Path-length-resolved dynamic light scattering in highly scattering random media: The transition to diffusing wave spectroscopy

Kostadinka K. Bizheva, Andy M. Siegel, and David A. Boas
Electro-Optics Technology Center, Tufts University, 4 Colby Street, Medford, Massachusetts 02155
(Received 19 May 1998)

We used low coherence interferometry to measure Brownian motion within highly scattering random media. A coherence gate was applied to resolve the optical path-length distribution and to separate ballistic from diffusive light. Our experimental analysis provides details on the transition from single scattering to light diffusion and its dependence on the system parameters. We found that the transition to the light diffusion regime occurs at shorter path lengths for media with higher scattering anisotropy or for larger numerical aperture of the focusing optics. [S1063-651X(98)11112-1]

PACS number(s): 82.70.Dd, 87.64.−t, 87.80.+s

Dynamic light scattering (DLS) has been used extensively during the past few decades for characterization of the structural and dynamical properties of materials that weakly scatter light [1,2]. DLS is based on measuring fluctuations in the intensity of the scattered light arising from phase and/or amplitude fluctuations induced by particle dynamics. This technique is applicable to media in which the detected light has scattered no more than once. In highly scattering materials the scattering angle and the polarization of the scattered wave are not well defined due to multiple scattering events and details about the sample properties are lost. However, the intensity of the multiply scattered light is accurately predicted by the photon diffusion equation and therefore the theory of diffusing wave spectroscopy (DWS) can be applied for quantitative analysis of the angle averaged dynamic properties [3,4]. Although both DLS and DWS provide information about the structural and dynamical properties of the sample, they are only valid in the two extreme cases of single scattered and diffusive light respectively. Durian [5] and Kaplan *et al.* [6] have studied conditions under which DWS is valid, but still little is known about the intermediate regime between DLS and DWS. Since the detection of multiply scattered light causes degradation of image contrast and resolution in confocal [7] and optical coherence microscopy [8], a clear understanding of the transition from ballistic to diffusive light will permit a quantitative analysis of scattering media that do not satisfy the single scattering or light diffusion criteria and can lead to the development of new techniques for image quality improvement.

In this paper we show how low coherence interferometry (LCI) can be used to make path-length-resolved measurements of particle Brownian motion within highly scattering media. LCI uses a coherence gate to select light that has traveled a specific path length in the medium. Thus it is possible to detect light that has scattered only once within a turbid medium and to apply DLS for the determination of the sample dynamical properties or to select only diffusive light and apply DWS theory. We experimentally demonstrate these two extremes in highly scattering samples of polystyrene microspheres, as well as the smooth transition between them. We show experimentally that this transition depends on the scattering properties of the medium and the measurement geometry. In particular, we find that the transition to the diffusing light regime occurs at shorter path lengths for either higher scattering anisotropy or a larger numerical aperture (NA) of the collection optics.

A schematic of our LCI system is shown in Fig. 1. The single-mode fiber optic interferometer is illuminated with an 850-nm superluminescent diode (25-nm spectral bandwidth, 1.2-mW output power). The optical properties of the sample generate a distribution of optical path lengths in the sample arm, while 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. Thus a coherence gate is used to select specific path lengths within the sample. The amplitude of the interference signal is therefore proportional to the path-length-dependent reflection/scattering properties of the sample. In the single scattering regime the axial resolution is determined by the source coherence length, while the lateral resolution depends on the focusing optics. The position of the reference mirror (retroreflector) is adjusted in such a way as to align the coherence gate with the beam waist, thus optimizing the rejection of multiply scattered light [8].

As demonstrated in our previous studies [9], particle dynamics of highly scattering media can be imaged and quantified in the single scattering regime with dynamic LCI by examining the intensity fluctuations of the backscattered light and extracting information from the photocurrent power spectrum. For a fixed position of the reference mirror of the

