'_{inc} can be measured in situations where $\sigma^2 \gg b^2$, e.g., in tracer experiments where all but a few of the particle amplitudes vanish. In principle, both parts of $S'(\mathbf{q}, \tau)$, and thus also S_D , can be inferred indirectly from a series of experiments with varying σ^2 and b^2 .³ Such a separation requires accurate knowledge of both parameters and is therefore rather tedious.⁶

The aim of the present paper is to demonstrate the possibility of measuring a *distinct* time correlation function directly in a single experiment. This requires the possibility of distinguishing between the particles of the system. The desired distinguishability is naturally offered when the particles are tagged by fluorescent dyes. The proposed method is a simple generalization of a technique called number-fluctuation spectroscopy.

The theoretical basis of the proposed technique is developed in Sec. II. In its present form the technique is particularly suited for investigations on colloidal suspensions. Their properties are briefly discussed in Sec. III. Section IV contains experimental details on the apparatus and the sample used in preliminary experiments. In Sec. V the results demonstrating the feasibility and usefulness of the method are discussed.

II. THEORETICAL BACKGROUND

A. Principles of number-fluctuation spectroscopy

The number-fluctuation spectroscopy encompasses two related techniques measuring signal fluctuations caused by movement of suspension particles with respect to the intensity profile of a probing light beam. In fluorescence correlation spectroscopy^{7,8} (FCS) such movements are the only source of signal fluctuations. In the laser light scattering variety of the technique⁹⁻¹¹ the signal contains

also the much faster interference fluctuations which can be suppressed by preaveraging over a sufficiently long time. Though both number-fluctuation techniques have been proposed as early as 1972,^{10,12} they never became as popular as the closely related quasielastic light scattering (QLS). We therefore review the basic principles, focusing on the example of FCS and keeping in mind the application to dynamics of interacting particles. We proceed along the lines discussed in Refs. 9 and 13, adapting the results to the needs of the present paper.

In a FCS experiment one excites fluorescent particles contained in a small open volume by a narrowly focused laser beam. The fluorescence light is collected in a solid angle as large as possible. Fluorescence is incoherent and the resulting detector signal can be written as a sum of contributions from individual particles:

$$I(t) = \int F(\mathbf{r}) \sum_i b_i \delta(\mathbf{r} - \mathbf{r}_i(t)) d\mathbf{r}. \quad (5)$$

The function $F(\mathbf{r})$ is the product of the position-dependent intensity of the illuminating beam and the col-

lection efficiency of the detection optics. $F(\mathbf{r})$ is an important characteristic of a FCS setup; it influences the form of the measured correlation functions. The particle visibilities b_i depend on the sensitivity of the detector to the chosen wavelength of the fluorescent light, on the amount of dye per particle, and on the photophysical properties of the dye. They correspond to the scattering amplitudes from Sec. I. Though in outlining the theory we focus on the fluorescence case, Eq. (5) applies also to a light scattering experiment, providing the detector intercepts an area much larger than the coherence area or the signal is preaveraged over times longer than the correlation time of interference fluctuations.⁹⁻¹¹

As the particles move around in the illuminated space the signal $I(t)$ fluctuates. The intensity fluctuations are processed to obtain an estimate of the correlation function

$$C(\tau) = \langle I(t)I(t+\tau) \rangle. \quad (6)$$

Expressing the δ functions in Eq. (5) as Fourier integrals one obtains

$$C(\tau) = \frac{1}{(2\pi)^6} \int_{\mathbf{q}} \int_{\mathbf{q}'} F(\mathbf{q})F(\mathbf{q}') \left\langle \sum_i \sum_j b_i b_j e^{+iq'r_j(t) + iqr_i(0)} \right\rangle d\mathbf{q} d\mathbf{q}', \quad (7)$$

where $F(\mathbf{q})$ is the spatial Fourier transform of $F(\mathbf{r})$. In a homogeneous isotropic and pairwise interacting system the bracketed average vanishes if not $\mathbf{q} + \mathbf{q}' = \mathbf{0}$ and therefore

$$\begin{aligned} C(\tau) &= \frac{1}{(2\pi)^3 V} \int_{\mathbf{q}} F(\mathbf{q})F(-\mathbf{q}) \left\langle \sum_i \sum_j b_i b_j e^{-iq[r_j(\tau) - r_i(0)]} \right\rangle d\mathbf{q} \\ &= \frac{\rho}{(2\pi)^3} \int_{\mathbf{q}} \Phi(\mathbf{q}) S'(\mathbf{q}, \tau) d\mathbf{q}. \end{aligned} \quad (8)$$

