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Standard Guide for
Measurement of Particle Size Distribution of Nanomaterials in Suspension by Nanoparticle Tracking Analysis (NTA)[1]

This standard is issued under the fixed designation E2834; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This guide deals with the measurement of particle size distribution of suspended particles, from ~10 nm to the onset of sedimentation, sample dependent, using the nanoparticle tracking analysis (NTA) technique. It does not provide a complete measurement methodology for any specific nanomaterial, but provides a general overview and guide as to the methodology that should be followed for good practice, along with potential pitfalls.

1.2 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.3 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

2. Referenced Documents

2.1 *ASTM Standards:*[2]
C322 Practice for Sampling Ceramic Whiteware Clays
E456 Terminology Relating to Quality and Statistics
E1617 Practice for Reporting Particle Size Characterization Data
E2490 Guide for Measurement of Particle Size Distribution of Nanomaterials in Suspension by Photon Correlation Spectroscopy (PCS)
2.2 *ISO Standards:*[3]
ISO 13320 Particle Size Analysis—Laser Diffraction Methods
ISO 13321 Particle Size Analysis—Photon Correlation Spectroscopy

ISO 14488 Particulate Materials—Sampling And Sample Splitting for the Determination of Particulate Properties
ISO 22412 Particle Size Analysis—Dynamic Light Scattering (DLS)

3. Terminology

3.1 *Definitions:*

3.1.1 *diffusion coefficient, n*—a measure to characterize the rate a particular molecule or particle moves in a particular medium when driven by random thermal agitation (Brownian motion).

3.1.1.1 *Discussion*—After measurement, the value is to be inputted into the Stokes-Einstein equation (Eq 1, see 7.2.1.2(3)). Diffusion coefficient units in nanoparticle tracking analysis (NTA) measurements are typically cm^2/s, rather than the correct SI units of m^2/s.

3.1.2 *repeatability, n*—in NTA and other particle sizing techniques, this usually refers to a measure of the precision of repeated consecutive measurements on the same group of particles under identical conditions and is normally expressed as a relative standard deviation (RSD) or coefficient of variation (CV).

3.1.2.1 *Discussion*—The repeatability value reflects the stability (instrumental, but mainly the sample) of the system over time. Changes in the sample could include dispersion, aggregation and settling.

3.1.3 *reproducibility, n*—in NTA and particle sizing this usually refers to a measure of the deviation of the results obtained from the first aliquot to that obtained for the second and further aliquots of the same bulk sample (and therefore is subject to the homogeneity or heterogeneity of the starting material and the sampling method employed). Normally expressed as a relative standard deviation (RSD) or coefficient of variation (CV).

3.1.3.1 *Discussion*—In a heterogenous and polydisperse (for example, slurry) system, it is often the largest error when repeated samples are taken. Other definitions of reproducibility also address the variability among single test results gathered from different laboratories when inter-laboratory testing is undertaken, or operator-to-operator, instrument-to-instrument, location-to-location, or even day-to-day. It is to be noted that the same group of particles can never be measured in such a

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[2] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.
[3] Available from American National Standards Institute (ANSI), 25 W. 43rd St., 4th Floor, New York, NY 10036, http://www.ansi.org.

system of tests and therefore reproducibility values may typically be considerably in excess of repeatability values.

3.1.4 *robustness, n*—a measure of the change of the required parameter with deliberate and systematic variations in any or all of the key parameters that influence it.

3.1.4.1 *Discussion*—For example, dispersion energy input (that is, ultrasound power and duration) almost certainly will affect the reported results. Variation in pH is likely to affect the degree of agglomeration and so forth. A useful discussion of robustness experiment considerations is found in the ICH Validation of Analytical Procedures Q2(R1) Guideline (**1**).[4]

3.1.5 *rotational diffusion, n*—a process by which the equilibrium statistical distribution of the overall orientation of molecules or particles is maintained or restored.

3.1.6 *translational diffusion, n*—a process by which the equilibrium statistical distribution of molecules or particles in space is maintained or restored.

