A Practical Guide to Analyzing and Reporting the Movement of Nanoscale Swimmers

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Abstract. The recent invention of nanoswimmers– synthetic, powered objects with characteristic lengths in the range of 10-500 nm - has sparked widespread interest among scientists and the general public. As more researchers from different backgrounds enter the field, the study of nanoswimmers offers new opportunities but also significant experimental and theoretical challenges. In particular, the accurate characterization of nanoswimmers is often hindered by strong Brownian motion, convective effects, and the lack of a clear way to visualize them. When coupled with improper experimental designs and imprecise practices in data analysis, these issues can translate to results and conclusions that are inconsistent and poorly reproducible. This Perspective follows the course of a typical nanoswimmer investigation from synthesis through to applications and offers suggestions for best practices in reporting experimental details, recording videos, plotting trajectories, calculating and analyzing mobility, eliminating drift, and performing control experiments, in order to improve the reliability of the reported results. *(end of Abstract)*

Powered motion at the micro- and nanoscales is an essential feature of life. Inspired by the various mechanisms of living organisms that swim at small scales,[1–3] the recent invention of nanoswimmers[4–9]—synthetic, powered objects with characteristic lengths in the range of 10–500 nm—has sparked widespread interest among scientists and the general public. For some, nanoswimmers are promising as synthetic machines that might cruise through the human body to diagnose and to cure disease (see ref 10 for our recent review and references therein). For others, the ways in which energy is converted to powered motion at small scales,[11,12] and how dynamic assemblies emerge,[13–20] reveal hidden physical rules that govern a broad range of natural phenomena that fall under the topic of active matter.[21–27] Motivated by these fundamental questions and possible applications, the study of nanoswimmers has advanced steadily over the past 15 years with a growing list of propulsion mechanisms, new materials, and fabrication techniques that endow diverse functionality, steering, and sensing strategies,[28,29] and reveal how simple, individual dynamics can give rise to complex multibody interactions.

As more researchers from different backgrounds enter the field, the study of nanoswimmers gains new opportunities but also significant experimental and theoretical challenges. With smaller swimmer size, Brownian motion becomes increasingly dominant in fluids such as water, and tracking by optical microscopy becomes more difficult. Moreover, understanding the propulsion mechanisms of nanoswimmers[30,31] may pose particular challenges for researchers who are new to colloid physics and chemistry, or to low-Reynolds-number hydrodynamics.[32,33] These challenges, elaborated in the next section and addressed throughout this Perspective, present obstacles to the correct interpretation of experimental data.

Drawing from our own experience working with nanoswimmers over the past 15 years, we present here a user guide for their study. In particular, we suggest practices that, when applied carefully and systematically, can significantly improve the reliability of analyses and prevent embarrassing mistakes. This guide is organized in the order of how a nanoswimmer study/research manuscript typically unfolds: sample preparation and experimental details, reporting nanoswimmer dynamics, analyzing propulsion mechanisms, and exploring applications. In each section, we describe key steps that should be taken to obtain meaningful results, and comment on possible artifacts and pitfalls that are common in reporting, analyzing, and understanding the movement of nanoswimmers.

Although the idea of a practical guide serving the nanoswimmer community has not been implemented previously, little of what we describe here is new knowledge, and a number of articles have already addressed the fundamental issues we discuss.[4,5,9,34–37] We hope that a closer look at the details of how each step is done will help to lower the barriers for researchers who are new to the field, and perhaps will help some experienced scholars as well. Note that this article is more useful for readers who already have some experience with nanoswimmers/micromotors. Readers completely unfamiliar with these topics, on the other hand, are directed to other resources that cover more basic concepts.[6,28,29,38]

The Two Principal Challenges of Nanoswimmer Studies

The two principal challenges in the study of nanoswimmers are rooted in the physics of their small size. First, living or synthetic nanoswimmers move in a tumultuous environment, constantly kicked and reoriented by the thermally driven, random movement of solvent molecules. This environment gives rise to Brownian rotation and translation,[39] and makes it challenging to confirm from observational data whether a nanoparticle is “active” or simply driven by thermal forces, because both result in diffusive behavior. The second fundamental challenge is that at least one dimension of a nanoswimmer is typically below the resolution limit of optical microscopy (~300 nm). As a result, it is challenging to observe them directly, and to record and to analyze their instantaneous speeds and trajectories. Super-resolution microscopy is one option,[40,41] and methods such as field gradient nuclear magnetic resonance and dynamic light scattering (DLS) have been used to provide indirect measurements of nanoswimmer diffusion (see ref 35 and references therein). But, practically speaking, the latter methods are often less available, less straightforward to use and/or to interpret, and are prone to errors and mistakes. The high spatial resolution required to locate a nanoswimmer precisely, and the high temporal resolution needed to differentiate its active propulsion from strong Brownian motion, combine to make the characterization of nanoswimmer propulsion challenging, much more so than for their micrometer counterparts.

Aside from these inherent limitations, researchers new to this field often encounter practical challenges, which can be tentatively grouped into three categories. The first challenge is how to make a nanoscale object move autonomously in water (or in an aqueous solution), which is surprisingly viscous to a nanoswimmer. The answer lies in different propulsion mechanisms, such as those relying on body twisting,[42–44] a chemical gradient,[31,45–48] or ultrasound.[49–52] These propulsion mechanisms are different from those that work efficiently with macroscopic machines. Finding the right materials to endow these properties, and constructing nanoscopic

objects of appropriate shapes and asymmetry, present significant challenges.

A second challenge, on which we focus in this Perspective, is finding the right tools to measure and to analyze the movement of nanoswimmers. For scientists new to the field, this question can demand a completely new set of tools, theories, and protocols, which we elaborate on below. Many things can go wrong at this stage, including video clips that are of poor quality, the presence of bubbles and drift, and incorrect calculation/interpretation of nanoswimmer mobility, to name a few.

These concerns are not hypothetical but are already widespread in the nanoswimmer literature. By surveying 40+ recent literature reports of nanoswimmers,[53–95] we see reoccurring inconsistencies that include blurry, drifting trajectories captured by optical microscopes,[58,60,61,65,68,73,79,88,92] inappropriate speed calculations and fitting of mean square displacement,[57,61,68,71–75,77,79,82,83,89] and lack of proper and complete control experiments.[68,70,72,89] These inconsistencies are often accompanied by other issues, such as an incomplete reporting of experimental details, a casual discussion of the propulsion mechanism of a nanoswimmer, and sometimes even a lack of supporting videos to confirm self-propulsion.[64,89] Moreover, since many of these studies aspire to apply nanoswimmers in a biomedical or environmental setting, mistakes in fabricating, measuring, and analyzing the movement of nanoswimmers continue to propagate and to undermine their utility in these applications.

A third challenge in studying nanoswimmers is understanding their dynamics and, in particular, their propulsion mechanisms. For example, how does surface catalysis convert the energy stored in a chemical into mechanical work, and with what efficiency does this process occur? How do ultrasonic waves operating at megahertz frequencies induce directional motion for a microrod that is orders of magnitude smaller than the wavelength? Why would a group of magnetic nanoswimmers aggregate and disperse in varying magnetic fields, and how does one control them? These fundamental questions are often difficult to unravel, yet their answers hold the keys not only to understanding why a particular nanoswimmer is effective in a certain applied setting, but, more importantly, how one can design nanoswimmers that swim faster, tow more strongly, deliver cargo controllably, and perform better overall. These questions are addressed in a companion article that focuses on the propulsion mechanisms of the major classes of nanoswimmers.[28]

Here, we embark on a virtual journey with an imaginary new researcher (Figure 1), who is working on a project that involves making a nanoswimmer that successfully self-propels, analyzing and reporting its motion, explaining its motion with a particular propulsion mechanism, and, finally, demonstrating a biomedical, analytical, or environmental application in which the presence of nanoswimmers improves the end result (be it pharmaceutical efficacy, cell sorting, or waste removal rate). A survey of the literature suggests that this is a typical story for nanoswimmer studies. Because each project involves different nanoswimmers, we provide general guidelines that emphasize scientific robustness and reproducibility, rather than listing all of the experimental details. However, we provide full descriptions of the tools and techniques needed to analyze nanoswimmer dynamics, because they are generically applicable to a wide variety of nanoswimmer experiments.

