

A comparison of atomic force microscopy (AFM) and dynamic light scattering (DLS) methods to characterize nanoparticle size distributions

Christopher M. Hoo · Natasha Starostin ·
Paul West · Martha L. Mecartney

Received: 30 July 2007 / Accepted: 27 May 2008 / Published online: 20 June 2008
© Springer Science+Business Media B.V. 2008

Abstract This paper compares the accuracy of conventional dynamic light scattering (DLS) and atomic force microscopy (AFM) for characterizing size distributions of polystyrene nanoparticles in the size range of 20–100 nm. Average DLS values for monosize dispersed particles are slightly higher than the nominal values whereas AFM values were slightly lower than nominal values. Bimodal distributions were easily identified with AFM, but DLS results were skewed toward larger particles. AFM characterization of nanoparticles using automated analysis software provides an accurate and rapid analysis for nanoparticle characterization and has advantages over DLS for non-monodispersed solutions.

Keywords Atomic force microscopy · Dynamic light scattering · Polystyrene nanoparticles · Size analysis · Nanotechnology · Instrumentation

C. M. Hoo · M. L. Mecartney (✉)
Department of Chemical Engineering and Materials
Science, University of California, Irvine, 916 Engineering
Tower, Irvine, CA 92697-2575, USA
e-mail: martham@uci.edu

N. Starostin · P. West
Technology Center, Pacific Nanotechnology, Inc.,
17981 Sky Park Circle Suite J, Irvine, CA 92614, USA

Present Address:
N. Starostin
Rosemount Analytical Inc., Irvine, CA 92606, USA

Introduction

Nanoparticles display a number of unique properties that directly correlate to their size. It has been found that properties such as melting temperature (Dick et al. 2001) and dissolution rate (Meulenkamp 1998) are dictated by the size of nanoparticles. The increased surface to volume ratio of nanoparticles is a key property that makes them ideal for nanosensors that depend on surface reactions. As a result, it is important to be able to accurately characterize size distributions within nanoparticle suspensions. While it has been shown that atomic force microscopy (AFM) and dynamic light scattering (DLS) can analyze complex colloidal systems such as latex colloids (Cadene et al. 2005), this study compares AFM and DLS measurements against reported NIST traceable polystyrene nanoparticle size distributions in order to determine an optimal method for size distribution characterization.

Atomic force microscopy

The atomic force microscope (AFM), developed in 1986 by Binnig et al. (1986), enables users to characterize nanoscale objects. The AFM utilizes piezoelectric ceramics to move a specimen in nanoscale increments in the X, Y, and Z directions. An AFM tip mounted on a cantilever is positioned above the specimen at a distance where the tip is repelled or attracted by the forces due to the interaction with the

specimen surface. As the specimen is moved below the tip, the cantilever bends due to topography changes as the tip maintains a constant force from the surface. A laser reflects off the cantilever to a photo-detector. Detector electronics reads the deflection of the laser, processes it through the feedback loop, and the data acquisition software turns the measured deflections into a 3-dimensional image.

The ability of an AFM to characterize nanoscale objects makes it an ideal characterization tool for determining particle size distributions as well as image complex arrays of nanoparticles (Juillerat et al. 2005; Liu et al. 2003; Xia et al. 2004). It has also been proposed that nanoparticles could function as a calibration standard for the atomic force microscope (Li and Lindsay 1991).

Dynamic light scattering

Dynamic light scattering (DLS), a technique often referred to as photon correlation spectroscopy (PCS), is a common technique for determining particle size in colloidal suspensions. Particles suspended in a liquid solvent undergo random Brownian motion. Light is scattered off the particles in suspension. Since the particles cause localized changes in the refractive index, intensity variations are produced by the particles and evaluated using the second order normalized autocorrelation function

$$g_2(\tau) = \frac{G_2(\tau)}{\langle I \rangle^2} \quad (1)$$

where $\langle I \rangle$ is the average intensity, τ is the correlation time, and $G_2(\tau)$ is the temporal correlation function. The second order normalized correlation function is then related to the first order correlation function $g_1(\tau)$ where $g_1(\tau)$ is expressed as

