Using dynamic low-coherence interferometry to image Brownian motion within highly scattering media

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Dynamic low-coherence interferometry was used to measure Brownian motion of submicrometer particles within highly scattering media. Strong rejection of multiply scattered light was obtained by combination of a coherence gate with a confocal microscope, thus allowing particle characterization methods generally reserved for optically dilute materials to be applied to optically concentrated suspensions. The Brownian diffusion coefficient of highly scattering media was determined with an accuracy better than 5%. Furthermore, we show that spatial variations in the Brownian diffusion coefficient can be imaged with an axial resolution determined by the coherence length of the light source (~30 μm). The experiments also show broadening of the power spectrum as a function of depth into the sample, most likely as a result of detecting multiply scattered light.

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Dynamic light scattering is a powerful tool for measuring translational, rotational, and internal motions of ~1-nm- to ~10-μm-sized particles in suspension[1] and is based on the fact that the phase and the amplitude of the scattered electric field are modulated by the dynamics of scattering particles. These modulations appear as intensity fluctuations of the scattered light, and information about the sample properties can be extracted from the power spectrum or the temporal correlation function of the photodetector current. In an optically dilute suspension, light scatters only once, so the scattering angle and the polarization are well defined, and detailed information on particle dynamics can be obtained. As the suspension becomes more concentrated, light scatters multiple times before detection, so the scattering angle and the polarization are no longer well defined, and details of particle dynamics are lost. Clearly it is desirable to have an optical technique that can separate singly from multiply scattered light, thus permitting detailed characterization of particle dynamics within turbid media; examples include in-line particle sizing and monitoring aggregation and gelation kinetics.

In this Letter we demonstrate how dynamic low-coherence interferometry (LCI) can measure detailed particle dynamics within highly scattering media. We achieve such measurement by combining a coherence gate with confocal discrimination, resulting in strong rejection of multiply scattered light. Our technique is based on LCI that uses a short-coherence-length light source in an optical heterodyne arrangement, which provides a coherence gate to achieve path-length selectivity.[2,3] By choosing a path length in the single-scattering regime, we are able to determine the Brownian diffusion coefficient of strongly scattering media with an accuracy of better than 5%. We also show that spatial variations in the Brownian diffusion coefficient can be imaged with a spatial resolution of ~30 μm.

A schematic of our single-mode fiber optic LCI system is shown in Fig. 1. The single-mode fiber optic (N.A. = 0.15) interferometer is illuminated by an 850-nm superluminescent diode. The optical properties of the sample generate a distribution of optical path lengths in the sample arm, whereas the path length in the reference arm is determined solely by the position of the retroreflector. Interference is observed only when the optical path-length difference between the reference and the sample arms is within the coherence length of the source. The coherence length of the light source defines the axial (path-length) resolution of the measurement, and the lateral resolution depends on the optics used to focus the probe beam in the sample.[3–6] The position of the reference mirror is adjusted to yield a path length equal to the path length to the focal point in the sample arm. By centering the coherence gate within the beam waist, we maximize the rejection of multiply scattered light [4] and maximize the detection of singly scattered light.

When the reference mirror is fixed, the photocurrent is proportional to[2,4–6]

$$i_d \propto I_r + I_s + I^{\text{fluct}}(z,t)\,, \tag{1}$$

where I_r (I_s) is the intensity from the reference (sample) arm and $I^{\text{fluct}}(z,t)$ is the cross term (or optical heterodyne term) that results from the coherent

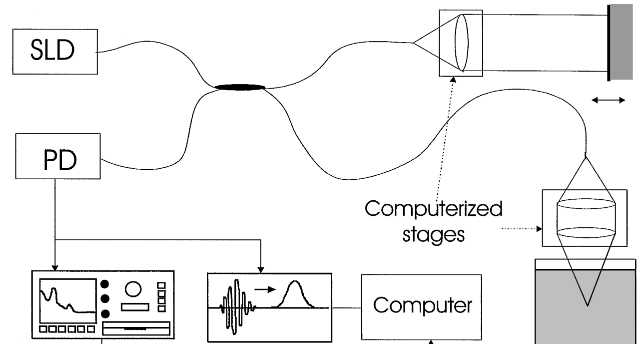

Fig. 1. Dynamic low-coherence interferometer system: PD, photodetector; SLD, superluminescent diode.