In the second part of the equation we have introduced ρ , the average number concentration of the particles and abbreviated $F(\mathbf{q})F(-\mathbf{q})$ by $\Phi(\mathbf{q})$. The function $\Phi(\mathbf{q})$ is a filter through which $S'(\mathbf{q})$ is viewed. The shape of the filter is a characteristic of the apparatus. Usually the filter is set to measure $S'(\mathbf{q}, \tau)$ around $\mathbf{q} = \mathbf{0}$ (a possibility of working at higher \mathbf{q} is discussed in Ref. 14).

The integration in Eq. (8) encompasses $\mathbf{q} = \mathbf{0}$, where²

$$S'(\mathbf{q} = \mathbf{0}, \tau) = (2\pi)^3 \rho b^2 \delta(\mathbf{q}).$$

As usual,² we remove this singularity redefining $S'(\mathbf{q}, \tau)$ as being analytically continued through $\mathbf{q} = \mathbf{0}$ [this corresponds to writing $\rho(\mathbf{r})$ as $\rho + \Delta\rho(\mathbf{r})$]. In contrast to scattering theories, however, we do not drop the $\delta(\mathbf{q})$ contribution. The final expression for the FCS correlation function becomes

$$C(\tau) = \frac{\rho}{(2\pi)^3} \int_{\mathbf{q}} \Phi(\mathbf{q}) S'(\mathbf{q}, \tau) d\mathbf{q} + \Phi(\mathbf{0}) \rho^2 b^2. \quad (9)$$

The second term, originating from the $\delta(\mathbf{q})$ contribution to S' , is the base line $\langle I \rangle^2$ of a FCS experiment.¹⁵ Equation (9) applies to pointlike particles but its generalization to finite-size fluorescent objects is straightforward.¹⁶

Defining an effectively observed volume V_{eff} as

$$V_{\text{eff}} = \frac{(\int F(\mathbf{r}) d\mathbf{r})^2}{\int F(\mathbf{r})^2 d\mathbf{r}} = (2\pi)^3 \frac{\Phi(\mathbf{0})}{\int \Phi(\mathbf{q}) d\mathbf{q}}, \quad (10)$$

the normalized FCS correlation function $c(\tau)$ can be written as

$$c(\tau) = \frac{1}{\rho V_{\text{eff}} b^2} \frac{\int \Phi(\mathbf{q}) S'(\mathbf{q}, \tau) d\mathbf{q}}{\int \Phi(\mathbf{q}) d\mathbf{q}} + 1. \quad (11)$$

The first term in Eq. (11) contains the information on particle dynamics. To make the dynamical term easily measurable in the presence of the base line, the number of particles in effective volume ρV_{eff} must be kept low.

Since $S(\mathbf{q}, 0)$ is the static structure factor, the amplitude $c(0) - 1$ of FCS correlation function can be written (assuming all particles are equivalent) as

$$c(0) - 1 = \frac{1}{V_{\text{eff}} \rho} + \frac{1}{V_{\text{eff}}} \frac{\int \Phi(\mathbf{q}) h(\mathbf{q}) d\mathbf{q}}{\int \Phi(\mathbf{q}) d\mathbf{q}}. \quad (12)$$

The second term originates from the distinct part of $S(\mathbf{q}, \tau)$; $h(\mathbf{q})$, defined by $S(\mathbf{q}) = 1 + \rho h(\mathbf{q})$, is the Fourier transform of the pair correlation function. Assuming the

correlation length of the particle suspension is smaller than the size of V_{eff} , the filter $\Phi(\mathbf{q})$ cuts out a narrow portion of $h(\mathbf{q})$ around $\mathbf{q}=\mathbf{0}$. Defining the correlation volume $V_c = \int h(\mathbf{r})d\mathbf{r} = h(\mathbf{0})$, Eq. (12) reduces to

$$c(0) - 1 = \frac{1}{V_{\text{eff}}\rho} + \frac{V_c}{V_{\text{eff}}} . \quad (13)$$

We recall that V_c is negative for repulsive interactions. When ρ and V_{eff} are known and the distinct term V_c/V_{eff} is sufficiently large compared to the self-term $1/\rho V_{\text{eff}}$, Eq. (13) can be used to determine V_c , a measure of the correlation length.

