Measurement of the Dynamic Structure Function of Fluorescently Labeled Complex Fluids by Fourier Imaging Correlation Spectroscopy

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Wave-number-resolved time-correlation functions of density fluctuations are determined from purely incoherent fluorescence signals using a new method, Fourier imaging correlation spectroscopy (FICS). We demonstrate the application of FICS to chemical and biological samples with ultrahigh signal sensitivity and time resolution superior to direct imaging methods. We outline the theory that establishes the connection between FICS observables and statistical mechanical quantities describing liquid state dynamics.

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Traditionally, experimental information about the structure of polymer, colloid, and biomembrane materials is obtained by light scattering from ordered arrangements of atoms, molecules, or larger scattering centers. The dynamics of these systems is studied by dynamic light scattering (DLS) measurements of the fluctuations of the scattered light intensity [1,2]. An important limitation of DLS is that insufficient scattering contrast restricts the possible systems that can be studied, prohibiting investigation of problems such as transport in live biological cells. Alternatively, more sensitive techniques are based on measurements of fluorescently labeled substituents in which emission is detected against a dark background. State-of-the-art imaging is capable of visualizing spatial chromophore distributions from ultralow fluorescence signals, in some cases from a single isolated molecule [3]. Perhaps the most important advantage of direct visualization over conventional fluorescence spectroscopies is its ability to measure distributions of physical quantities (such as particle positions) rather than just their mean values. Nevertheless, what imaging experiments gain in spatial information is compensated for by a loss in temporal resolution; relatively slow video frame rates [4] limit the dynamic range.

Here we present a new, highly sensitive, approach to measure time-dependent spatial distributions of fluorescently labeled particles, Fourier imaging correlation spectroscopy (FICS). Unlike conventional fluorescence correlation spectroscopy [5], FICS is based on the detection of modulated fluorescence signals and measures temporal fluctuations of both amplitude and phase of a spatial Fourier component of the sample particle number density. The amplitude and the phase sensitivity of FICS arises from the sweeping of a patterned photoexcitation profile across the particle density at a much greater velocity than the speed at which a particle can travel the interference distance. Other fluctuation methods using similar experimental geometries have been applied to measure the bulk diffusion constant [6].

While microscopy simultaneously records information on all spatial scales, FICS measurements isolate fluctuations of an N-body system at a single wave number, \( k \). Execution of independent measurements at successive \( k \) values provides the statistically relevant information to determine the two-point spatial and temporal distribution functions of the fluid. Furthermore, by using established methods of photon correlation spectroscopy [2], the spatial information contained in the FICS observable is collected more efficiently in time than over the full spectrum of \( k \) using commercial charge-coupled device (CCD) cameras [4]. Depending on the choice of the data acquisition device, the dynamic range accessed by FICS experiments can exceed \( \sim 10^{-7} - 10^2 \) sec.

The dynamics of complex fluids is characterized by wave-number-dependent time-correlation functions [7]. In particular, the dynamic structure function,

\[
F(k, \tau) = \langle \hat{C}^*(k, t) \hat{C}(k, t + \tau) \rangle, \tag{1}
\]

is the time-correlation function of the Fourier components of the fluid particle number density, \( C(r, t) \):

\[
\hat{C}(k, t) = \left( \frac{1}{\sqrt{N}} \right) \int d^3r \; e^{-i\mathbf{k} \cdot \mathbf{r}} C(r, t) \tag{2}
\]

and

\[
C(r, t) = \sum_{i=1}^{N} \delta[\mathbf{r} - \mathbf{r}_i(t)],
\]

where the index \( i \) enumerates the N “particle” positions, \( \mathbf{r}_i(t) \), in the fluid at time \( t \). Information about the time averaged spatial distribution of chromophores is contained in the limit \( F(k, \tau = 0) = \bar{S}(k) \).

