Rotational and translational diffusion of copper oxide nanorods measured with holographic video microscopy

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Abstract: We use holographic video microscopy to track the three-dimensional translational and rotational diffusion of copper oxide nanorods suspended in water. Rayleigh-Sommerfeld back-propagation of a single holographic snapshot yields a volumetric reconstruction of the nanorod's optical scattering pattern, from which we estimate both its dimensions and also its instantaneous position and orientation. Analyzing a video sequence yields measurements of the freely diffusing nanorod's dynamics, from which we estimate the technique’s resolution.

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References and links
Optical methods are increasingly widely used to manipulate [1, 2, 3, 4] and track [4] nanostructured materials. The high-numerical-aperture optics required for such studies offer optimal spatial resolution, but severely restrict the accessible depth of focus to within a few micrometers. Confocal and deconvolution microscopies overcome this limitation by scanning through the sample and assembling the resulting axial slices into a volumetric data set. Scanning takes time, however, and so is of limited utility for studying dynamic processes that evolve in three dimensions. Some implementations also require the sample to be fluorescently labelled, which may not be desirable. Scanning probe microscopy and electron microscopy both have superior spatial resolution, but typically are not compatible with three-dimensional micromanipulation techniques, particularly under environmental conditions.

Holographic video microscopy addresses all of these concerns by providing high-resolution volumetric information at video frame rates [5, 6, 7], even for non-fluorescent samples. When applied to colloidal spheres, holographic video microscopy can yield individual particles’ three dimensional positions with nanometer resolution [7, 8, 9, 10], to characterize particles’ optical

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**Fig. 1.** (a) Schematic representation of holographic video microscopy. The sample scatters light from a collimated laser beam. Both the scattered and unscattered laser light are collected by an oil-immersion objective lens and relayed to a video camera, which records the intensity of their interference pattern. (b) Unprocessed holographic micrograph $I(r)$ of an inclined CuO nanorod in water. (c) Normalized image $b(r)$ of the nanorod.
properties [7, 10, 11] and to measure their dimensions with sub-nanometer precision [7, 10, 11]. Such extremely high-resolution analyses have been applied to homogeneous [5, 6, 7, 9, 10, 11] and coated [10, 11] spheres, but not previously to nanostructured materials with other shapes. Here we demonstrate high-resolution holographic tracking of copper oxide nanorods diffusing freely in three dimensions. The results extend recently reported investigations of rotational and translational diffusion of ellipsoids [12] and other rod-like colloidal particles [13, 14, 15] by providing three-dimensional tracking information at video frame rates.

In-line holographic microscopy [5, 6] replaces the incandescent illuminator of conventional bright-field microscopy with a collimated coherent light source. Our implementation, shown schematically in Fig. 1(a), illuminates the sample with a continuous-wave solid state laser (Coherent Verdi 5W) operating at a vacuum wavelength of $\lambda = 532$ nm. Light scattered by the object interferes with the unscattered portion of the laser beam in the focal plane of an objective lens (Zeiss S Plan Apo, 100×, oil immersion, numerical aperture 1.4) mounted in an inverted optical microscope (Zeiss Axiovert TV 100 S). The magnified interference pattern is projected by a video eyepiece (1×) onto a video camera (NEC TI-324AII), which records its intensity at 30 frames per second. This system provides a total magnification of 0.101 $\mu$m/pixel. Each holographic snapshot contains comprehensive information on the scatterers’ shape, composition, position and orientation within the laser beam. How to retrieve that information is clarified by considering how the image is formed.

We model the incident field as a plane wave

$$E_0(\mathbf{r}, z) = u_0(\mathbf{r}) e^{i k z} \hat{\varepsilon}_0$$  \hspace{1cm} (1)$$

propagating along $\hat{z}$ with wavenumber $k = 2\pi n_m/\lambda$ in a medium of refractive index $n_m$. Its amplitude $u_0(\mathbf{r})$ may vary with position $\mathbf{r} = (x, y)$, but we assume that its polarization $\hat{\varepsilon}_0$ does not. An object located upstream of the microscope’s focal plane scatters some of this incident beam, thereby creating the scattered field