B. Fluorescence cross correlation

An important feature of FCS, distinguishing it from the light scattering methods, is its selectivity. Once the chemistry of labeling is mastered it is easy to equip the detector with an appropriate light filter to detect only the fluorescent light. An application of this feature to interacting particles has been suggested already in the early days of FCS.¹⁷ By mixing labeled and plain particles one can vary b^2 and σ^2 , varying thus the contributions of S_{coh} and S_{inc} . In this section we exploit further the selectivity, introducing a new technique—the fluorescence cross correlation.

Consider a mixture of fluorescent particles some of which are labeled by, say, a blue dye and the remaining labeled green. Consider further a FCS apparatus equipped with two detectors, one responding ideally to the blue light only (detector B) and the other responding to green (detector G) (see Sec. IV and Fig. 2 for an experimental realization). Assume for simplicity that the detection optics is realized in a way that we have $F_B(\mathbf{r}) = F_G(\mathbf{r}) = F(\mathbf{r})$. With the two detectors, three different correlation functions can be measured.

(1) The autocorrelation function of the blue detector (superscript i stands for “ideal”) reads

$$\begin{aligned} C_{BB}^i(\tau) &= \frac{\rho_b b_B^2}{(2\pi)^3} \int \Phi(\mathbf{q}) \frac{1}{N_b} \left\langle \sum_{jb}^{N_b} \sum_{ib}^{N_b} e^{-i\mathbf{q}[\mathbf{r}_{jb}(\tau) - \mathbf{r}_{ib}(0)]} \right\rangle d\mathbf{q} \\ &= \frac{\rho_b b_B^2}{(2\pi)^3} \int \Phi(\mathbf{q}) S_{bb}(\mathbf{q}, \tau) d\mathbf{q} + \Phi(\mathbf{0}) \rho_b^2 b_B^2 . \end{aligned} \quad (14)$$

Here b_B is the visibility of blue particles as seen by the detector B (in the ideal case $b_G=0$) and ρ_b is the blue concentration. By $S_{bb}(\mathbf{q}, \tau)$ we denote the partial dynamic structure factor of the blue particles, keeping in mind that it is modified by the presence of the green ones.

(2) An analogous equation applies for the ideal autocorrelation function measured in the green channel,

$$C_{GG}^i(\tau) = \frac{\rho_g g_G^2}{(2\pi)^3} \int \Phi(\mathbf{q}) S_{gg}(\mathbf{q}, \tau) d\mathbf{q} + \Phi(\mathbf{0}) \rho_g^2 g_G^2 . \quad (15)$$

The visibility of green particles in the green channel is denoted by g_G (again $g_B=0$).

(3) A third correlation function one can measure is the cross correlation function of fluctuating signals of the blue and green detectors,

$$\begin{aligned} C_{GB}^i(\tau) &= \frac{g_G b_B}{(2\pi)^3} \frac{\rho_b \rho_g}{\rho} \int \Phi(\mathbf{q}) S_{gb}(\mathbf{q}, \tau) d\mathbf{q} \\ &\quad + \Phi(\mathbf{0}) \rho_b \rho_g b_B g_G . \end{aligned} \quad (16)$$

Here ρ is the total particle concentration $\rho = \rho_b + \rho_g$. $S_{gb}(\mathbf{q}, \tau)$ is the purely distinct dynamic structure factor describing the blue-green space-time correlations,

$$S_{gb}(\mathbf{q}, \tau) = \frac{N_b + N_g}{N_b N_g} \left\langle \sum_{ib} \sum_{jg} e^{-i\mathbf{q}[\mathbf{r}_{jg}(\tau) - \mathbf{r}_{ib}(0)]} \right\rangle . \quad (17)$$