In FICS experiments, the motion of chromophore labeled species is detected as slow fluctuations in fluorescence intensity from the intersection of an oscillatory excitation pattern (an optically generated grating) with a microscopically heterogeneous distribution of chromophores, \( C(r, t) \). The excitation grating is created by the optical interference of two linearly polarized laser beams brought to a focus at the sample plane of a fluorescence microscope. The excitation source is the cw frequency doubled output of a Nd:YAG laser (\( \lambda_{ex} = 532 \) nm); its output power (measured just before sample incidence) is set to minimize photodegradation during data acquisition. One of the two excitation beams travels through an
The ability to measure time and wave-number-dependent distributions is demonstrated on systems composed of monolayer suspensions of rhodamine labeled 1 μm polystyrene spheres (∆em = 605 nm) confined between the glass walls of a thin capillary cell. The slow drifts in laser alignment, particles moving in and out of the illuminated region and the mean fluorescence level, and a band of small-decay of the spatial Fourier components represented by integrated fluorescence intensity reflect the growth and the illuminated region. Temporal fluctuations in the in addition to a dG grating is thus swept across the illuminated sample at a FIG. 1. (A) Fluorescence micrograph of a dense monolayer sample using a long focal length lens (f = 40 cm).

The period of the excitation grating, dG, depends on the intersection angle, φ, between the beams [8], and can be varied continuously from tens of microns to λex/2, according to dG = λex/2sin(φ/2). Figure 1 displays the resulting fluorescence image of a dense suspension before [Fig. 1(A)] and after [Fig. 1(B)] it is illuminated with the excitation grating. At any instant in time, the excitation grating picks out a small set of spatial Fourier components of the labeled particle density. There is a primary component at wave number kG = |kG| = 2πdG−1, in addition to a |k| = 0 component associated with the mean fluorescence level, and a band of small-k contributions associated with the Gaussian envelope of the illuminated region. Temporal fluctuations in the integrated fluorescence intensity reflect the growth and decay of the spatial Fourier components represented by the image. Slow fluctuations of the chromophore density associated with the small-k spatial components arise from particles moving in and out of the illuminated region and slow drifts in laser alignment.

Fluctuations of the signal due solely to number density fluctuations at wave number kν are selectively measured using the lock-in detection method [9]. A frequency generator (Keithley) modulates the phase of the excitation grating from 0 to 2π at the frequency ωG = 10 kHz. The grating is thus swept across the illuminated sample at a velocity νG (= ωG/kG) greater than that with which a particle can travel the distance 2πkG−1. The resulting fluorescence intensity that emerges from the image is also modulated at the frequency ωG. The modulated fluorescence signal, Ig(t), is collected using a fused silica oil-immersion objective (Leica, Plan Fluotor, 100X, N = 1.3) and eyepiece (Zeiss, 5×) and then coupled into a multimode optical fiber (3M). The emission from the transmitting end of the fiber is imaged onto a thermoelectrically cooled photomultiplier tube (PMT) (Hamamatsu, R3896) after filtration by an interference bandpass filter (CVI Laser, central wavelength 590 nm, bandwidth 10 nm) and an excitation barrier filter. The analog signal from the PMT is detected using a lock-in amplifier (Stanford Research Systems), which is referenced to the waveform used to drive the phase modulator. A computer, which controls an analog-to-digital data acquisition board (National Instruments), records separately (i) the average background fluorescence intensity [kI ref 0 Ĉ(0), defined below], (ii) the complex components of the demodulated signal, X(t) and Y(t), and (iii) the laser excitation power.

The instantaneous fluorescence intensity, Ig(t), is given by the spatial overlap of the excitation grating, Ig0(r), and the time-dependent distribution of fluorescently labeled species, C(r,t):

\[
I_g(t) = \kappa \int C(r, t) I_{ex}(r) d^3r = \frac{1}{(2\pi)^3} \kappa \int \hat{C}(\mathbf{k}, t) \hat{I}_{ex}(\mathbf{k}) d^3k ,
\]

where \(\hat{C}(\mathbf{k}, t)\) is the Fourier transform of \(C(r, t)\) [Eq. (2)] and \(\kappa\) is the proportionality factor that accounts for the luminescence efficiency of the system. Since the \(e^{-2}\) laser beam waist, w (= 100 μm), is much larger than \(d_G\) we approximate \(I_{ex}(x)\) by an infinite two-dimensional fringe pattern modulated along the x axis:

\[
I_{ex}(x) = I_0 [1 + \cos(\mathbf{k}_G \cdot \mathbf{x} + \phi(t))],
\]

where \(I_0\) is a constant intensity. The time-dependent phase of the excitation grating, \(\phi(t) = \omega_G t + \theta_G = k_G \cdot v_G t + \theta_G\), completes a 0 to 2π cycle at frequency \(\omega_G\), where the direction of \(\mathbf{k}_G\) is parallel to \(v_G\), and \(\theta_G\) is a constant.

