Interferometric Scattering Enables Fluorescence-Free Electrokinetic Trapping of Single Nanoparticles in Free Solution

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Supporting Information

ABSTRACT: Anti-Brownian traps confine single particles in free solution by closed-loop feedback forces that directly counteract Brownian motion. Extended-duration measurements on trapped objects allow detailed characterization of photophysical and transport properties as well as observation of infrequent or rare dynamics. However, this approach has been generally limited to particles that can be tracked by fluorescence emission. Here we present the Interferometric Scattering Anti-Brownian Electrokinetic (ISABEL) trap, which uses interferometric scattering rather than fluorescence to monitor particle position. By decoupling the ability to track (and therefore trap) a particle from collection of its spectroscopic data, the ISABEL trap enables confinement and extended study of single particles that do not fluoresce, only weakly fluoresce, or exhibit intermittent fluorescence or photobleaching. This new technique significantly expands the range of nanoscale objects that may be investigated at the single-particle level in free solution.

KEYWORDS: Interferometric scattering, single-nanoparticle, single-molecule, anti-Brownian electrokinetic trap, ABEL trap

The consequence of Brownian motion in solution-phase single-molecule fluorescence spectroscopy experiments is that nanoscale objects quickly diffuse out of focus or through a confocal observation volume, resulting in brief bursts of signal that can reveal population-wide heterogeneity or submillisecond dynamics.1–5 In order to access longer time scales or record infrequent events, it is necessary to employ methods that prolong observation of individual particles by overcoming diffusion. One such strategy is to actively maintain the position of the particle within an observation volume in free solution using closed-loop feedback, either by quickly following the trajectory of the particle with a positioning stage6–10 or by applying forces that counteract the effects of Brownian motion.11 The latter class of approaches, collectively known as anti-Brownian traps,12–14 can circumvent the potential risks of perturbation due to interactions with or alterations of the local nanoenvironment that may accompany immobilization of particles by tethers, surface attachment, or encapsulation.15–21 All implementations of anti-Brownian traps can be distilled to two essential aspects of the closed-loop feedback: first, real-time tracking provides the location of a particle relative to the target position and may be determined using fluorescence6,11 or bright- or dark-field imaging2,8,22,23 in combination with either camera-based tracking5,24 or timed movement of the excitation beam and one or more point detectors.6,9,26–28 Second, a feedback force must be quickly applied to move the particle back toward the target, which may be implemented using electric fields to induce electrophoresis or electro-osmosis,28–30 thermal gradients to induce thermophoresis,31 optical forces,32 or differential pressure to induce hydrodynamic flow.33 These steps must be implemented quickly enough to overcome the diffusive motion of the particle, and significant recent progress has been made toward optimizing this control loop to enable trapping of individual small organic fluorophores.26,34 Trap implementations that utilize either intrinsic or label-based fluorescence to track emissive particles have been employed to characterize time-varying photophysical states,35–40 molecular dynamics and kinetics,41–47 and more.48–50 However, the trapping duration and associated data collection in anti-Brownian traps are typically limited by photobleaching or blinking because dark particles cannot be tracked and are quickly lost. Bechhoefer and co-workers successfully demonstrated trapping of nonfluorescent particles using a dark-field signal,23 but the unfavorable scaling of scattering intensity with particle size limits this approach to relatively large nanoscale objects (>100 nm) with large scattering cross sections.

We introduce here the Interferometric Scattering Anti-Brownian ELectrokinetic (ISABEL) trap, a new anti-Brownian device to track and trap small, nonfluorescent nanoscale...
objects by using the interference signal between scattered light from the particle and a constant reference field to rapidly estimate a nanoparticle’s position. Closed-loop electrokinetic feedback can then be used to control the position of the particle. We demonstrate trapping of gold, polystyrene, and semiconductor nanoparticles as small as 15–20 nm in diameter and show that the ISABEL trap completely decouples the ability to trap a nanoparticle from measurements related to its fluorescence. Because the interferometric scattering signal scales favorably with particle size relative to scattering alone, the ISABEL trap significantly broadens the range of trappable objects to include small nanoparticles with weak, highly variable, or even no fluorescent signal.

The ISABEL trap is conceptually similar to its anti-Brownian electrokinetic (ABEL) trap predecessors, with the key modification that the particle is tracked interferometrically. Recently, interferometric scattering microscopy techniques that utilize a coherent reference (or local oscillator) field, $E_r$, to homodyne-detect a scattered electric field, $E_s$, have been developed for detection and tracking of single weak scatterers. The favorable scaling of an interferometric scattering signal (commonly abbreviated as “iSCAT”54) enables detection and high-speed tracking of nanoscale particles55–57 and has been used to directly detect biological molecules, including viruses,59 cell secretions, and microtubules, and to weigh single proteins.60 The recent progress in this field motivated us to develop interferometric scattering into a useful signal for active-feedback nanoparticle trapping.

