Optical tracking of nanoscale particles in microscale environments
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Optical tracking of nanoscale particles in microscale environments

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The trajectories of nanoscale particles through microscale environments record useful information about both the particles and the environments. Optical microscopes provide efficient access to this information through measurements of light in the far field from nanoparticles. Such measurements necessarily involve trade-offs in tracking capabilities. This article presents a measurement framework, based on information theory, that facilitates a more systematic understanding of such trade-offs to rationally design tracking systems for diverse applications. This framework includes the degrees of freedom of optical microscopes, which determine the limitations of tracking measurements in theory. In the laboratory, tracking systems are assemblies of sources and sensors, optics and stages, and nanoparticle emitters. The combined characteristics of such systems determine the limitations of tracking measurements in practice. This article reviews this tracking hardware with a focus on the essential functions of nanoparticles as optical emitters and microenvironmental probes. Within these theoretical and practical limitations, experimentalists have implemented a variety of tracking systems with different capabilities. This article reviews a selection of apparatuses and techniques for tracking multiple and single particles by tuning illumination and detection, and by using feedback and confinement to improve the measurements. Prior information is also useful in many tracking systems and measurements, which apply across a broad spectrum of science and technology. In the context of the framework and review of apparatuses and techniques, this article reviews a selection of applications, with particle diffusion serving as a prelude to tracking measurements in biological, fluid, and material systems, fabrication and assembly processes, and engineered devices. In so doing, this review identifies trends and gaps in particle tracking that might influence future research. © 2016 Author(s). All article content, except where otherwise noted, is licensed under a Creative Commons Attribution 3.0 Unported License.
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I. INTRODUCTION

The initial condition and subsequent interaction of a particle with the surrounding environment determines the spatiotemporal trajectory of the particle. This interaction varies with the composition and motion of the environment, as well as the properties of the particle, recording useful information about both the environment and the particle in the trajectory. Therefore, apparatuses and techniques for tracking particles and obtaining information from the resulting trajectories, in different contexts and by various modalities, comprise an evolving area of research. Optical tracking of nanoscale particles in microscale environments is at the vanguard of such research due to the convergence of several trends in science, technology, and commerce.

Vision is the dominant sense in humans. As a natural consequence, apparatuses for creating, transmitting, and sensing visible light have received much attention throughout the history of technology. Optical microscopy, a classical technique ordinarily permitting observation of the microscale world with submicrometer spatial resolution, forms the basis of optical tracking of nanoscale particles in microscale environments. In recent decades, microscopists have improved the spatial resolution that is achievable with optical microscopy as they have better understood and subsequently circumvented previously perceived limits of the technique. Such advances have enabled the application of optical microscopy at the nanoscale, revealing the structure and function of the microscale world in extraordinary detail by tracking nanoscale emitters. Complementing these new techniques, ongoing advances in semiconductor manufacturing and digital imaging are increasing the performance and decreasing the cost of optoelectronic devices for creating and sensing light, improving both the accessibility and capability of optical microscopes. Contemporaneously, a diverse enterprise has developed to control and exploit the properties of nanoscale particles, which often interface through microscale environments to the macroscale world. A useful complementarity of length scales and physics couples this enterprise to particle tracking, as nanoparticles are natural probes of microenvironments, which are in turn natural media in which to manifest and measure the properties of nanoparticles. Using an optical microscope, an experimentalist can track nanoparticles in a variety of microenvironments with a reasonable input of effort, providing an often rich source of information as an output. For all of these reasons, diverse applications involving the optical tracking of nanoparticles in microenvironments are emerging. This review article focuses on fundamental limitations and practical implementations of optically tracking nanoparticles in microenvironments.

Observing a signal from a particle and estimating the spatiotemporal coordinates of the particle from this signal over a relevant spatiotemporal interval defines the process of particle tracking. This definition encompasses a broad range of measurements even within the focused scope of this review article that Sec. II specifies through optical, spatiotemporal, and particulate parameters. One purpose of this review is to serve as an initial guide to experimentalists seeking to design tracking systems, consisting of apparatuses or techniques to optically track nanoparticles in microenvironments. In a review of such tracking systems, explicitly mapping the capabilities of different apparatuses or techniques to specific applications is complex. A discussion of the optimality of such a map will have to consider issues ranging from physical limits to hardware costs to sample perturbations. Secs. III–VI address the more manageable portions of this map, collectively serving as a useful guide but not an exhaustive set of prescriptions. In particular, Sec. III presents a measurement framework that uses information theory to identify the parameters that determine the capabilities of optical microscopes and describes how experimentalists can trade off these parameters to design tracking systems for particular applications. Sec. IV discusses the practical aspects of hardware used to assemble tracking systems, including the influence of sources and sensors of light on measurement errors, and the many factors influencing the choice of the particle being tracked. Within the limitations that Secs. III and IV describe, Sec. V reviews various tracking apparatuses and techniques. This review focuses on structuring illumination and improving detection, implementing feedback and confinement, and using prior information to track multiple and single particles. Particle tracking enables remarkably diverse measurements on experimental systems ranging from lipid membranes to cryogenic superfluids, and from photore sist films to micromechanical devices. Sec. VI reviews a selection of illustrative examples from these different domains of science and technology in the context of the measurement framework and review of apparatuses and techniques. This approach provides a broad perspective in a comparison of tracking systems and applications, whereas other review articles focus more on particular applications, for example, biological applications. The ultimate purpose of this review is to help experimentalists design tracking systems for different applications, to serve as a reference for current measurement capabilities, and to focus attention on gaps in current capabilities that additional research and development could close.

II. SCOPE

This article reviews measurements made in the far field, typically using an optical microscope equipped with a digital optoelectronic sensor, of visible to near-infrared radiation emanating from nanoparticles. Such measurements are highly relevant, as optical microscopes are widely accessible to experimentalists in many fields of research and development, and a large infrastructure of optoelectronic devices is readily available to sense light under many different conditions. These devices, usually integrating silicon photodiodes, convert incident photons with wavelengths from 0.4 \( \mu m \) to 1 \( \mu m \) into electrons with high quantum efficiency and data throughput, and low noise.

This review focuses on the tracking of particles with radii from 0.5 \( nm \) to 500 \( nm \), ranging from single fluorophores to colloidal nanoparticles, within experimental volumes with dimensions from 0.1 \( \mu m \) to 100 \( \mu m \), encompassing numerous microenvironments of interest that are consistent
with measurement by optical microscopy. Physical processes in such microenvironments occur over time scales that range at least from 1 ns to 10 s, extending from the fluorescence lifetimes of single fluorophores to the diffusion times of colloidal nanoparticles across the micrometer scale.

Unless specified otherwise, the term emitter denotes a particle detected by an optical signal. In cases that it is relevant, this article notes differences in the mechanism by which the particle produces the optical signal, for example, fluorescence or scattering, and the effects of the mechanism on experimental design. This article focuses on the isotropic emission of light from particles, with occasional deviations. This focus is not limiting, even in experimental systems with some degree of emission anisotropy. For example, the mean emission of an ensemble of dipole emitters, such as organic fluorophores oriented randomly within a polymeric particle, is approximately spatially isotropic. In addition, single dipole emitters can, due to diffusion, rotate over large angles during the integration time of a light sensor, resulting in a mean emission that is approximately spatially isotropic. Such spatial or temporal averaging to approximate isotropic emission simplifies routine tracking measurements involving the localization of anisotropic emitters. Emission anisotropy is an area of active research for many applications involving single fluorophores.4

Spatial precision and temporal bandwidth cannot exclusively determine the suitability of a particular tracking technique for an application of interest. Sec. III elaborates on this point and develops a measurement framework by considering an optical microscope for nanoparticle tracking as an apparatus for information transmission. However, spatial precision and temporal bandwidth still comprise a useful starting point in specifying the measurement capability of a tracking system. Therefore, as an initial guide to this review article, Fig. 1 maps a variety of applications of particle tracking, in one or more spatial dimensions, along axes denoting the spatial precision and inverse temporal bandwidth of the respective measurements. These axes, in units of meters and seconds, simultaneously denote other scales of length and time that are relevant to some of the hardware, processes, and applications that this article reviews. The quantities that this article reports are nominal and in some cases approximate as the details of uncertainty evaluation are beyond the scope of this review. Similarly, this article does not attempt to impose a strict metrological nomenclature where it does not exist in the literature, instead using the terms precision and uncertainty, and the terms error and inaccuracy, synonymously.

This article reviews canonical applications of nanoparticle tracking such as estimating the diffusion coefficient of a colloidal particle in a fluid, as well as emerging applications involving superresolution imaging.14 The main difference between such measurements is the way in which the techniques use prior information about the emitters, as Sec. III explains. Therefore, this review does not distinguish between nanoparticle tracking and superresolution imaging in the broad sense, describing both within a common measurement framework. However, Sec. VI maintains a focus on applications involving moving nanoparticles.

Finally, while this article reviews the ultimate limits of tracking precision and the relationship of those limits to other parameters in a tracking system, this review does not focus on how specific tracking algorithms approach those limits. Maximum likelihood estimation approaches the ultimate limit of achievable precision but is not the only metric of algorithm performance. Computational cost is also important. Another article thoroughly reviews issues relating to algorithm performance.15 However, the present article does review one algorithm, compressed sensing, in some detail. This is because the design of tracking systems can, and increasingly does, explicitly incorporate the assumptions underlying the use of prior information in this algorithm.

III. MEASUREMENT FRAMEWORK

Implementing a tracking system consists primarily of choosing nanoparticles that are appropriate for an application and designing an apparatus or technique to track the nanoparticles. The optical properties of the nanoparticle naturally influence the design of the apparatus or technique. However, optical properties do not solely determine the choice of nanoparticle, for example, if the nanoparticle is the object of interest, or if the nanoparticle is to interact in a specific way with the surrounding microenvironment to probe a sample. Sec. IV discusses the factors affecting the choice of nanoparticle separately from the considerations influencing the design of the apparatus or technique. This section describes a measurement framework, primarily based on information theory, that identifies the parameters of optical microscopes influencing the design of an apparatus or technique for particle tracking.

A. Optical microscopes and information theory

Whether tracking the diffusive motion of an isolated nanoparticle in a homogeneous fluid or imaging an immobile section of biological tissue of heterogeneous composition, an optical microscope detecting an object modulates the optical field emanating from the object. Ultimately, the microscope detects only this modulated field, limiting what information about the object is available using the microscope. This determines the capability of a tracking system to perform a useful measurement. Many parameters influence this modulation and the resulting limits of an optical microscope for nanoparticle tracking.

An optical microscope functions as a low-pass filter of spatial information in a far-field measurement. For the incoherent imaging of an object, light with spectral components corresponding to spatial frequencies exceeding 2NA/λ, where NA is the numerical aperture of the microscope and λ is the wavelength of light, diffracts away from the aperture of the microscope.16 The corresponding finer structure of the object is not visible to the observer. Thus, the spatial resolution of an optical microscope is ordinarily 2πNA/λ, where the factor 2π represents a minimum acceptable level of contrast. For incoherent illumination, z has a value of 0.61 for the commonly used Rayleigh criterion and a value of 0.5 for the closely related Abbe limit.17 For nearly a century, microscopists considered the diffraction limit to be the minimum distance
FIG. 1. A map serving as an initial guide to the optical tracking of nanoscale particles in microscale environments. Horizontal and vertical axes, in units of meters and seconds, respectively, denote various scales of length and time that are relevant to some of the applications, hardware, and processes that this article reviews. Red data markers with parenthetical letters indicate specific applications, with the horizontal axis denoting the reported spatial precision and the vertical axis denoting the inverse of the reported temporal bandwidth. The values of temporal bandwidth correspond to (a)–(e) the times to update a sensor for tracking single particles and (f)–(h) the times to acquire a series of images for tracking multiple particles. Applications of particle tracking are remarkably diverse. (a) Ballistic-to-diffusive transition of a particle in a fluid. (b) Viscoelastic response of diluted polyethylene oxide. (c) Dynamics of unbound myosin head. (d) Hop–diffusion of lipid in cell membrane. (e) Kilohertz rotation of nanorods propelled by ultrasound. (f) Mapping structural heterogeneity of a polymer film. (g) Highest temporal bandwidth for photoactivated localization microscopy in three dimensions. (h) Chromosome motion during cell division. Red line segments perpendicular to axes indicate specifications of common hardware for nanoparticle tracking. Red line segments, rays, and lines along axes indicate spatial or temporal ranges of interest. Visible and near-infrared (vis–NIR) wavelengths range between 0.4 μm and 1 μm. The localization precision of an isolated isotropic emitter extends down from approximately 200 nm to below 1 nm. The conformations of proteins involve dynamics at time scales between 100 ns and 1 ms. The red line within the white space of the figure indicates the Stokes-Einstein estimate for the mean squared displacement in one dimension of a nanoparticle diffusing freely in water at an absolute temperature of 300 K. Any point on this line corresponds to the time for diffusion to displace the particle by a length equal to the diameter of the particle. Transitions from diffusive to ballistic motion occur at shorter scales of length and time, depending on the properties of the particle and fluid, and deviate from this line.
between identical and stationary point objects that is necessary to resolve the objects. The diffraction limit remained a barrier until microscopists realized that prior information about the objects, such as a low temporal bandwidth, enables superresolution techniques to overcome this limit. A recent example is photoactivated localization microscopy (PALM) for superresolution imaging. This technique relies on prior information about the stationarity of adjacent emitters on a static structure, in both position and mean value of emission of photons, to resolve the emitters to within a fraction of the Rayleigh limit. The stochastic, but sequential, activation and localization of these emitters provides spatial information about the static structure below the diffraction limit. Importantly, this technique makes a trade-off between finer spatial resolution and lower temporal bandwidth.