$$g_1(\tau) = \exp(-q^2 D \tau) \quad (2)$$

for particles subject to Brownian diffusion where q is the magnitude of the scattering vector and D is the translational diffusion coefficient. The scattering vector q is expressed as

$$q = \frac{4\pi n}{\lambda_o} \sin\left(\frac{\theta}{2}\right) \quad (3)$$

where n is the refractive index of the solution, λ_o is the wavelength of incident light in vacuum, and θ is

the scattering angle of light. The sizes of particles in solution are determined using the Stokes–Einstein equation

$$D = \frac{kT}{6\pi\eta R_h} \quad (4)$$

where D is the diffusion coefficient, k is Boltzmann's constant, T is the temperature, η is the solvent viscosity, and R_h is the hydrodynamic radius of the particles in solution (Cao 2003; Finsy et al. 1992; Flamberg and Pecora 1984; Leung et al. 2006; Provder 1997).

While DLS works well for monomodal samples, for bimodal samples, it has been reported that conventional DLS measurements are unable to accurately measure particle mixtures with a large difference in the ratio between the diameters of the particles. The size ratio limit beyond which bimodal size differences cannot be accurately detected has been reported to range from 2:1 to 3:1 (Bryant and Thomas 1995; Elizalde et al. 2000; Provder 1997).

Experimental procedure

Twenty nm and 100 nm polystyrene nanoparticles were obtained from Duke Scientific Corporation. Particles are suspended in a water-surfactant suspension with a pH of 7 with an initial concentration of 0.015 vol.%. Vendor reported size values for the 100 nm polystyrene nanoparticles, as determined by transmission electron microscopy (TEM), are as follows: mean diameter: 102 ± 3 nm, size distribution of 4.4 nm, and standard deviation of 4.3%. Vendor reported size values for the 20 nm polystyrene nanoparticles as determined by dynamic light scattering are as follows: mean diameter: 21 nm, size distribution not reported, and standard deviation ± 1.5 nm.

AFM sample preparation and analysis

Four nanoparticle dilutions were made for atomic force microscopy (AFM) analysis. The 20 and 100 nm stock solutions were diluted with de-ionized water to 5×10^{-3} vol.%. For the third and fourth dilution, 20 and 100 nm stock solutions were combined in an 8:1 and a 5:1 ratio, respectively, and diluted with de-ionized water to 5×10^{-3} vol.%.

Diluted solutions were deposited onto Nanoflat mL-base substratesTM from Pacific Nanotechnology, Inc. These substrates are similar in chemistry to poly-L-lysine functionalized mica substrates. Diluted solutions were allowed to sit for 30 s prior to spin coating at 2,000 rpm for 30 s. Dry samples were removed after spin coating and room temperature evaporation placed in an AFM.

AFM analysis was conducted using a Nano-RpTM AFM from Pacific Nanotechnology, Inc. in close contact mode. Instrument was calibrated on NIST traceable VLSI standard with a pitch size $2.99 \pm 0.02 \mu\text{m}$ and a step height of $19.5 \pm 0.8 \text{ nm}$. Six $2 \mu\text{m}$ area scans were conducted over the substrates surface to ensure a large statistical sampling of polystyrene nanoparticles. Pacific Nanotechnology, Inc. Nano Rule+TM software was used to compute the morphological parameters of polystyrene nanoparticles. Nanoparticle height (z direction) was used to determine nanoparticle diameter. Tip artifacts introduced into AFM images can make in-plane measurements (x – y direction) larger than the actual nanoparticle width, requiring deconvolution methods to remove artifacts (Villarrubia 1997).

Dynamic light scattering sample preparation and analysis

De-ionized water was filtered through a $0.2 \mu\text{m}$ filter to remove any large impurity particulates. The 20 and 100 nm polystyrene nanoparticle stock solution from Duke Scientific Corporation was diluted with the filtered de-ionized water to produce four dilutions. The 20 nm polystyrene nanoparticles were diluted to 4.4×10^{-4} v/v and the 100 nm polystyrene nanoparticles were diluted to 5×10^{-4} v/v. The 20 and 100 nm polystyrene stock solution was mixed in an 8:1 and a 5:1 ratio, respectively, and diluted to 4.0×10^{-4} v/v.