$$E_s(\mathbf{r}, z) = E_s(\mathbf{r}, 0) \hat{\varepsilon}(\mathbf{r}, z)$$  \hspace{1cm} (2)$$

at position $\mathbf{r}$ and height $z$ relative to the center of the focal plane. The scattered wave’s complex amplitude $E_s(\mathbf{r}, z)$, and polarization $\hat{\varepsilon}(\mathbf{r}, z)$ depend on the sample’s shape, size and composition as well as its position and orientation relative to the coordinate system centered on the focal plane [16]. The image in the microscope’s focal plane at $z = 0$ is therefore

$$I(\mathbf{r}) = u_0^2(\mathbf{r}) + 2 \Re \{ u_0(\mathbf{r}) E_s(\mathbf{r}, 0) \hat{\varepsilon}_0 \cdot \hat{\varepsilon}(\mathbf{r}, 0) \} + |E_s(\mathbf{r}, 0)|^2.$$  \hspace{1cm} (3)$$

If the scattered field’s dependence on the sample’s position and composition are known, Eq. (3) may be fit to an experimentally obtained hologram to locate and characterize the sample [7, 10]. In the particular case of isotropic homogeneous colloidal spheres, such fits yield the position of each particle in a holographic snapshot to within a nanometer, and their radii and refractive indexes to within a part in a thousand [10]. A holographic video sequence then provides time-resolved sequences of such single-particle measurements. This approach, however, is computationally intensive and requires an accurate and numerically stable model for light scattering by the sample. When such a model is not available, quantitative information still may be obtained by reconstructing a time-resolved three-dimensional snapshot of the scattered field from each recorded hologram [5, 6].

Unprocessed holograms, such as the example in Fig. 1(b), are marred by nonuniform illumination and artifacts due to light scattered by fixed objects and surfaces in the optical train. Previous studies addressed these imperfections either by subtracting a previously recorded background image [5] or by normalizing with an estimate for the background’s amplitude [6]. We
instead normalize by the illumination’s intensity \([7, 10]\), \(I_0(\mathbf{r}) = u_0^2(\mathbf{r})\), to obtain

\[
b(\mathbf{r}) = 1 + 2\Re\left\{ \frac{E_s(\mathbf{r}, 0)}{u_0(\mathbf{r})} \hat{\mathbf{e}}(\mathbf{r}, 0) \right\} + \frac{|E_s(\mathbf{r}, 0)|^2}{u_0^2(\mathbf{r})}.
\]

This normalization reduces additive artifacts to an additive constant and substantially suppresses multiplicative artifacts. The qualitative improvement can be seen in the normalized hologram in Fig. 1(c). In practice, we obtain the background image for a moving object by computing the median intensity at each pixel over a time window long compared with the object’s residence at any point. A running median filter then provides an updated estimate for \(I_0(\mathbf{r})\) even if the background itself were to change over time.

The third term in Eq. (4) is likely to be smaller than the other two because the scattered wave diverges as it propagates to the focal plane, but the illuminating beam does not. Neglecting it is best justified for small samples located well above the focal plane. In this limit, out-of-plane rotations of the polarization also may be considered to be small. Assuming, furthermore, that the sample is optically isotropic, we may approximate \(\hat{\mathbf{e}}(\mathbf{r}, 0) \approx 1\). Finally, if the illumination does not vary too substantially across the field of view, the reduced amplitude \(\hat{\mathbf{E}}(\mathbf{r}, 0) \equiv E_s(\mathbf{r}, 0)/u_0(\mathbf{r})\) is merely the scattered amplitude in the focal plane normalized to unit intensity. These considerations then yield

\[
b(\mathbf{r}) \approx 1 + 2\Re\left\{ \hat{\mathbf{E}}(\mathbf{r}, 0) \right\}.
\]