In the ISABEL trap, particles of interest are diluted and loaded into a quartz microfluidic cell (Figure 1a,b), across the trapping region of which a low-coherence-length laser is scanned in a 32-point grid pattern to create the incident trapping region of which a low-coherence-length laser is loaded into a quartz microfluidic cell (Figure 1a,b), across the trapping region of which a low-coherence-length laser is scanned in a 32-point grid pattern to create the incident trapping region (Figure 1b). The reflected light from the quartz–water interface forms a reference field, $E_r$, which, with the scattered field, $E_s$, is collected by a high-NA objective lens and detected on a photodiode, as shown in Figure 1a,c. The reflected and scattered fields are both separated from the excitation beam using a linear polarizing beamsplitter in combination with a quarter-wave plate. The beam scanning in $x$ and $y$ is produced by a pair of acousto-optic deflectors (AODs) controlled by a field-programmable gate array (FPGA), so that the beam position is precisely known as a function of time. Thus, the signal detected on the photodiode over the course of the beam scan can be directly mapped to the scan grid. In addition to detection of scattered and reflected light, fluorescence signals may be simultaneously acquired in a separate detection channel on an avalanche photodiode.

In order to track the particle and thereby determine the appropriate feedback forces necessary to trap it, the particle position is estimated after each complete beam scan, and the detected signal is organized into a single 32-point frame (typical frame rates 1–10 kHz). To locate the particle, the photodiode signal at each scan position is first recorded, as shown in Figure 2a. The expected intensity incident upon the detector, $I_{det}$, depends upon the amplitudes of the reflected and scattered fields, $E_r$ and $E_s$, as well as their relative phase, $\theta$:

$$I_{det} \propto |E_r + E_s|^2 = |E_r|^2 + 2|E_r||E_s|\cos \theta + |E_s|^2$$ (1)

$E_r$ and $E_s$ are generated by interactions of the incident field, $E_i$, with an interface and the scattering particle, respectively. $E_r$ is determined by the reflectivity of the interface, $r$, so that $E_r = rE_i$. Typical values for $r^2$ are on the order of 1%; glass–air and glass–water interfaces reflect 4% and 0.4% of normally incident light, respectively. $E_s$ is determined by the complex scattering coefficient, $s$, so that $E_s = sE_i$. In the Rayleigh limit, $s$ is proportional to the complex bulk polarizability of the particle, which is described by the particle’s volume, $V$, and the complex bulk polarizability of the material relative to the surrounding medium, $\alpha(\lambda)$, where $\lambda$ is the wavelength of the incoming light: $s \propto \alpha(\lambda)V$. The most commonly reported metric for scattering objects is the scattering cross section, $\sigma_{scat} \propto |s|^2$, which includes all dependencies on particle size and particle and medium bulk polarizability.

Figure 1. ISABEL trap schematic. (a) A focused incident beam ($E_i$) with low coherence length illuminates a particle in a shallow microfluidic cell (700 nm height), and the backscattered light ($E_s$) is collected by a high-NA objective. A coherent back-reflection from the quartz–water interface is also collected ($E_r$). (b) Top view of a microfluidic cell showing the scan pattern of the excitation beam, controlled via a field-programmable gate array (FPGA) and two acousto-optic deflectors (AODs), at the center of two crossed microfluidic channels. The trap center, marked by an “X”, can be programmatically placed anywhere on this grid. (c) Schematic of optical excitation and detection paths for the ISABEL trap. Linearly polarized excitation light passes through a quarter-wave plate, so that backscattered and reflected light can be redirected with a polarizing beamsplitter (PBS) and detected at a fast photodiode. Emitted fluorescence can be collected in a separate emission channel. Closed-loop feedback voltages are calculated by the FPGA and applied to the solution using platinum electrodes.
It is important to note that $E_i$ (which varies spatially in both magnitude and phase because of the focused excitation beam) generates $E_s$ only at the position of the scattering particle, while the reflected field is generated across the entire beam profile. Considering for the moment an on-axis particle position, where the magnitude of $sE_s$ would be greatest, eq 1 can be rewritten as

$$I_{det} \propto |rE_i + sE_s|^2 = |E_i|^2(r^2 + 2rsl\cos \theta + |l|^2)$$

(2)