Light scattering analysis was conducted using a Dawn[®] Heleos light scatter from Wyatt Technology Corp at 25 °C. Ten mL of filtered de-ionized water was placed in a 10 mL disposable syringe with a $0.2 \mu\text{m}$ filter. Two mL of de-ionized water was flowed through the internal flow cell to ensure that residual particulates were flushed from the system. The flow rate was stopped and light scattering data was collected for 2 min to produce a baseline scattering intensity measurement. About 0.8 mL of

the polystyrene solution was placed in a 1 mL disposable syringe and flowed into the flow cell after a baseline intensity was established. Of the 0.8 mL of polystyrene solution, 0.7 mL of solution was pumped through the flow cell to determine that scattering intensity was within the detector limit. After 0.7 mL of solution was flowed through the cell, the flow rate was stopped and light scattering measurements were taken for 25 min. Filtered de-ionized water was then pumped into the cell to purge the polystyrene solution and re-establish a baseline for nanoparticle size analysis. The size distribution of polystyrene nanoparticles was determined using Wyatt Technology's ASTRA cummulants algorithm software.

Results and discussion

Monomodal size distribution characterization

100-nm Polystyrene particles

Dynamic light scattering analysis conducted on the 100 nm polystyrene nanoparticles yielded a nominal diameter of 114.6 nm with a standard deviation of 2.3 nm (Fig. 1).

Vendor reported size values for the 100 nm polystyrene nanoparticles as determined by transmission electron microscopy (TEM) are as follows: mean

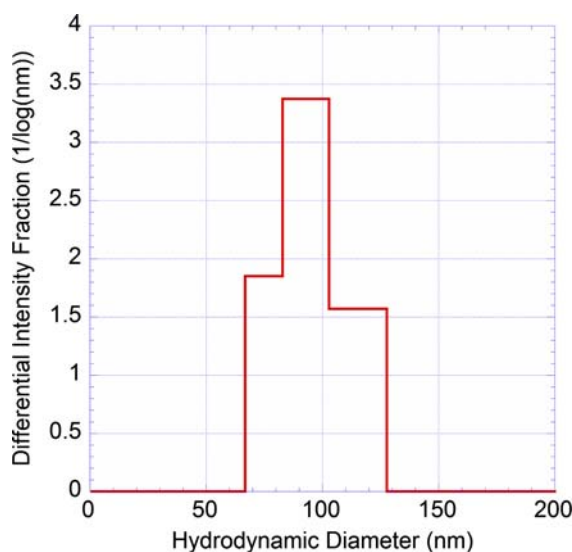
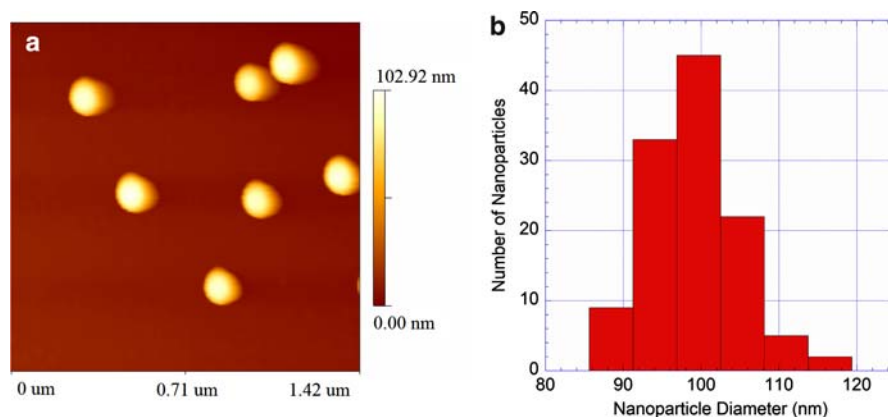