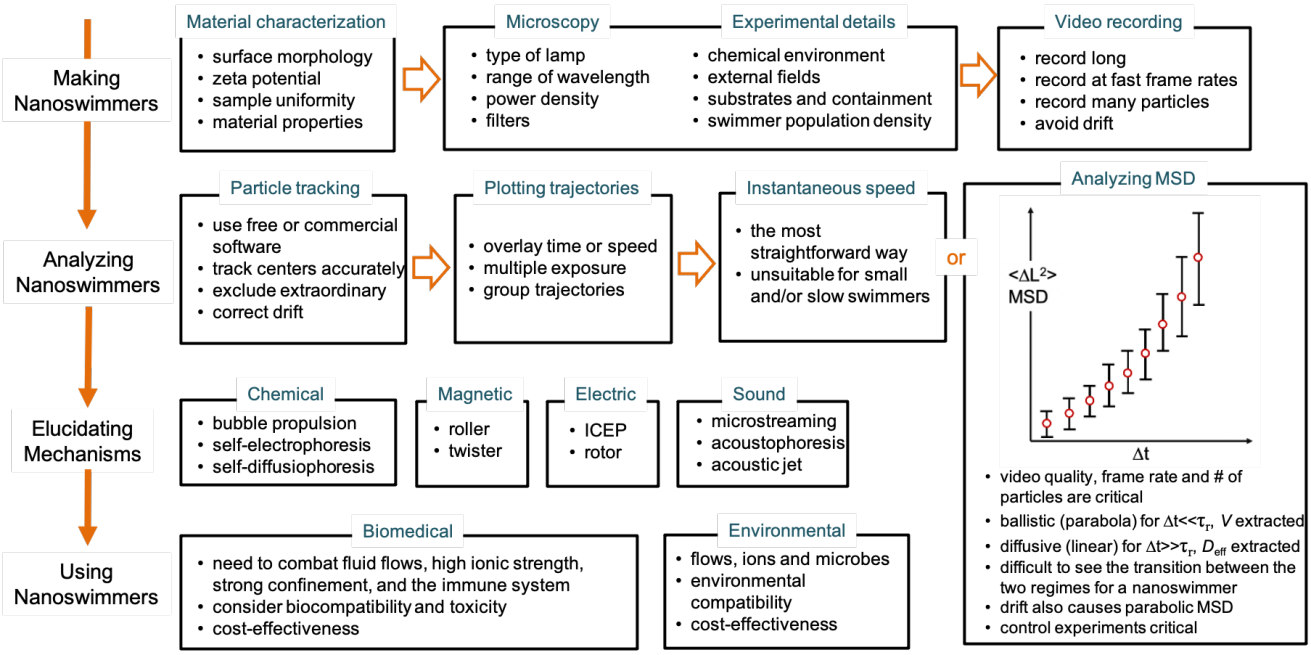

Figure 1. General scheme for analyzing and reporting the movement of nanoswimmers. ICEP stands for induced charge electrophoresis. MSD stands for mean squared displacement. τ_r, V and D_{eff} are the characteristic time scale for the rotational diffusion of a nanoswimmer, its ballistic speeds and effective diffusivity, respectively (see the section on "Reporting Speed/Mobility" for details).

Sample Preparation and Experimental Details

Fabrication and Material Characterization

A nanoswimmer experiment almost always starts with making the nanoswimmers, which is often done *via* chemical synthesis, physical deposition, or micro/nanofabrication.[96–99] Fabrication details are one place where issues of reproducibility can arise. For the majority of papers published by chemists, physicists, and material scientists, the standard protocol is to report the synthesis/fabrication procedure, followed by a set of characterization experiments (often including electron microscopy, spectroscopy, and X-ray diffraction) that describe the shape, size, and material composition of the samples. In addition to these data, we suggest that it is equally important to characterize and to report the following:

- Surface morphology, because many nanoswimmers rely on surface catalysis to propel, and are sensitive to surface roughness[100,101] as well as the spatial arrangement of the active components.[102,103]
- Zeta potential (related to the surface charge of a colloid),[104] because its magnitude and sign largely determine the speeds of phoretically powered nanoswimmers.[105]
- Sample uniformity (*e.g.*, size distribution), because the performance of a few selected nanoswimmers is often not representative of the entire population, especially when their

speeds are sensitive to shapes and sizes.

- Measurement of material properties, such as the photochemical current and/or light absorbance for a photo-responsive nanoswimmer, or the magnetic hysteresis for a magnetically powered nanoswimmer. These measurements are critical in achieving a quantitative understanding of propulsion.

Microscopy

An optical microscope is often the central piece of equipment used for studying nanoswimmers if their dimensions are comparable to or larger than the diffraction limit.[106,107] Beyond the common practice of reporting the type of microscope (upright or inverted) and the camera, as well as the frame rate at which videos were recorded, we strongly suggest that authors report details about the light source, such as the type of lamp (halogen, mercury, or light-emitting diodes), the range of light wavelengths, power density, and whether any filters are used, regardless of whether the samples are light-sensitive.

Reporting these details is not only important for reproducing results, but also because a lack of such details could lead to confusion or inconsistency. For example, mercury lamps are often used as a bright source of ultraviolet (UV) light in nanoswimmer studies. However, because the mercury lamp spectrum contains sharp lines (at 365, 405, 436, 546, and 579 nm[108]), its use as a UV light source requires visible light to be properly filtered or at least acknowledged. In addition, accurately measuring the power density (typically in mW/cm^2) of the light source is critical for cross-comparing results obtained in different labs, or even different samples from the same lab. Furthermore, it is often difficult to rule out light-related effects (photothermal, photochemical, *etc*.) on the movement of nanoswimmers that are assumed to be non-photoresponsive without complete knowledge of the light that is reaching the sample. In the same spirit, control experiments in the dark are helpful for eliminating possible light-induced artifacts (see discussion around Figure 5 for more details on control experiments).

Experimental Details

In order to improve reproducibility, to minimize artifacts, and to facilitate understanding of the results, we recommend including the following information in the experimental details of a nanoswimmer experiment:

- *Energy input.* For chemically powered nanoswimmers, it is critical to know what chemical species are present in the solution, and at what concentrations. One obvious reason is that nanoswimmers perform differently at different fuel concentrations (for examples, see refs 109,110). But more subtly, many types of chemical nanoswimmers, especially those powered by chemical gradients, could be sensitive to local pH (which changes their surface charge and, thus, their mobility), and to ionic strength (which changes the thickness of their electrical double layer and consequently their speeds[111,112]). For nanoswimmers powered by electromagnetic fields, critical details include the magnitude of the local electric or magnetic field and whether there is a gradient, or the way light is applied and at what power density. For ultrasonically powered nanoswimmers, it is important to report the details of the experiment setup, whether bulk or surface acoustic waves were used, whether experiments were conducted

at the levitation plane, a measurement of the local amplitude of the acoustic field,[113] and any sign of global or local acoustic streaming.[114] Furthermore, because the performance of a nanoswimmer under any powering mechanism can be time-dependent because of the degradation of materials or depletion of chemical fuels, it is useful for the sake of reproducibility to report the duration of an experiment and possibly the waiting time before a recording is made.