The suffixes b and g guarantee the summation encompasses only distinct terms. We note that the normalized amplitude of $C_{GB}^i(\tau)$ now contains only the distinct contribution and is thus directly proportional to V_c ,

$$c_{GB}^i(0) - 1 \approx \frac{V_c}{V_{\text{eff}}} . \quad (18)$$

The perfect selectivity expressed by $g_B=0$ and $b_G=0$ cannot be expected in a real experiment. Fortunately the stringent fulfillment of this condition turns out to be unnecessary. Using a simple algebra and employing the stationarity of the observed processes the measured correlation functions C_{BB}^m , C_{GB}^m , and C_{GG}^m can be shown to be linear combinations of the ideal functions defined in Eqs. (14)–(16). The linear relationship can be expressed in closed matrix form as

$$\begin{pmatrix} C_{BB}^m \\ C_{GB}^m \\ C_{GG}^m \end{pmatrix} = \underline{M} \begin{pmatrix} C_{BB}^i \\ C_{GB}^i \\ C_{GG}^i \end{pmatrix} , \quad (19)$$

$$\underline{M} = \begin{pmatrix} 1 & 2\gamma & \gamma^2 \\ \beta & 1 + \gamma\beta & \gamma \\ \beta^2 & 2\beta & 1 \end{pmatrix} .$$

The ratios $\gamma = g_B/g_G$ and $\beta = b_G/b_B$ appearing in the transformation matrix \underline{M} characterize the selectivity of the experiment. They can be measured easily. Providing $\gamma\beta \neq 1$ the matrix \underline{M} can be inverted and the ideal correlation functions calculated from the set of the measured correlation functions.

III. COLLOIDAL SUSPENSIONS WITH COULOMB INTERACTIONS

To make the distinct correlation function readily observable with a typical FCS apparatus the ratio V_c/V_{eff} [Eq. (18)] should be at least 0.01. For the apparatus described in Sec. IV this implies a correlation length of approximately $0.5 \mu\text{m}$. On the hand, the particle diameter should be smaller than $0.1 \mu\text{m}$ to avoid complications with the particle form factor.¹⁶ Both requirements can be met with suspensions of charged colloids.

The static structure factor of such suspensions resembles closely the structure factor of a simple liquid (see Fig. 1 for an illustrative example). Since this fact has been recognized¹⁸ the charged colloidal systems have become the subject of numerous experimental and theoretic-

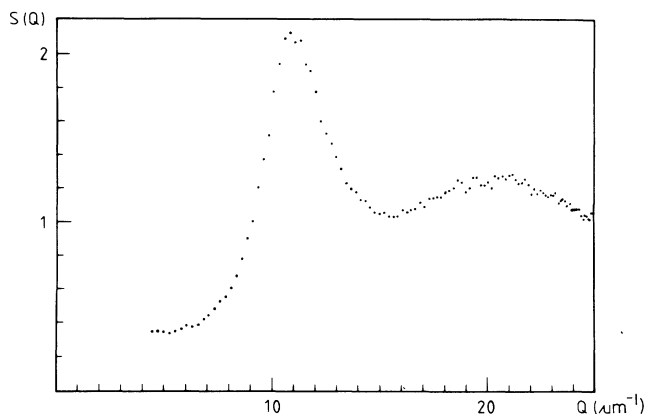


FIG. 1. Static structure factor of a deionized latex suspension. An illustrative example. (Particle diameter 100 nm, concentration approximately 2 particles/ μm^3 .)

cal studies. Two recent reviews of the subject are Refs. 4 and 19. Unlike a simple fluid the dynamics of suspended particles is strongly influenced by the viscous drag of the surrounding solvent. The term "Brownian dynamics" was coined to characterize this behavior.^{20,21} The Coulomb interactions can easily be controlled by changing the ionic strength of the solution and the concentration of the particles. This makes the charged colloids a convenient model system for studying the Brownian dynamics in two important limits. In dilute solutions only the Coulomb pair interactions are important, whereas at higher concentrations the hydrodynamic interactions between the particles have to be taken into account.