More generally than the diffraction limit, communication theory describes how the degrees of freedom that an optical microscope can transmit govern the modulation of a tracking system. The logarithm of the total number of messages that can pass through an optical microscope defines this transmission capability, or the information capacity $N$ of the tracking system, which the signal-to-noise ratio ($SNR$) also affects. The information capacity is then

$$N = (2L_x B_x + 1)(2L_y B_y + 1)(2L_z B_z + 1) \times (2TB_T + 1) \log(1 + SNR),$$

(1)

where $L_x$, $L_y$, and $L_z$ denote the spatial range along the $x$, $y$, and $z$ axes of the tracking system, $B_x$, $B_y$, and $B_z$ denote the spatial bandwidths of the system along those axes, $T$ is the observation time, and $B_T$ is the temporal bandwidth of the tracking system. This definition accounts for a factor of two that corresponds to the two possible polarization states of the optical signal. The spatial and temporal bandwidth products in Eq. (1) reflect the requirement of the Nyquist sampling theorem for an optical microscope in the absence of noise. These parameters are collectively the degrees of freedom of an optical microscope. In the presence of additive noise, different signals are reliably distinguishable if the signal strengths differ by at least the mean intensity of the noise. In this way, the $SNR$ affects the reliability with which an optical microscope can transmit messages.

A note about terminology—for a conventional optical microscope, the spatial ranges $L_x$ and $L_y$ correspond to the field of view, and $L_z$ corresponds to the depth of field. If the configuration of such a microscope remains fixed during the course of the measurement, then these parameters do not change with respect to a frame of reference that is fixed to a laboratory. However, for tracking systems that actively reconfigure during an experiment, the field of view and depth of field can change in time with respect to a frame of reference fixed to a laboratory. To avoid any preconceptions about such systems, this article uses the term spatial range for these three quantities, with $L_x$ and $L_y$ denoting the lateral range and $L_z$ denoting the axial range.

The number of degrees of freedom of an optical microscope is finite. However, by using prior information about the sample, an experimentalist can focus on any of the above parameters determining this number at the expense of other parameters. Particular applications of particle tracking inform such trade-offs in the rational design of the capabilities of a tracking system. The importance of prior information in any imaging measurement follows from the fact that although the degrees of freedom of an optical microscope are finite, those of the object under observation are, in the classical approximation, infinite. In other words, an optical microscope maps a signal of infinite dimensionality to a signal of finite dimensionality. This implies that a potentially infinite number of objects could be responsible for any single image. Even an Airy pattern, commonly considered as the image of a single point emitter, could, due to the finite degrees of freedom of an optical microscope, be the result of an infinite number of point emitters separated by $\lambda/(2NA)$, the distance set by the Nyquist criterion. While this surprising result occurs only by a conspiratorial arrangement of the phase and amplitude among all the emitters, this scenario illustrates the potential for degeneracy in estimating the object responsible for an image. This degeneracy is independent of detector ideality. Non-idealities such as pixelation and noise add further degeneracy. Prior information can break such degeneracy by helping to select a single estimate from an infinite number of possibilities. For example, in superresolution imaging of a static structure, the mean intensity of a nominally single emitter informs of whether the signal is the result of multiple emitters instead. In applications of particle tracking in which the emitters are moving, there is a strong justification, after the experiment, for assuming a certain number of emitters. If there were a large number of independent emitters, then an undetected independence in motion and intensity of these emitters would imply an extraordinary correlation between the emitters, invalidating the assumption of independence. However, a lack of prior information remains confounding even if the emitters are moving, for example, in the case of adjacent particles sharing the same lateral, but different axial, positions in the observation volume of an optical microscope. Interpretation of such results requires care. Techniques for particle tracking can make use of prior information in many other ways. For example, compressed sensing enables image reconstruction using subtle information about the sparsity underlying the representation of an image. Subsequent sections discuss these examples in specific contexts.

With a known number of particles being tracked, what are the measurement limits of a tracking system? To answer this question, it is instructive to delve deeper into the use of a tracking system to measure a distribution of nanoparticles in a microenvironment, which can inform of the properties of either or both measurands.

A pedagogical description of the microenvironment is as a field varying in space and time, $F(\vec{r}, t)$, originating from a distribution of some quantity of interest. For example, the field might be the microstructural variation of a distribution of nerve fibers or the microrheological variation of a distribution of macromolecules in a cell. Tracking the interaction of nanoparticles with the field provides information about the relevant distribution. This description assumes, for simplicity, that the field is scalar, analytic, and has only linear
spatiotemporal variations. The field at a spatiotemporal point near \((r', t)\) is then \(F(\mathbf{r} + \delta \mathbf{r}, t + \delta t) = F(\mathbf{r}, t) + \nabla F(\mathbf{r}, t)\delta \mathbf{r} + F(\mathbf{r}, t)\delta t\). A superresolution imaging experiment, for example, often assumes a temporally invariant sample. The field \(F(\mathbf{r}, t)\) then shows the structural variation of a distribution of nerve fibers and \(F(\mathbf{r}, t) = 0\). This prior information factors into the design of the experiment and the reconstruction of the nerve fibers from a tracking measurement. Single fluorophores probe the microstructure, and tracking the positions of the fluorophores provides the distribution of interest. In another example, the field \(F(\mathbf{r}, t)\) in a microelectrochemical experiment has a temporally varying but spatially constant viscosity, so that \(\nabla F(\mathbf{r}, t) = \vec{0}\). That prior information factors into the design of the experiment and the reconstruction of the viscosity dynamics by tracking the diffusive motion of a colloidal nanoparticle in a small volume, provided the temporal variation is not too large. Prior information about the field constrains the variations of the field. As such, the field need not have zero spatial or temporal variation, as in these two examples, or have a sparse representation as in the earlier allusion. In an experiment in which the particle properties are of interest, for example, in estimating the hydrodynamic size of a colloidal nanoparticle by tracking the diffusive motion of the particle in water at room temperature, the experimentalist may have prior information about the field and the particle. The main point is that understanding and using the constraints associated with prior information is important for rationally designing a tracking system and correctly interpreting experimental results.

A common assumption is that the accuracy and precision associated with localizing a particle are sufficient statistics for parameterizing the distributions of interest in applications of particle tracking. This article proceeds with those statistics as the metrics of the theoretical limits of a tracking system. The following discussion focuses on the spatial localization of a particle and assumes isotropic motion. The localization accuracy along the \(x\)-axis is \(\delta x = \langle x_i \rangle - x_i\), where \(\langle x_i \rangle\) is the mean value of the estimated positions \(x_i\), with each estimate made on the basis of a measurement of the position of the particle and \(x_i\) is the true, unknown, position of the particle. With \(n\) independent measurements, the localization precision along the \(x\)-axis is most generally

\[
\sigma_x = \sqrt{\frac{\langle (x_i - \langle x_i \rangle)^2 \rangle}{n}}.
\]

Analogous definitions for the localization accuracy and precision hold for the \(y\) and \(z\) axes.

The properties of the sample and the optical microscope influence the ultimate limit of precision in estimating the position of a particle. This limit is independent of the algorithm for performing the estimation, which allows for an unambiguous characterization of the measurement capability of the tracking system, based on the Fisher information content in the measured random variables. In particular, the distribution of the detected photons contains information about the position of the particle, and the Cramer-Rao lower bound identifies the minimum theoretical variance of an unbiased estimate of the position of the particle with the inverse of the Fisher information matrix. Since this bound is a function of the distribution of detected photons, any factor that influences this distribution also affects the bound. These factors include the intensity of emission from the particle and resulting shot noise, point spread function (PSF) of the imaging apparatus, and readout noise, pixelation, and size of the light sensor. Assuming a sufficiently representative set of unbiased estimates of position, the localization precision obeys the same lower bound as the theoretical variance. With this assumption, the Cramer-Rao lower bound for the localization precision along the \(x\)-axis of a single point emitter in the simple case of an imaging apparatus with a symmetric point spread function is

\[
\sigma_x \geq (\mathbf{I}_{xx})^{-1/2},
\]

where \(\mathbf{I}\) is the Fisher information matrix having only diagonal entries, with the entry

\[
\mathbf{I}_{xx} = N_p \int \int \frac{1}{f(x, y)} \left(\frac{\partial f(x, y)}{\partial x}\right)^2 dx dy,
\]

where \(N_p\) is the mean number of detected photons, assuming a Poisson emission process. The intensity point spread function \(f(x, y, z)\), which is unit normalized, depends on the coordinate \(x\) defining the position of the point object, and on the coordinates \((x_s, y_s)\) of the region of the light sensor imaging the point spread function. For brevity, Eq. (3) shows only the \(x\)-dependence of the position of the point object while the integral spans the spatial extent of the sensor. This ideal analysis assumes that the emitter is isolated, stationary, and emitting isotropically and that the sensor is a continuous two-dimensional planar region that is free of noise. Analogous expressions define the Cramer-Rao lower bound for localization precision along the \(y\) and \(z\) axes, and related expressions account for sensor pixelation and noise. In the simplest case for Eq. (3), the square root transform \(g(x; x_s, y_s) = \sqrt{f(x; x_s, y_s)}\) shows that the integrand is, equivalently, \(4 \left(\frac{\partial g(x; x_s, y_s)}{\partial x}\right)^2\). This shows how better localization precision is possible with steeper gradients in the amplitude \(g(x; x_s, y_s)\). Reducing the size of the point spread function increases this gradient, which is a topic of considerable interest. Importantly, Eq. (3) also shows that the localization precision of an emitter improves as the number of detected photons \(N_p\) increases.

The above expressions apply only to isolated particles but are still valid in tracking and localizing multiple particles simultaneously, as long as the images of the particles do not spatially overlap. Overlap does not occur if the nanoparticles moving through a microenvironment are sufficiently dilute, or if the temporal activation of a high concentration of fluorophores in a superresolution imaging experiment occurs at a low enough rate to spatially resolve single emitters during an acquisition interval, or if the illumination or detection region is small enough to observe only a single emitter.
The Cramer-Rao lower bound is difficult to calculate analytically. However, this bound is accessible through numerical computations, which frequently guide the design of an apparatus. In principle, the maximum likelihood estimate, which is computationally expensive but unbiased, approaches the Cramer-Rao lower bound for localization precision in the limit of $N_f$ growing to infinity. Subsequent sections review the Cramer-Rao lower bound for specific apparatuses. The remainder of this section gives examples of typical trade-offs between the parameters that govern the information capacity and measurement capabilities of tracking systems.

B. Spatial bandwidth

Any energy-limited and bandwidth-limited function, such as the point spread function of an optical microscope, has an upper bound on the maximum value of the gradient of the function. Thus, with a fixed number of photons from the emitter, the Cramer-Rao lower bound cannot decrease indefinitely by shaping the point spread function, as the gradient term in the integrand of Eq. (3) would otherwise suggest. The wavelength of emitted light, as well as the size of the aperture that limits the transmission of photons to the sensor, restricts the spatial bandwidth of the apparatus. In general, increasing the numerical aperture and using shorter wavelengths of light increases the spatial bandwidth. Subsequent sections review related techniques.

C. Shaping the point spread function

The high Fresnel number of a conventional, unaberrated optical microscope makes the point spread function nearly symmetric about focus. This renders the apparatus imprecise for axial localization, since the associated Cramer-Rao lower bound is infinitely large—at the focal position, the symmetry makes the gradient term vanish in the version of Eq. (3) for the axial Cramer-Rao lower bound. Sec. V discusses techniques for improving axial localization by shaping the point spread function, within the spatial bandwidth and energy bounds that constrain the point spread function, and breaking this symmetry using additional optics.