Fig. 1 DLS size distribution of 100 nm polystyrene nanoparticles

Fig. 2 (a) AFM scan of the 100 nm polystyrene nanoparticles. (b) 100 nm polystyrene nanoparticle detailed AFM size distribution data



diameter: 102 ± 3 nm, size distribution of 4.4 nm, and standard deviation of 4.3%. The vendor reported that the size distribution in the range of one standard deviation is 90.5–114.1 nm. DLS results suggest that the 100 nm polystyrene nanoparticles are closer to the upper limit reported value. Atomic force microscopy analysis conducted on the 100 nm polystyrene nanoparticles yielded a nominal diameter of 99.1 nm with a standard deviation of 5.6 nm. Values determined by the AFM were closer to the TEM determined diameter calculated by the vendor (Fig. 2a, b).

Analysis conducted by the AFM places a nominal size range from 93.5 to 104.7 nm within one standard deviation. Vendor reported nominal sizes range from 90.5 to 114.1 nm within one standard deviation, and thus AFM analysis yields a size range within the limits of the specified range.

20-nm Polystyrene particles

DLS analysis conducted on the 20 nm polystyrene nanoparticles yielded a nominal diameter of 22.8 nm with a standard deviation of 0.4 nm (Fig. 3).

The vendor reported sizes range from 19.5 to 22.5 nm. The DLS deviation from the reported value is less than 15%, which is not surprising given that vendor sizes were determined by DLS. AFM analysis conducted on the 20 nm polystyrene nanoparticles yielded a nominal diameter of 15.6 nm with a standard deviation of 4.6 nm (Fig. 4a–c).

While size distribution data gathered using the Dawn® Heleos DLS agreed with vendor DLS data, AFM scans show that not only are particles present within the reported size range but also particles that

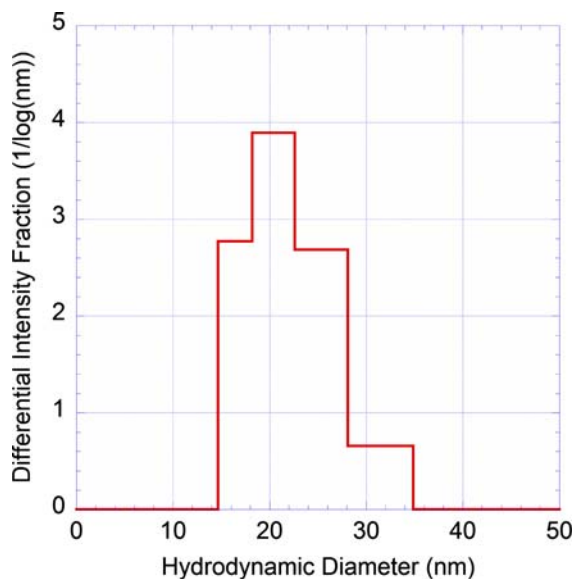


Fig. 3 DLS size distribution of 20 nm polystyrene nanoparticles

are more than half the nominal size are present. DLS fails to detect the smaller particles, but AFM scans show a more accurate representation of the entire system since each individual particle can be accounted for to determine a size distribution.

Bimodal 20 nm, 100 nm mixture size distribution characterization

DLS analysis conducted on the 8:1 20 nm, 100 nm polystyrene nanoparticle mixture yielded a nominal diameter of 245 nm with a standard deviation of 17.6 nm (Fig. 5). Since the size difference between the two particles was more than 2:1, it was expected that the DLS would be unable to confirm two separate

Fig. 4 (a) AFM scan of 20 nm polystyrene nanoparticles. (b) AFM scan of 20 nm polystyrene nanoparticles. (c) 20 nm polystyrene nanoparticle detailed AFM size distribution data