"Drosophila" among the charged colloidal systems are lattices consisting of sulphonated polystyrene particles having a typical diameter of 100 nm.²²⁻²⁴ In commercially available aqueous suspensions the Coulomb interactions are screened by ionic impurities; they are barely strong enough to prevent the aggregation of the particles. By means of a rigorous deionization the interactions can be "turned on." The range of the interaction grows to several particle diameters. Fluorescent polystyrene lattices are commercially available and therefore we have chosen this system to demonstrate the feasibility of the proposed cross correlation technique.

IV. EXPERIMENT

A. The sample

Fluorescent polystyrene lattices of the desired diameter of ≈ 100 nm can be purchased at Polysciences Inc. (Fluoresbrite particles). Two colors are available. While the green fluorescing particles (dye unknown) were found to be suitable for present needs, the orange particles proved very unsatisfactory. The dye is only adsorbed to the particles instead of being covalently bound. In a mixture of the green and orange particles the orange dye quickly redistributes, contaminating strongly the green particles. Fortunately Eq. (19) permits us to employ stan-

dard nonfluorescent latex particles as the "blue" sort in the system and observe the scattered light instead of fluorescence in the blue channel. However, one has to make sure the interference fluctuations can be neglected (see Sec. IV B).

The investigated suspensions contained mostly the blue particles, i.e., polystyrene latex (Dow Chemical Co.) of 100-nm diameter (approximately 20 particles/ $10 \mu\text{m}^3$), whereas the green particles of 64-nm diameter were added as tracers (approximately 2 particles/ $10 \mu\text{m}^3$). The densities are estimated from the nominal concentrations of original suspensions. To deionize the sample 0.1 g of a mixed ion exchange resin (Biorad AG 501-X8) was added to 2 ml of the suspension in a quartz cell. The cell was sealed and gently shaken for approximately 48 h. The success of the procedure was checked by observing the static structure factor. All measurements were performed at room temperature.

B. Apparatus

The experiments were performed using a FCS apparatus described in detail in a previous publication.²⁵ The apparatus consists of a fluorescence microscope (Fig. 2) into which a laser beam is directed through a monomode optical fiber. A semitransparent (usually dichroic) mirror M_1 reflects the beam into the objective which in turn focuses it into a narrow spot of approximately 1- μm diameter. The fluorescent light is collected by the same objective. A pinhole in the image plane of the microscope determines the axial size of the observed region. The function $F(r)$ of such an apparatus can be well approximated by a prolate Gaussian

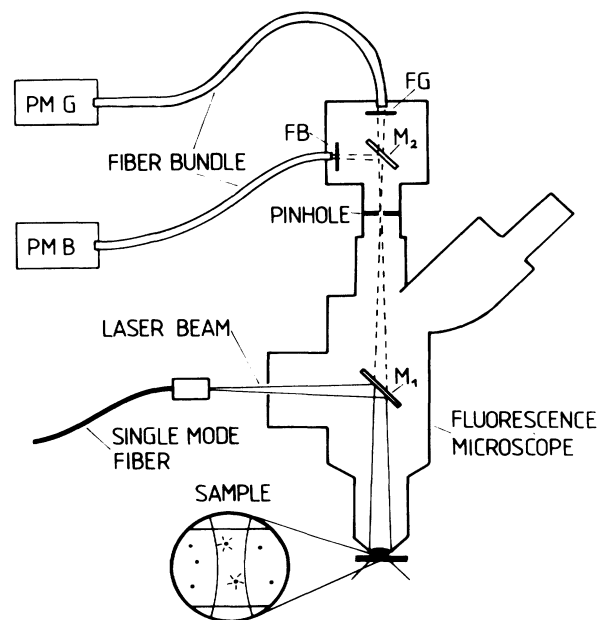


FIG. 2. Schematics of the fluorescence cross correlation apparatus. M_1 , dichroic mirror reflecting the laser light and transmitting fluorescence light; M_2 , dichroic mirror separating two wavelengths of fluorescence; FB, FG filter completing the wavelength selection. (B and G refer to blue and green light.)