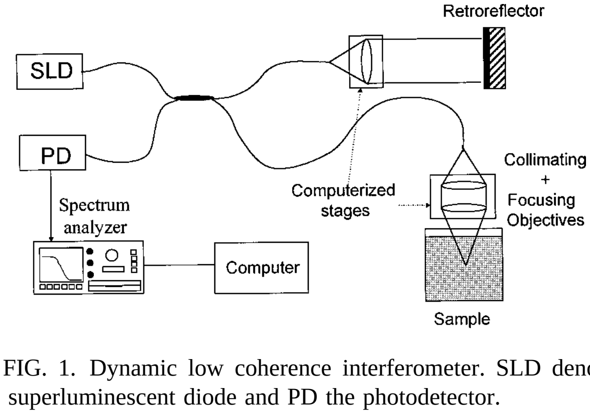

FIG. 1. Dynamic low coherence interferometer. SLD denotes the superluminescent diode and PD the photodetector.

interferometer and a sample consisting of a monodisperse suspension of scattering particles undergoing Brownian motion, the photocurrent power spectrum is a Lorentzian [1,2]

$$P(f)=\frac{1}{\Omega}\frac{A}{1+\left(\frac{2\pi f}{\Omega}\right)^2}, \tag{1}$$

where A is the amplitude of the power spectrum and Ω is the spectrum linewidth.

In an optically dilute suspension, light scatters only once before detection; therefore, the scattering angle and polarization are well defined. For the case of single scattered light, assuming weak scattering and noninteracting particles, the power spectrum linewidth is proportional to the particle self-diffusion coefficient D_B:

$$\Omega=q^2D_B, \tag{2}$$

where $D_B=k_BT/3\pi\eta a$, k_B is the Boltzmann constant, T is the temperature, η is the viscosity of the suspending liquid (in our case of H_2O, $\eta=1.0$ cps), and a is the hydrodynamic diameter of the scattering particle. Here q denotes the photon momentum transfer, $q=2k\sin(\theta/2)$, where k is the wave vector in the scattering medium and θ is the scattering angle. Note that Eq. (2) is valid for optical heterodyne detection, while in the case of homodyne measurement, the expression is multiplied by a factor of 2.

In an optically dense medium where light scatters multiple times before detection, the scattering angle, the polarization of the scattered wave, and consequently the momentum transfer q change with each scattering event. In this case Eq. (2) no longer describes the observed power spectrum. According to the DWS theory, for the case of light diffusion through a turbid medium undergoing Brownian motion, the power spectrum of the detected diffuse light is also a Lorentzian, but with a linewidth dependent on the scattering properties of the medium and the photon path length within it [3,4,10]:

$$\Omega=2k^2D_B\frac{S}{l^*}. \tag{3}$$

Here s is the path length traveled by the light in the medium, $l^*=l_s/(1-g)$ is the photon random walk length, l_s is the photon scattering length, and g is the scattering anisotropy of the medium defined as an average of the cosine of the scattering angle. For polystyrene microspheres we were able to calculate l^* and l_s using Mie theory and the Percus-Yevick structure factors for hard spheres [11].

To explore the effect of multiply scattered light on the spectrum linewidth we measured the power spectrum as a function of path length in a highly scattering, monodisperse suspension of 0.22-μm polystyrene microspheres in water. The particle concentration (4% volume fraction) was chosen to yield a photon scattering length ~100 μm. The power spectrum was measured in steps of 33 μm inside the sample and the spectrum linewidth and amplitude were determined by fitting the experimental data with a Lorentzian function [Eq. (1)]. Figure 2(a) shows the power spectra measured with the focal point positioned 33 and 1000 μm below the surface