3.1.7 *visualization, n*—as it relates to the NTA technique, the particles themselves are not imaged, being below the diffraction limit. Each particle acts as a point scatterer, meaning that the imaging system only sees the scattered light from the particle. This allows the position of each particle to be identified and followed with respect to time. See 7.2.

3.1.7.1 *Discussion*—The intensity and shape of the scattered light pattern for each particle may vary, and some additional information may be obtained from these differences, at least qualitatively, but is outside the scope of this guide.

3.1.8 *percentile, n*—a statistical measure of the distribution of sizes. The size below which a certain percent of the distribution falls. For example, the 10th percentile is the size below which 10 percent of the particles may be found. Expressed in ISO form as x_{10}, x_{50}, x_{90}, and also commonly expressed as D10, D50, D90. The 50th percentile is the median.

3.1.9 *coefficient of variation, n*—in statistics, a normalized measure of dispersion of a distribution. Defined as the standard deviation divided by the mean value. (Note: CV = SD/Mean)

3.1.10 *relative standard deviation, n*—in statistics, the absolute value of the coefficient of variation, expressed as a percentage. (Note: RSD = 100·SD/Mean)

NOTE 1—Other common statistical measures are defined in Terminology E456.

3.2 *Acronyms:*

3.2.1 *CV*—coefficient of variation

3.2.2 *CCD*—charge-coupled device

3.2.3 *CMOS*—complementary metal–oxide–semiconductor

3.2.4 *DLS*—dynamic light scattering

3.2.5 *EMCCD*—electron-multiplying charge-coupled device

3.2.6 *NTA*—nanoparticle tracking analysis

3.2.7 *PCS*—photon correlation spectroscopy

3.2.8 *RSD*—relative standard deviation

4. Summary of Guide

4.1 Nanoparticle tracking analysis (NTA) is a method for the direct and real-time visualization and analysis of nanoparticles in liquids. Particles in suspension are illuminated with a focused laser beam. Light scattered from each particle is visible through magnifying optics fitted to a digital camera such as a CCD. The software analyzes the video stream from the camera, identifying and tracking the motion of each particle with time. Because each particle in the field of view is being simultaneously but separately tracked and analyzed, the particle size distribution profile obtained by NTA is a direct number-based distribution.

4.2 The laser beam is focused such that only particles in the focal plane of the magnifying optics are illuminated. Particles out of the focal plane are not illuminated and at the size range under discussion are not visible to the camera. This yields a high signal to noise image, allowing particles as small as 10 nm to be visualized, depending on sample material. While outside the scope of this document, the technique is generally able to measure particles as large as approximately 1 μm.

4.3 The average distance each particle moves in the image is automatically calculated by the software. From this value, the particle diffusion coefficient can be obtained and through the use of the Stokes-Einstein equation, particle size can be determined.

4.4 This Guide discusses the scientific basis for the technique, size limits, concentration ranges, sampling and sample preparation considerations, condition and analysis selection, data interpretation and comparison to other complementary techniques.

5. Significance and Use

5.1 NTA is one of the very few techniques that are able to deal with the measurement of particle size distribution in the nano-size region. This Guide describes the NTA technique for direct visualization and measurement of Brownian motion, generally applicable in the particle size range from several nanometers until the onset of sedimentation in the sample. The NTA technique is usually applied to dilute suspensions of solid material in a liquid carrier. It is a first principles method (that is, calibration in the standard understanding of this word, is not involved). The measurement is hydrodynamically based and therefore provides size information in the suspending medium (typically water). Thus the hydrodynamic diameter will almost certainly differ from size diameters determined by other techniques and users of the NTA technique need to be aware of the distinction of the various descriptors of particle diameter before making comparisons between techniques (see 8.7). Notwithstanding the preceding sentence, the technique is routinely applied in industry and academia as both a research and development tool and as a QC method for the characterization of submicron systems.