Our large-area point detector measures the integrated value of the intensity image $I_{det}$, denoted $S_{det}$ for each grid position, and after each complete scan these values are used to reconstruct a single ISABEL image frame, as shown in Figure 2b, where each pixel is assigned the recorded raw signal value from the corresponding scan location. It is clear from eq 2 and the definition of $s$ above that the dark-field term, $|E_i|^2|l|^2$, will scale with the square of the particle volume, or with the sixth power of the diameter (i.e., $d^6$), and therefore will become negligible in comparison to the homodyne term, $2|E_i|^2rsl\cos \theta$, for small particles. The reference term, $|E_i|^2r^2$, is generated from the quartz–water interface of the microfluidic cell and usually dominates the measurement. This reflection should remain constant over time, so in order to isolate the desired homodyne term in eq 2, a background frame containing only the reflection, $S_{bkg}$, is subtracted and used for normalization. For each pixel in the ISABEL scan, the absolute fractional contrast, $C_f$, is defined as

$$C_f = \frac{|S_{det} - S_{bkg}|}{S_{bkg}}$$

(3)

The same frames depicted in Figure 2b are shown in Figure 2c as fractional contrast before the absolute value is taken, and they demonstrate that the homodyne term may take either positive or negative values at different points in the scan. The homodyne term can also change sign as a result of motion of the particle and subsequent change in the relative phase $\theta$. Therefore, to overcome these issues and reliably identify the scan point that deviates most from the background, the absolute value of the fractional contrast is calculated by the FPGA (eq 3 and Figure 2d), and the location of max($C_f$) is used as the particle position in that frame. We define the value of the absolute fractional contrast for each frame as the value at that pixel. It is worth noting that the residual Brownian motion of the nanoparticle (even in the axial direction) causes various relative phases to be sampled during each scan position. In spite of this, the absolute fractional contrast in terms of $s$ and $r$ still scales approximately as $|l|^2/r^2$.

After the estimated particle position is determined, the displacement vector from the particle to the trap center is calculated, and appropriate feedback voltages are immediately applied to the electrodes in the microfluidic cell by the FPGA for the duration of one frame. As in the previous ABEL trap designs, the resulting applied field is locally uniform with no gradient. The resulting drift force directs the particle toward the pixel marked with a white $\times$ in Figure 2d, and the amplitude of the applied field is scaled linearly with the distance between the estimated pixel position and the target pixel position. If the estimated position is the same as the target, no voltages are applied. Depending upon surface treatment and zeta potentials, the applied voltages generate either an electrophoretic force (dependent upon the particle charge) or an electroosmotic flow (no requirement on particle charge) that biases the random diffusion of the scatterer in solution toward the target in the middle of the trapping region.

To test the ISABEL trap, we trapped a series of gold nanoparticles of various sizes <100 nm in diameter. Typical results for trapping of nominally 40 nm diameter beads can be seen in Figure 3. For each trapped particle, the key variables are the absolute fractional interferometric scattering contrast and the position of the pixel with the highest value of contrast, defined by $x$, $y$, and the radius $R$ from the software-defined trap center (Figure 3a). In the gray-shaded regions, feedback is off and the trap is typically empty, except for occasional diffusion of a bead through the trap, as seen here at $\sim$34.3 s. During these intervals, the values of $x$, $y$, and $R$ are generally random because the algorithm is showing the position of the maximum interferometric scattering signal from noise, which could occur anywhere within the frame. A background frame is typically

Figure 2. Image reconstruction and trapping algorithm. (a) The photodiode voltage contains the information about interference between the scattered and reflected beams, and the signal is digitized at the times $P_i$ shown, after multiple photodiode time constants. (b) A representation of the signals recorded from the various scanning beam positions (pixels). The background signal with no bead in the trap is also shown. (c) Flat-fielded fractional contrast signals after removal of background for two complete frames. (d) Illustration of the trapping algorithm. After each 600 $\mu$s frame, the largest absolute value is used to define the force direction (red arrow) needed to move the particle to the trap center (white $\times$).
collected and saved under operator control during this time, for example, at the time ∼32.5 s in Figure 3a. An xy plot of the absolute fractional contrast in this background frame is shown in the upper panel of Figure 3b. When feedback is on, a bead is quickly trapped after it diffuses into the trapping region. An example of the spatial distribution of the signal (the “image” of the particle in the trap) for a trapped bead is shown in the lower panel of Figure 3b. It is important to note that for a bead of this size (∼40 nm diameter), the trapping is typically so robust that the feedback must be turned off to release the trapped particle. The real-time plot of fractional contrast in Figure 3a (top) demonstrates that each trapping event exhibits slightly different contrast. These differences are likely due to heterogeneity in bead diameter, which was also observed and quantified by transmission electron microscopy (TEM) (Figure 4; also see Note S1 and Figure S5).

