Differential Dynamic Microscopy: Probing Wave Vector Dependent Dynamics with a Microscope

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(Received 6 January 2008; revised manuscript received 30 March 2008; published 5 May 2008)

We demonstrate the use of an ordinary white-light microscope for the study of the \( q \)-dependent dynamics of colloidal dispersions. Time series of digital video images are acquired in bright field with a fast camera, and image differences are Fourier analyzed as a function of the time delay between them. This allows for the characterization of the particle dynamics independent of whether or not they can be resolved individually. The characteristic times are measured in a wide range of wave vectors and the results are found to be in good agreement with the theoretically expected values for Brownian motion in a viscous medium.

Microscopy and light scattering are widely used in physics, chemistry, biology, and medical laboratories to access information on the structure and dynamics of mesoscopic systems. While microscopy gives direct access to real space images, scattering techniques work in reciprocal space, where information on the structure and dynamics of the system is obtained, respectively, from the angular and time dependence of the scattered light intensity [1]. These two complementary techniques have in general very different experimental requirements. White-light sources are usual choices in microscopy, while a certain degree of coherence of the illuminating beam is required in scattering experiments; this is usually achieved by using a laser. In the past many attempts have been made to build a scattering apparatus based on a microscope; all of these attempts involved the use of a laser as an illumination source [2–6]. In some cases special care was taken to ensure the capability to perform microscopy and scattering experiments simultaneously, thereby allowing for a powerful combination of the complementary information obtained by both techniques [2,3]. More recently, microscope-based laser dynamic light scattering (DLS) experiments have been developed to study the dynamic properties of samples of biological interest, such as living macrophage and red blood cells [5,6]. In practice, because of the intrinsic difficulties in building such instruments, the use of such techniques has been restricted to those laboratories, where a sufficient expertise in the realization of optical instrumentation was at hand.

In this Letter, we present a conceptual scheme to interpret and analyze microscopy images that are obtained from samples containing moving entities. This technique, which we term differential dynamic microscopy (DDM), does not entail any special experimental requirements, being based on the use of a standard light microscope with a normal illumination source and a digital video camera. By using the tools of Fourier optics [7] we provide the means to access information about the sample dynamics that are equivalent to the one obtained in multiangle DLS experiments [8]. We test DDM by analyzing time sequences of microscopy images obtained with an aqueous dispersion of colloidal particles with diameter 73 nm, well below the resolution limit of the microscope. Additional tests are performed with larger particles (diameter 420 nm). Our results are in good agreement with the predictions from the theory of Brownian motion in a viscous medium, thereby validating the proposed analysis. We believe that DDM is an interesting complement to the well-established DLS [9] and microscope video tracking [10] techniques.

Our experimental setup simply consists of an inverted microscope (LEICA DM IRB) equipped with a complementary metal-oxide-semiconductor (CMOS) camera (IDT X-Stream XS-3, 1280 \( \times \) 1024 pixels, pixel size = 12 \( \mu \)m). No modifications are made to the microscope. The sample is illuminated by focusing white light on the sample with a condenser lens (numerical aperture 0.9); for detection we use a standard objective (magnification \( M = 63 \times \), numerical aperture \( NA = 0.7 \)). The effective pixel size, accounting for the magnification \( M \), is then given by \( d_{\text{pix}} = 0.19 \, \mu \text{m} \), a value smaller than the microscope resolution limit imposed by diffraction. A capillary tube with a rectangular section (Vitrocom, Inc.) is filled with an aqueous dispersion of polystyrene spheres of diameter 73 nm (Duke Scientific, part no. 3070 A). The concentration is 1% by weight fraction, sufficiently low to neglect the effect of interactions between individual spheres. The thickness of the capillary tube along the microscope optical axis is 100 \( \mu \)m and a region at the center of the capillary tube is imaged onto the camera sensor.