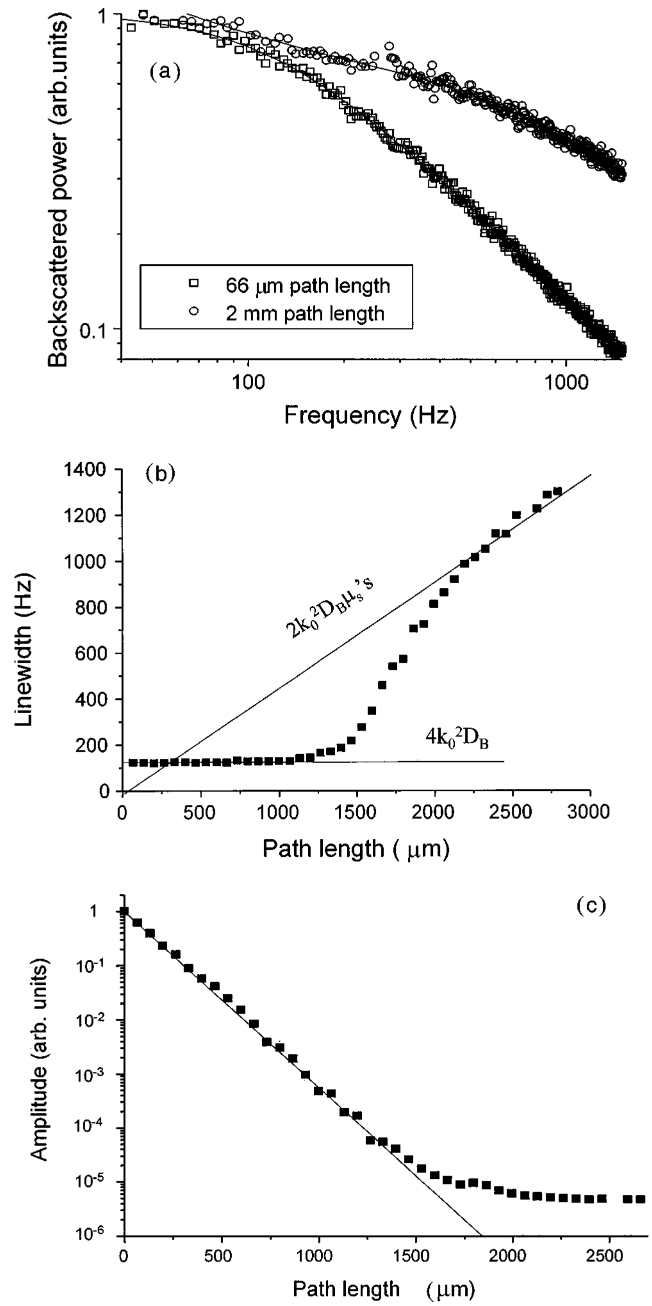

FIG. 2. (a) Power spectrum measured at depths of 66 μm and 2 mm below the sample surface. The solid curves indicate the Lorentzian fits. (b) Lorentzian linewidth as a function of the path length through the scattering medium. The solid lines indicate the expected linewidth behavior in the single scattering and light diffusion regimes, respectively. (c) Lorentzian amplitude as a function of the path length through the scattering medium. The solid line indicates the expected exponential decay in the single scattering regime.

of the sample, corresponding to path lengths of 66 μm and 2.0 mm, respectively. The solid lines through the data points represent the expected behavior according to Eqs. (1) and (3), respectively. The spectra were normalized to facilitate their comparison. Note that for the measurement at the longer path length, it was necessary to account for the $1/f$ electronic noise that was significant at lower frequencies. This was done by directly measuring the $1/f$ spectrum and including it in the Lorentzian fit. As Fig. 2(a) shows, the power spectrum measured at a path length of 20 photon scattering lengths (2.0 mm) through the sample is considerably broader than the spectrum measured close to the suspension surface, where the detected light is scattered only once. This broadening of the power spectrum can result from changes in either the particle diffusion coefficient or the total accumulated momentum transfer or both. Since the sample was a homogeneous, monodisperse suspension held at constant temperature, the observed increase in the linewidth could only be attributed to changes in the accumulated momentum transfer resulting from detection of multiply scattered light, as predicted by DWS theory.

To examine how well our measurements agreed with the predictions of the DWS theory we measured the Lorentzian linewidth as a function of path length in the sample. In Fig. 2(b) we plot the linewidth versus the path length in the medium. As previously observed [9], the spectrum linewidth changed very little (within 5% of the value predicted by the DLS theory) up to 6 scattering lengths through the suspension. This indicated that dynamic LCI is detecting only single scattered light. In this region we measured a linewidth of 122 Hz corresponding to a Brownian diffusion coefficient $D_B = 1.98\times10^{-8}$ cm^2/s, which was in good agreement with the expected value of 2.00×10^{-8} cm^2/s. For path lengths greater than 20 scattering lengths, we observed a linear broadening of the Lorentzian linewidth with photon path length in the media as predicted by the DWS theory [Eq. (3)]. For a path length of 2.2 mm and random walk length l^* of 0.133 mm we measured a linewidth of 986 Hz corresponding to a Brownian diffusion coefficient of $D_B = 1.99\times10^{-8}$ cm^2/s, which was in excellent agreement with the expected value of 2.00×10^{-8} cm^2/s. In the transition region for path lengths ranging from 6 to 20 scattering lengths we observed a nonlinear broadening of the linewidth corresponds to detection of light that has scattered more than once but is not yet diffuse.