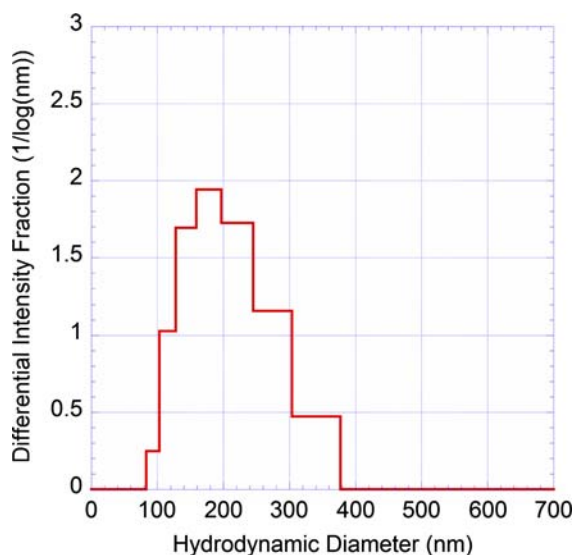
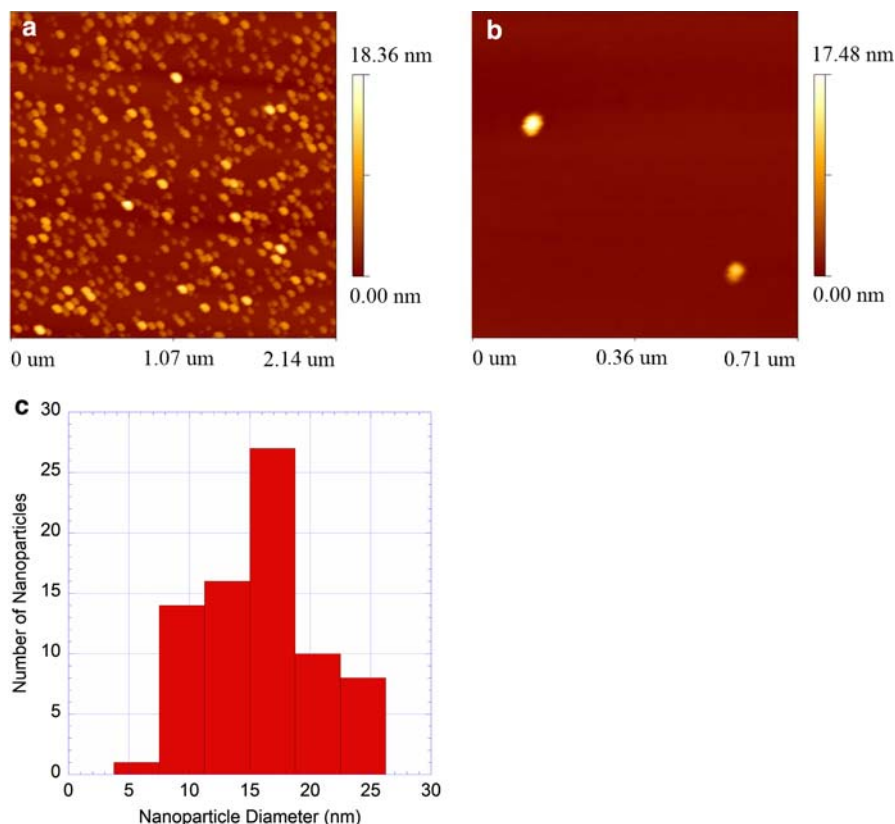


Fig. 5 DLS size distribution of the 8:1 20 nm, 100 nm polystyrene nanoparticle mixture

distributions. The nominal diameter of 245 nm reported by the DLS shows that particle mixtures with a size difference greater than 2:1 yields

statistical data not representative of the mixture. The anomalously large 245 nm value could be due to agglomeration or impurities.

AFM analysis conducted on the 20 nm, 100 nm polystyrene nanoparticle mixture yielded two distributions. The first distribution had a nominal diameter of 15.8 nm with a standard deviation of 4.7 nm, the second had a nominal diameter of 98.2 nm with a standard deviation of 6.4 nm.

As illustrated in Fig. 6a, AFM analysis of the mixed sample was able to easily produce two distinct distributions with nominal sizes similar to the nominal sizes found for monodisperse samples. Both the 20 and 100 nm nanoparticles can be easily identified and counted to form a statistical analysis (Fig. 6b, c).