6. Reagents

6.1 In general, no reagents specific to the technique are necessary. However, dispersing and stabilizing agents often are

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[4] The boldface numbers in parentheses refer to the list of references at the end of this standard.

required for a specific test sample in order to preserve colloidal stability during the measurement. A suitable diluent is used to achieve a particle concentration appropriate for the measurement. The apparent hydrodynamic size or diffusion coefficient may undergo change on dilution, as the ionic environment, within which the particles are dispersed, changes in nature or concentration. This is particularly noticeable when diluting a monodisperse latex. A latex that is measured as 60 nm in 1×10^{-3}M NaCl can have a hydrodynamic diameter of over 70 nm in 1×10^{-6}M NaCl (close to deionized water).

6.2 In order to minimize any changes in the system on dilution, it is common to use the "mother liquor". This is the liquid in which the particles exist in stable form and is usually obtained by centrifuging of the suspension or making up the same ionic composition of the dispersant liquid if knowledge of these components is available. Many biological materials are measured in a buffer (often phosphate buffered saline), which confers the correct (range of) conditions of pH and ionic strength to assure stability of the system. Instability (usually through inadequate zeta potential—see (**2**)) can promote agglomeration leading to settling or sedimentation in a solid-liquid system or creaming in a liquid-liquid system (emulsion). Such fundamental changes interfere with the stability of the suspension and need to be minimized as they affect the quality (accuracy and repeatability) of the reported measurements. These should be investigated in a robustness experiment.

7. Procedure

7.1 *Verification:*

7.1.1 The instrument to be used in the measurement should be verified for correct performance, within pre-defined quality control limits, by following protocols issued by the instrument manufacturer. These confirmation tests normally involve the use of one or more NIST-traceable spherical particle size standards. In the sub-micron ($< 1 \times 10^{-6}$ m) region, these standards (for example, NIST, Duke Scientific - now part of ThermoFisher Scientific) tend to be nearly monodisperse (that is, narrow, single mode distribution, CV < 17 %) and, while confirming the *x* (size) axis, do not verify the *y* (or quantity) axis of the size distribution. Note that NTA is a first principles measurement and thus calibration in the formal sense (adjustment of the instrument to read a true and known value) need not be undertaken. In the event of a "failure" at the verification stage, then the issues to check involve quality of the dilution water, state of dispersion and stability of the standard under dilution plus instrumental issues such as thermal stability, cleanliness and alignment of optical components. The raw video data can be examined during and after acquisition. Such examination can provide useful qualitative information about the sample condition, particle concentration, and system operation. During data acquisition one looks for a consistent number of particles in the field of view, well-separated particles, a low level of random or background noise, and particle tracking lengths sufficient for accurate measurements of each particle. Manufacturers also provide other means of assuring the reliability of the data and it is recommended that these protocols are consulted, as appropriate.

7.1.2 Given the likelihood that the size standard has been certified by electron microscopy, care needs to be exercised in direct comparison of the results. While electron microscopy is carried out under high-vacuum conditions, NTA performs the analysis with particles in suspension. NTA measures the diffusion coefficient of the particle and the diameter given by the Stokes-Einstein relation (Eq 1) is that of the sphere-equivalent, hydrodynamic diameter (the particle itself plus the molecular-scale layer of solvent associated with the particle surface). This solvent layer may be significant relative to the size of the standard particles being analyzed, increasing the apparent size of the particles. For larger particles, however, the effect of this hydrodynamic layer is minimal.

7.1.3 Note that verification of a system only demonstrates that the instrument is performing adequately with the pre-scribed standard materials. Practical considerations for real-world materials (especially "dispersion" if utilized in sample preparation or if the distribution is relatively polydisperse) mean that the method used to measure that real-world material needs to be carefully evaluated for precision (repeatability).