A sequence of \( N = 1000 \) images is acquired with a sampling rate of 100 samples/s and with an exposure time of 1 ms. In Fig. 1(a) we show a typical image: the image appears as a bright background with some dark spots. These dark spots are dust particles located mainly on the protective glass of the camera sensor. The signal due to the particles is barely visible as its contribution is small compared to the large background signal. However, the
of the difference signal D on statistically uncorrelated. This dependence of the variance to monitor the particle dynamics. The question then arises whether it is possible to extract quantitative information on the dynamics of the system from the difference images. The answer is yes, but its justification requires the use of two steps.

The first one is to consider that the variance, defined in Eq. (1), can be calculated in the Fourier space as well. If we define the 2D Fourier transform of D(x, y; Δt) as

$$F_D(u_x, u_y; Δt) = \int D(x, y; Δt) \exp[-i2\pi(u_x x + u_y y)] dx dy,$$

the use of the Parseval theorem [7] guarantees that

$$\sigma^2(Δt) = \int |F_D(u_x, u_y; Δt)|^2 du_x du_y. \quad (2)$$

The equivalence of Eq. (1) and (2) shows that the total energy content of D in real space is the same as the one of FD in Fourier space. However, in Fourier space it is possible to isolate every Fourier component and study the growth of its amplitude as a function of Δt.

To clarify whether this mode decomposition provides insight about the dynamics of the system, we need to go through the second step. This step consists in realizing that a Fourier component of the microscope image is associated in a simple way with a scattering angle and in turn with a scattering wave vector. According to Abbe’s theory of microscope image formation [7,12], the object to be imaged on the camera sensor can be thought of as the superposition of different Fourier components characterized by a spatial frequency u_{obj}. Each frequency component of the object acts as a grating and diffracts light at an angle θ = sin⁻¹(λu_{obj}) with respect to the microscope optical axis, where λ is the wavelength of light. On the camera sensor, the diffracted plane wave will produce a sinusoidal fringe pattern, characterized by a spatial frequency u_{det} = sin(θ)/λ = u_{obj} independent of the wavelength λ. This guarantees that we can associate, in a unique way, a spatial frequency u_{det} on the detector to a scattering angle θ.

To investigate the angular dependent dynamics of our system, we thus use the Fourier power spectrum.
as the wave vector over to the plateau value systematically shifts to smaller 

is then easy to show that

\[ \exp \text{concentration mode decays exponentially in time,} \]

are due to concentration fluctuations in the sample. For 

consider that fluctuations in the intensity of the original images 

with \( q \) instead of spatial frequencies \( u \). Processing the data at 

several \( \Delta t \) enables us to report the one-dimensional power spectrum 

\[ |F_D(u_x, u_y; \Delta t)|^2, \] 

for easy comparison with scattering experiments we use wave vectors \( q = 2\pi u \) 

as simple adjusting parameters and extract the characteristic time \( \tau(q) \) by 

the term \( \tau(q) = 1/D_m q^2 \), where \( D_m \) is the mass diffusion coefficient 

of the particles [9]. For the analysis of the difference images it 

is then easy to show that

\[ |F_D(q; \Delta t)|^2 = A(q)[1 - \exp(-\Delta t/\tau(q))] + B(q). \]  (3)

This equation contains two main contributions: the term \( B(q) \) is due to the power spectrum of the camera noise and 

is present even in the absence of the particles. The term \( A(q)[1 - \exp(-\Delta t/\tau(q))] \) describes the contribution 

associated with the particles. A quantitative description of 

the term \( A(q) \) requires the knowledge of the exact relationship 

between intensity and concentration fluctuations, which is beyond the scope of this Letter. However, once 

\( q \) is fixed, we can treat \( A(q) \) and \( B(q) \) as simple adjusting parameters and extract the characteristic time \( \tau(q) \) by 
simply considering the \( \Delta t \) dependence of \( |F_D(q; \Delta t)|^2 \). This is very similar to what has been done in Ref. [14] 

where an expression similar to Eq. (3) has been proposed to analyze 

sequences of shadowgraph images for the characterization of nonequilibrium concentration fluctuations in a binary mixture.