To quantify the functional relationship between bead diameter and absolute fractional contrast and to assess whether the relationship follows the expected linear trend with the cube of the diameter (the relationship follows the expected linear trend with the cube diameter and absolute fractional contrast and to assess whether scaling for the scattering-only signal alone is robust that the feedback must be turned off (indicated by the “Trapped” arrow in a). The white x marks the center pixel of the trap.

Figure 3. Trapping of a 40 nm gold nanoparticle. (a) Absolute fractional contrast signal during real-time operation of the trap, showing times when the feedback is on (unshaded) and when it is off (gray-shaded). For a trapping event, the ISABEL signal is shown per frame and also averaged over 10 ms. The asterisk shows a particle diffusing through the trap. The lower curve shows the x, y, and radial estimated positions of the particle in a 10 ms window. (b) Empty trap background image averaged over 250 ms (indicated by the “Background” arrow in a) and image of the trapped object (indicated by the “Trapped” arrow in a). The white x marks the center pixel of the trap.

To a gold bead with a diameter of ∼20 nm, and consistent with the observed contrast of 0.2%. Although the fluorescence signal from the bead photobleaches in just a few seconds under high excitation, the bead remains trapped almost indefinitely—illustrated here by an additional 30 s of trapping. In this case, the scattering signal and the fluorescence excitation are produced by the same laser, but it is possible to use two different wavelengths as needed to excite the fluorescence signal in an optimal way. An accompanying video demonstrating continuous trapping of a 50 nm gold particle for several minutes is available in the Supporting Information.

Figure 4. The experimentally measured interferometric scattering contrast from trapped gold nanoparticles scales as $d^3$. Gold nanoparticles of nominal diameters 20, 30, 40, 50, and 60 nm were trapped (contrast values from Figure S6). The diameters of these samples were determined from TEM images (selected images are inset, with scale bars representing 50 nm; values are from Figure S5). The symbols and error bars represent means and standard deviations. The mean calculated scattering cross sections (σ) for the smallest and largest of the gold bead samples are 0.6 and 800 nm$^2$, respectively.
much smaller than the polystyrene beads. In this batch of particles, the observed fluorescence intensity is highly heterogeneous from particle to particle and exhibits significant emission fluctuations within individual trapping events. Although variability is also observed in the interferometric scattering signal among particles, consistent with the heterogeneous particle morphology (see Figure S7), within each trapping event the interferometric scattering signal remains constant.

In summary, we have demonstrated a single-nanoparticle device, the ISABEL trap, which utilizes interferometric scattering to enable fast position detection and closed-loop feedback trapping of nanoscale particles in solution. In contrast to a dark-field detection approach, the ISABEL trap readily confines objects <100 nm diameter. With future optimization of trap design and stability, it should be possible to trap single objects as small as those that have been tracked using other interferometric scattering techniques. This would require higher illumination power, increased integration time, and an optimized illumination pattern combined with optimized detection and feedback algorithms. Critically, the ISABEL trap decouples the ability to trap a particle from spectroscopic or other observations of its nature and photophysical behavior and therefore permits trapping of a broadly expanded range of nanoscale particles to include those that either do not fluoresce or fluoresce only weakly or intermittently. We therefore anticipate that this approach will prove useful for a wide range of future applications in single-molecule biophysical and single-nanoparticle studies in free solution.

**ASSOCIATED CONTENT**

Supporting Information
The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.nanolett.9b01514.

Notes S1–S5, Figures S1–S8, and video captions (PDF)
Video S-1: beam scan pattern (slow) for the ISABEL trap (MP4)
Video S-2: ISABEL trap with 40 nm gold beads (MP4)
Simultaneous flat-fielded video recording of 40 nm gold beads in the ISABEL trap (MP4)
Video S-4: minutes-long trapping of a single 50 nm gold bead (MP4)

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**Notes**
The authors declare no competing financial interest.

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**ABBREVIATIONS**
ISABEL trap, interferometric scattering anti-Brownian electrokinetic trap; ABEL trap, anti-Brownian electrokinetic trap; iSCAT, interferometric scattering; FPGA, field-programmable gate array

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**Figure 5.** The ISABEL trap can confine and measure very weakly fluorescent objects. (a) Simultaneous fluorescence and ISABEL signals for a trapped 46 nm fluorescent polystyrene bead. (b) Simultaneous fluorescence and ISABEL signals for trapped CdSe/CdS nanoparticles.