D. Spatial range and signal-to-noise ratio

Spatial range and signal-to-noise ratio have related effects in determining the measurement limits of a tracking system. The spatial range of an optical microscope with an imaging sensor affects the Cramer-Rao lower bound through the magnification of the microscope optics and the spatial extent of the sensor array. The magnification is implicit in the definition of the point spread function, while the size of the sensor limits the field of view for particle tracking. The use of an imaging sensor with pixels and additive background noise modifies the expression for the Cramer-Rao lower bound. This modification shows that, at an overly low magnification, the point spread function extends on an area smaller than a single pixel, leading to a position estimate with a precision that is no smaller than the pixel. Therefore, it is necessary to image the point spread function onto multiple pixels for localization with subpixel resolution. But extending the point spread function over too many pixels can bury the signal from the emitter in background noise and result in a localization precision that is less than optimal. Background noise can be optical, arising from background fluorescence or diffuse scattering, or electronic, arising in the sensor. Increasing illumination intensity to boost optical signal can perturb or even damage the microenvironment.

E. Lateral range and temporal bandwidth

Lateral range and temporal bandwidth have related effects in determining the measurement limits of an apparatus for particle tracking. This limitation is commonly evident in the frame rate of an imaging sensor. Reducing the region of interest on the imaging sensor can increase the frame rate, the trade-off being a decrease in lateral spatial range. The limit of this reduction process is a point sensor, such as an avalanche photodiode (APD), which can count single photons at nanosecond scales but provides no spatial information absent additional position sensors, for example, in a moving stage.

F. Axial range, lateral range, and temporal bandwidth

The axial range of an apparatus for particle tracking is typically smaller than the lateral range and scales with the depth of field, $\approx \lambda/NA^2$. Increasing the axial range by reducing the numerical aperture broadens the point spread function and worsens lateral precision. If only a single particle is of interest for tracking, then a moving stage, for example, can position that particle in or near focus, improving both axial localization precision and axial range for that particle. The trade-off is a loss in lateral range, since such a measurement system positions and tracks only one particle at a time rather than collecting information from multiple particles across the entire sample. The finite temporal bandwidth of the stage, in other words, how fast it can move, effectively increases the size of the point spread function through motion blur and limits localization precision.

G. Temporal range and temporal bandwidth

Unintended motion of the microscope and loss of the signal from the particle frequently limit the temporal range of an apparatus for particle tracking. Vibration and drift of a microscope can move the sample, potentially out of the field of view or depth of focus during the acquisition of data. This is a significant issue in tracking measurements with long intervals of data acquisition. If the microscope motion is not so severe, then referencing the sample position in each frame to a precisely localized fiducial marker near the sample can account for this motion after the experiment. Another technique is to use a moving stage to compensate for unintended motion during the experiment, using a signal derived from position measurements of a fiducial marker. Whether a correction for unintended motion of the microscope occurs during or after the experiment, errors in this correction worsen localization precision. Even when the particle remains in the field of view, loss of signal from the
particle due to photobleaching can limit the temporal range of the measurement. Sec. IV D discusses this and other effects resulting from the choice of nanoparticle.

H. Polarization states

Polarization is a vectorial attribute of light that can cause anisotropic emission of light from a particle. Emission anisotropy can serve as an additional source of information or bias an estimate of the position of the particle without a correction. Due to averaging effects, as Sec. II discusses, such a correction may not be necessary for particles containing many dipole emitters or dipole emitters that are rotationally diffusing. One use of polarization in positional tracking is as a filter for improving the signal-to-noise ratio. For example, if the polarization of the optical response of the background is dominant in a fixed direction, while the polarization of the emission of the particle is dependent on the orientation of the particle, then a polarizer placed in the imaging path can reduce the background. The trade-off is a reduction of signal intensity and worse localization precision as the particle diffuses rotationally from orientations from which the emission is polarized perpendicular to the easy axis of the polarizer.

In summary, this section discusses the degrees of freedom of an optical microscope, and some of the possible trade-offs among these parameters to gain useful information about a sample. Such gains derive from designing a tracking system to incorporate prior information, for example, that the sample is static or the need to track only a single particle, that is specific to the application. Sec. IV discusses the hardware that is available for constructing tracking systems, placing limits on the leverage of prior information.

IV. TRACKING HARDWARE

Sec. III presents the theoretical limitations of an optical apparatus for particle tracking. This section reviews some of the practical limitations imposed by hardware, determining what is possible to measure at the current state of the art and what might be possible to measure in the future. Light sources, light sensors, moving stages, and nanoscale particles are critical components of tracking systems. The requirements of specific applications, especially the particle properties, influence the selection of these components. Commercial trends in performance and cost are also important factors in enabling the widespread use of these components. In particular, semiconductor manufacturing is increasing the performance and decreasing the cost of many light sources and sensors, which drives trends in the design of apparatuses and techniques for particle tracking.

A. Light sources

The critical characteristics of light sources for particle tracking are power output and dynamic response, which respectively limit signal-to-noise ratio and temporal bandwidth in a tracking measurement. The spectral purity of a light source is also important in many applications of particle tracking. For example, a gold nanoparticle scatters all wavelengths of a broadband light source. The longest wavelength in the emission spectrum of the nanoparticle limits the spatial bandwidth determining the localization precision. Fluorescence introduces other considerations, such as the need to prevent overlap of the excitation spectrum of the light source with the emission spectrum of the particle, to decrease spectral crosstalk and resulting background noise. Sec. IV D discusses these mechanisms in more detail, which motivate the use of lasers and light emitting diodes in tracking systems, as these technologies have advantages in spectral purity and dynamic response. These light sources are the focus here.

Lasers are common sources of light in tracking systems for structuring illumination profiles ranging from spots, to beams, to sheets, as Sec. V describes. The pulse-to-pulse jitter of <1 ps for commercially available lasers has a lesser effect on the practical value of temporal bandwidth than the frame-to-frame jitter of typical imaging sensors. Temporal stability is necessary for synchronization and repeatability in pulsed illumination. Lasers in tracking systems commonly have output powers from 0.1 W to 1 W, linewidths of 1 nm in the visible range, and temporal modulation capability exceeding 1 MHz.

Light emitting diodes are also common in tracking systems and will probably become more so as the current density and luminous efficacy of these devices increase. This is particularly important for widefield imaging, as a large focal area decreases irradiance. Light emitting diodes are generally not as monochromatic as lasers, however, having spectral peaks with widths of tens of nanometers. A broad spectral peak can reduce effective irradiance, for example, by illuminating a fluorophore at wavelengths differing from the peak absorption wavelength of the fluorophore. Light emitting diodes in tracking systems commonly have output powers from 0.1 W to 10 W and temporal modulation capability exceeding 100 kHz.

B. Light sensors

Semiconductor photodetectors have revolutionized optical measurements, in general, and are essential to optical microscopes for particle tracking. Large numbers of photodetectors arrayed in an imaging sensor are common in tracking systems. Point sensors are also useful for tracking single particles, with additional hardware such as a moving stage providing spatial range and position information. Other articles comprehensively review the limits of light sensors, so this section briefly reviews only a few of the most salient characteristics of light sensors for tracking systems.

Digital cameras are typical for imaging and tracking particles. In a charge-coupled device (CCD) camera, the quantum efficiency of the sensor and the readout noise sets the noise level. In an electron multiplying CCD (EMCCD) camera, on-chip gain multiplication doubles the noise variance and halves the effective quantum efficiency, worsening localization precision. Complementary metal–oxide–semiconductor (CMOS) cameras have emerged as alternatives to EMCCD cameras, with lower costs, wider fields of view, better performance at higher intensity signals.
Moreover, the parallel readout architecture of a CMOS camera enables higher frame rates than a CCD camera or EMCCD camera.\cite{38} Commercially available CMOS cameras have data rates approaching 1 gigabyte per second with frame rates depending on the number of pixels being read. It can be important to calibrate interpixel variation in gain and noise to prevent loss in localization accuracy and precision.\cite{39} This variation can have a complex relation, depending on the signal-to-noise ratio of the measurement.\cite{40}

An avalanche photodiode is the semiconductor analogue of a photomultiplier, using a high reverse bias voltage, potentially above the breakdown voltage of the semiconductor, to create a high gain and sensitivity. Avalanche photodiodes typically detect single photons at a single point, which is useful for particle tracking in combination with moving stages, which Sec. IV C discusses, although arrays of avalanche photodiodes with temporal bandwidths of $>10$ MHz are becoming commercially available for imaging.\cite{41}

Balanced photodetectors are point sensors that are essentially two ideally identical photodiodes which report a difference of light intensity that is incident on the pair. Different nonlinearities\cite{42} in the intensity response of the two photodiodes can, absent proper calibration, result in localization errors in tracking experiments. This type of error is of concern in applications requiring high temporal bandwidths, in which two opposing considerations compete—the signal intensity must be high to improve the Cramer-Rao lower bound, yet high intensities might amplify any deviations from ideality in the detector.

**C. Moving stages**

A moving stage can position a sample or optic in three spatial dimensions with nanoscale precision. This capability is useful in several ways in tracking systems. First, stages can be part of an experimental calibration which, for example, maps the point spread function of the optical apparatus by moving a point particle adsorbed to a coverslip across a region of interest. For such a calibration, the positioning range and precision of the stage are important, but the temporal bandwidth of the calibration is usually unimportant. Second, a stage can scan either a sample or optic during an experimental measurement to form an image, in which case the positioning range and precision and temporal bandwidth of the stage are all important. Third, a stage can be part of an active system, in which the stage moves an element of the system during the experiment in response to some feedback signal. In this case, the positioning range and precision and temporal bandwidth are critical to the apparatus, as will become apparent in Secs. V and VI that review techniques and applications involving a moving stage to enable feedback control.

Flexure guidance of piezoelectric actuation is common in electromechanical stages, due to the high positioning precision afforded by the piezo element, and the inter-axis decoupling of the stage motion provided by the flexural element. Stages with positioning precision of $<1$ nm, spatial range of $10 \mu m$ to $100 \mu m$, and temporal bandwidth in the kilohertz range are commercially available. A careful experimental design considers that these values, particularly the temporal bandwidth, will change depending on the mass of the object that the stage moves. Objects loading a stage range from a coverslip in a calibration measurement to an objective lens in a tracking system enabled by feedback. A comprehensive review of the performance limits of stage motion is available elsewhere.\cite{43}

**D. Nanoparticles**

A primary concern in particle tracking is the capability of a nanoparticle to indicate its interaction with a microenvironment, either to probe the microenvironment or to manifest properties of the nanoparticle. Selection of a nanoparticle for a specific application involves determining the optimum combination of a number of properties. Table I tabulates six types of nanoscale particles used as optical emitters and their essential optical properties.

The size, shape, structure, and surface properties of nanoparticles are all important in tracking applications. Relevant sizes range from single fluorophores with

| TABLE I. Types and properties of nanoscale particles used as optical emitters. |
|-----------------|--------|--------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                | Radius (nm) | Lifetime (ns) | Photostability | Quantum yield | Absorption cross-section (nm$^2$) | Emission cross-section (nm$^2$) |
| Organic fluorophore$^a$ | 0.5 to 1 | 3 to 4 | Low | 0.93 | 0.028 | 0.026 |
| Fluorescent protein$^b$ | 1 to 2.5 | 1 to 10 | Low | 0.6 | 0.03 | 0.02 |
| Semiconductor crystal$^c$ | 2.5 to 25 | 10 to 30 | Moderate | 0.1 to 0.9 | 0.04 to 0.4 | 0.004 to 0.36 |
| Nitrogen–vacancy center$^d$ | 2.5 to 25 | 25 | Excellent | 0.7 to 0.8 | 0.003 | 0.002 |
| Fluorescent bead$^e$ | 5 to 50 | 3 to 4 | Moderate | 0.93 | 0.75 to 750 | 0.69 to 690 |
| Noble metal particle$^f$ | 20 and 40 | $10^{-6}$ | High | 0.04 and 0.36 | 3 015 and 30 200 | 125 and 11 000 |

$^a$Fluorescein.\cite{44}

$^b$Green fluorescent protein.\cite{45}

$^c$Quantum dot—The properties vary with size, composition, and structure.\cite{46}

$^d$Diamond particle containing negatively charged nitrogen–vacancy centers.\cite{47}

$^e$Polymeric particle containing multiple fluorescein molecules—The number of fluorophores in a bead of radius $R$ determines the absorption and emission cross-sections of the bead. These values assume that the number of fluorophores in a bead scales as $R^3$, and that a bead with $R = 5$ nm contains 25 fluorophores.