A second bimodal distribution was obtained by mixing 20 and 100 nm polystyrene nanoparticles in a 5:1 ratio.

DLS analysis conducted on the 20 nm, 100 nm polystyrene nanoparticle mixture with a 5:1 ratio yielded a nominal diameter of 109.4 nm with a standard deviation of 2.0 nm (Fig. 7). DLS analysis of the 5:1 size ratio mixture yielded a distribution

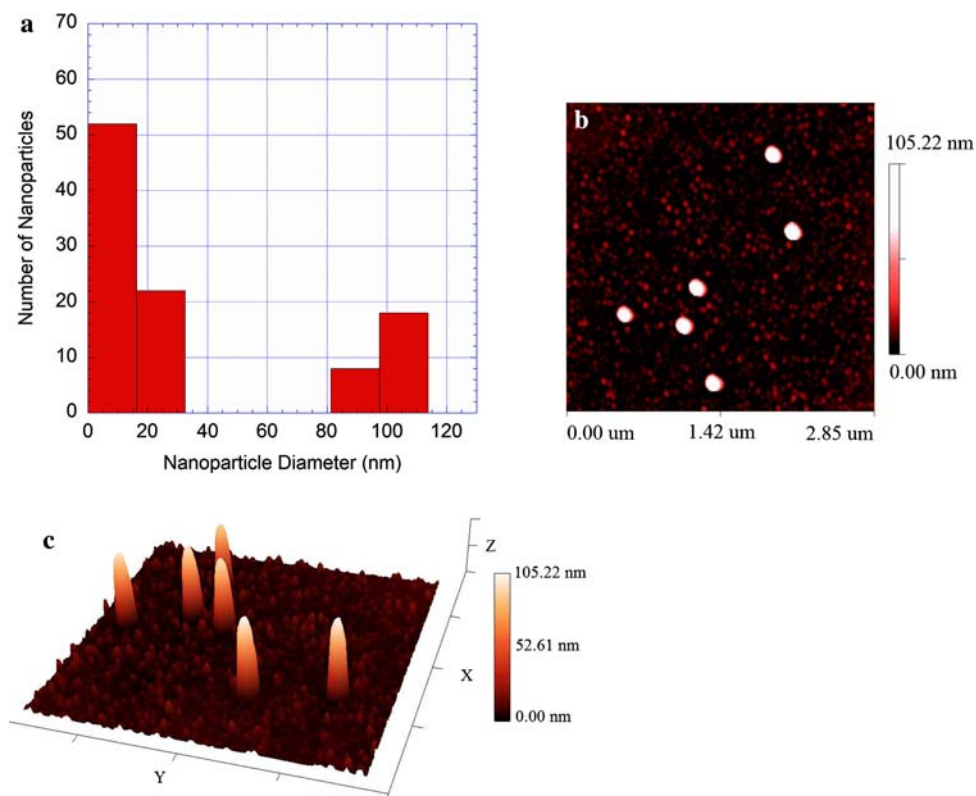


Fig. 6 (a) AFM distribution of 20 nm, 100 nm polystyrene nanoparticles in an 8:1 mixture. (b) AFM scan of 20 nm, 100 nm polystyrene nanoparticle mixture and AFM size

distribution data. (c) 3 dimensional AFM image of 20 nm, 100 nm polystyrene nanoparticle mixture. 20 nm particles can be seen in the background. Scan area is $2.14 \times 2.14 \mu\text{m}$