$$F(r) \approx \exp(-2r^2/w^2 - z^2/\xi^2)$$

(cylindrical coordinates, z axis coincides with the optical axis). The waist w is determined by measuring dilute solutions of fluorescent latex spheres calibrated by QLS. The effective volume V_{eff} is calculated from the amplitude $c(0)-1$ obtained with a dilute dye solution of known concentration. Having a Gaussian $F(\mathbf{r})$ with $\xi \gg w$ the normalized FCS correlation function of noninteracting Brownian particles reads⁷

$$c(\tau) = 1 + \frac{1}{\rho V_{\text{eff}}} \frac{1}{1 + \frac{4D\tau}{w^2}}, \quad (20)$$

where D is the diffusion coefficient of the particles. The exponential dynamic structure factor $\exp(-\mathbf{q}^2 D \tau)$ is transformed into a hyperbola upon application of Eq. (8).

For cross-correlation measurements the apparatus described in Ref. 25 was modified as indicated in Fig. 2. The light transmitted through the pinhole is split by a long-pass dichroic mirror M_2 and coupled into two optical-fiber bundles leading to two photomultipliers (PM B, PM G). The wavelength selection is completed by appropriate filters FG and FB selecting the green or blue light, respectively.

The apparatus was not designed for scattering experiments. One has to cope with the back reflection of laser light on the optical surfaces of the microscope. A makeshift solution to the problem was to shift the laser beam slightly out of the optical axis. The resulting distortion of $F(\mathbf{r})$ (an increase in V_{eff} to $17 \mu\text{m}^3$ and w to $0.5 \mu\text{m}$) and a decreased stability of the setup were accepted in these preliminary experiments. Some residual stray light was taken into account in evaluation of parameters β and γ and the correlation functions.

The light source was a stabilized argon laser; the line 459 nm was chosen for the present experiments. A satisfactory detection wavelength selection was achieved by the following combination: Dichroic mirror M_1 (acting as a semitransparent mirror for the laser light), long pass, edge 460 nm; dichroic mirror M_2 , long pass, edge 510 nm; filter B , interference filter 459 nm; filter G , $2 \times$ Schott KV 520. The values of the selectivity parameters $\gamma < 0.001$ and $\beta = 0.13$ were found to be small enough to observe the relevant effects even before the correction of the correlation functions.

Knowing the parameters of the setup one can estimate the influence of interference fluctuations in scattering measurements. The correlation time of these fluctuations reads $\tau_i = 1/2Q^2D = \lambda^2/32\pi^2D$ (backscattering), whereas the correlation time of number fluctuations from Eq. (20) is $\tau_n = w^2/4D$. Since $w \approx \lambda$, $\tau_n/\tau_i \approx 100$. Working with sample times in the order of $\tau_n/10$ the interference fluctuations will contribute significantly only to the first channel of the measured correlation function.

V. RESULTS AND DISCUSSION

Figures 3–5 show the three resulting correlation functions $c_{GG}(\tau)-1$, $c_{BB}(\tau)-1$, and $c_{GB}(\tau)-1$. They are the

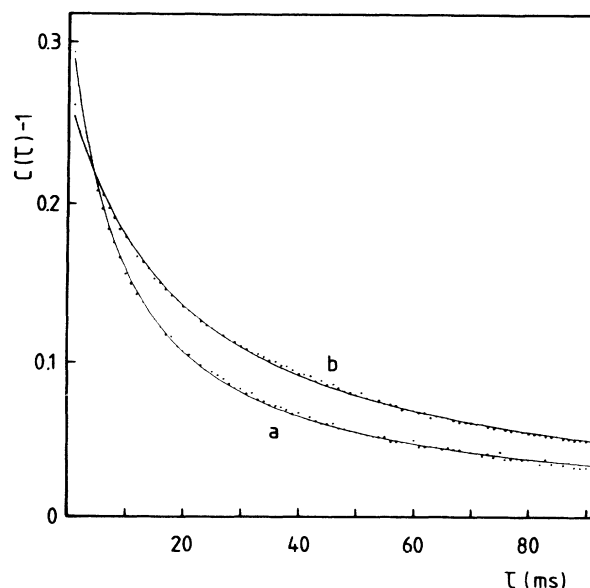


FIG. 3. Corrected [Eq. (19)] correlation function $c_{GG}(\tau)-1$ of the green particles. Curve a , prior to deionization; curve b , after the deionization. Amplitudes: $a_a=0.31$, $a_b=0.26$. Correlation times: $\tau_a=9.4$ ms, $\tau_b=20.3$ ms. The concentration of green particles is low; the self-contribution dominates.