$^f$Gold—Mie theory provides the calculated absorption and emission cross-sections for spherical gold particles of $R < 50$ nm.
subnanometer radii, to polymeric particles with radii of hundreds of nanometers. Various structural properties, including the particle core, and any surrounding shell, ligands, and functional groups, determine the effective size of a particle. Interaction of a particle with the surrounding media can significantly influence the hydrodynamic size of the particle. For example, brush layers or shrink as a function of pH, salt concentration, temperature, or, in the case of DNA, hybridization. Anisotropic particles have different apparent sizes, depending on the direction of motion and interaction with the local microenvironment, determining the observed optical and hydrodynamic properties of the particles. A probe particle must be much smaller than the critical dimensions of a microenvironment or microstructure, particularly if the latter is in motion, if the former is not to perturb the sample and bias the measurement through size effects. Otherwise the probe particle can, for example, increase the hydrodynamic diameter of the sample. Other interactions between the probe particle and microenvironment or microstructure can also influence a tracking measurement. Chemical functionalization of particles allows the probing of specific interactions, such as the attachment of particles to, or uptake of particles by, biological cells. The charge of a particle is important in such interactions, as well as in investigations of colloids and interfaces in which strong electrostatic interactions occur. Such interactions depend on the surface chemistry of the particle and the ionic strength of the medium. Considering these various issues, it is clear that the choice of particle is not always straightforward, and assessing specific perturbations to experimental systems can be complicated. But the primary considerations in choosing a particle for tracking, in that the performance of the particle determines the accuracy and precision of the tracking system, relate to the optical emission and associated properties of the particle.

For an optical irradiance $I$ and sensor integration time $t$, the particle emits a number of photons $N_p$, enabling localization of the position of the particle to a precision $\sigma$. A specific example makes the relation between these parameters more concrete and introduces a canonical application—tracking the diffusion of a spherical particle in free solution, while targeting a lateral localization precision of $\sigma = 10 \text{ nm}$. Assuming a detector with infinitesimally small pixels that are free of noise, the Cramér-Rao lower bound gives the minimum number of photons $N_p = (\lambda/(2\pi NA\sigma))^2$ that enables this localization precision. At a typical detection wavelength $\lambda = 600 \text{ nm}$ and numerical aperture $NA = 0.5$, $N_p \approx 350$ photons are necessary. For a power $P$ incident on a sample area $A_{\text{ilum}}$, an estimate of the irradiance $I = P/A_{\text{ilum}}$ that results in $N_p$ photons incident on the sensor is

$$I = \alpha R^3,$$

where $\alpha$ is the Planck constant, $c$ is the speed of light, $\lambda$ is the incident wavelength, $\sigma_{\text{em}}$ is the emission cross section of the particle, $Y$ is the quantum yield of the particle, $\eta_{\text{QE}}$ is the quantum efficiency of the sensor, and $s$ is the fraction of the light emitted by the particle and transmitted through the optics. For ideal transmission through the optics, $\eta_{\text{QE}} = 1$ for negligible light absorption, and $s = \frac{1}{2}(1 - \cos(\theta))$, where $\theta = \arcsin(NA/n)$ is half of the angle subtended by the objective lens at the particle and $n$ is the refractive index of the immersion medium for the objective lens.

![Figure 2](image-url)  
**FIG. 2.** A simulation estimating the irradiance necessary to localize a static fluorescent bead to a target precision, as well as the motion blur of a diffusing bead, both varying as a function of time. Eq. (4) relates the irradiance $P/A_{\text{ilum}}$ to the integration time $t$ to achieve a static lateral localization precision of 10 nm, with parameter values $c = 3 \times 10^8 \text{ m} \cdot \text{s}^{-1}$, $\lambda_p = 350$, $\lambda = 600 \text{ nm}$, $\sigma_{\text{em}} = 690 \text{ nm}^2$, $Y = 0.92$, $\eta_{\text{QE}} = 0.7$, and $s = 0.067$ corresponding to an objective lens having $NA = 0.5$ that is immersed in air. The motion blur in one dimension due to diffusion is $\sqrt{2Dt}$, where $D = 4 \mu \text{ m}^2 \cdot \text{s}^{-1}$ is the diffusion coefficient of a spherical bead with radius $R = 50 \text{ nm}$ in water at an absolute temperature of 300 K. With all other parameters held constant, the irradiance needed to localize a particle to a target precision varies as $R^{-1/2}$ while the motion blur varies as $R^{-1/5}$.
fluorescence lifetime limits the maximum number of photons that a particle emits per unit time, which then limits the localization precision for a certain integration time. Fluorescence lifetimes are sensitive to a large number of microenvironmental parameters, including temperature, pH, viscosity, magnetic field strength, and dielectric constant. If the fluorescence lifetime is measurable at the level of a single emitter, then this value can provide additional information about the local microenvironment. Many fluorescent emitters also exhibit temporal effects at longer time scales. Two of the most problematic for particle tracking applications are blinking, when an excited electron transitions into a dark state with a long lifetime, and bleaching, when the system undergoes an irreversible chemical change and becomes non-fluorescent. Photostability, which is related to the maximum dose of illumination before a particle goes dark, limits the maximum duration of a tracking measurement. Fluorophore engineering for improved photostability, particularly while maintaining biological compatibility, is an active area of research. Peak emission wavelengths of fluorophores range from blue to near-infrared. Longer excitation wavelengths often decrease absorption by the sample, reduce autofluorescence, and increase photostability of the sample. Shorter detection wavelengths yield higher spatial resolution for a given energy budget and often higher quantum efficiency of the detector but might result in more damage to the sample. Absorption and emission cross-sections and quantum yield influence the irradiance necessary for a target number of signal photons and localization precision. Phototoxic effects from free radicals generated by fluorophores are an issue in biological measurements. Intense illumination or long exposure at visible wavelengths to maximize signal-to-noise ratio can produce oxygen species that react with cellular components and damage cells. Apart from reducing illumination intensity and exposure time, the use of free radical scavengers can mitigate phototoxic effects, as well as bleaching of organic fluorophores. Sec. VA2 discusses a specific example in a biological application of fluorophore tracking.

Organic fluorophores are among the smallest optical emitters, enabling the quasistatic tracking or superresolution imaging of a variety of biological and material microenvironments. Single fluorophores are the only optical emitters that live cells can express as parts of heritable fluorescent proteins as probes for in vivo measurements. Otherwise, chemistries exist to functionalize and bind single fluorophores to specific proteins or polymers. The length and flexibility of linkers binding single fluorophores to a microstructure potentially complicates the analysis of tracking data. If the microstructure is static, such as the illustrative distribution of nerve fibers in Sec. IIIA, then the rigidity of the linker determines the resulting type of localization error. If a rigid linker fixes the orientation of a fluorophore to the extent that the resulting emission is anisotropic, then localization accuracy and precision worsen absent a detailed analysis. While this situation is not the focus of this article, Sec. VIC reviews a few exceptions involving the superresolution imaging of materials. In contrast, a short, flexible linker confines the diffusion of a fluorophore to a volume limited by the length of the linker. Localization precision worsens in this particular case. The length of the linker limits the degradation of localization accuracy as the fast rotational diffusion of a typical fluorophore, largely unimpeded by a flexible linker, results in a more isotropic emission during the integration time of the light sensor. For a linker of intermediate flexibility, a detailed analysis may be necessary to accurately estimate the loss of localization precision. Models of confined diffusion in potential wells are useful to determine any resulting degradation of localization precision. Organic fluorophores are not the most reliable of fluorescent emitters, being prone to bleaching.

Nanoscale crystals of semiconductor materials, or quantum dots, are bright and have tunable emission spectra. Quantum dots often have longer fluorescence lifetimes than organic fluorophores and blink, which is problematic for some tracking applications. Quantum dots have numerous applications, due to the combination of a broad absorption spectrum and a narrow emission spectrum, which enables effective spectral multiplexing.

Nanoscale particles of diamond containing the negatively charged nitrogen–vacancy center (NV−) are of great interest. In comparison to many other fluorescent emitters, a nitrogen–vacancy center in a diamond nanoparticle is resistant to blinking and bleaching. The absorption peak at 560 nm and emission band from 630 nm to 800 nm make the nitrogen–vacancy center suitable for applications in biological imaging. Magnetic fields modulate the fluorescence intensity of this emitter, enabling the use of diamond nanoparticles as local sensors of magnetic fields in microenvironments. In contrast to organic fluorophores or quantum dots, the emission of the diamond nanoparticle with a nitrogen–vacancy center is not tunable over a wide range. Although the concentration of nitrogen–vacancy centers in natural diamond is low, irradiating and annealing the material can dramatically increase the number of fluorescent point defects, to the extent that a particle with a radius of 5 nm can contain multiple fluorescent defects. Diamond nanoparticles are chemically inert in many microenvironments of interest but oxidation processes can terminate the surfaces of the nanoparticles with a variety of oxygen species, enabling the functionalization of diamond nanoparticles. A recent technique for coating diamond nanoparticles with a thin shell of silica allows the use of standard attachment chemistries which can potentially enable new applications in particle tracking.

Multiple fluorophores in a polymeric particle, also known as a fluorescent bead, are common probes of microenvironments. Such beads are optically bright, isotropic, and fairly stable. The brightness of the bead scales with the number of fluorophores, giving a nominal dependence of $R^3$ for spherical particles of geometric radius $R$ for volumetric distributions.

Light scattering is another mechanism to produce an optical signal in particle tracking. Particle size determines scattering regime, from Rayleigh scattering for particles with sizes much smaller than the wavelength of light, to Mie scattering for larger particles with sizes comparable to the wavelength of light. In Rayleigh scattering, scattering intensity varies as the square of the volume of the particle, giving an
$R^6$ dependence for spherical particles of geometric radius $R$. In this case, absorbed and scattered light have the same wavelength, and perfectly spherical particles maintain the polarization of light. Darkfield microscopy is a common technique to increase signal-to-noise ratio in such measurements. Light scattering has some advantages for particle tracking. The short effective lifetime of the excited state results in an emission rate with a high value of saturation. Noble metal nanoparticles scatter light strongly and have particularly large scattering cross-sections at plasmon resonance. Illumination at a wavelength corresponding to this resonance peak maximizes the scattering signal of the nanoparticle relative to the background noise. The high photostability of such particles means that loss of signal is not usually a concern. Unlike fluorescent nanoparticles requiring low irradiance or control over oxygen concentration to limit photobleaching, the practical limit on the maximum useful irradiance for scattering particles results from particle heating. Heat transfer to the local environment of the particle can perturb the sample and bias the measurement, as Sec. VI A discusses.

The statistics of the emission of photons from a particle is another optical property that can improve spatial resolution. In particular, a sub-Poissonian distribution of photon emission allows resolution of adjacent emitters below the diffraction limit. Such a distribution, for example, Bernoulli, has a smaller variance than a Poisson distribution with the same mean value. Quantifying missing photon coincidences between emitters improves the resolution in comparison to the classical Poisson photon distribution. Sub-Poissonian anti-bunched emission from quantum dots allows superresolved images of a static sample. The missing three-photon coincidence signal enables a spatial resolution that is two thirds of the diffraction limit of a tracking apparatus with an EMCCD camera. These results indicate the possibility of improved temporal bandwidth in comparison to photoactivated localization microscopy and comparable spatial resolution by using even higher order missing coincidences. However, this technique requires further suppression of sensor noise to achieve such improvements. The computational expense of localization algorithms for the above measurement and other kinds of non-classical photon statistics might receive further attention with ongoing advances in the signal-to-noise ratios of light sensors.

V. APPARATUSES AND TECHNIQUES

Sec. III discusses a measurement framework in terms of the degrees of freedom of an optical microscope, determining the theoretical limits of a tracking measurement. Sec. IV reviews the hardware currently used to construct tracking systems, determining the practical limits of a tracking measurement. This section reviews, within these limits, the implementation of different apparatuses and techniques. An important consideration in this review is that no single apparatus or technique is universally applicable to, let alone optimal for, different experimental measurements. For example, building on the discussion of nerve fibers in Sec. III A, the technique of scanning a focused illumination volume across a population of single fluorophores probing a biological microstructure produces an image with spatial precision at the nanometer length scale. However, even with a fast photodetector, the scanning time of the stage limits the temporal bandwidth of the measurement. Therefore, the fluorescence lifetimes of the fluorophores at the nanosecond scale, for example, are not simultaneously accessible using the same apparatus.

The following discussion of apparatuses and techniques bifurcates into those for tracking multiple particles, and for tracking single particles. This bifurcation mirrors two basic designs of optical microscopes—with widefield illumination and an imaging sensor, and with focused illumination and a point sensor. In this context, the number of light sensors is the first consideration in designing an apparatus or implementing a technique for particle tracking. The number of light sensors varies from over $10^6$ down to 1, limiting the number of particles being tracked, as Fig. 3 shows. These two basic designs of optical microscopes emphasize the importance of structuring illumination and improving detection, and this section reviews the trade-offs made in these...
Processes. The use of feedback—coupling systems to modify their behavior—is another typically distinguishing characteristic between apparatuses and techniques for tracking single particles and multiple particles. The use of feedback typically trades off degrees of freedom to gain information about the motion of a specific particle at the expense of a loss of information about the rest of the sample. Confinement and compressed sensing are other techniques which improve tracking measurements for both single and multiple particles.