7.2 *Measurement:*

7.2.1 *Introduction:*

7.2.1.1 The measurement of particle size distribution in the nano- (sub 100 nm) region by nanoparticle tracking analysis depends on the interaction of light with matter and the random or Brownian motion that a particle exhibits in liquid medium in free suspension (**3**). There must be an inhomogeneity in the refractive indices of a particle and the medium within which it exists in order for light scattering to occur. Without such an inhomogeneity (for example, in so-called index-matched systems) there is no scattering and the particle is invisible to light and no measurements can be made by the NTA or any other technique making use of light scattering. A coating or functionalization of the primary particle may affect this refractive index sufficiently to impact the light scattering properties. While some physical phenomena used by the NTA measurement technique are in common with the dynamic light scattering technique (photon correlation spectroscopy), as defined in Guide E2490 and ISO 22412, NTA is a distinctly different technique.

7.2.1.2 For particles <100 nm, as considered in this guide, several facts hold true:

(1) The amount of scattering is weak in relative terms and depends highly on the size of the particle as well as particle composition. In the Rayleigh approximation region (typically d < λ/10 in which d is the diameter of particle and λ is the wavelength of light employed), then this intensity of scattering is proportional to d^6—or (volume)^2 or (relative molecular mass)^2. With a commonly utilized diode laser (638 nm), then the upper size limit of this Rayleigh scattering behavior is approximately 64 nm. This means, in practice, that a 60 nm particle scatters 1 million times as much light as a 6 nm particle of the same composition. Thus, for a sample that contains a wide range of particle sizes or includes contaminating particles (for example dust) that are often present in the local environment and are usually considerably larger than the material that requires measurement, caution must be exercised in selecting the appropriate measurement conditions to properly analyze all

particles. NTA is able to measure over a wide range of sizes, but it may be difficult to find a set of instrument settings to measure all particles in a single analysis. This would mean filtering liquids used to contain or dilute the particles to at least the same level as the size of the particles that require characterizing unless the user is conscious of the inclusion of these particles in the final result. Alternatively, two separate analyses may be conducted, with the conditions optimized for either the smaller or larger particles, then the results from each added together. Unless the two populations are totally distinct, caution must be exercised in interpreting this combined result particularly in the region where the two analyses overlap.

(2) The intensity of scattering in the Rayleigh region is inversely proportional to the fourth power of the wavelength of light employed. Thus, if the wavelength of incident light could be halved then the intensity of scattering that would be observed is increased by a factor of 16. It is possible to use lasers of a lower wavelength than 638 nm to increase the amount of scattering and, hence, signal. This is usually preferable to increasing the power of the laser with possible undesired effects (for example, heating, convection currents). However, note that lower wavelengths sometimes overlap an absorption edge for some molecular species leading to a loss of signal intensity. The detector is a digital video camera (such as CCD, higher-sensitivity EMCCD, or CMOS) of an appropriate frame rate and spectral response to the laser wavelength being used. Sensitivity of the camera varies with manufacturer and together with the other variables discussed such as laser wavelength and power, particle and liquid refractive indices, and optical configuration will determine the lower detection limit of the system.

(3) The measurement of size in the sub-100 nm region relies on the measurement of the amount of Brownian motion (in particular the diffusion coefficient) of the particle as formulated in the Stokes-Einstein equation:

$$d_H = \frac{K_B T}{3\pi\eta D_t} \qquad (1)$$

Where d_H is the particle hydrodynamic diameter, K_B is the Boltzmann constant, T is absolute temperature in Kelvin, η is viscosity in centipoise and D_t the (measured) translational diffusion coefficient in cm^2/s.

(4) Note that, in Eq 1, the density of the particle plays no role in Brownian motion (although, of course, it does in settling; see Point 9 below), even though this appears to be counterintuitive. Note also that a hydrodynamic diameter is derived. This refers to an equivalent size in spherical terms to that of a particle moving with the same diffusion coefficient as the observed particle. Thus, for an irregularly shaped particle or one with significant external morphology or surface coatings (or both), then the derived diameter is not likely to correspond to any measured axis of the image of the particle (**4**). The viscosity refers to the medium in which the particle is dispersed. In a dilute system it is assumed that the particles do not interact, so the viscosity can be assumed to be that of the medium or diluent.