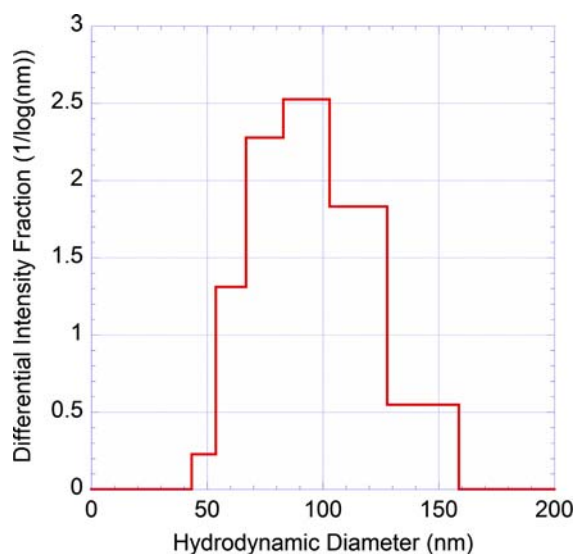


Fig. 7 DLS analysis of 20 nm, 100 nm polystyrene mixture 5:1 ratio

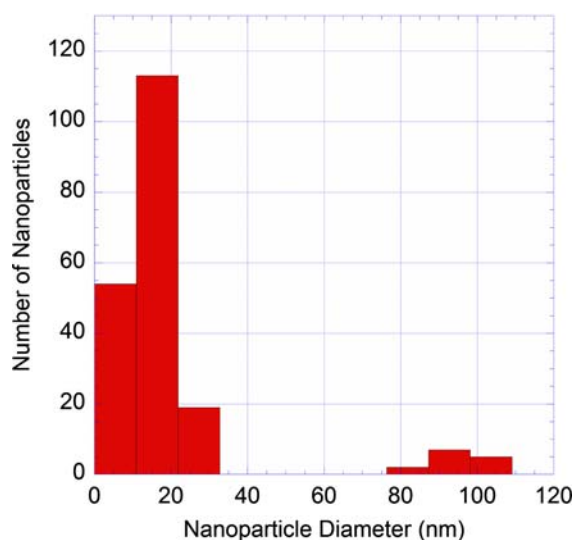


Fig. 8 AFM distribution of 20 nm, 100 nm polystyrene nanoparticles in a 5:1 mixture

Table 1 AFM and DLS size distribution data

Nanoparticle sizes (nm)	Volume ratio	Nominal size determined by vendor (nm)	DLS (nm)	AFM (nm)
100	–	102 ± 3	114.6 ± 2.3	99.1 ± 5.6
20	–	21 ± 1.5	22.8 ± 0.4	15.6 ± 4.6
20,100	5:1	–	109.4 ± 2.0	14.9 ± 3.8 95.3 ± 3.8
20,100	8:1	–	245 ± 17.6	15.8 ± 4.7 98.2 ± 6.4

closer to the monodispersed value obtained for the 100 nm polystyrene particles. AFM results (Fig. 8) yield a more accurate representation with a bimodal size distribution of 14.9 ± 3.8 nm and 95.3 ± 3.8 nm in the nanoparticle mixture.

A bimodal distribution could not be obtained using conventional DLS due to the intensity difference of scattered light from the 20 nm particles versus the 100 nm particles. Since the intensity I of a spherical particle with radius r is proportional to r^6 , the scattered intensity of the 100 nm polystyrene particles contributes heavily to the DLS measurement whereas the scattered intensity from the 20 nm polystyrene particles are lost in the background signal. A summary of the data comparing measurement values obtained by conventional DLS and AFM is presented in Table 1.

An attempt to quantify the ratio of 20–100 nm particles deposited using the spin coating technique was unsuccessful. Taking the volume ratio between 20 and 100 nm particles, there should be 125 20-nm particles of every 1 100-nm particle. Over a 2 μm by 2 μm area, the 8:1 mixture deposited 153 20-nm particles and 6 100-nm particles. For the 5:1 mixture over the same sized area, 241 20-nm particles and 24 100-nm particles were deposited. Since the spin coating method deposits nanoparticles randomly over a smooth surface, the ratio of 20–100 nm particles could not be determined since a 2 μm by 2 μm area shows a random arrangement of particles.