- *Substrates.* Most types of nanoswimmers are denser than the surrounding fluid, so they readily settle to a substrate that can decrease[115] or increase[116] their speeds, cause them to tilt,[117–120] or otherwise alter their trajectories.[121,122] Charged substrates typically produce electrokinetic flows in the presence of an electric field at a local (*e.g.*, a self-electrophoretic nanoswimmer[8,123]) or global (*e.g.*, the electrohydrodynamic (EHD) effect[124,125]) level, and also cause a nanoswimmer to float slightly above the substrate.[126,127] For example, the most common substrate used is a glass slide (or cover slip), the surface properties of which depend on the sample history and adsorption of molecules in the laboratory environment. Varying such surface properties, intentionally or not, can lead to surprising differences in nanoswimmer behaviors. As a result, experimentalists should be aware of these wall effects and measure the surface zeta potential, wettability, and roughness of the substrate used, among other relevant properties. However, if appropriate surface characterization is not available, researchers should adopt and report a systematic protocol for cleaning and preparing their substrates, in order to ensure consistency at least within their own laboratory.

- *Containment.* A nanoswimmer experiment is often conducted in containment (*e.g.*, a drop of liquid sandwiched between two pieces of glass and encircled by a polymer spacer). When constructing such a chamber, one needs to ensure that i) it is properly sealed from all sides to minimize convection and evaporation; ii) no contaminant is introduced from the container, either from its construction or prolonged exposure to the liquid; and iii) the entire setup, including the microscope stage and the table it is sitting on, is even and flat. These requirements are self-explanatory, but easier said than done. Carefully conducted control experiments with inert tracers (*e.g.*, polystyrene microspheres) can help confirm proper seals and minimal drift, and that the experiment is free from other unwanted effects, such as heating from a microscope lamp or air flow in the lab. Despite the critical roles of control experiments, it is unfortunate that properly designed positive and negative control experiments are not yet widely adopted in all nanoswimmer studies (see discussion around Figure 5 for more suggestions on control experiments).

- *Population density.* By varying the mixing ratio of a colloidal suspension with its medium, nanoswimmer suspensions of different number densities can be obtained. This quantity is often represented as the coverage percentage in the two-dimensional (2D) plane, ϕ. Although this value is most relevant in studies of dynamic assembly and the collective behavior of nanoswimmers, we argue that it is necessary even if swimmer–swimmer interactions are not the focus of the study. For one, it forces one to consider how dilute the suspension truly is, and the consequences of many-body effects as well as the consumption of chemical fuels over time. In addition, more than a few

nanoswimmers in the field of view are needed to acquire statistically significant tracking data, especially when calculating mean square displacement (MSD, see below). Importantly, examining the trajectories of many nanoswimmers is the easiest way to tell if global effects exist, such as convective drift, electroosmotic flows, or a gradient (*e.g*, in the case of chemotaxis). For these reasons, we do not recommend merely recording and reporting the trajectories and speeds of only one or two (or even a handful of) nanoswimmers (see refs 66, 70, 74, and 89 for examples). In the same spirit, we suggest reporting the population density of tracers as well.

Video Recording

Whether for the purpose of data analysis or visualization, a high-quality video clip displaying the self-propulsion of a nanoswimmer without drift is standard for confirming its successful operation. Beyond the typical advice given for acquiring high-quality videos, such as improving the optical resolution with a good camera and lens and optimizing the light path, we offer the following suggestions for acquiring scientifically robust video data from nanoswimmers:

- *Record a video that is as long as possible*. Although a short video clip (*e.g*., a few seconds) is often long enough to see that a nanoswimmer is moving about, a much longer video clip is needed to confirm the absence of drift, to generate enough data for plotting MSD with minimal errors (see ref 37, discussed in more detail below), or to examine collective behavior that often takes a while to develop. Beware though that recording a nanoswimmer for an extended period of time is often accompanied by unwanted side effects such as a gradual decrease in fuel concentration or a change in the nanoswimmer properties, irreversible sticking of the swimmer to the substrate, accumulation of gas bubbles that obstruct the view or cause drift, or nanoswimmers leaving and entering the field of view. Carefully designed experiments can balance these practical challenges with the requirement of recording a video of adequate duration.

- *Record as many particles as possible.* As we argued above, it is critical to track many nanoswimmers, even if a dilute suspension is used, to generate enough data for statistical analyses (Novotný *et al.* suggested a minimum of 90 tracks be used to calculate MSD at a confidence level of 95%[37]). However, for dilute suspensions, only a few nanoswimmers can typically be found in the field of view, especially when using a high magnification lens so that small nanoswimmers can be seen. To record more nanoswimmers, data can be collected by either moving around the field of view between recordings (at the risk of nanoswimmers changing their behavior over time), or by repeating the same experiment multiple times (at the risk of poor reproducibility across multiple runs).

- *Record at as fast a frame rate as possible.* As we elaborate below, videos at high frame rate are essential for identifying nanoswimmer dynamics at small time intervals, which is in turn critical for extracting their ballistic speed (*e.g*., a frame rate of >1000 frames per second, fps, is needed for a nanoswimmer 200 nm in diameter). However, because of the limited speeds of data transfer between cameras and computers and the limited buffer memory on cameras, high frame rates often require reduced frame size or resolution when recording videos, which in turn compromises its quality.

Analyzing and Reporting Nanoswimmer Dynamics

A milestone in any experimental study of nanoswimmers is demonstrating their successful operation. For fundamental studies, a successfully activated nanoswimmer is a prerequisite for studying how it interacts with its environment or its neighbors, or for understanding how and why it moves. For applications such as delivering drugs with improved efficacy,[61,62,75,78] or decontaminating waste water more effectively,[89] the fact that a nanoswimmer can self-propel is what makes it better than its passive counterparts.

In the case of optically resolvable nanoswimmers, their successful operation is often first described qualitatively, with trajectories of a few nanoswimmers over a period of time, then quantitatively, showing "mobility" acquired under various experimental conditions (most commonly by varying the fuel concentration or field intensity). Here, "mobility" can refer to the speed (either instantaneous speed or ballistic speed, see below) or the diffusivity of a nanoswimmer.

In this section, we cover the various methods of quantifying and analyzing nanoswimmer movement, highlighting potential pitfalls and recommended protocols that yield statistically significant and physically meaningful results. In particular, we focus on the calculation of the MSD as a way of quantifying nanoswimmer movement, because it is the most common method in the literature and where most misinterpretations arise. Visualization of nanoswimmers is typically performed in a literature report using an optical microscope (such as ordinary optical microscopy,[60,62–64,66,68,73,74,76,78,80–84,86,87,89,94] confocal microscopy,[58,65,75] or a specialized commercial setup such as a Nanosight[58,61,64,67,70,79,85,88,90,92]) for nanoswimmers with 100 nm or larger dimensions. In the case of smaller nanoswimmers, optical microscopy fails to capture their movement. In this case, their diffusivity is often inferred indirectly by techniques such as DLS (see refs 54, 70, 81, 88, and 95 for examples in the nanoswimmer literature), a topic that is discussed in details elsewhere.[35]

Note that this section deals exclusively with the movement of a nanoswimmer in 2D, which is the most commonly reported scenario in the literature. However, these 2D dynamics are, in most cases, a projection of the three-dimensional (3D) movement of a nanoswimmer due to strong Brownian motion. Tracking and capturing the true 3D dynamics requires more sophisticated setups and techniques, such as holographic microscopy[121,128–130] or defocusing-based tracking algorithms,[131,132] that are beyond the scope of this tutorial.