Fourier transformation of Eq. (4), followed by substitution into Eq. (2) and using the fact that \(\hat{C}(\mathbf{r}, t)\) is real, leads to

\[
I_g(t) = \kappa I_0 [\hat{C}(0) + \hat{C}(\mathbf{k}_G, t)] \cos(\phi(t) + \alpha(\mathbf{k}_G, t)),
\]

where \(\alpha\) is the phase angle associated with \(\hat{C}\), namely, \(\alpha = \tan^{-1}[\text{Im}\hat{C}/\text{Re}\hat{C}]\). Figure 2 shows a schematic of the signal together with the reference waveform used to generate the modulation: sin(\(\phi_{ref}(t)\)), where \(\phi_{ref}(t) = \omega_{ref} t + \theta_{ref}\) and \(\omega_{ref} = \omega_G\). The phase shift between the signal and reference is \(\theta_{ref} - \theta_G + \alpha\). Equation (5) shows that the total signal is composed of a stationary (dc) and modulated (ac) component. Because fluctuations of \(\hat{C}(\mathbf{k}_G, t)\)
The mean fluorescence level, \( \kappa I_0 \hat{C} \) (0), is time independent as is the phase shift, \( \theta = \theta_{\text{ref}} - \theta_G \), which can be set to any arbitrary value.

Fluctuations of greatly exaggerated speed and amplitude are shown. The mean fluorescence level, \( \kappa I_0 \hat{C} \) (0), is time independent as is the phase shift, \( \theta = \theta_{\text{ref}} - \theta_G \), which can be set to any arbitrary value.

Equations (7) and (8) show that explicit knowledge of the phase angle is not necessary to determine \( F(k, \tau) \) and \( S(k) \).

In practice, the mean fluorescence intensity, after being corrected for drifts in laser power (less than \( \pm 1\% \)), is used to normalize the modulated signal, effectively removing the influence of photodegradation. The ability to detect this signal is determined by the signal-to-background ratio, \( S/B ~ \langle \hat{C}^2 \rangle / \langle \hat{C}(0) \rangle ~ N^{1/2} / N = 1 / N^{1/2}. \) Typically, for each experiment, 16,000 data points are collected at a variable acquisition frequency. Time-correlation functions of these trajectories are computed and averages are constructed from at least eight individual data sets.

Figure 3(A) shows a comparison between FICS measurements [solid curves, Eq. (7)] and the results of a video microscopy analysis [10] to determine \( F(k, \tau) / S(k) \) for a dense monolayer colloid suspension (areal density, \( \rho^* = N a^2 / A = 0.51 \)). For this system, the dynamics is complicated by multiexponential decays that vary with \( k \). An analogous comparison is shown for \( S(k) \), where the FICS...
Particles are highly correlated. For systems that exhibit this and spatial regimes where the motions of neighboring par-

vant two-point spatial distributions yielded by microscopy. While real-space trajectories contain all of the dynamical 
information that characterizes the system, this informa-
tion suggests the possibility of an alternative ap-
proach to direct imaging methods, particularly at shorter 

wavelengths where CCD cameras do not operate. Were 
we to perform measurements at many values of k simulta-

neously on the same sample, it would be possible to 
Fourier transform the resulting fluctuation data to recover 
real-space particle trajectories. This aspect of the FICS 
method is similar in spirit to DLS experiments in which 

heterodyne optical mixing is used to measure the scattered 

electric-field–field-correlation function [1]. For these 
reasons, the FICS method holds promise as a new tool to study 

a wide range of problems where fluorescence is extremely 
weak, such as intracellular transport and polymer dynami-

cs in ultrathin films.

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