The scattered field at height \(z\) above the focal plane then can be reconstructed from Eq. (5) with \([18, 19, 6]\)

\[
\hat{\mathbf{E}}(\mathbf{r}, -z) \approx \frac{e^{-ikz}}{4\pi^2} \int_{-\infty}^{\infty} B(\mathbf{q}) H(\mathbf{q}, -z) e^{i\mathbf{q}\cdot\mathbf{r}} d^2q,
\]

where \(B(\mathbf{q})\) is the Fourier transform of \(b(\mathbf{r}) - 1\) and where

\[
H(\mathbf{q}, -z) = e^{-c(k^2-q^2)^{1/2}}
\]

is the Fourier transform of the Rayleigh-Sommerfeld propagator \([17, 18, 19]\). Although Eq. (7) applies in the paraxial approximation, it yields more accurate results than the Fresnel approximation that is often applied to numerical reconstruction of holograms \([19]\). The associated intensity \(I_0(\mathbf{r}, z) = |\hat{\mathbf{E}}(\mathbf{r}, z)|^2\) is an estimate for the image that would be observed at \(\mathbf{r}\) and \(z\).

We now use this general formalism to track the translational and rotational motions of cylindrical nanorods diffusing in water. Copper oxide nanorods were prepared with the simple hotplate technique method \([20]\). A substrate of Cu foil (99.99% purity, Sigma-Aldrich) was polished to remove the native oxide layer on the surface. It then was heated in a Thermolyne 4800 box furnace at 400 °C for 24 hr. The Cu substrate was returned to room temperature over 8 hr before being removed from the oven. After this treatment, the foil is covered with a uniform film of CuO nanorods, each less than five hundred nanometers in diameter and up to 100 micrometers long. The film can be peeled off of the remaining copper substrate and the nanowires separately dispersed by sonication in deionized water for 5 min.

A small droplet of this aqueous dispersion was sealed in the 100 μm thick gap between a glass microscope slide and a glass cover slip whose edges were bonded with Norland Type 81 optical adhesive. This sample then was mounted on the microscope for observation at room temperature. Less than 100 mW of light was projected into the sample over the 3 mm diameter of a Gaussian beam. This illumination was too weak to raise the temperature of the aqueous sample appreciably, to alter the nanorods’ structure \([21]\), or to exert measurable forces on the individual nanorods.
Fig. 2. (a) Numerically refocused version of Fig. 1(c) in the plane of best focus. (c) Intensity profile along the nanorod’s axis indicating a length of 4.9 µm. (d) Intensity profile transverse to the nanorod’s axis indicating a diameter slightly less than 200 nm.

The image in Fig. 2(a) shows the numerically refocused image \( \tilde{I}_s(r,z) \) of a freely floating nanorod whose axis was aligned approximately along the midplane of the sample cell, roughly 50 µm from either wall. The nanorod appears as a dark feature in \( \tilde{I}_s(r,z) \) because it both absorbs and scatters the green illumination. The false-colored three-dimensional reconstruction was computed with axial steps of 101 nm, consistent with the in-plane resolution. The rays converging from the bottom of the reconstruction toward the center should be a faithful representation of the nanorod’s light scattering pattern. Those features that continue upward through the focus are artifacts that arise because Eq. (6) assumes the medium to be homogeneous, an assumption that breaks down at the position of the nanorod. The field upstream of the nanorod should be featureless. Faint artifacts also are evident radiating upward and outward from the lower edge of the reconstruction, which are due to the twin image. For these reasons, \( \tilde{I}_s(r,z) \) should not be considered a straightforward image of the nanorod, but nevertheless is useful for tracking its position and orientation in three dimensions.

The intensity profile along the nanorod’s axis is plotted in Fig. 2(b) and suggests that it is \( 4.9 \pm 0.3 \) µm long. Nonuniformities in this axial trace recur in all of this nanorod’s holograms, independent of its position and orientation. Consequently, they appear to be ascribable to irregularities in the nanorod itself rather than to nonuniformities in the illumination or artifacts of the holographic reconstruction. Fig. 2(c) shows a transverse intensity profile through the middle of the nanorod in the horizontal plane. The dip in the intensity has a full width at half-maximum of \( \sigma_0 = 280 \) nm, which reflects the nanorod’s actual diameter \( \sigma \) broadened by diffraction. A simple estimate based on Gaussian broadening,

\[
\sigma^2 \approx \sigma_0^2 - \frac{\lambda^2}{2 \pi n^2 m}
\]

yields \( \sigma \approx 200 \pm 20 \) nm for the rod’s diameter, which is consistent with results obtained for similar samples by electron microscopy.