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mixing of the electric field in the reference arm, E_r, with the path-length distribution of $E_s(z)$. Temporal fluctuations in the cross term result from changes in the amplitude, the phase, or both of either E_r or E_s. When the reference mirror is held fixed, the amplitude and the phase of E_r do not change over time. Any dynamics in the sample, such as flow and Brownian motion, causes Doppler shifts in the frequency of the propagating light, resulting in phase fluctuations in $E_s(z)$. The power spectrum derived for a system undergoing Brownian motion is a Lorentzian[1]:

$$P(f) \propto \frac{[I_r I_s(z)]^{1/2}}{1 + \left[\dfrac{2\pi f}{D_B q^2(\theta)}\right]^2}, \qquad (2)$$

where $I_s(z)$ is the intensity of light that has traveled a path length of $2z$ through the sample; D_B is the self-diffusion coefficient of the scattering particles and is given by $D_B = k_B T/(3\pi\eta a)$, where k_B is the Boltzmann constant, T is the temperature, η is the viscosity of the suspending liquid, and a is the hydrodynamic diameter of the scattering particle. The momentum transfer of the light-scattering event is $q = 2k \sin(\theta/2)$, where θ is the light-scattering angle defined by the geometry of the optical instrument. To increase sensitivity to the photocurrent fluctuations, our detector is ac coupled to a band-pass filter with a cut-on frequency of 5 Hz and a cutoff frequency of 1 kHz. For the results presented in Figs. 2–4 the signal was integrated for 30 s to improve the signal-to-noise ratio, although a sufficient signal-to-noise ratio to characterize the linewidth is typically obtained in less than 1 s for depths of less than three scattering lengths.

For our experiments we examined suspensions of polystyrene (PS) microspheres of mean diameters $d = 0.22\ \mu$m and $d = 1.02\ \mu$m (Bangs Labs, Inc.), in distilled water. The particle volume fraction in each monodisperse suspension (4% and 0.5%, respectively) was chosen to provide a photon scattering length of $\sim$100 μm. The sample holder was made of plastic [poly(methyl methacrylate)], and the temperature of the suspensions was measured before and after each complete depth scan.

To demonstrate that LCI can be used to image and quantify Brownian motion within highly scattering media, we measured the power density spectra for two separate monodisperse PS microsphere suspensions at a sample depth of 200 μm (Fig. 2). This depth was chosen to avoid Fresnel reflections from the glass–suspension interface and to ensure detection of only singly scattered light. The experimental data were fitted with a Lorentzian [relation (2)], as indicated by the solid curves through the data points. We used the linewidths obtained from these fits to determine the experimental Brownian diffusion coefficients and the corresponding particle sizes. The calculated microsphere diameters (0.222 and 0.997 μm) were within 5% of the values cited by the manufacturer. Multiple experiments with different-sized PS spheres showed the same accuracy. The inset of Fig. 2 shows the amplitudes, determined from the Lorentzian fits as a function of the depth in the sample, for the same two monodisperse suspensions. As expected, in both cases the amplitude decayed exponentially with the path length in the medium, with an extinction coefficient equal to the scattering coefficient of the suspension. The solid curves through the data points represent the expected amplitude decay as calculated from Mie theory. The agreement between theory and experiment was always within 10%.

Because the power spectrum for a system undergoing Brownian motion depends on the scattering angle [relation (2)] and the geometry of our experimental setup permits collection of light over a range of angles (determined by the N.A. of the focusing objective), one would expect the measured power spectrum

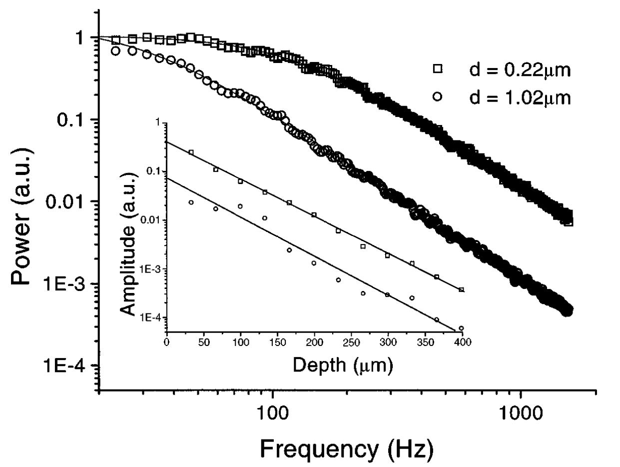

Fig. 2. Power spectra measured at a depth of 200 μm for two different suspensions. The solid curves show the Lorentzian fits to the experimental data. Inset, amplitude as a function of depth in the sample for the same samples; the solid curves represent the expected amplitude decays.

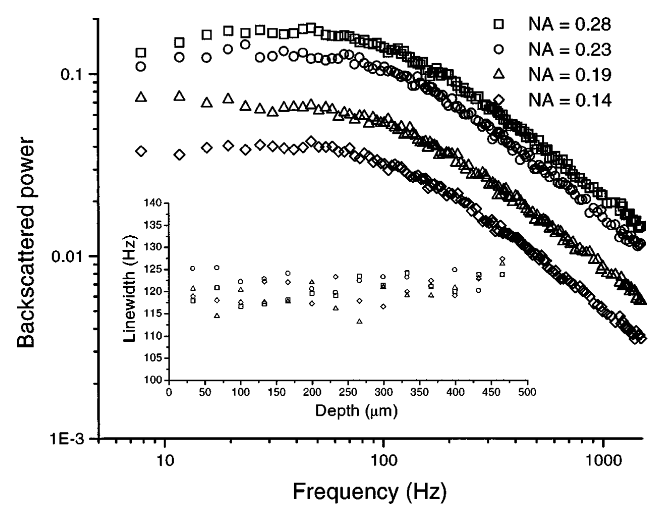

Fig. 3. Power spectra for a 4% monodisperse solution of 0.22-μm-diameter PS microspheres for different N.A.'s of focusing objective. Inset, Lorentzian linewidth as a function of depth for different N.A.'s of the focusing objective.