A. Tracking multiple particles

Structuring illumination and improving detection of a sample, often independently, can increase the amount of information gained from the sample. Fig. 4 shows a few examples of such techniques. This independence, as an application permits, allows for a modular design of a tracking apparatus. The following discussion of techniques to track multiple particles clarifies the choices available for such a modular approach.

1. Structuring illumination

For applications requiring spatial precision, in particular, an ideal measurement localizes each emitter, no matter how densely packed in a microenvironment, with the targeted precision. A conventional, unaberrated optical microscope with widefield illumination precludes such ideality by exciting other emitters in the vicinity of the targeted emitter, including emitters above and below focus. For a high concentration of emitters, the resulting widefield emission increases background noise and worsens the localization precision of the target. Viewed as an aspect of improving localization precision, illumination design reduces background noise and increases prior information about the sample, which aids in particle tracking and image reconstruction.

Confocal microscopy is a classical technique which increases signal-to-noise ratio by restricting both the illumination volume and the detection volume of the sample. In the original implementation of confocal microscopy, light from a pinhole aperture focuses at a point $P$ on the sample. Before detection, light emitted from that point passes through a second pinhole aperture placed at a plane conjugate to $P$. The pinhole aperture in the detection path blocks any out-of-focus light emitted from the sample. Scanning the focal point of the illumination builds up an image of the sample but trades off temporal bandwidth. Subsequent discussions revisit this technique in different contexts.

Total internal reflection (TIR) microscopy is another classical technique which permits measurement of a sample volume within a few hundred nanometers of a surface. As light moves from a medium with a higher refractive index into a medium with a lower index, the light undergoes total internal reflection within the high index medium, as the angle of incidence at the interface exceeds the critical angle. At visible wavelengths, the resulting evanescent wave extends approximately 200 nm from the interface into the low index medium, illuminating only this shallow volume of the sample. Calibration of the intensity profile of evanescent illumination allows axial localization of nanoparticles in the low index medium near the interface. This approach confines...
the illumination volume to an interface, however, with the intensity profile depending on the wavelength and the angle of incidence. Sec. VI E reviews applications of particle tracking in nanofluidic devices which can make use of, or take the place of, total internal reflection microscopy and other techniques of structuring illumination.

Bessel beams, focused with a separate objective lens and scanned through the sample, can overcome this limitation by structuring light sheets as thin as 500 nm spanning the entire lateral extent of the sample.12 This technique mitigates scattering and shadowing artifacts in biological media due to the self-reconstructing properties of the central core of a Bessel beam.73 Scanning these sheets along different axial planes at the rate of 200 planes per second results in an isotropic precision of 300 nm and an isotropic range of 10 μm and the ability to resolve cellular organelles. Light sheets also mitigate phototoxic effects in biological specimens because of the reduction in irradiance.12 This technique trades temporal bandwidth for the ability to image in three dimensions.

Multiphoton microscopy, an established technique in fluorescence microscopy, exploits the emission arising from the low probability of simultaneous absorption of multiple photons to create a smaller effective point spread function,74 as Fig. 4 shows for the two-photon case with confocal imaging. For a gold nanoparticle that scatters light strongly, resonant interactions with plasmons on the surface of the nanoparticle enhance the signal from two-photon luminescence.75 This allows tracking of gold nanorods with an isotropic spatial precision of <10 nm, while the absence of blinking or bleaching enables a temporal range of 30 min.76 To improve temporal bandwidth in comparison to scanning confocal microscopy with a single illumination volume in typical applications of two-photon luminescence, a diffractive optical element creates an array of illumination volumes on a planar sample region of 30 μm × 30 μm, scanning laterally at a bandwidth of 20 Hz. Although this apparatus enables axial imaging, scanning multiple focal planes across an axial range of 5 μm limits the temporal bandwidth to 2 Hz. Phase masks for axial imaging could increase this value by reducing the number of scanned focal planes, without reducing axial localization precision. Sec. VA 2 discusses the design of phase masks in more detail.77 The absence of blinking and bleaching enables a temporal range of 30 min.

Stimulated emission depletion (STED) is a class of techniques14 to structure illumination by using two beams of light that overlap spatially but are distinct spectrally. Stimulating the sample with the usual Gaussian beam, and simultaneously quenching all emission from fluorophores outside of a central region with a concentric annular beam shifted towards the red region of the visible spectrum, limits the lateral diameter of the central excitation region to 20 nm.14 The annular beam quenches the fluorophores at a faster rate than the spontaneous fluorescence decay from the excitation profile of the Gaussian beam. The speed with which the combined stimulating and quenching beams scan across the sample limits the temporal bandwidth of this technique. Such explicit control of temporal bandwidth versus scanning area makes stimulated emission depletion a good candidate for tracking moving particles. The quenching beam need not have an annular shape,14 meaning that the excitation region need not be circular. Smaller excitation regions require higher powers, however, which may perturb or damage the sample.

Adaptive control of the wavefront illuminating a sample is useful in heterogeneous microenvironments, such as biological specimens, in which inhomogeneity in refractive index aberrates the wavefront passing through the sample. Emerging techniques in optical microscopy build on existing techniques in stellar imaging for correcting aberrations due to atmospheric turbulence.78 A spatial light modulator (SLM) actively modulates the phase of the wavefront to correct for microenvironmental inhomogeneity in refractive index.79 The spatial light modulator focuses an aberrated wavefront, divided into approximately 100 beamlets, into a static biological sample to a depth of 400 μm. Since the pixels of the spatial light modulator individually correct the phase of each of the beamlets, there is a necessary loss in temporal bandwidth due to the combined latency of the spatial light modulator and the imaging algorithm that provided the feedback signal. Shaping the wavefront using feedback is a trade-off that an experimentalist can make if a certain degree of freedom, such as temporal bandwidth, is unimportant for a particular application.

2. Improving detection

Lateral localization of an emitter is often of primary importance in particle tracking. Related improvements in optical microscopes, localization algorithms, and experimental systems have resulted in impressive tracking measurements.

FIG. 5. A schematic showing that apparatuses and techniques using imaging sensors typically provide more lateral range than axial range. (a) At focus, the image from a conventional microscope provides no localization precision out of the image plane due to the near symmetry of the point spread function in the axial direction. (b) Astigmatic modification of the point spread function with a cylindrical lens increases axial range without reducing the lateral range. (c) A microscope with diffractive optical elements simultaneously images multiple focal planes across the sensor array, reducing lateral range and increasing axial range for a fixed localization precision.
For example, fluorescence imaging with one-nanometer accuracy (FIONA), an early demonstration of particle localization by fitting a two-dimensional Gaussian function to the point spread function of a conventional, unaberrated optical microscope, enables lateral localization of a single fluorophore with a precision of 1 nm from 14,000 photons per image. To detect this many photons from a single fluorophore, an oxygen scavenging environment increases the photostability of the dye and enables a temporal range of minutes.

Localization along the optical axis presents a different challenge. The point spread function of a conventional, unaberrated optical microscope with a single objective lens is axially elongated and nearly axially symmetric about focus, reducing the value of the axial gradient, which increases the Cramer-Rao lower bound for axial localization. Other strategies are necessary to improve axial localization precision, which can reduce the lateral range from which information is available if using an imaging sensor for detection. Fig. 5 shows this schematically.

Microscopists have studied techniques for reducing the axial size and axial symmetry of the point spread function for two decades, beginning with the use of a cylindrical lens to provide an astigmatic modification of the point spread function of a conventional microscope. This lens, or another phase mask that modifies the point spread function, is usually placed in the Fourier plane of a telescopic system comprising two lenses. This pair of lenses relays the image of an emitter from the focal plane of the tube lens of the microscope, where an imaging sensor is conventionally placed, to a conjugate plane where the imaging sensor is placed instead. Modification of the point spread function using a cylindrical lens or other phase mask is subject to a trade-off between axial range and precision. Infinitely steep gradients, ideal for reducing the Cramer-Rao lower bound, violate theoretical limits on the bandwidth and energy of the optical signal, which, for a fixed bandwidth, determine the photon budget. Phase masks can implement point spread functions targeting certain values of axial range or signal-to-noise ratio. A variational framework for the systematic design of phase masks treats the Cramer-Rao lower bound as a functional to minimize by an optimal point spread function. Using a numerical routine, this technique results in a design for a phase mask that yields an experimental axial precision of 15 nm to 40 nm over a range of 3 μm, with a budget of 3500 photons in the visible part of the spectrum. This experimental value of axial precision differs from the theoretical value of <15 nm over the entire range, possibly due to microscope aberrations. The geometry of the non-convex functional of the Cramer-Rao lower bound, in the presence of energy and bandwidth limits and camera noise, is not yet clear. Clarification of this issue will inform the design of point spread functions with reduced model mismatch and that also target certain values of orientational localization precision. Lateral and axial localization precisions vary across the field of view, depending on the spatial gradients of the point spread function. An objective function, based on the Fisher information, that penalizes these variations improves this aspect of the design of the point spread function. Experimental studies do not always report these variations, which can be insignificant over a small field of view. The design and fabrication of phase masks is not trivial but the relative ease of incorporating these optics into microscopes for superresolution imaging is an incentive for future research.

Increasing the size of the aperture for light collection increases spatial bandwidth, which improves localization precision. If only one objective lens collects light, then the point spread function of the image elongates along the optical axis, resulting in a shallower gradient in the point spread function and a higher Cramer-Rao lower bound. A 4Pi apparatus, so named for the two opposing objective lenses that collect light, overcomes this limitation. Both confocal and widefield versions exist, although the latter implementation requires axial scanning of the point spread function to achieve an improved axial range. The principle, as Fig. 4 shows, is that a constructive interference pattern from wavefronts emerging from the two objective lenses reduces the axial size of the point spread function and consequently increases the axial gradient of the point spread function, improving axial precision. Simulations for localizing a point particle using a 4Pi apparatus show that a factor of 6 improvement in axial precision is possible in comparison to a conventional apparatus with one objective lens, resulting in an isotropic precision of 10 nm over an axial range of 1 μm for 250 photons detected per objective lens. Variations of this theme exist. The combination of photoactivated localization microscopy with an interference technique with three phases increases the axial range by eliminating dead zones of local intensity maxima and minima in interference techniques relying on inversion of a single phase, while also affording a more uniform positional precision through the axial range. The combination of 4Pi microscopy with multiphoton microscopy also improves axial precision. Multiphoton microscopy reduces the size of the illumination point spread function, as Sec. VA1 discusses. In combination with 4Pi microscopy, there is a further reduction in the size of the point spread function of the image. This is mainly due to a diminution of the side lobes of the point spread function, which is a result of the solid angle of light collection in 4Pi microscopy being, due to practical considerations, less than 4π. Issues related to the complexity of the apparatus remain a barrier to widespread adoption. One solution to the problem of maintaining stability at the nanometer scale of interference arms with lengths of several centimeters is to use a mirror in place of the second objective lens. However, precise control of the distance between the emitter and mirror is necessary. Another solution is to incoherently detect the signal from the two objective lenses, increasing the number of photons detected with the second objective lens. This is relatively simpler to implement due to the lack of an interference arm. But incoherent detection offers less precision than techniques based on interference which provide steeper gradients in intensity of point spread functions. Similarly, using a mirror to generate a simultaneous image from a side view increases the photon count and localization precision but does not provide steeper gradients in the intensity of the point spread function.
Beyond structuring illumination and improving detection, tracking systems can use feedback and confinement to focus on the specific degrees of freedom that are of interest in an experiment. This approach is particularly relevant in applications that involve tracking only a single particle at a time, which Sec. VIB discusses.

B. Tracking single particles

If information about the motion of only a single particle is most relevant, then rearranging the tracking apparatus with respect to the target particle during the experiment can be a useful technique. Such a rearrangement relates to an ordinary measurement. If the length of a particular object in a room is the measurand, then an experimentalist brings a ruler closer to that object or vice versa for an optimal measurement. But such rearrangement necessarily involves an element of feedback, in that the experimentalist must first identify the location of the object relative to the ruler before repositioning one or both to make the measurement. In tracking a single particle, feedback of the position of the particle controls rearrangement of the sample relative to the apparatus. In this technique, the motion of the target particle at a fine scale matters more than that of the other particles in the sample volume. So, the spatial and temporal bandwidths that are necessary to track the target particle are important, while the spatial range that contains useful information is only in the immediate vicinity of the particle. This assumes that any optical signal from other particles in proximity to the target particle does not perturb the target signal. Recognizing the potential trade-off of the spatial range of the image for spatial and temporal bandwidth, a reasonable design for the apparatus is to illuminate only the volume containing the target particle, increasing the photon count and the Fisher information for localization precision. This is similar to the light sheet technique12 that Sec. VA2 describes, except that a focused laser spot in a feedback loop often suffices to illuminate and track a single particle. A moving stage in combination with a fast photodetector is useful for this measurement, with the stage guiding the illumination volume along the trajectory of the particle. Depending on the temporal bandwidth relevant to the measurement, the stage provides some or all of the positional information, as Fig. 6 shows schematically.