We quantified the nanorod’s three-dimensional position and orientation relative to the coordinate system centered on the focal plane by analyzing the deviation from background intensity of volumetric reconstructions such as those in Fig. 2. Estimates for the nanorod’s axis were computed by intensity-weighted skeletonization [22] of \( \tilde{I}_s(r,z) \). Those points identified as lying on the axis then are fit by linear regression to a line segment whose center is taken to be the
estimate for the rod’s position \( \mathbf{R}(t) = (x(t), y(t), z(t)) \) at time \( t \) and whose orientation is the estimate for the nanorod’s orientation \( \hat{s}(t) = (\cos \theta(t) \sin \phi(t), \sin \theta(t) \sin \phi(t), \cos \phi(t)) \), where \( \theta(t) \) and \( \phi(t) \) are polar and azimuthal angles respectively. The fit segment’s length is found to be substantially independent of orientation even when the nanorod is oriented axially, as in Fig. 3(b). This provides support both for the use of Rayleigh-Sommerfeld back propagation to reconstruct the three-dimensional intensity distribution and also for skeletonization as a means to locate the nanorod within reconstructed volumetric data.

Analyzing the volumetric reconstruction in this way is complementary to direct analysis of the hologram itself, which has proved fruitful for tracking colloidal spheres [7, 8, 10]. It has the advantage of not requiring a specific model for light scattering by the rod, it is less sensitive to details of the scattering geometry, and also is far less computationally intensive. Consequently, Rayleigh-Sommerfeld volumetric imaging offers much-needed real-time feedback for optical micromanipulation techniques that increasingly are being used to assemble nanorods and nanowires into three-dimensional functional structures [2, 3]. It also makes possible real-time analysis of nanorods’ three-dimensional rotational and translational Brownian motion.

A Brownian rod’s rotational diffusion generally is independent of its translational motion and can be quantified through displacements of the orientational unit vector [23]

\[
\Delta s^2(t) \equiv \langle |\mathbf{s}(t) - \mathbf{s}(0)|^2 \rangle = 2 \left[ 1 - (1 - \varepsilon_s^2) \exp \left( -2D_r \left( t - \frac{\tau}{3} \right) \right) \right],
\]

where the rotational diffusion coefficient is given by [23]

\[
D_r = \frac{3k_B T}{\pi \eta L} \left[ \ln \left( \frac{L}{\sigma} \right) - \gamma \right]
\]

in a fluid of viscosity \( \eta \). The constant \( \gamma \approx 0.45 \) depends on the detailed shape of the cylindrical rod and is known analytically only for special cases, such as prolate ellipsoids [23]. Equation (9) also includes terms accounting for the mean-squared error \( \varepsilon_s^2 \) in measurements of \( \mathbf{s}(t) \) [24, 25] and for blurring during the \( \tau = 1 \) ms exposure time of the camera [25]. Measurements of \( \Delta s^2(t) \) not only provide information on the nanorod’s structure and dynamics, they also enable us to estimate the measurement error inherent in our holographic rod-tracking procedure.

Fig. 4(a) shows the evolution of \( \Delta s^2(t) \) for the nanorod in Fig. 3 obtained from a continuous 5 min trajectory recorded at 1/30 s intervals. The data agree well with the prediction of Eq. (9) and yield a rotational diffusion coefficient of \( D_r = 0.0846 \pm 0.0004 \) s\(^{-1} \), which is two orders

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Fig. 3. Volumetric reconstruction of a diffusing nanorod (a) inclined at roughly 45° to the focal plane and (b) oriented nearly perpendicularly to the focal plane.
Fig. 4. (a) Evolution of the nanorod’s mean-square orientational fluctuations together with a fit to Eq. (9). Shaded region indicates statistical uncertainty in $\Delta s^2(t)$. Inset: Location of $\hat{s}(t)$ at 1/3 s intervals, colored by time. (b) Evolution of the mean-square displacement along and transverse to the nanorod’s axis. Shaded region indicates the statistical uncertainty in $\Delta R^2_{||}(t)$. The similar error range for $\Delta R^2_{\perp}(t)$ is omitted for clarity. Inset: Displacement of the nanorod’s center of mass $R(t)$ during the first 20 s of the 5 min trajectory, plotted as a three-dimensional ribbon extended along the nanorod’s orientation $\hat{s}(t)$. Scale bar indicates 2 $\mu$m.

of magnitude greater than the largest value accessible by confocal microscopy of nanorods in viscous media [13]. The fit estimate for the error, $\varepsilon_s = 0.02$, corresponds to an error in orientation of roughly 3$^\circ$. The associated characteristic rotation time $(2D_s)^{-1} = 5.9$ s is short enough that the rod explores all orientations over the duration of the measurement, as can be seen in the inset to Fig. 4(a).