Particle Tracking

With a suitable video clip recorded, the first step is to extract the positional information of all nanoswimmers with a tracking program, which includes commercial software that often comes with a high-end camera, open-source programs/codes (such as TrackMate,[133,134] Video spot tracker,[135] or Physmo[136]), or homemade codes compiled in MATLAB or Python (many of the existing codes for particle tracking can be traced to an early IDL code by Crocker and Grier, see refs 137 and 138). Regardless of which program is used, the general principle is often the same: a computer algorithm finds the center of the nanoswimmer being tracked, and outputs a spreadsheet with its x and y coordinates at each time step. These data are then used to calculate

its instantaneous speed or mean squared displacement (MSD).

A few issues are worth noting. First, it is important to track the center of the nanoparticle as accurately as possible. Tracking can be improved by optimizing the brightness and contrast of images and removing noise from the background with tools such as imageJ.[139] Second, given sample inhomogeneity, one needs to decide if particles of extraordinary behavior (too fast, too slow, or stuck) need to be filtered, and what those criteria will be. Third, because convective drift is difficult to eliminate in the experiment, the tracking algorithm must be able to correct for drift.[140,141] Such a correction protocol is often available as open-source code,[142-144] or is embedded in commercial tracking packages (*e.g.*, the NanoSightNTA program[145]). See the Supporting Information for a copy of and instructions for the MATLAB codes that we have used for drift correction (Permission granted by Himanagamanasa Kandula from University of Massachusetts and Prof. Yongxiang Gao from Shenzhen University).

Plotting Trajectories

Particle tracking typically yields a nanoswimmer's x and y coordinates, which are critical data for reporting and analyzing nanoswimmers. Often, a plot of trajectories is made to visualize how active (or inactive) a group of nanoswimmers is. Although the process of plotting trajectories is seemingly as straightforward as connecting dots, we note a few variations that improve clarity and convey more information (Figure 2).

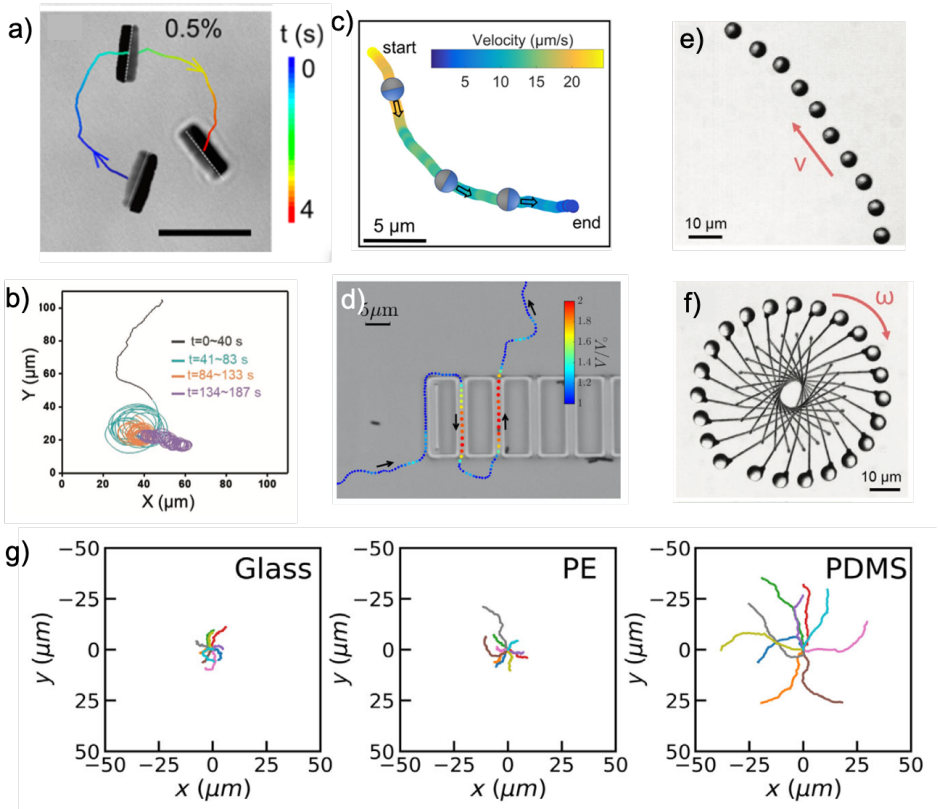

Figure 2. Plotting nanoswimmer trajectories. a–d) Overlaying a trajectory with time (a, b) or instantaneous speeds (c, d). e, f) Time lapse optical micrographs made by multiple exposures. g) Grouping the trajectories of multiple nanoswimmers to a single origin. Panel a reprinted from ref 146. Copyright 2020 American Chemical Society. Panel b, e and f reprinted with permission from ref 147.

The data points (or the line connecting them) can be made in colors that scale with time or speed. Trajectories overlaid with time (Figure 2a and b) are particularly helpful if the way a nanoswimmer moves changes over time, such as a switch from linear to circular motion,[146] or a change in orbiting radius.[147] This overlay also serves as a time stamp that indicates the start and finish of a trajectory. Trajectories overlaid with speeds are helpful if swimmer speeds change over time, either as chemicals are consumed (Figure 2c),[148] or as a nanoswimmer encounters new environments (Figure 2d).[149] These plots often require specialized computer programs to make, rather than common plotting software. See the Supporting Information for a copy of the MATLAB codes for generating overlay plots (courtesy of Hepeng Zhang from Shanghai Jiaotong University).

Two additional techniques are worth noting. First, instead of plotting x and y coordinates, the actual micrographs of a moving nanoswimmer at different instances can be superimposed together to yield a single plot (Figure 2e and f [147]). This technique is known as multiple exposure in photography, and it conveys additional information such as nanoswimmer speed in a visually striking manner. Such a micrograph can be easily made with imageJ by loading an image sequence and choosing the "z-project" under the "image→stacks" tool. Second, the head of multiple trajectories (plotted by connecting x and y coordinates) can be combined (Figure 2g[126]), so that all trajectories radiate from one common origin instead of being scattered over a wide area. This technique is particularly helpful in visualizing the performance of nanoswimmers operating under different conditions, in conjunction with the speed analysis that we introduce below.

Reporting Speed/Mobility

There are typically two ways to quantify how fast a nanoswimmer moves (*i.e.*, its "mobility"). One can either calculate the instantaneous speed V by dividing the displacement (ΔL) by the time interval (Δt) (for examples, see refs 53, 71–74, 76, and 83), or extract the ballistic speed V or diffusivity D from the MSD (for examples, see refs 54, 60, 63, 69, 72, 74–76, 78, 80, 81, 83, 86–90, 93, and 95). Caution is needed with either method to avoid artifactual claims, as we detail below.