Instead of moving the illumination volume, another technique, if the application permits the perturbation of the microenvironment, is to confine the particle near a static illumination volume. The following discussion proceeds along these two lines—tracking a free particle with feedback and tracking a confined particle.

1. Tracking a free particle with feedback

One technique that uses feedback to track a single particle is to rapidly scan a focused laser in an orbit around a fluorescent nanoparticle in a fluid cell.99 The phase of the fluctuations in the fluorescence intensity of the nanoparticle provides information on the position of the nanoparticle relative to the center of the orbit, scanning at 300 kHz in this tracking system. As the nanoparticle moves away from the center of the orbit, the measured fluctuation serves as an input to a feedback loop, actuating a piezo stage containing the fluid cell to reposition the nanoparticle near center of the orbit. The flow induced by the relatively slow motion of the stage does not significantly perturb the motion of the nanoparticle. The record of the stage motion contains the temporal dependence of the motion of the nanoparticle to the precision of the feedback loop of 50 nm. A related technique relies on the actuation of multiple elements enabled by feedback.98 A mirror and an objective lens mounted to piezoelectric actuators follow a nanoparticle in a feedback loop with a focused laser. The feedback uses images from two planes near the nanoparticle in object space, separated axially by 750 nm, on different regions of 5 pixels × 5 pixels of an EMCCD camera. This small region of interest on the camera allows imaging of a lateral region of 750 nm × 750 nm surrounding a fluorescent bead with a radius of 100 nm with a temporal bandwidth of 3.2 kHz. This technique results in a lateral precision of <10 nm and an axial precision of <30 nm with a mean value of 3 500 photons per image. After the experiment, analysis of images of the local microenvironment enables correction of artifacts related to multiple nanoparticles in proximity. The size of the region of interest on the camera and the bandwidth of 300 Hz of the objective lens mounted on a piezoelectric stage limit the maximum velocity of a particle that the system can track. These capabilities are sufficient to track the diffusive motion of a sphere with a radius of 1 nm in water at room temperature, based on

FIG. 6. A schematic showing how systems for tracking single particles can combine moving parts or confining devices with a small number of light sensors. (a) A piezoelectric stage provides nanometer resolution and kilohertz bandwidth, relying on a light sensor and feedback control for guidance. (b) A tracking system combines a piezoelectric actuator guiding an illumination volume and an EMCCD camera with a region of interest of tens of pixels to track fluorescent beads in three dimensions.96 (c) A technique combines a nanofluidic channel with a sequential pair of illumination volumes and avalanche photodiodes to track and superresolve fluorescent DNA molecules in one dimension.97 (d) A single avalanche photodiode senses light at the nanosecond scale of the lifetime of an organic fluorophore, relying on a piezoelectric actuator for spatial information.
the axial range of 750 nm. Axial focusing based on adaptive optics overcomes this bandwidth limitation.\(^{100}\) Reducing the inertia of the optomechanical system for axial tracking\(^{101}\) or exploiting recent improvements in the design of diffraction optics optimized for axial range\(^{77}\) can conceivably provide the improvement of a few fold in axial range that is necessary for tracking single fluorophores with this apparatus.

2. Tracking a confined particle

Ideally, the design of an apparatus optimally distributes the demands of an application among the various components of the apparatus with minimal perturbation of the sample, as in a previous example.\(^{98}\) In some applications, however, implementing a controlled perturbation of the microenvironment that confines a particle for tracking is a more appropriate technique. Tracking the transition from ballistic to diffusive motion of a colloidal microparticle in a fluid is one such application near the edge of the map in Fig. 1. Tracking this transition for a particle with a radius of 500 nm requires spatial precision of \(<1\) nm and temporal bandwidth of \(>1\) MHz, according to hydrodynamic calculations.\(^{102}\) The typical frame rates of cameras, which are slower than 1 MHz, preclude the use of such imaging sensors for detection over a wide field. However, a spatial range of only a few tens of nanometers is relevant to this application, so that balanced photodetectors become a viable alternative for tracking the centroid of a particle with a radius of 500 nm.\(^{102}\) This technique trades off reduced spatial range for increased temporal bandwidth. To track the ballistic-to-diffusive transition, a pair of counter-propagating laser beams forms a shallow optical trap,\(^{102}\) confining the center of a dielectric particle to a spherical volume with a diameter of 50 nm. The perturbation of the motion of the particle is minimal near the center of the potential well of the trap, so that thermal fluctuations from collisions with fluid molecules dominate the velocity of the particle. Statistical variation of the intensity of these impacts governs the ballistic-to-diffusive transition. Deflection of one of the two trapping beams by the particle indicates a one-dimensional component of the motion of the particle on the balanced photodetector. The confined range of motion of the particle enables the reduction of spatial range necessary to reach the spatial and temporal bandwidth that are necessary for this application. The temporal bandwidth of 75 MHz of the balanced photodetectors limits this tracking system.\(^{102}\) Nonetheless, this measurement enables validation of the equipartition theorem at short length scales, as Sec. VI A discusses. Fluidic devices implement confining microenvironments for particle tracking. One technique uses a nanofluidic channel to transport single molecules of fluorescent DNA through a sequential pair of illumination volumes and avalanche photodiodes for tracking in one dimension with a temporal bandwidth of 100 kHz.\(^{103}\) This technique allows uniform analysis of each DNA molecule with high throughput. Without feedback, however, this technique is sensitive to microscope drift, requiring submicrometer alignment of two focused lasers and a nanoscale channel in free space. This limitation emphasizes the utility of imaging techniques that use fiducials to correct for microscope drift, as well as techniques with feedback that enable active reconfiguration of tracking systems during measurement. Sec. VI E discusses the intersection of fluidic devices and particle tracking in more detail, enabling techniques for tracking single and multiple particles, and applications involving strongly confining microenvironments.

C. Compressed sensing

Sampling a signal at the Nyquist rate enables the reconstruction of the signal without assuming any prior information, apart from the bandwidth, of the signal. Over the last decade, mathematicians realized that identifying a natural basis, such as time, in which the set of candidate signals is sparse, enables the reconstruction of the true signal by measuring far fewer samples in a sufficiently incoherent measurement basis, such as frequency, than Nyquist sampling requires. Here, incoherence measures the maximal correlation between the natural sparsity basis and the measurement basis—see this introductory article\(^{104}\) for the definitions. This concept, termed compressed sensing,\(^{105}\) has motivated a vast amount of research, recently extending to particle tracking.\(^{106}\) Compressed sensing is relevant to this article because of the power of this algorithm in using prior information and the interest in explicitly incorporating compressed sensing into the design of optical microscopes for particle tracking. Such use of prior information is not limited to techniques related to tracking single or multiple particles.

A puzzle builds intuition of how to exploit sparsity—how can an experimentalist identify one heavy marble among a set of \(2^N\) otherwise identical marbles by weighing the marbles against each other using a balance scale? For ease of exposition, this puzzle assumes exact information of the sparsity structure—the existence of only one heavy marble—but this assumption is not necessary for more general measurements. The efficient solution involves weighing subsets of \(2^{N-1}\) marbles against their complementary subsets in each measurement. By relying on the sparsity of the number of heavy marbles, the experimentalist designs each measurement to extract a unique bit of information, enabling the identification of the heavy marble in just \(N\) measurements. A less sophisticated experimental design that requires \((2^N – 1)\) pairwise measurements comparing, for example, the first marble to each of the rest, is less efficient in the number of measurements since this design does not exploit the inherent sparsity. Using the definition of incoherence\(^{104}\) and representing the possible set of marbles and measurements of weight with appropriate sparsity and measurement bases, the more efficient design involves a maximally incoherent pair of bases.

Reconstruction algorithms for inferring more general sparse signals are analogous to the inference process for the efficient solution to this puzzle and are robust to incomplete information of the sparsity structure. Moreover, such algorithms can use additional assumptions about the signal to reduce computational expense and increase robustness to measurement noise.\(^{106}\) Recent work using such algorithms and prior information of the imaging point spread function
of a single fluorophore demonstrates the possibility of localizing up to an order of magnitude more emitters per superresolution image in two and three dimensions, in comparison to the ordinary requirement that the images of emitters do not overlap. This enables superresolution imaging of a sample with higher temporal bandwidth. Such algorithms, in combination with an imaging architecture that incorporates the requisite incoherence, capture two-dimensional images of aperiodic events at the surprisingly high rate of $10^{11}$ Hz.

In summary, if single particles or particular aspects of a microenvironment are of primary interest in an application, then a tracking system can use feedback or confinement to focus on those particles or aspects while trading off other measurement capabilities. Prior information constrains the size of the class of possible objects that are attributable to the detected signal. Though subtle, such information is powerful in reconstructing the relevant distribution of the quantity of interest in the sample and in designing an apparatus.

VI. APPLICATIONS

Applications of particle tracking extend across a broad spectrum of science and technology, reflecting the accessibility of optical microscopes, the utility of nanoparticles as probes of microenvironments, and the interest in measuring and exploiting the properties of nanoparticles. In the context of the measurement framework and review of apparatuses and techniques, this section reviews applications of particle tracking in five broad categories. These categories overlap due to the multidisciplinary nature of many of the applications. Sec. VI A reviews the diffusive motion of particles in fluids, which is commonly present in the following applications. Sec. VI B reviews applications of nanoparticle tracking in biological systems, extending the historic interest in imaging biological systems by optical microscopy to probing the structure and function of the systems at the nanoscale. Sec. VI C reviews applications of particle tracking in fluid and material systems, which are evolving from classic measurements of flow fields and strain fields into diverse studies of the nanoscopic details of microscopic materials. Sec. VI D reviews applications of particle tracking involving fabrication and assembly processes at the microscale and nanoscale, which are useful for constructing functional devices. Sec. VI E reviews applications of particle tracking in, on, or around engineered devices to characterize either the particles or the devices.

A. Particle diffusion

In many applications of particle tracking, the experimental system of interest involves a fluidic microenvironment, in which the thermal motion of fluid molecules imparts diffusional motion to particles. Because of the common presence of diffusion, this topic merits a separate discussion as a prelude to the following review of applications.

Particles exhibit free diffusion in an isotropic fluid that is free of any obstacles. This is probably the most common stochastic motion in applications of particle tracking and emphasizes the duality of particles as probes and measurements. Specifically, the Stokes-Einstein relation connects the temperature and viscosity of the fluid environment to the hydrodynamic size of a particle. In the case of free diffusion, the mean squared displacement (MSD) of the particle increases linearly with time. Analysis of this trend gives the diffusion coefficient, however, a careful analysis is necessary to obtain an unbiased estimate of the diffusion coefficient from tracking data with finite trajectories and motion blur. In addition to the localization precision that is achievable for static particles, as Inequality (2) states, additional localization errors result from motion of a particle. Diffusional motion blurs the signal from the particle over a larger area of the detector. For an ordinary microscope–camera apparatus, the localization error from motion blur becomes comparable to the localization precision for a static particle, as the exposure time of the camera in a single frame becomes comparable to the time for the particle to diffuse a distance equal to the standard deviation of the point spread function of the microscope. This additional error, along with the exact nature of the camera exposure and the resulting motion blur—see Equation (5), and related discussion, in Ref. 109—can bias the estimate of the diffusion coefficient. The underlying reason for this bias is that such estimates often assume the independence of mean squared values of particle displacements in successive time steps. The motion of the particle can, however, introduce correlations between successive displacements. The lowest uncertainties in an estimate of diffusion coefficient occur in the regime where such correlations are known as a function of the length of the trajectory, using optimal algorithms that approach those estimates.

Anomalous diffusion requires a more involved analysis. This term applies when the mean squared displacement of a particle grows non-linearly with time. Sub-diffusion is a common occurrence in applications of particle tracking that probe biological systems with a heterogeneous microenvironment. Identification of the underlying source of this heterogeneity is challenging due to certain confounding aspects of the predictions of physically different models. Continuous-time random walks (CTRW) and fractional Brownian motion (fBM) are different physical models that nonetheless predict a superficially similar sub-diffusive motion from the trajectory of a single particle. Continuous-time random walks, in contrast to fractional Brownian motion, assume a long-tailed distribution of waiting times between consecutive jumps of a diffusing particle and are non-ergodic, meaning that a return of the particle to a previous position does not ensure the same waiting time before the subsequent step. Evaluation of higher order moments of the particle displacements can allow identification of the physical model responsible for the anomalous diffusion. Analytical methods can also extract the effect of localization errors in anomalous diffusion. Tracking spherical microparticles with radii of 0.5 μm to 5 μm in nematic fluids at 1 Hz shows that the diffusion of the particles becomes anomalous at time scales corresponding to the orientational relaxation times of the constituents of the nematic fluids.
Experiments of this type reveal the heterogeneous properties of a host of artificial and biological microenvironments.