ideal correlation functions obtained from the raw data by application of Eq. (19). Figure 3, curve a , is the correlation function of the green particles as measured prior to the deionization. We recall that the concentration of the green particles is low (V_c is estimated further below). Therefore the self-term dominates [see Eq. (13)] and the

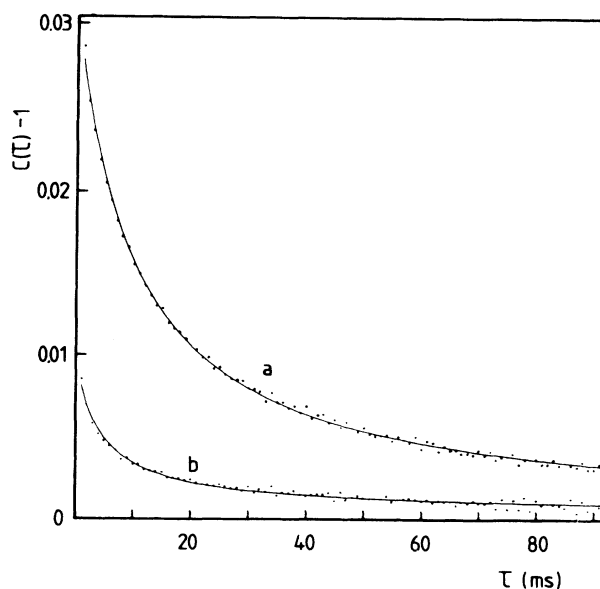


FIG. 4. Same as Fig. 3 but for the blue particles. Amplitudes: $a_a=0.03$, $a_b=0.01$. Correlation times: $\tau_a=10.5$ ms, $\tau_b=4.6$ ms. The concentration of blue particles is high; c_{BB} is a collective correlation function.

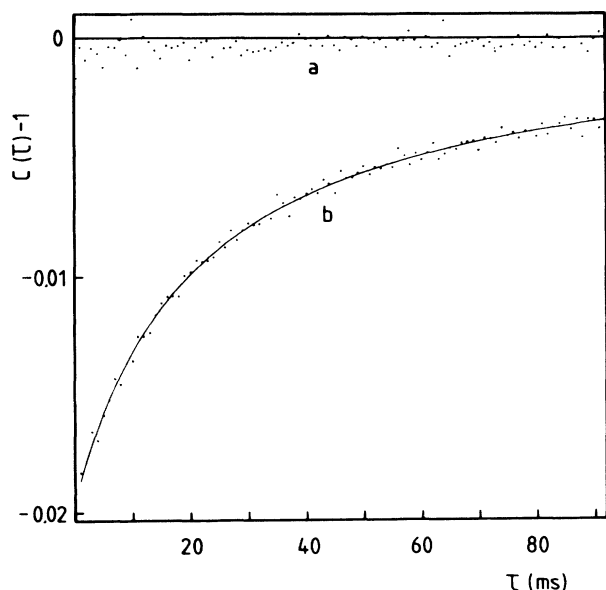


FIG. 5. Distinct correlation function $c_{GB}(\tau) - 1$. Prior to the deionization (curve *a*) motions of the blue particles do not correlate with the green ones. After the deionization (curve *b*) there occurs a strong negative correlation, indicating repulsive interactions. $a_b = -0.02$, $\tau_b = 21.4$ ms.

correlation function $c_{GG}(\tau)$ is essentially a self-correlation function. The amplitude $1/\rho V_{\text{eff}} = 0.31$ corresponds to the concentration of particles estimated from the concentration of original suspensions. The solid line is a fit by a hyperbola $1/(1 + 4D\tau/w^2)$. The correlation time of 9.4 ms is somewhat longer than the 8.7 ms expected for a freely diffusing particle with a radius of 32 nm. The curve *b* shows the result after the deionization. The amplitude is slightly diminished, reflecting the presence of a small negative distinct contribution. The functional form of a hyperbola is preserved but the relaxation time is approximately doubled to 20.3 ms. These findings are in accordance with the known results on the self-diffusion in charged suspensions.⁴ Note that we are observing the particles moving across the 1- μm diameter beam, i.e., over many particle diameters and measure thus the correlation functions in the long-time limit.