Because a rod’s viscous drag coefficient depends on its orientation, its translational diffusion is coupled to its rotational diffusion when viewed in the laboratory frame [12, 23]. Translational fluctuations are separable from rotations in the nanorod’s proper frame of reference, however. Consequently, the axial and transverse projections of the center-of-mass translations satisfy the standard Einstein-Smoluchowski relations,

$$\Delta R^2_{||}(t) \equiv \left\langle |R(t) - R(0)| \cdot \hat{s}(0) |^2 \right\rangle = 2D_{||} \left(t - \frac{\tau}{3}\right) + 2\varepsilon^2_{||}$$ and

$$\Delta R^2_{\perp}(t) \equiv \left\langle |R(t) - R(0)| \times \hat{s}(0) |^2 \right\rangle = 4D_{\perp} \left(t - \frac{\tau}{3}\right) + 4\varepsilon^2_{\perp},$$

with diffusion coefficients [23]

$$D_{||} = \frac{k_BT}{2\pi\eta L} \left[\ln \left(\frac{L}{\sigma}\right) - \gamma\right] \quad \text{and} \quad D_{\perp} = \frac{k_BT}{4\pi\eta L} \left[\ln \left(\frac{L}{\sigma}\right) + \gamma\right].$$

Equations (11) and (12) are corrected for the camera’s exposure time [25]. They also account for measurement errors $\varepsilon_{||}$ and $\varepsilon_{\perp}$ along and normal to the nanorod’s axis under the assumption that these errors are independent of orientation, $\hat{s}$. They omit higher-order dependence on $\varepsilon_s$.

Fitting Eqs. (11) and (12) to the data plotted in Fig. 4(b) yields $D_{||} = 0.3691 \pm 0.0004 \mu m^2/s$ and $D_{\perp} = 0.2426 \pm 0.0003 \mu m^2/s$. Given the experimental temperature, $T = 295.3 \pm 0.5$ K, and the associated viscosity of water, $\eta = 0.955 \pm 0.001$ cP, Eq. (13) then suggests that $L = 5.05 \pm 0.09 \mu m$ and $\sigma = 0.18 \pm 0.02 \mu m$, both of which are consistent with results obtained
directly from holographic imaging. From the same fits we obtain \( \epsilon_\parallel \approx \epsilon_\perp \approx 100 \text{ nm} \). This suggests that our procedure tracks the center of the nanorod to within roughly one pixel in all three dimensions.

We have demonstrated that individual holographic video snapshots may be interpreted with Rayleigh-Sommerfeld back-propagation to measure the instantaneous three-dimensional position and orientation colloidal nanorods. Dynamical information obtained from sequences of holographic images agrees well with the predicted behavior of Brownian cylinders and confirms a measurement resolution of 100 nm in all dimensions. The technique’s time resolution is limited only by the frame rate of the video camera. Rayleigh-Sommerfeld back-propagation has the advantage of providing a model-free approach to reconstructing the light field scattered by microscopic objects, and thus lends itself to high-speed processing and imaging. Holographic video microscopy thus can provide real-time feedback for three-dimensional micromanipulation of nanowires and nanorods. It also is useful for studying the rotational and translational motions of nanorods subjected to external forces. The present study takes advantage of the comparative simplicity of single isolated nanorod’s diffusion when viewed in the co-oriented frame of reference. This advantage is lost when studying the coupled motions of multiple nanorods, so that measurements of nanorods’ hydrodynamic and electrostatic interactions will be substantially more challenging than corresponding measurements on colloidal spheres. This complexity, however, arises from the underlying physics, rather than the technique, and constitutes an interesting and potential fruitful area of application for the methods described here.

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