Active tracking techniques are useful for studies of diffusional motion. Sec. V B 2 reviews an active technique based on a combination of particle trapping and tracking to study the statistical physics and hydrodynamics in the ballistic-to-diffusive transition of the motion of colloidal microparticles in a fluid.5,114 This technique enables measurement of particle displacements of 10 pm in one dimension at intervals of <50 ns,5 allowing observation of the effect of the Basset force in hydrodynamic interactions between the particle and the fluid. This force is a result of the motion of a particle creating vortices in the fluid115 that affect the particle motion at later times, a phenomenon of fundamental importance in the behavior of biological media116 and non-Newtonian fluids.117 In another active tracking technique, a focused laser follows particles of varied sizes diffusing at an oil–water interface. This measurement is relevant to the characterization of emulsions and other complex media. In this interfacial microenvironment, the scaling of diffusion with respect to nanoparticle size shows an unexpected deviation, with several possible causes.118

A perturbation to particle diffusion occurs if the optical illumination significantly heats the particle. Heat transfer from the particle to the surrounding microenvironment increases the temperature around the particle, increasing the diffusivity of the particle and potentially confounding related analysis. Ignoring this effect, for example, can lead to an underestimate of the size of the particle due to the increased diffusivity of the particle. But a correct analysis is complicated, as the physical models describing the heat that the particle absorbs and transfers to the fluid, and that the fluid dissipates, all vary with the properties of the particle and fluid. Some of the related subtleties for gold nanoparticles, for which local heating is of great interest,119 are as follows. The absorption cross-section of a gold nanoparticle at the wavelength of illumination influences the amount of heat that the nanoparticle absorbs. The time scale of the conduction of heat across the nanoparticle is much faster than the time scale of the diffusion of the nanoparticle by a distance equal to the diameter of the nanoparticle.119 Therefore, the nanoparticle is effectively a local hot spot with a temperature set by the heat capacity of the nanoparticle and the heat transfer to the fluid. This heat transfer depends on the interfacial resistance to heat conduction and sets the temperature at the boundary layer of the fluid. The temperature of the boundary layer can substantially differ from the temperature of the nanoparticle,120 depending on the properties of the interface, which also vary with temperature. The temperature at the boundary layer, along with a steady state continuum model for the hydrodynamics, specifies a physical model describing the increased diffusivity. Experimental measurements validate this model for gold nanoparticles.119 This model does not directly depend on the type of particle but does require the temperature at the boundary layer as an input to estimate the increased diffusivity. In addition to increasing diffusivity, particle heating, as well as direct heating of the microenvironment from optical illumination, has the potential to perturb or damage sensitive biological or cryogenic microenvironments.

In biological samples, the diffusion of nanoscale particles occurs in microscale fluidic environments that are structurally complex and that manifest additional transport phenomena and biochemical interactions. Sec. V I B reviews such applications of particle tracking.

B. Biological systems

Apart from a core set of quantum mechanical principles involved in all chemical reactions, classical descriptions of macromolecules and larger structures explain the organizational principles that govern most,121 but not all,122 known physiological processes. However, there is a relative scarcity of measurement tools for probing in vivo processes123 without significantly perturbing the relevant physiological conditions. Particle tracking can partly, and relatively conveniently, address this issue by the use of functionalized nanoparticles.124 More generally, particle tracking is useful for probing physiological processes at the nanoscale and microscale, in vivo and otherwise. This section reviews applications that realize these advantages, starting at the level of biomolecules, with a technique relying on tracking the combined signal from an ensemble of particles.

Dissociation rate constants between ligands and receptors are important in cellular signaling. Fluorescence correlation spectroscopy (FCS) is a classical technique to infer molecular binding by monitoring fluctuations in the fluorescence signal from a presumably uniform ensemble of fluorescent ligands. The fluorescence intensity from each ligand depends on whether the ligand is bound to the receptor. Instead of passively monitoring an ensemble, an active tracking technique125 enables measurement of the temporally varying charge and size of a single biomolecule undergoing conformational changes during binding and unbinding. An electrokinetic trap with position feedback weakly confines such a biomolecule to a planar region with a width of several micrometers. Within each interval of 20 μs of the feedback loop, the apparatus scans a laser across the trap and localizes the photon emission from the biomolecule using an avalanche photodiode. In this way, the apparatus tracks the residual drift and diffusion of the biomolecule in the trap, providing the charge and size of the molecule. Confining the biomolecule to the trap allows for long observation times. Though perturbative, the electrokinetic trap is potentially less perturbative than surface interactions that could alternately confine the molecule but also more severely confine the conformational degrees of freedom of the molecule and obscure the binding kinetics. In general, combining tracking signals and actuation signals for trapping particles tethered to biomolecules is a useful approach for measuring the mechanical properties of biopolymers such as DNA.126

Sec. V A 2 reviews the technique of fluorescence imaging with one nanometer accuracy, showing that the myosin dimer, which is responsible for transporting cargo within the cell, moves in a hand-over-hand motion inside the cell using.57 Tracking a gold nanoparticle bound to a myosin dimer7 with darkfield microscopy at frame rates of >3 kHz...
reveals that the hand-over-hand steps of 75 nm of the dimer consist of several sub-steps, including a rotational diffusion of the heads of the myosin dimer.

Tracking the diffusion of lipid molecules in a cell membrane at high frame rates over a small region of interest of an imaging sensor elucidates the structure of a cell membrane in two dimensions. Gold nanoparticles bound to the lipid molecules provide the high signal-to-noise ratio necessary for precise localization at intervals of 25 μs within the cell membrane. Analysis of the tracking data shows that the cell membrane, rather than being a homogenous entity, consists of compartments with widths of approximately 200 nm. The value of the mean squared deviations of the lipid molecules at long time intervals results from a hop–diffusion motion of the molecules between adjacent compartments, with each compartment weakly trapping the molecules. Estimates of diffusion coefficients provide residence times in the trap, which differ depending on the duration of the time intervals for analysis of the tracking data. Other studies have investigated aspects of hop–diffusion behavior in cells, although the precision of the estimates of such behavior at short time intervals could benefit from more rigorous analysis. The use of confocal microscopy to track the intracellular random walks of fluorescent granules of insulin with radii of 150 nm at a bandwidth of 1 Hz reveals non-ergodic motion of the granules during the insulin secretion process.

The structure of biological membranes and the resulting influence on biological processes are of significant interest, with advancing measurements enabling revisions of existing models of membrane structures. There is a particular need for techniques to measure membranes with a spatial uncertainty of 1 nm and a temporal uncertainty of 1 μs. A wide field of view is useful for tracking multiple interactions within a membrane, but the typically lower frame rate of an imaging sensor limits temporal bandwidth. The combination of temporal structuring of illumination and spectral resolution of detection is a potential solution to this problem. In one example, two fluorophores that are spectrally distinct probe a biomolecule, and two lasers on a time delay excite the fluorophores. A dichroic wedge disperses the two fluorescent emissions onto separate portions of the imaging sensor. The time delay of the laser determines the temporal bandwidth of localization of the biomolecule. Correlation of the two signals after the experiment enables tracking of the diffusion of the biomolecule in two dimensions at a bandwidth of 10 kHz.

Apart from reducing background noise, spatially structuring illumination plays an important role in reducing phototoxicity in biological samples. The combination of Bessel beam scanning with two-photon microscopy reduces the axial thickness of a light sheet to 0.5 μm. Rapid scanning of this sheet enables the tracking of chromosome motion during cell division at a bandwidth of 1 Hz over 10 min without stopping cell division.

An advantage of photoactivated localization microscopy and related techniques that rely on sparse switching of emitters, and differ essentially in the states that are switched off, is the ability to combine these techniques with other imaging techniques. For example, the introduction of astigmatism into the imaging path of a superresolution apparatus improves axial precision. The combination of sparse switching with 4Pi detection enables imaging of intracellular structures with an isotropic precision of <10 nm. The combination of stimulated emission depletion with 4Pi detection enables imaging of intracellular structures.

Optical tracking of nanoparticles is a relatively new approach towards measuring biological microenvironments. In contrast, related techniques have a longer history of measurements of fluids and materials, and new applications are emerging as the techniques advance, as Sec. VI C discusses.

C. Fluids and materials

Particle tracking has an extensive record of application to the measurement of velocity fields that inform of fluid dynamics and strain fields that evolve during material testing. More recently, experimentalists have taken advantage of the ability to functionalize nanoparticles to interact with specific materials or respond to local variations in material properties to probe nanoscopic interactions and effects in microscopic material systems in greater detail.

An attractive feature of tracking the diffusion of particles in a fluid is that the intrinsic energy scale of such measurements is $k_B T$, where $k_B$ is Boltzmann’s constant and $T$ is the absolute temperature. This enables precise measurement of the interaction potentials between multiple colloidal particles, which has led to many discoveries in colloidal science. There is also an associated history of algorithms for inferring such potentials.

Tracking the motion of multiwalled carbon nanotubes using a total internal reflection microscope with a CCD camera at a frame rate of >100 Hz reveals the mutual and surface interaction potentials of the nanotubes. A confining fluidic device with a depth of 300 nm controls the potential between nanotubes with a length of 200 nm and the surfaces of the device, and the deposition of the nanotubes on the surface in the presence of the potential. Scanning probe techniques would otherwise introduce additional potentials that could confound the measurement. This technique allows the inference of excluded volume interactions between charged species that are of interest in biological systems and in self-assembly processes mediated by fluids. Sec. VI E discusses confining microfluidic and nanofluidic devices in more detail.

Magnetic forces are useful for externally controlling the transport of nanoparticles in microfluidic environments, without otherwise perturbing a nonmagnetic microenvironment. Darkfield microscopy allows tracking of nanoparticles with iron oxide cores and gold shells to differentiate between magnetic, viscous, and thermal forces for applications in living cells and in microfluidic devices. In this study, the magnetic potential energies of the nanoparticles range from zero, corresponding to motion due only to diffusion, to $>10 k_B T$. Sec. VI E discusses an application of particle tracking combining magnetic forces and microfluidic confinement for nanoparticle magnetometry.

Plasmonic interactions between electromagnetic fields and free electrons in metals are of intense interest for
numerous applications. Engineering plasmonic interactions in metallic nanostructures allows control over the direction, intensity, and frequency of the light exiting the structure. By electrokinetically trapping quantum dots near silver nanowires and imaging the residual motion of the quantum dots in two dimensions with a precision of \( \approx 10 \text{nm} \), intensity fluctuations of quantum dots provide an estimate of the local density of optical states along different positions of the nanowire axis. This technique can presumably reveal plasmonic interactions with emitters near other kinds of nanostructures.

Cryogenic fluids have interesting material properties such as low kinematic viscosity and superfluid dynamics. Microscale particles of frozen hydrogen probe the velocity distribution of quantum mechanical vortices in superfluid helium. These velocities differ from the velocities of particles in the classical background flow, clarifying the fluidic connection between vortices. This study motivated further research into experimental techniques for reliably dispersing fluorescent nanoparticles into cryogenic fluids. These brighter nanoparticles allow for lower irradiance and less thermal perturbation of the cryogenic environment. Theories predicting non-classical diffusion characteristics at short time scales for particles that are not trapped in the quantum vortices, have not yet been experimentally validated. Investigation of zero-point fluctuations in the quantum regime with particle tracking is also possible.

Particle tracking can provide information on interfacial stresses, which are important in many material systems. Traction force microscopy enables stress measurement at the interface of a sample and a known substrate by using strain measurements obtained from tracking particles in or on the substrate. A typical application is to measure the stress applied by a structure undergoing deformation, for example, a collagen network in a cell, on an elastic substrate with embedded fluorescent particles. Motion of the particles, in combination with prior information of the nature of the elastic response of the substrate, provides information about stresses in structures spanning many length scales in biology and materials science. For a soft substrate, the strain measured with particle tracking gives the stress exerted by the sample by solving a Hookean boundary value problem.

The viscoelastic response of a material depends on critical scales of length and time. The scales at which the bulk response sets in is a topic of active research. Tracking the motion of nanoparticles within a viscoelastic material gives the frequency dependence of stress–relaxation at the microscale. In certain frequency ranges, the structures in a polymer solution that cause the viscoelastic response are much smaller than the nanoparticles that probe the material. This separation of length scales justifies the assumption that the stress–relaxation behavior of the fluid strained by a moving nanoparticle is identical to that of the bulk fluid subjected to a macroscopic strain. Monitoring the motion of nanoparticles by light scattering provides information about the stress moduli of the solution at kilohertz frequencies. Light sensors limit the temporal bandwidth in this study, but the versatility of this technique has motivated further research, including studies of its theoretical limits.