(5) Note the term diffusion coefficient. There are two types of diffusion to be considered for particles in free suspension:

(a) Translational, where the so-called Stokes-Einstein relationship given in Eq 1 applies. Rewriting with the diffusion coefficient on the left:

$$D_t = \frac{K_B T}{3\pi\eta d_H} \qquad (2)$$

(b) Rotational, where the Stokes-Einstein-Debye relation applies:

$$D_r = \frac{K_B T}{\pi\eta d_H{}^3} \qquad (3)$$

(6) Association of particles (or molecules) leads to changes in the rotational diffusion coefficient, and also affects the translational diffusion coefficient. Hence, interactions between particles can complicate the interpretation of the observed diffusion coefficient, which for nonspherical particles, is a combination of the translational and rotational diffusion coefficients. These particle-particle interactions tend to be concentration rather than size dependent, and both translational and rotational diffusion coefficients are dependent on the viscosity of the surrounding fluid. For the concentrations appropriate to NTA measurements, the particle concentration is generally too dilute for these effects to be significant.

(7) Brownian translational motion occurs in three dimensions but NTA observes motion only in two dimensions. It is possible to determine a diffusion coefficient based on the measurement of one, two or three dimensions (and theoretically up to six if rotation could be measured). By measuring a higher order of $D_{r,t}$ a more accurate approximation can be made of the particle size for a given number of steps that contribute towards a track. D_t is derived from measuring the mean squared displacement of a particle in one, two or three dimensions (Eq 4-6 respectively) (**5, 6**).

$$\overline{(x^2)} = \frac{2TK_B t}{3\pi\eta d} \qquad (4)$$

$$\overline{(x,y)}^2 = \frac{4TK_B t}{3\pi\eta d} \qquad (5)$$

$$\overline{(x,y,z)}^2 = \frac{2TK_B t}{\pi\eta d} \qquad (6)$$

Where t is the time between sequential displacement measurements, in this case the frame to frame period.

(a) In the case where measurement of movement in two dimensions is made:

$$\frac{\overline{(x,y)}^2}{4t} = D_t = \frac{TK_B}{3\pi\eta d} \qquad (7)$$

and results thus obtained by NTA are shown as a function of particle diameter, d.

(8) The motion of the particles must be random. Nonrandom particle motion is the main reason for apparent failure or nonapplicability of the technique. Such nonrandom motion can occur through convection currents being present in the system, through particles (too large or dense for the technique) settling during the measurement sequence, or through particles interacting due to chemical reaction or electronic charge. While the system can measure and compensate for this motion, it is preferable to reduce this through accurate temperature measurement and stabilization where possible. If settling/sedimentation occurs in the measurement, other than to a very

minor extent, then the result is almost certainly compromised, as it will reflect a changing and unstable system. If visible settled solid is present at the bottom of a container, then it is very likely that the NTA technique is not recommended. In this case conventional laser light scattering (laser diffraction) is likely to be the preferred technique. If settling can be observed either in the sample container or in the measurement chamber, then it is certain that the original material being measured is not "nano" or is unstable during the measurement time frame.

(9) With respect to size and density, consider the calculations in Table 1 using Stokes' Law:

(10) It can be deduced from Table 1 that if a material is truly < 100 nm, it tends to remain in suspension and exhibits little if any settling tendency. In many situations, for example a gel, the particle density is significantly lower due to incorporation of water into the particle matrix and thus the settling time increased further.

(11) Sometimes it is thought that placing the particles in a material of higher viscosity reduces or even eliminates any settling tendency. This is true, but the Brownian motion is also reduced accordingly and no gain is achieved.

(a) Most dry powder materials are difficult to fully disperse back to a primary size and thus size measurements from diffusion reflect the state of agglomeration of the system rather than to a primary "as produced" size. Hence this Guide assumes that the reader has access to a well dispersed liquid suspension or preparation of nano-size particles for the measurement or that the measurement of the agglomerates is of interest.

(12) Note from Eq 1 the obvious points that:

(a) As the size of particle increases then the amount of Brownian motion decreases.

(b) As the viscosity of the medium increases then the amount of Brownian motion decreases.