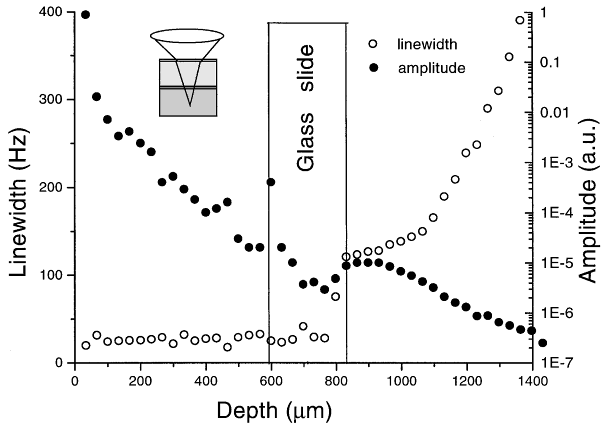

Fig. 4. Lorentzian linewidth and amplitude as functions of depth in the two-layer system. Inset, schematic of the cell used to image spatial variations in the dynamic properties of turbid media. Top layer, 0.5% suspension of 1.02-μm PS spheres; bottom layer, 4% suspension of 0.22-μm PS spheres.

to be a weighted sum of Lorentzians, i.e.,

$$P(f) \propto \int_0^{\pi} \frac{W(\theta)\mathrm{d}\theta}{1 + \left[\dfrac{2\pi f}{D_B q(\theta)^2}\right]^2}, \qquad (3)$$

where $W(\theta)$ is the distribution of scattering angles. We explored this idea by varying the N.A. of the focusing objective from 0.14 to 0.28, keeping all other parameters constant. The results for a 4% monodisperse suspension of 0.22-μm PS spheres are shown in Fig. 3. The power spectrum amplitude decreased with N.A. because light was collected over a smaller solid angle. Contrary to our expectations, the experimental data showed excellent agreement with a single Lorentzian fit, and furthermore the linewidth was independent of N.A. This contradiction may result from the fact that most of the detected light is backscattered within the beam waist where the wave front is planar.

To demonstrate the ability of LCI to map spatial variations in the particle Brownian diffusion coefficient, we used the two-layer cell shown in the inset of Fig. 4. A 580-μm-thick layer of 1.20-μm PS microspheres (0.5% volume fraction) was contained between two 150-μm-thick cover slips with a spacer, and this chamber was positioned above a 4% solution of 0.22-μm PS spheres. Both the linewidth and the amplitude, determined from the fits to the experimental data, are graphed as functions of sample depth (Fig. 4). The two layers are clearly distinguished by the Fresnel reflections at the glass–solution interfaces that appear in the amplitude data and also by the abrupt change in the Lorentzian linewidth. Note that the thickness of the glass slide appears to be greater than the physical thickness because we did not correct for the slide's refractive index. Within the single-scattering regime no signal was expected within the cover slip, so the observed signal was probably due to multiply scattered light.[4] The experimentally determined amplitude decay for the top layer agreed well with Mie theory calculations. The anomalous light attenuation of the first two data points in the bottom layer may be due to aberrations caused by the glass slide. For depths between 900 and 1200 μm the decay rate was within 10% of the expected value. Beyond 1200 μm the decay rate decreased, corresponding to detection of multiply scattered light.[4] The linewidth measured within the 1.02-μm suspension (top layer) agreed very with the expected value. In the 0.22-μm suspension (bottom layer) the Lorentzian linewidth initially agreed with the expected value; however, it then broadened with depth. The Brownian diffusion coefficient determined for the first two data points was within 5% of the expected value, which showed that spatial variations in particle dynamics can be discriminated with LCI. Further experimentation is needed to reveal the origin of the observed linewidth broadening as measurement depth increases. This broadening most likely results from the detection of multiply scattered light. For single scattering the linewidth is $D_B q^2$. As the number of scattering events increases, the linewidth also increases, as has been described by Bonner and Nossal.[7] This anomaly, although it is not desirable for most applications, may be a useful tool for the analysis of the effect of multiple scattering on LCI and optical coherence tomography.[4,8]

In summary, we have shown that LCI can image and quantify Brownian motion within highly scattering media. The technique has potential applications as both a tool for noninvasive characterization of the dynamic and structural properties of optically thick materials as well as a method for studying the effect of multiple scattering on image contrast and resolution in confocal and optical coherence microscopy.

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