Tracking single molecules enables characterization of silica gel nanostructures and correlation of diffusion pathways with morphological information from electron microscopy. Such measurements provide insight into the heterogeneities that develop in thin polymer films as solvent exposure plasticizes the films. The combination of photoactivated localization microscopy and astigmatic imaging allows inference of the conformations of a single polymer, a determinant of the macroscopic properties of the polymer, with lateral precision of \(<20 \text{nm}\) and axial precision of \(<40 \text{nm}\). Ideally, experimental studies would report such values for precision along with the relative positions of the emitters, as these values vary across the field of view of an astigmatic imaging apparatus.

Fabrication and assembly processes exploit the properties and dynamics of microscale and nanoscale fluids and materials to form functional devices and assemblies. Sec. D reviews applications of particle tracking to probe such processes.

### D. Fabrication and assembly

Top-down fabrication and bottom-up assembly processes enable the production of functional devices and assemblies at the microscale and nanoscale. This article briefly reviews emerging applications of particle tracking to elucidate the spatial properties and temporal dynamics of these processes.

During the formation of a latent image in a chemically amplified photoresist film, the spatial distribution of photoacid molecules is a critical factor in the final quality of a photolithographic pattern. Photoactivated localization microscopy enables superresolution imaging of the spatial distribution of photoacid molecules in two dimensions with a precision of 50 nm limited by unintended motion of the microscope. Both ultraviolet light and protonation can activate the organic fluorophores attached to the photoacid molecules.

Beyond semiconductor nanofabrication, DNA nanotechnology enables the manufacture of complex nanostructures integrating multiple components, and particle tracking can provide insight into such assembly processes. A focused laser spot moving in a feedback loop tracks the binding of quantum dots to nanoscale DNA origami scaffolds. Limiting the tracking volume and increasing the tracking signal allow identification of distinct origami–quantum dot conjugates by simultaneously extracting diffusion coefficients and analyzing the statistics of photon emission.

Particle tracking elucidates mechanisms determining the fluidic assembly of particles. The coffee ring effect, a description of how colloidal particles deposit at the receding contact line of an evaporating fluid, is a model assembly process for study by particle tracking. The coffee ring effect is a non-equilibrium process that, apart from fluid properties, depends on particle size, and externally applied fields. This complexity motivates tracking systems that simultaneously measure a variety of the degrees of freedom of optical microscopes. Optical coherence tomography tracks microparticles in three dimensions in a sessile
drop with a height of 300 μm, achieving a spatial resolution of 1 μm, and a temporal bandwidth of 1 kHz.  

These and other fabrication and assembly processes are useful for constructing microscale and nanoscale devices with engineered functions. Tracking particles in, on, and around the devices can characterize the particles and test the devices, as Sec. VIE reviews.

**E. Engineered devices**

Microscale and nanoscale devices are complementary to particle tracking in several ways. In one manifestation of device function, fluidic devices use solid–fluid interfaces to structure confining microenvironments in which hydrodynamic, electrostatic, electrokinetic, entropic, and steric interactions are useful. Through such interactions, fluidic devices control the motion of nanoparticles, while nanoparticles also probe the confining microenvironments. For other device technologies, the motion of a device enables the function of sensing or actuating the surrounding microenvironment. However, such motion may be too small, fast, or complex to measure using existing techniques. In such applications, particles serve as optical probes of the motion of a device to test the performance and reliability of the device. This again shows the duality of nanoparticles as probes of microenvironments, and microenvironments as media to manifest the properties of nanoparticles.

In a simple but useful combination of microfluidic confinement and particle tracking, a slit confines nanoparticles to an observation volume with a depth of 10 μm, which is approximately three times greater than the depth of field of an objective lens with a low magnification. This prolongs the tracking by darkfield microscopy of the diffusion in two dimensions of gold nanoparticles, which otherwise diffuse far out of focus. This increased temporal range confers improved statistics in the analysis of diffusion, and a more precise estimate of nanoparticle size from the Stokes–Einstein relation. For an accurate estimate of the hydrodynamic size of a nanoparticle from free diffusion, the slit must be deep enough such that occasional interactions of the nanoparticle with the slit surfaces do not strongly bias the transport of the nanoparticle.

Reducing the dimensions of a microfluidic device results in decreasing diffusivity of a nanoparticle through hydrodynamic interactions with the confining interfaces. Such interactions become more important as the complexity of confinement increases. For example, arrays of nanoscale posts, spaced from 10 μm to 1 μm apart in a microfluidic slit, are representative of a complex confined medium. As the spacing between posts decreases and confinement increases, the diffusion of the nanoparticles slows and multiple relaxation times emerge, which is consistent with the onset of dynamic heterogeneity and the approach to vitrification.

As the characteristic scale of confinement decreases further into the submicrometer length scale, hydrodynamic interactions become even stronger, and, depending on the ionic strength of an electrolyte solution, electrostatic interactions also become stronger. In a manifestation of these interactions, nanofluidic slits with depths from 300 nm to 600 nm electrostatically confine gold nanoparticles around the centers of the slits. Total internal reflection microscopy allows tracking of nanoparticle diffusion in three dimensions. Diffusion coefficients of gold nanoparticles in these slits are substantially lower than expected after accounting for hydrodynamic interactions, possibly due to electroviscous effects. In an application of these interactions, modification of the geometry of nanofluidic confinement traps nanoparticles. The confining geometry of a trap and the ionic strength of the electrolyte solution modify the trap stiffness. Interferometric light scattering allows tracking of the distribution of gold nanoparticles in three dimensions in these traps. This measurement gives an estimate of both the size and charge of nanoparticles. Electrokinetic forces are useful to transport charged nanoparticles in fluidic microenvironments. Total internal reflection microscopy allows tracking of the electrophoresis of fluorescent beads in three dimensions in a nanofluidic slit with a depth of 100 nm. This measurement shows the effects of hydrodynamic interactions and electrical double layer interactions on the mobility and distribution of nanoparticles. Electrokinetic forces are useful to transport charged nanoparticles in fluidic microenvironments. Total internal reflection microscopy allows tracking of the electrophoresis of fluorescent beads in three dimensions in a nanofluidic slit with a depth of 100 nm. This measurement shows the effects of hydrodynamic interactions and electrical double layer interactions on the mobility and distribution of nanoparticles. In one technique, a sequential pair of illumination volumes and avalanche photodiodes enables tracking of DNA molecules in one dimension with a temporal bandwidth of 100 kHz. Analytical modeling of bursts of fluorescence allows measurement of DNA length with an optimal resolution of 100 nm and an analysis time of 20 ms per molecule. In comparison, an EMCCD camera enables direct imaging of DNA transport with a frame rate of 0.4 kHz.

In an a different application of hydrodynamic and electrostatic interactions in confinement, a microstructured solid–fluid interface guides the magnetophoresis of ferromagnetic nanorods for quantitative magnetometry. Through-focus scanning optical microscopy enables tracking of particles and surfaces in three dimensions, allowing simultaneous measurement of particle velocity and particle–surface proximity to accurately determine viscous drag in a force balance. Nanoparticle shape influences this force balance and the resulting accuracy of analytical magnetophoresis.

As the dimensions of nanofluidic devices decrease further to the sizes of nanoparticles, and below, steric and entropic interactions strongly influence the transport of nanoparticles and reveal relevant properties of the nanoparticles. Size exclusion in a nanofluidic device with a depth profile approximated by a staircase function separates nanoparticles. The nanofluidic staircase functions as a separation matrix and reference material. Tracking fluorescent beads and mapping positions of size exclusion to step depths gives the size distribution of the nanoparticles with a resolution of approximately 20 nm. In a shallower nanofluidic staircase with step depths of approximately 10 nm, DNA molecules with a chain diameter of several nanometers, a nominal persistence length of 50 nm, and a radius of gyration of several hundred nanometers, exhibit a different type of motion related to conformational entropy. Rather than being excluded from the shallowest regions of the nanofluidic staircase, DNA
molecules enter these regions under an applied electrokinetic force. Upon cessation of the applied force, the DNA molecules recoil out of strong confinement to relieve the increased free energy of confinement, biasing the diffusion of the DNA molecules over a spatial range of greater than one hundred micrometers and over a temporal range of tens of minutes. There are other techniques to implement fluidic devices that strongly confine nanoparticles and biomolecules for tracking and analysis, while avoiding nanofabrication. For example, pressing a convex lens into a coverslip creates an observation volume with a height varying from hundreds of nanometers down to zero nanometers. Fluidic devices with dimensions in this spatial range structure confinement, rather than illumination, to reduce the observation volume of an optical microscope with widefield illumination.

Acoustic actuation of nanoscale and microscale particles in microfluidic devices is a topic of considerable interest due to the ease of integration and the ability to transport particles and mix liquids in such devices. Many questions remain about related interactions between particles and liquids. Darkfield microscopy enables the tracking of polymeric beads with radii of 200 nm beads, advecting in microvortical flows around gold nanorods in a microfluidic acoustic resonator. Analysis of hydrodynamic interactions allows classification of the beads as tracer particles that do not significantly perturb the microsystem. Prior information about the approximately circular orbit of the beads allows analysis of motion in three dimensions, revealing the kilohertz axial rotation of the nanorods.

Finally, micromechanical devices are increasingly useful for sensing and actuating microenvironments. Depositing and tracking particles on a rigid micromechanical device yields kinematic information without having to modify the design or fabrication of the device, or having to solve a Hookean boundary value problem, as in traction force microscopy on soft substrates. In the first demonstration of this technique, a sparse constellation of fluorescent nanoparticles indicates the motion of a scratch drive actuator over a surface. Fitting rigid transformations to successive images of the particle constellation reveals aperiodic variations in the stepwise motion of the device with a lateral precision of 2 nm and 100 μrad at a bandwidth of 1 Hz. Particle tracking of rigid microstructures complements edge tracking and introduces new capabilities, enabling simultaneous measurement of multiple degrees of freedom, improving centroid precision and orientation precision by scaling up constellation size, and building on the existing theoretical foundation of localization microscopy for rigorous evaluation of measurement uncertainty.

VII. CONCLUSIONS AND OUTLOOK

Optical microscopes are widely accessible and nanoparticle tracking is broadly applicable. Consequently, experimentalists from diverse backgrounds might initially approach a variety of measurements without necessarily having a clear understanding of the capabilities of optical microscopes for nanoparticle tracking. To clarify these capabilities, this article describes the theoretical and experimental limitations of tracking systems, and in this context reviews apparatuses and techniques for, and applications of, nanoparticle tracking. In this way, this article aims to make the initial approach towards designing tracking systems more systematic, and to provide a common basis for considering the many aspects of this topic.

Several trends will drive future research in particle tracking. The ongoing development of apparatuses and techniques making use of prior information will continue. These apparatuses and techniques might include engineering optical emission in non-classical ways to improve localization precision, as well as identifying and incorporating sparse sampling schemes in algorithms and in hardware to improve both spatial precision and temporal bandwidth. Adaptive optics is more mature in astronomy than in microscopy and will enable clearer access to microenvironments within macroenvironments that induce optical aberration. On the hardware front, the continuing development of technologies for illumination, modulation, and detection will translate into improved capabilities for particle tracking. Light emitting diodes might become more prevalent for sample illumination due to the increasing ratio of performance to cost of these optoelectronic devices. Considering the issues of loss of sensitivity and ease of implementation, there is a gap between the capabilities of spatial light modulators and nanofabricated optical elements. The development of more efficient spatial light modulators or easier techniques for rapidly prototyping nanofabricated optical elements might close this gap. The incorporation of neuromorphic computing architectures into light sensors—for example, commercial imaging sensors already track the edges of objects—might enable the implementation of algorithms that automatically track particles at usefully fast rates. Practical implementation of recent theoretical techniques for optical computation using metamaterials might also enable faster tracking capability. Imaging sensors are becoming more economical, while the size of the pixels is scaling down towards, and perhaps eventually below, the diffraction limit. Placing a microenvironment in close proximity to such a sensor might obviate the need for the traditional hardware of an optical microscope to achieve superresolution imaging. While this article focuses on visible wavelengths, apparatuses, and measurements, other modalities of nanoparticle tracking are also emerging. For example, electron microscopy uses the much shorter de Broglie wavelengths of electrons to achieve an inherently higher imaging resolution. This has the advantage of allowing the tracking of translation of nanoparticles at the nanometer scale without the need for any superresolution technique and the disadvantage of requiring an enclosure to contain the fluidic environment within a vacuum system for measurement. The ongoing development of such technologies might eventually eliminate this disadvantage.

As a final point, there is an increasing understanding of optical microscopes not as imaging systems with diffraction limits, but as apparatuses to transmit information at visible wavelengths. Further, optical tracking of nanoparticles enables measurements of remarkably diverse microenvironments and microstructures, extending into disparate applications such as biological systems, cryogenic physics,
and nanofabricated devices. Therefore, it seems reasonable to expect the unexpected as particle tracking finds use in the future.

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