To understand this point, we need to revisit how a nanoswimmer moves. A nanoswimmer typically (but not exclusively) moves along a particular body axis in a directional way, regardless of its shape, size, or propulsion mechanism. In the absence of any environmental obstacles or thermal noise, its trajectory would be simple and smooth (and linear if without torque). However, as noted above, thermal kicks from solvent molecules constantly nudge a nanoswimmer. Occasionally, these nudges give the nanoswimmer a nonzero torque, reorienting it on a rotational timescale $\tau_r = 8\pi\eta a^3/k_BT$ where k_BT is the thermal energy, η the viscosity of the medium, and a the particle radius (or a characteristic size, for a nonspherical swimmer). As a result, in an observational window much shorter than τ_r, a nanoswimmer does not rotate but rather undergoes ballistic, directional motion with a speed V. When the swimmer

is observed over a much longer time than τ_r, however, its direction of motion is randomized and it appears to diffuse with an effective diffusion coefficient D_{eff}. For a powered swimmer, D_{eff} can be much larger than D_0, which is the diffusion coefficient for the same object with the power source removed.

This transition from ballistic to diffusive motion, around a time scale τ_r, is a defining feature of the dynamics of a nanoswimmer, and the extraction of V and D_{eff} out of this convoluted dynamic is the focus of this section. Note in particular that the reorientation time τ_r varies as the third power of the size of the swimmer (recall: $\tau_r = 8\pi\eta a^3/k_BT$), and can be as small as milliseconds for a nanoswimmer (*ca*. 1 ms for a = 50 nm, 50 ms for a = 200 nm). Such a short time scale for a nanoswimmer poses fundamental challenges that we describe below.

Calculating instantaneous speeds (*V*). This method is often considered the gold standard for assessing the mobility of *microscopic* swimmers. The idea is simple: divide the displacement of the object (projected in two dimensions) by the time interval between video frames (Δt) to obtain the instantaneous speed at that particular step. Plotting the acquired instantaneous speed over time yields the speed profile of one nanoswimmer along its trajectory. One can then calculate the average speed of a single nanoswimmer throughout its journey, the average speed of many nanoswimmers for a single time step, or the average speed of many nanoswimmers throughout the entire video clip. Note that because of thermal kicks and sample inhomogeneity, there is often a large error associated with this average speed.

Although this method can be quite useful for microswimmers with diameters in the 1–10 μm range (τ_r = 1–1000 s), it quickly becomes incorrect as the size decreases, especially when the frame rate for capturing the video is slow (*i.e*., 30 fps). The reasons are twofold. First, accurately tracking a nanoparticle is inherently challenging, leading to systematic errors in its x and y coordinates, which translate to errors in calculating its speed. This problem is aggravated for slow-moving swimmers with small displacements between individual steps. Second, the instantaneous speed is only meaningful over the timescale during which a nanoswimmer is moving directionally (*i.e*., within its "persistence length"). For a nanoswimmer with a reorientation time τ_r on the order of milliseconds, measuring instantaneous speeds with cameras typically operating at 30 fps or lower is essentially and incorrectly calculating the "speed" of diffusion, and the required frame rate increases with the inverse third power of nanoswimmer size. This issue was carefully elaborated in an article by Ebbens and Howse,[36] who showed that, when plotted as discrete steps, the trajectory of a Brownian nanoparticle is indistinguishable from that of a self-propelled nanoswimmer. A similar augment has also been made by Mestre and Palacios, et al.[34]

We therefore strongly recommend against calculating/reporting the instantaneous speeds of nanoswimmers (see refs 53, 55, 60, 62, 65, 71, 73–75, 82, 83, 94, and 150 for examples), unless a high-speed camera is used, or the nanoswimmer (for good and known reasons) experiences very limited Brownian rotation.

Calculating the mean squared displacement (MSD). A more appropriate way of quantifying the mobility of a nanoswimmer is to calculate its MSD, the general principle of which has been amply explained in the literature.[34,36,37,151,152] To put it simply (see Figure 3a and b for an illustration), one takes the average value of the square of the displacement $(\Delta L)^2$ within a

certain time interval Δt and plots the averages ($\langle \Delta L^2 \rangle$) over increasing Δt. This calculation is often done with specialized programs or codes, and some are open-source.[153] We emphasize a few important caveats below for plotting (and, in the next section, analyzing) the MSD, because these results often hold the key to confirming the successful operation of a nanoswimmer, especially one that is beyond optical resolution, and for understanding how nanoswimmers operate.

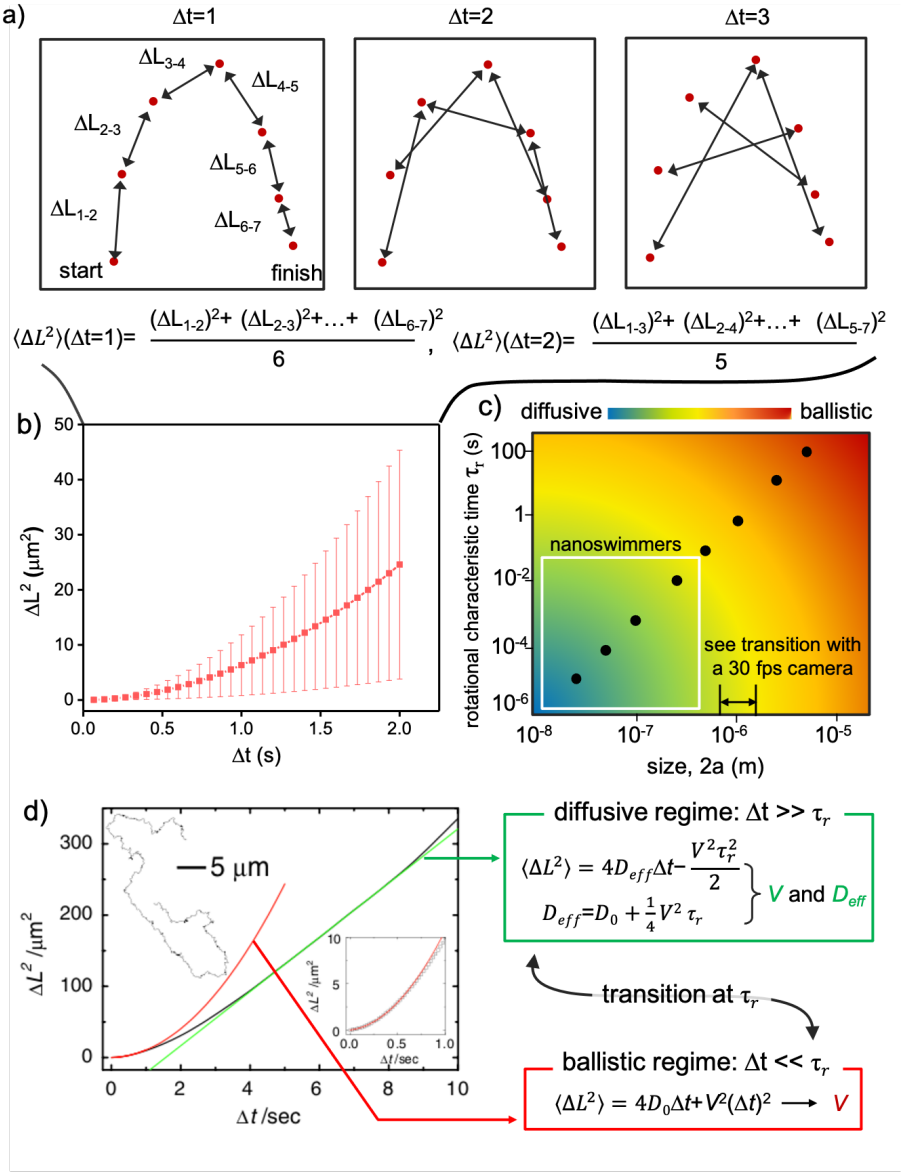

Figure 3. Mean squared displacement (MSD) of a nanoswimmer. a) Principles of calculating MSD (< ΔL^2>). ΔL is the displacement of a nanoswimmer in a time interval Δt. b) An example of an MSD plot with error bars representing standard deviations, acquired from the self-propulsion of a SiO_2-Pt Janus micromotor of 5 μm diameter in 5% H_2O_2. c) The rotational time scale τ_r of a swimmer is proportional to the third power of its size (radius=a). For a typical camera recording at 30 frame per seconds (fps), the obtained MSD plot only exhibits the diffusive (linear) regime for a nanoswimmer smaller than ~500 nm, and only the ballistic (parabolic) regime for a microswimmer larger than a few μm. d) An example of the transition between two regimes on an MSD plot, and the corresponding fitting equations.