Figure 4 shows the results for blue particles. These particles make up the gross of the suspension. The correlation function $c_{BB}(\tau)$ is therefore approximately the full (collective) correlation function of the suspension (i.e., S_S and S_D may become comparable). Prior to the deionization (curve *a*) the results are qualitatively the same as in Fig. 3(a). However, whereas the amplitude of 0.03 is consistent with the concentration of the blue particles, the relaxation time of 10.5 ms is considerably smaller than the 13.5 ms expected for particles of 50-nm radius. This may indicate that repulsive interactions are present even

in the nondeionized sample (the suspension was diluted by high quality deionized water) or that the interference contribution to the signal cannot be completely neglected. A drastic change is observed after the deionization (Fig. 4, curve *b*). The amplitude decreases three times and the relaxation time is shortened by a factor of 2.3. Again this behavior is consistent with the known features of deionized lattices. The decrease of the amplitude reflects the decrease of $S(\mathbf{q})$ at $\mathbf{q} = 0$ which is caused by repulsive interaction (see Fig. 1). The relaxation time follows approximately the relation $\tau_c = \tau_0 S(\mathbf{q})$ (τ_0 is the correlation time of a free particle) established for the first cumulant of a collective correlation function.⁴ The hyperbola fit still provides a reasonable approximation to the form of the correlation function; some deviation, however, can be observed at short times.

While both types of correlation functions, the collective correlation function of the suspension [here, approximately equal to $c_{BB}(\tau)$] and its self-contribution [here, approximately equal to $c_{GG}(\tau)$] can be obtained with conventional techniques, the results in Fig. 5 are new. Figure 5 shows the directly measured distinct correlation functions describing the correlations in movements of the blue and green particles. Prior to the deionization (curve *a*) the particles move almost uncorrelated. After the turning on of the repulsive interaction (curve *b*) we observe a strong anticorrelation. While there are many blue particles in the observed volume, the green ones are excluded and vice versa. From the amplitude we estimate the correlation volume V_c to be $-0.35 \mu\text{m}^3$. Comparing with the concentration of the particles we find there are approximately 0.6 particles in the correlation volume, slightly less than the limit of 1 for repulsive interactions. Lacking a theoretical basis for the time dependence of the distinct correlation function we simply state that the shape is well approximated by a hyperbola and the relaxation time is somewhat longer than the self-relaxation time of the deionized green particles.

The observations illustrate nicely the behavior of repulsively interacting Brownian particles. For a quantitative interpretation a better characterized system where all the particles are of the same size and carry the same charge would be desirable. Also the complication of measuring fluorescence in one channel and light scattering in the other should be avoided in future work.

Despite the preliminary nature of the present experimental work the results demonstrate clearly the possibility of measuring directly a truly *distinct* correlation function. Moreover, when the concentrations of the two sorts of fluorescent particles are suitably chosen the corresponding *self*-contribution as well as the *collective* correlation function of the suspension can be obtained simultaneously in the same experiment.

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¹⁵In general, the second term should be written as

$$\Phi(0)\rho^2 \left\langle \frac{1}{N^2} \left| \sum_i \sum_j b_j(\tau) b_i(0) \right|^2 \right\rangle.$$

- Since N is very large, statistical independence of $b_i(0)b_j(\tau)$ is sufficient to obtain the simple form in Eq. (8).
¹⁶A straightforward generalization of the concept to particles of finite size yields (all particles having the same visibilities b)

$$C(\tau) = \frac{\rho b^2}{(2\pi)^3} \int \Phi(\mathbf{q}) R(\mathbf{q}) S(\mathbf{q}, \tau) d\mathbf{q} + \Phi(0)\rho^2 b^2,$$

- where $R(\mathbf{q})$ is a form factor describing the distribution of fluorescence over the particle.
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