Figure 2(c) illustrates the effect of the detection of multiply scattered light on the Lorentzian amplitude. As expected, in our practically nonabsorbing sample, in the single scattering regime the amplitude decayed exponentially with the path length with an extinction coefficient equal to the scattering coefficient μ_s of the medium ($\mu_s = 1/l_s$). The solid line through the data points represents the decay predicted from the single scattering theory. The agreement between the experimentally determined scattering coefficient and the theoretically predicted value was within 10–15 %. The existence of a systematic deviation due to spatial coherence has been discussed by Schmitt *et al.* [12]. The deviation of the experimental data from the straight line for path lengths through the suspension greater than 1.3 mm (13 scattering lengths) has been observed previously and is generally attributed to detection of multiply scattered light [8].

Figure 2(b) clearly demonstrates that with dynamic LCI we can make measurements in the single scattering and diffusion regimes that agree well with the predictions of DLS and DWS theories, respectively. Currently, the transition between these two regimes is not yet well characterized. Dynamic LCI offers the opportunity to acquire detailed information on this transition. This knowledge is important since detection of multiply scattered light causes image degradation in confocal microscopy and optical coherence microscopy. A quantitative experimental analysis followed by a theoretical model of this transition will set the basis for developing new computer algorithms that minimize the effects of multiply scattered light. In order to characterize this intermediate regime we used a reflectance mode geometry with a single fiber for emission and collection of light. In this case we expected the transition (normalized by the scattering length) to depend only on the NA and the sample scattering anisotropy.

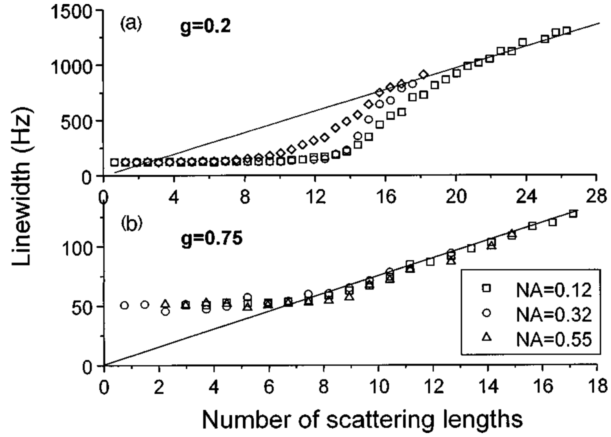

FIG. 3. Linewidth as a function of the number of photon scattering lengths ($\mu_s s$) in the medium for three different NAs of the focusing objective: (a) sample scattering anisotropy $g = 0.2$ and (b) sample scattering anisotropy $g = 0.75$.

To explore the dependence on the NA of the focusing optics, we performed measurements on the same sample with three different microscope objectives (NA = 0.12, 0.32, and 0.55). The experimental results are shown in Fig. 3(a). These results clearly demonstrate that increasing the NA causes the transition to occur at shorter path lengths. Repeated measurements on monodisperse suspensions of particles with mean diameters 0.3, 0.55, and 1.06 μm revealed that this behavior of the linewidth is typical for media with low scattering anisotropy (observed for $a = 0.22\ \mu$m with $g = 0.2$ and $a = 0.3\ \mu$m with $g = 0.39$). However, in suspensions with a larger scattering anisotropy ($a = 0.55\ \mu$m with $g = 0.75$ and $a = 1.06\ \mu$m with $g = 0.9$) the effect of the NA on the line-