τ_r, V and D_{eff} are the characteristic time scale for the rotational diffusion of a nanoswimmer, its ballistic speeds and effective diffusivity, respectively. Panel d reprinted with permission from ref 151. Copyright 2007 American Physical Society.

Calculating an MSD requires the coordinates of the nanoswimmer *versus* time and, thus, a video clip of high quality, long duration, and high frame rates. The length requirement arises because the error in calculating the MSD increases for longer time intervals (see error bars in Figure 3b), because fewer data points are available.[34] To improve the statistical robustness, only 1/10 or 1/5 of the maximum time interval can be used to plot the MSD. For example, even though a 10 s video clip generates an MSD plot of a maximum Δt of 10 s, only the first 1–2 s of Δt are plotted (note that these data are still produced from the *entire* 10 s video). The data at Δt=9 or 10 s, however, are limited and prone to error, because the displacement between two steps so far from each other is infrequent. Finally, the frame rate requirement arises because the smallest Δt in an MSD plot is given by the finest temporal resolution of the camera being used. For a typical camera operating at 30 fps, the smallest Δt in an MSD plot is 0.033 s, and any dynamics occurring on a finer timescale are lost. Therefore, videos recorded at a much higher frame rate are needed to capture the ballistic motion of a nanoswimmer.

Taking the average over many nanoswimmers is also critical for an MSD plot. Because each individual nanoswimmer yields a significantly different MSD, using only a few of them can give an incorrect interpretation of the ensemble dynamics, such as over- or underestimation of their mobility, or even whether or not they are active. This last point has been presented explicitly and convincingly in a recent article by Novotný and Pumera,[37] and again by Mestre and Palacios, et al.[34]

Analyzing the mean squared displacement. Once an MSD plot is generated, the next step is to analyze it to extract the mobility (V or D_{eff}) of the nanoswimmers. This extraction is based on the idea that at small Δt (below the rotational time scale τ_r), the nanoswimmer undergoes ballistic, directional motion, and thus its displacement ΔL is proportional to Δt (*i.e.*, $(\Delta L)^2$ scales as $(\Delta t)^2$). On an MSD plot, this regime of $\Delta t << \tau_r$ shows a parabolic shape that can be fitted to $\langle \Delta L^2 \rangle = 4D_o \Delta t + V^2 \Delta t^2$, which yields the ballistic speed V of a nanoswimmer. At $\Delta t >> \tau_r$, however, Brownian motion renders a nanoswimmer diffusive, with its $(\Delta L)^2$ scaling with Δt (instead of $(\Delta t)^2$). This diffusive regime is then manifested as a linear MSD curve that can be fitted by $\langle \Delta L^2 \rangle = 4D_{eff} \Delta t$, the slope of this linear fit being the "effective diffusivity" D_{eff} of the nanoswimmer over a longer observational window. Note that the entire MSD plot can be fitted with a full equation[36,151] or its higher order variations for better accuracy,[34] but in practice the above two limiting forms are more popular. Figure 3d gives an example of how this is done from the literature.[151]

Both V and D_{eff} are useful measures of a nanoswimmer's mobility, and extracting these quantities from either the ballistic or the diffusive regime of an MSD plot then becomes the main target of analyzing the MSD. However, there are a few important caveats to remember, which unfortunately account for the majority of mistakes in the nanoswimmer literature.

- *Not all MSD plots contain both regimes.* As we emphasized earlier, because τ_r is small

for nanoswimmers, the transition from the ballistic to the diffusive regime occurs at a Δt on the order of ms. For data acquired from a camera operating at a frame rate lower than 1000 fps, only the diffusive regime (*i.e.*, the linear part) of a nanoswimmer is sampled in the MSD plot (see, for example, refs 66 and 80), which can be fitted with the corresponding equation to yield D_{eff}. Extracting the ballistic speed V from such data, on the other hand, is difficult and inappropriate for nanoswimmers. Even for larger swimmers with sizes close to 1 μm (reorientation time ~ 1 s), fitting the parabolic part (Δt <~0.2 s) of an MSD plot is still subject to large fitting uncertainties, because of how short the segment being fitted is. As the size of the swimmer continues to grow into the micrometer range, τ_r quickly rises well into seconds, so that only the ballistic but not the diffusive regime on an MSD plot can be observed, unless minutes-long videos are used. This principle is illustrated in Figure 3c.

- *Be careful with parabolas.* Although a parabolic MSD for a large microswimmer makes the extraction of V straightforward, seeing a parabola on the MSD plot of a nanoswimmer is alarming and not expected, because, as we have mentioned a few times, their small size and small τ_r dictate that their MSD contains only the linear regime unless a camera of a high frame rate was used. The most likely source for seeing such an anomaly with a nanoswimmer is the presence of convective drift,[58,60,61,65,68,73,79,88,92] which could arise from a number of possible sources: an uneven stage, leaks in containment, growth and collapse of bubbles, and/or disturbance to the fluid from adding solutes or solvents, to name a few. Difficult to eliminate completely, drift collectively moves all nanoswimmers in the field of view in the same direction and, as a result, superimposes a directional force on a diffusive nanoswimmer, rendering its *entire* MSD plot parabolic, even for $\Delta t >> \tau_r$. If carelessly done, the parabolic MSD plot can be interpreted as the ballistic regime of a nanoswimmer, and can be fitted with the corresponding fitting equation to yield a ballistic speed that, unfortunately, originates entirely from drift (see also ref. 34 for discussion). To prevent this misinterpretation, the original tracking data must be drift-corrected (see description and methods introduced above). A straightforward way to check whether drift has been successfully corrected is to plot the corrected trajectories of a few nanoswimmers within the same frame to see if they diffuse independently (see refs 63 and 67 for good examples) or all in the same direction. The above principles and operations are illustrated in Figure 4, where we deliberately introduced drift into a population of polymer tracers and then had the drift corrected.

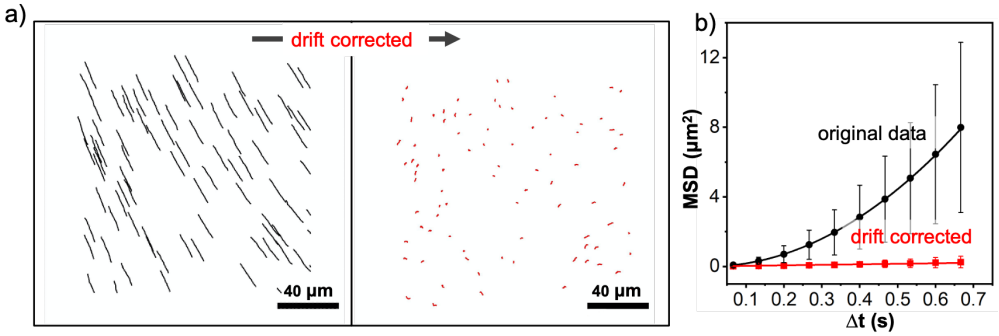

Figure 4. Drift correction. a) Drift is intentionally introduced into a suspension of polystyrene microspheres (5 μm in diameter) by blowing air. Their original trajectories over 6 s and those after drift correction are compared. The correction is not perfect for such a strong drift. b) Mean square displacement (MSD) plots obtained from the data in a).