(c) As the temperature is increased then the amount of Brownian motion increases correspondingly.

7.3 *Measurement and Analysis of Diffusion Coefficient:*

7.3.1 It is necessary to measure the diffusion coefficient to input into Eq 1 in order to derive a particle size. The diffusion coefficient for each particle in the field of view is determined individually and the resulting sizes are summed to produce the final particle size distribution. This section deals with the measurement of the diffusion coefficient and the objective of providing a particle size distribution from the measured data.

7.3.2 In viewing the scattered light from a group of suspended moving particles over a period of time, each particle moves under Brownian motion. Each particle is identified and tracked for the duration of its presence in the sampling volume, which is defined by the field of view of the camera, the dimensions of the illumination beam, and the focal plane of the magnification optics. The mean squared displacement of each particle is directly measured from the video file images. Dimensions of the displacement (in pixels) are translated into actual displacement through knowledge of the magnification of the system and dimensions of each pixel. This should be calibrated or verified to improve accuracy of the displacement measurement. From the mean squared displacement, the diffusion coefficient is calculated and entered into Eq 1.

7.3.3 Each particle must be tracked for a sufficient length of time required to accurately determine the average displacement. The software sizes particles based on analysis of their Brownian motion by identifying and tracking the centers of the spots of light scattered by the individual particles and then measuring the distance moved between frames. Track lengths of only 0.5 seconds duration generate statistically useful data for most particles of interest, assuming a camera frame rate of

TABLE 1 Settling Calculations Based on Stokes' Law as a Function of Size and Density (T = 298K)

Diameter µm	Diameter nm	ρ (Material) kg/m³	ρ (Water) kg/m³	η (Water) 298K, Poise	Time to settle 1 cm (1 × 10⁻² m) in water		
					Minutes	Hours	Days
0.01	10	2500	997	0.008955	1815494.39	30258	1261
0.1	100	2500	997	0.008955	18154.94	302.58	12.61
1	1000	2500	997	0.008955	181.55	3.03	0.126
10	10000	2500	997	0.008955	1.82	0.03	0.001
100	100000	2500	997	0.008955	0.02	0.00	0.000
0.01	10	3500	997	0.008955	1089296.64	18154.94	756
0.1	100	3500	997	0.008955	10892.97	181.55	7.56
1	1000	3500	997	0.008955	108.93	1.82	0.076
10	10000	3500	997	0.008955	1.09	0.02	0.001
100	100000	3500	997	0.008955	0.01	0.00	0.000
0.01	10	4200	997	0.008955	851013.00	14183.55	591
0.1	100	4200	997	0.008955	8510.13	141.84	5.91
1	1000	4200	997	0.008955	85.10	1.42	0.059
10	10000	4200	997	0.008955	0.85	0.01	0.001
100	100000	4200	997	0.008955	0.01	0.00	0.000
0.01	10	5500	997	0.008955	605164.80	10086.08	420
0.1	100	5500	997	0.008955	6051.65	100.86	4.20
1	1000	5500	997	0.008955	60.52	1.01	0.042
10	10000	5500	997	0.008955	0.61	0.01	0.000
100	100000	5500	997	0.008955	0.01	0.00	0.000

30 frames/s. The software may automatically select the optimum minimum number of steps on which size estimate is based by continuously monitoring estimated particle size (**7**).

7.3.4 Particles must be detected by the camera in order to be tracked and the lower limit to particle size which can be analyzed by NTA is determined primarily by particle composition and relative refractive indices. The amount of light scattered by a particle in any given direction is a function of many variables including incident illumination power, wavelength, angle and polarization and particle size, complex refractive index and shape, as well as solvent refractive index (**8**). Similarly, the amount of light falling on a detector and strength of the resultant signal is dependent, of course, on the efficiency of the collection optics (in particular Numerical Aperture) and the spectral response and sensitivity of the camera. Instrument settings, whether manual or automatically adjusted, will have a direct effect on the system response to scattered light.