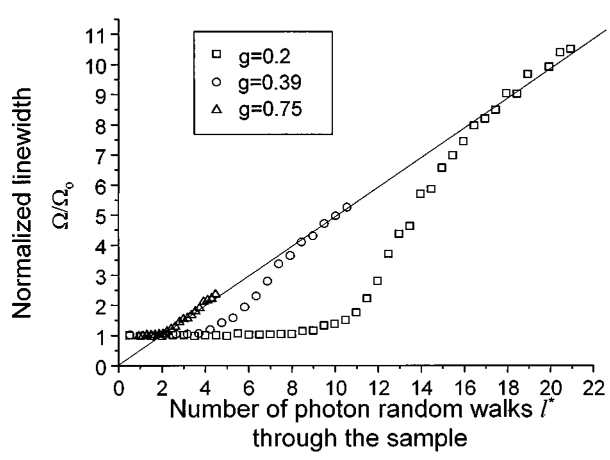

FIG. 4. Normalized linewidth as a function of the number of photon random walks through the medium for NA = 0.32 and $g = 0.2$, 0.39, and 0.75.

width broadening was either very small (undetectable with the used experimental method) or nonexistent [see Fig. 3(b)].

The results of the NA experiments indicated that the transition depends on scattering anisotropy. To examine the dependence on g, we performed measurements with three different diameter microspheres, 0.22, 0.3, and 0.55 μm, while keeping the NA of the focusing optics constant (NA $=0.32$). The results from these experiments, presented in Fig. 4, clearly show a dependence on sample scattering anisotropy. Specifically, for smaller scattering anisotropy diffusive light is detected after a greater number of photon random walks in the sample. The results in Fig. 4 are graphed versus photon random walk steps (l^*) rather than scattering lengths (l_s) to utilize the s/l^* scaling predicted by DWS [Eq. (3)].

One possible explanation for the observed trends in the results is that in a turbid medium with high scattering anisotropy the lateral spread of the backscattered photon flux due to multiple scattering events is smaller than the acceptance cone of the collection optics, even for small NA objectives. Therefore, the detection of multiply scattered or diffusive light in this case is practically independent of the NA of the focusing optics. On the other hand, in a medium with low scattering anisotropy the lateral spread of the backscattered photon flux is much greater; therefore, better rejection of multiply scattered light is obtained by using a low NA objective. Since the NA determines also the image spatial resolution, selecting an objective for imaging in turbid media with low scattering anisotropy will require a compromise between image spatial resolution and depth penetration.

In summary, we have shown that dynamic LCI permits path-length-resolved measurements of particle dynamics in highly scattering media with the ability to separate singly scattered, multiply scattered, and diffusive light. Our results showed excellent agreement with the predictions of the DLS and DWS theories in the single scattered and diffusion regimes, respectively. We also found that the transition from ballistic to diffusive light takes fewer scattering events for samples with high scattering anisotropy and for collection optics with a larger NA. We anticipate that the experimental results presented in this paper will stimulate the development of a theoretical model describing the few-scattering-event regime.

[1] P. J. Berne and R. Pecora, *Dynamic Light Scattering* (Wiley, New York, 1976).

[2] R. Pecora, *Dynamic Light Scattering: Applications of Photon Correlation Spectroscopy* (Plenum, New York, 1985).

[3] D. J. Pine, D. A. Weitz, P. M. Chaikin, and Herbolzheimer, Phys. Rev. Lett. **60**, 1134 (1988).

[4] G. Maret and P. E. Wolf, Z. Phys. B **65**, 409 (1987).

[5] D. J. Durian, Phys. Rev. E **51**, 3350 (1995).

[6] P. D. Kaplan, M. H. Kao, A. G. Yodh, and D. J. Pine, Appl. Opt. **32**, 3828 (1993).

[7] M. Kempe, A. Z. Genack, W. R. Dorn, and P. Dorn, J. Opt. Soc. Am. A **14**, 216 (1997).

[8] M. J. Yadlowsky, J. M. Schmitt, and R. F. Bonner, Appl. Opt. **34**, 5699 (1995).

[9] D. A. Boas, K. K. Bizheva, and A. M. Siegel, Opt. Lett. **23**, 319 (1998).

[10] A. G. Yodh, P. D. Kaplan, and D. J. Pine, Phys. Rev. B **42**, 4744 (1990).

[11] P. D. Kaplan, A. D. Dinsmore, A. G. Yodh, and D. J. Pine, Phys. Rev. E **50**, 4827 (1994).

[12] J. M. Schmitt, A. Knuttel, A. H. Gandjbakhche, and R. F. Bonner, Proc. SPIE **1889**, 197 (1993).