- *D_{eff} is tricky.* D_{eff}, the effective diffusivity of a nanoswimmer, is extracted from the linear part of an MSD plot. It is a good indication of how mobile a nanoswimmer is and, thus, is often used to compare nanoswimmers with less mobile or immobile counterparts. However, the proper extraction and use of D_{eff} requires i) enough data to enable a robust MSD calculation, because D_{eff} values can vary greatly even for a subset of the entire swimmer population,[37] and ii) the application of linear fitting only to nanoswimmers that move in a straight line during their ballistic runs, with velocities pointing along their propulsion axes. If these conditions are met, D_{eff} can be further decomposed into $D_{\text{eff}}=D_0+ V^2\tau_r/4$, so that the ballistic speed V is obtained. This method has been used in some experimental studies to calculate the ballistic speed of nanoswimmers from an MSD plot in the absence of any parabolic fitting.[54,95] The key to its successful implementation lies in accurately fitting the linear regime of the MSD plot and determining the value of τ_r.

Importance of control experiments. Carefully designed control experiments are critical, given that MSD calculations are error-prone and inactive and active nanoswimmers are difficult to distinguish visually. At minimum, three control experiments are needed (Figure 5): a nanoswimmer in the absence of any activation (*e.g.,* no fuel or no external power), an inert nanoswimmer (*e.g*., a nanobowl without a Pt nanoparticle,[85] or a nanosphere without Pt coating) in the presence of activation, and an inert nanoswimmer without any activation. The effective propulsion of an activated nanoswimmer, and the absence of propulsion for all three controls, should become clear by plotting their respective trajectories (Figure 5a), calculating their drift-corrected MSD (Figure 5b), or extracting their respective mobility values (Figure 5c). Results from these control experiments are extremely powerful for eliminating errors associated with convective drift, and for providing a base value of nanoparticle diffusivity with which the enhanced diffusion of an activated nanoswimmer can be compared.

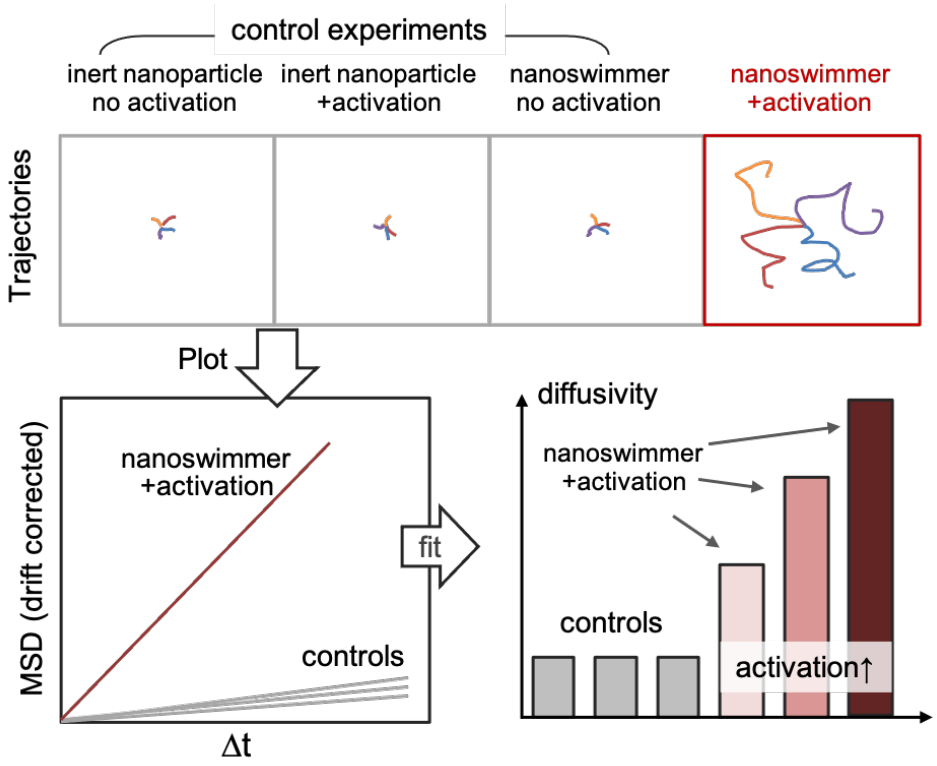

Figure 5. In addition to showing the results of an activated nanoswimmer, results from three control experiments are required to confirm the self-propulsion of a nanoswimmer.

Elucidating Propulsion Mechanisms

After the successful fabrication and activation of a nanoswimmer, the next order of business is often a discussion of how it moves. Depending on the nature and emphasis of the study, this mechanistic discussion can be extensive or brief. Either way, for the sake of scientific clarity and advancing knowledge, we offer a simple guide for identifying the dominant operating mechanism of a nanoswimmer (see our recent review articles and references therein for more details[28,29]). Note that, even though the exact mechanism by which a micro- or nanoswimmer is propelled is sometimes subject to controversy (*e.g.*, as in the case of Pt-coated microswimmers[112,154–160]) and it is inherently challenging to resolve optically how a nanoswimmer moves, there are often simple ways to eliminate some mechanisms as candidates.

For externally powered nanoswimmers, identifying the mechanism is often more straightforward than for their chemical counterparts. Typically, a magnetically powered nanoswimmer twists its body or rolls on a surface in a rotating magnetic field;[161–164] an electrically powered Janus nanoswimmer moves on a 2D plane sandwiched between two conductive electrodes by a mechanism known as induced charge electrophoresis (ICEP);[165] and an acoustically powered nanorod moves in a levitation plane created by resonating ultrasonic waves,[166,167] or by resonant oscillation of an on-board bubble.[168,169] There are certainly variations and caveats to each of these mechanisms but, in general, there is some consensus as to how they work.

Chemical nanoswimmers (often containing catalysts such as Pt[76,81,88] or enzymes[69,77,78,80,86,87,90]) are the subject of the majority of nanoswimmer studies, but identifying their detailed propulsion mechanism can be challenging. For example, although it is easy to visualize a trail of bubbles left in the wake of a microscopic motor operating by bubble propulsion, it is challenging to see any bubbles created by a nanoswimmer.[67,70] This sometimes leads to the speculation that nanobubbles are generated, even though nanobubbles are thermodynamically unstable and their existence has been contested.[170,171] The other major categories of chemical propulsion are self-electrophoresis and self-diffusiophoresis. Both refer to the transport of colloidal particles in a self-generated chemical gradient,[31,45,172] with major differences being whether the chemically active particle is a combination of source and sink of chemicals (self-electrophoresis) or just a source (self-diffusiophoresis). The particular case of self-diffusiophoresis, which is the most commonly invoked mechanism for a chemical nanoswimmer,[59–62,66,79] can be further divided into ionic and neutral types, depending on the nature of the released chemicals. Ionic self-diffusiophoresis occurs when the released ions have different diffusivities or different interactions with the particle, and it weakens as the solution ionic strength increases. Neutral self-diffusiophoresis, however, arises from the interaction of neutral molecules with the particle surface, and does not vary with solution conductivity. The simple experiment of adding salt (typically at 0.1–1 mM concentration) to the solution then becomes a useful way of distinguishing between ionic and neutral self-diffusiophoresis.