Thus, we fit our data to Eq. (3) to obtain \( \tau(q) \), which we 

report in Fig. 4 for \( q \) ranging from 0.4 \( \mu m^{-1} \) to 5 \( \mu m^{-1} \) 

(black open circles). On the same graph the dotted black line denotes the expected behavior calculated by 

using the formula \( \tau(q) = 1/D_m q^2 \), where \( D_m \) is estimated 

using the Stokes-Einstein relation \( D_m = k_B T/(3\pi \eta d) = 5.98 \mu m^2/s; \) \( k_B \) is the Boltzmann constant, \( T \) the absolute 

temperature, \( \eta \) the solvent viscosity, and \( d \) the diameter of 

the particles. The experimental results are in good agreement 

with the theoretically expected values over more than 

one decade in \( q \), showing that DDM is a reliable means to 
determine the dynamical properties of the sample. To our 

knowledge, this result provides the first experimental evidence 

that white-light, bright-field microscopy can be used to 
monitor the \( q \)-dependent dynamics of particles.

We also perform experiments by using particles with a 

diameter of 420 nm, value comparable to the point spread 

function of the microscope. In Fig. 5 we present two 

images of this sample separated in time by 10 s. In contrast 
to the previous case, the particles are fairly visible, though 

the static signal due to dust along the optical path still 

represents a significant contribution. However, the subtraction 

procedure described above can still be applied to 
visualize the particles’ motion and to eliminate the static 
noise, as shown in the movie clip referred to in [11]. The 
image sequence is analyzed as described above and the 
results are presented in Fig. 4 as blue open squares.

![FIG. 4 (color online). Characteristic decay time \( \tau \) versus the wave vector \( q \) for the 73 nm (black circles) and the 420 nm (blue squares) particles. Fitting of the data gives, respectively, the values \( D_m = 6.2 \pm 0.3 \mu m^2/s \) and \( D_m = 1.1 \pm 0.2 \mu m^2/s \). The lines are theoretical predictions calculated by using the Stokes-Einstein relation with no adjustable parameters.](http://doc.rero.ch)
agreement with the theoretically expected behavior ($D_m = 1.04 \ \mu \text{m}^2/s$, continuous blue line) is also found in this case, demonstrating that DDM is capable of monitoring the dynamics of particles that can either be resolved individually or not. This suggests that DDM could be a valid complement to tracking techniques [10] for relatively big particles, with the advantage of also being applicable to concentrated systems, where tracking becomes difficult, and to fluctuating systems, which are not composed by particles. Typical examples are membranes, interfaces, or liquid crystals. For small particles DDM can be subsidiary to DLS experiments [9], because it can operate at small wave vectors where stray light makes the use of DLS notoriously difficult. The range of accessible time scales is limited by the inverse of the acquisition frame rate on the lower side and by the overall duration of the experiment on the higher end.

A possible extension of this work could be the investigation of dynamically heterogeneous systems, where a space resolved investigation of the dynamics is of fundamental importance [15]. Our technique could also be applied to absorbing samples and to fluorescence microscopy, which is widely used for the investigation of biological samples [16]. Finally, we believe that DDM can be profitably used to perform microrheology experiments as pioneered in Ref. [17].

The authors thank M. Carpineti, F. Giavazzi, and A. Vailati for stimulating discussions and for critical reading of the manuscript. T. Bellini, P. Cicuta, F. Croccolo, M. Giglio, R. Piazza, C. Takacs, and D. Weitz are thanked for fruitful discussions. R.C. acknowledges financial support from the European Union (Marie Curie Intra-European Fellowship, Contract No. EIF-038772) and V.T. financial support from the Swiss National Science Foundation.

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