7.3.5 The derived particle size distribution from an NTA analysis is naturally a number-based distribution. Given the greater likelihood that smaller particles will diffuse in or out of the field of view due to their more-rapid diffusion, relative to larger particles, during the duration of the measurement, the smaller particles would be over-represented if the final result were reported as a count of particles measured. This effect can be negated by counting each step a particle moves as a single event rather than counting a particle's complete track as a single event. This can be normalized to the total number of frames analyzed. With a known sampling volume, the total concentration is also measured and the size distribution reported as a concentration of particles in each size class (**3**).

8. Test Method

8.1 Several optical arrangements are used by different manufacturers for NTA based measurement systems. Essentially, the laser source is focused and aligned within the sample cell, while magnifying optics (microscope) equipped with a video camera capture the images. Different approaches to beam focusing and alignment may be used. A generic diagram of an emerging-beam optical arrangement is shown in Fig. 2. A through-cell illumination method is shown in Fig. 3.
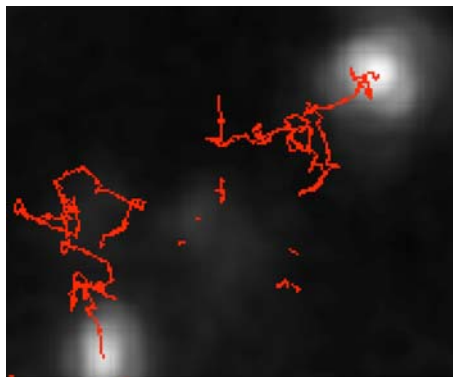
8.2 *Instrument Components:*

FIG. 1 Tracking of Brownian Motion

8.2.1 Sample analysis module for emerging-beam illumination—which comprises a suitable housing to contain the sample dispersion, the optical element, and the light source, typically a solid-state, single-mode laser diode. The beam is caused to refract at the interface between the liquid sample and the optical element through which it is passed such that it describes a path close to parallel to the glass-sample interface. This angle will be dependent on the dispersion liquid used. This results in a wide, shallow light path that is matched to the depth of field of the optics. It is imperative that only particles within the magnification optics' depth of field be illuminated, otherwise out-of-focus particles will interfere with the measurement by reducing the image signal to noise ratio. The light beam then scatters from the particles in suspension. The optical element may have a metalized surface or be comprised of black glass in order to provide a dark background for high image contrast.

8.2.2 Sample analysis module for through-cell illumination—which comprises a suitable housing to contain the sample dispersion and the light source, typically a solid-state, single-mode laser diode. The beam is focused into the focal point of a microscope lens at a 90° angle. The light beam then scatters from the particles in suspension.

8.2.3 Particles in the beam are visualized by a conventional optical microscope aligned normally to the beam axis and which collects light scattered from each and every particle in the field of view. The total magnification of the system may be quite modest as the system only needs to see the scattered light, not the particles themselves. The particles are seen through the microscope as small points of light moving under Brownian motion. The camera is a digital video camera. Due to the significant variation of the intensity of the image of each particle with size, some adjustment of camera settings (for example gain and shutter speed) is provided for adjustment, either manually or automatically by the software. Care must be taken such that the settings are sufficient to visualize the lower intensity particles while maintaining adequate image quality for the full range of particles in the field of view. This range of settings should be part of any robustness experiments.

8.2.4 A video of typically 30-90 seconds duration is taken of the moving particles by the camera mounted to the optics. The number of particles measured depends on sample concentration, scattering volume, particle size (as this affects diffusion rate), and analysis time. The greater this number, the more representative the analysis will be of the overall samples and the more reproducible the result will be. Wider size distributions will require more total particle measurements, as a statistically significant number of particles need to be measured in each of the size classes of the distribution. Total particle measurements of less than 100 particles may be sufficient for mono-disperse materials while broad distributions may require several hundreds to thousands of particles. The user must define the required reproducibility and the statistical measures for the desired end-purpose. For example, if accurate characterization of the minority parts of the distribution is required, then significantly more particles must be measured than if only the mean/median/mode size is of interest. This total number will depend on polydispersity and shape of the