Conclusions

For monosize 100 nm polystyrene nanoparticles, DLS and AFM size distributions were similar to vendor data obtained by TEM. For 20 nm polystyrene nanoparticles, DLS confirmed vendor data but

AFM scans show that particles much smaller than the reported nominal size are also present within the sample. An accurate size distribution for the 20 nm, 100 nm polystyrene nanoparticle 8:1 and 5:1 mixtures could not be determined using DLS. AFM scans of the mixed sample showed that both the 20 nm and 100 nm polystyrene nanoparticles were detected and two separate size distributions were formed. However, AFM scans cannot always be used to accurately calculate the ratio of nanoparticle sizes used in the nanoparticle mixtures, as deposition methods may skew the size distribution. DLS is a fast and accurate measurement tool for size distribution analysis of monosized particles in suspension. While intensity mode DLS alone may be insufficient for analyzing polydisperse mixtures, number mode as well as additional light scattering techniques and hybrid techniques are available for analyzing particle mixtures with broad particle size distributions, as illustrated by Provder (1997). Since AFM scans have the ability to identify large bimodal size distributions regardless of particle shape, AFM analysis of an unknown particle mixture serves as an excellent screening technique prior to DLS analysis.

Acknowledgments The authors would like to thank Dr. Hirotaka Sasaki and Dr. Stephen White of the University of California, Irvine School of Medicine for access to their dynamic light scattering analyzer. This work was supported by the U.S. Navy under contract # N00244-06-P-2341 and funding from Pacific Nanotechnology, Inc.

References

- Binnig G, Quate CF, Gerber C (1986) Atomic force microscope. *Phys Rev Lett* 56:930–934
- Bryant G, Thomas J (1995) Improved particle size distribution measurements using multiangle dynamic light scattering. *Langmuir* 11:2480–2482

- Cadene A, Durand-Vidal S, Turq P, Brendle J (2005) Study of individual Na-montmorillonite particles size, morphology, and apparent charge. *J Colloid Interface Sci* 285:719–730
- Cao A (2003) Light scattering. Recent applications. *Anal Lett* 36:3185–3225
- Dick K, Dhanasekaran T, Zhang Z, Meisel D (2001) Size-dependent melting of silica-encapsulated gold nanoparticles. *J Am Chem Soc* 124:2312–2317
- Elizalde O, Leal GP, Leiza JR (2000) Particle size distribution measurements of polymeric dispersions: a comparative study. *Part Part Syst Charact* 17:236–243
- Finsy R, De Jaeger N, Sneyers R, Gelade E (1992) Particle sizing by photon correlation spectroscopy. Part III: mono and bimodal distributions and data analysis. *Part Part Syst Charact* 9:125–137
- Flamberg A, Pecora R (1984) Dynamic light scattering study of micelles in a high ionic strength solution. *J Phys Chem* 88:3026–3033
- Juillerat F, Solak HH, Bowen P, Hofmann H (2005) Fabrication of large-area ordered arrays of nanoparticles on patterned substrates. *Nanotechnology* 16:1311–1316
- Leung AB, Suh KI, Ansari RR (2006) Particle-size and velocity measurements in flowing conditions using dynamic light scattering. *Appl Opt* 45:2186–2190
- Li Y, Lindsay SM (1991) Polystyrene latex particles as a size calibration for the atomic force microscope. *Rev Sci Instrum* 62:2630–2633
- Liu FK, Chang YC, Ko FH, Chu TC, Dai BT (2003) Rapid fabrication of high quality self-assembled nanometer gold nanoparticles by spin coating method. *Microelectron Eng* 67–68:702–709
- Meulenkamp EA (1998) Size dependence of the dissolution of ZnO nanoparticles. *J Phys Chem B* 102:7764–7769
- Provdor T (1997) Challenges in particle size distribution measurement past, present and for the 21st century. *Prog Org Coat* 32:143–153
- Villarrubia JS (1997) Algorithms for scanned probe microscope image simulation, surface reconstruction and tip estimation. *J Res Natl Inst Stand Technol* 102:425–454
- Xia D, Biswas A, Li D, Bruck S (2004) Directed self-assembly of silica nanoparticles into nanometer-scale patterned surfaces using spin coating. *Adv Mater* 16:1427–1432