Beyond the superficial and qualitative assessment described above, an in-depth investigation of the operating mechanism of a chemical nanoswimmer often requires measuring the chemical, electrical, and flow profiles around a nanoswimmer, as well as a full characterization of its shape, size, material composition, and surface morphology. An example of how involved this process can become is the archetypical case of Pt-catalyzed nanoswimmers, which move by catalytic decomposition of H_2O_2 into water and O_2. Owing to their simple fabrication and reliable performance, this type of nanoswimmer has gained popularity among chemists, material scientists, and physicists. As a result, much effort has been dedicated to elucidating their operating mechanism(s), including measuring the local pH and flow fields around a Pt micropump or micromotor;[156,173,174] measuring the catalytic mobility of a Pt cap;[155] measuring the separation distance from a bottom wall;[126] and investigating how particle size,[175] surface morphology,[157] or ionic strength[154,155] affect their speed. Yet, the dust has not completely settled on the propulsion mechanism.

Unraveling the operating mechanism of a microscopic swimmer is inherently challenging, and clarifying it at the nanometer scale is even more so, not only because of the increasing requirement of spatiotemporal resolution of all the measurements and characterization, but also because of the difficulty in seeing and quantifying how the nanoswimmers move and, more critically, which way they move. For those interested in the details of nanoswimmer propulsion, control experiments and careful measurements often give useful information that can eliminate unlikely mechanisms. In addition, it is helpful for experimentalists to work with theorists and modelers, combining experiments, simulations, and theory to understand how nanoswimmers move.

Using Nanoswimmers

The emphasis of many nanoswimmer studies is to use them in an applied scenario, most often for biomedical or bioanalytical tasks or for environmental remediation or separations, taking advantage of the fact that nanoswimmers are capable of actively seeking out targets, moving to places inaccessible to passive nanoparticles, mixing well with their environment, and performing specific tasks that depend on material properties. Although we are hopeful about the future prospects of nanoswimmers in real-world applications, we caution against rushed claims of nanoswimmer success that are not translational. Joining recent calls from others in our community (see refs 10, 176–178, and references therein), we make two brief and sober comments about responsible reporting of nanoswimmers in applications.

First, the success of a nanoswimmer operation must be assessed in its final, unaltered environment, even if preliminary experiments are conducted in a model system, for example *in vitro* or *ex vivo* experiments for a biological test, or in synthetic/diluted wastewater for environmental applications. In a real biomedical setting, hostile environments such as fast flows, high ionic strength, strong confinement, and a vigilant and efficient immune system, can quickly disable most types of nanoswimmers, especially those powered by surface chemical reactions.[10] Similar problems occur in environmental sensing and remediation applications, where the presence of flows, ions (including heavy metals that poison catalysts[179]), and microbes could render nanoswimmers inoperative. In addition to how well nanoswimmers deal with real environments, we also need to consider if they release unwanted chemicals or bubbles to their surroundings, *i.e.*, the issue of biocompatibility, toxicity, stability, and recyclability.[180,181] Recent studies have aimed to address some of these issues, such as understanding how the immune system responds to microswimmers of different shapes,[182] and how to design chemical microswimmers with high salt tolerance,[183] but the best practice of considering the realistic environment of a nanoswimmer application, and even offering potential solutions, is still far from being widely adopted in the literature.

Second, we must assess the cost-effectiveness of a nanoswimmer operation, and compare it to a practical benchmark. For example, nanoswimmer studies often show modest increases (sometimes as small as a few tens of percent) in drug delivery efficiency or animal life expectancy over nonmotile nanoparticles of the same formulation. Yet, incremental improvement in efficacy with nanoswimmers over existing technologies is rarely enough, both from the standpoint of technology transfer and investment, and from the cost of manufacturing, characterizing, and activating nanoswimmers. For environmental problems that exist on a much larger length scale than biomedical applications, the issue of cost becomes a much more serious problem and requires a stronger case to justify the use of nanoswimmers. As a result, bold, forward-looking ideas and methods are needed to enable nanoswimmer technologies that are orders-of-magnitude better than existing ones. Otherwise, there will be little market for commercializing nanoswimmers, and little hope of moving nanoswimmer research forward from its current phase.

Conclusions and Prospects

With sizes in the nanometer range and endowed with useful functionalities, nanoswimmers that move autonomously in fluids have the potential to contribute substantially to the fields of biomedicine, sensing, separations, microfabrication, and environmental remediation. However,

the accurate characterization of nanoswimmers is often hindered by strong Brownian motion and the lack of a clear way to visualize them. When coupled with improper experimental design and imprecise practices in data analysis, these issues can translate to results and conclusions that are inconsistent and poorly reproducible. In light of the increasing popularity of nanoswimmer research and its challenges, we have offered suggestions of best practices for reporting and analyzing their movement:

- When describing sample preparation and motility experiments, report the details of the surface morphology, zeta potential, and uniformity of the sample; the lighting conditions and camera frame rate; the chemicals present in the experiment; and the experimental setup including the substrate, container, stage, and particle population density.
- When analyzing nanoswimmer mobility, calculate instantaneous speeds only for fast micromotors larger than 1 μm, and calculate MSDs for those moving at the nanometer scale (it is rarely necessary to calculate both for the same sample). In particular, because the calculation of the MSD is error-prone, a long video at high frame rates containing many nanoswimmer trajectories is preferred (and must be included in the supporting information), and caution is needed to decide which equation to use to extract the appropriate mobility of a nanoswimmer from MSD data. Above all, drift must be accounted for and corrected, and proper control experiments are needed.
- Identifying the propulsion mechanism of a nanoswimmer often requires careful measurements of the chemical, electrical, and flow fields around it, as well as collaborative efforts from simulations and theory. It is important to familiarize ourselves with the key features of the most common propulsion mechanisms and keep up with the latest progress in understanding them.
- Evaluating the success of nanoswimmers in biomedical or environmental applications needs to be done in their real-world environments, and with a consideration of cost-effectiveness.

Finally, we emphasize that, among the nanoswimmer studies we have examined, a recurring issue is to claim self-propulsion for a "nanoswimmer" out of problematic data. This issue arises from two sources. First, some studies claim "nanoswimmers" simply from parabolic MSD curves, which are often incorrectly fitted to give the "speed" values. But as we have elaborated above, these parabolic curves can also easily arise from drift that is hard to eliminate completely. Compounding the problem, many such studies are haunted by videos of poor quality, or videos that show the collective drift of nanoparticles. A second source of misinterpretation is insufficient data, which can lead to MSD values that are incorrectly assigned to enhanced diffusion when in fact, they only support Brownian motion. In some cases, the entire argument of "nanoswimmer" is built on videos (or trajectories) that display a single nanoparticle moving about, or, in more extreme cases, no experimental evidence of their self-propulsion whatsoever. These sources of errors can certainly be eliminated by careful experimental designs and data analysis, and by an improved understanding of the basic operating principles of nanoswimmers.

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Suggested pull quotes

The two principal challenges in the study of nanoswimmers are rooted in the physics of their

small size.

Whether for the purpose of data analysis or visualization, a high-quality video clip displaying the self-propulsion of a nanoswimmer without drift is standard for confirming its successful operation.

An in-depth investigation of the operating mechanism of a chemical nanoswimmer often requires measuring the chemical, electrical, and flow profiles around a nanoswimmer, as well as a full characterization of its shape, size, material composition, and surface morphology.

TOC graphic: