Out-of-Equilibrium Dynamics of Colloidal Particles at Interfaces

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Out-of-Equilibrium Dynamics of Colloidal Particles at Interfaces

A dissertation presented
by
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to
The School of Engineering and Applied Sciences
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Doctor of Philosophy
in the subject of
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Out-of-Equilibrium Dynamics of Colloidal Particles at Interfaces

Abstract

It is widely assumed that when colloidal particles adsorb to a fluid-fluid interface, they reach equilibrium rapidly. Recently, however, Kaz et al. [Nature Materials, 11, 138-142 (2012)] found that a variety of functionalised latex microspheres breaching an aqueous phase-oil interface relax logarithmically with time toward equilibrium. The relaxation is so slow that the time projected for the particles to reach the equilibrium contact angle of 110° is months – far longer than typical experimental timescales.

In this thesis, we seek to understand the out-of-equilibrium behaviour of particles near interfaces. Because contact line pinning is likely an extra source of dissipation at interfaces, we start with experiments to elucidate the origins of contact-line pinning and find that polymer hairs on aqueous dispersed polymer particles strongly pin the contact-line. For particles without polymer hairs, nanoscale surface roughness can also pin the contact-line, though with a lower energy.

We then extend our digital holography capabilities to track non-spherical particles. We demonstrate that we can track the centre-of-mass of a colloidal spherocylinder to a precision of 35 nm in all three dimensions and its orientation to a precision of 1.5°. Furthermore, the measured translational and rotational diffusion coefficients for the spherocylinders agree with hydrodynamic predictions to within 0.3%. This new functionality enables us to track colloidal ellipsoids and spherocylinders as they breach interfaces. By comparing the adsorption trajectories of the non-spherical particles to what is predicted from energy minimisation, we learn that contact-line pinning affects not just the timescales of breaching, but also the pathway to equilibrium.

In fact, a particle’s path to equilibrium can have complications even before the particle breaches the interface. Some particles are attracted to the interface, but stay within a few nanometers without
ever breaching. We refer to this binding-mode as ‘non-capillary binding’, and we investigate when this binding mode is present, what causes it, and how interparticle interactions depend on the binding mode.

The last few chapters in this thesis are extensions of ideas developed in the first part. We track the run and tumble of *E.coli* to demonstrate the potential of digital holographic microscopy as an imaging tool for active particles. Taking all of the particle-interface literature into account, we also outline some simple design principles for making particle-stabilised Pickering emulsions.
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Prior Publications

Parts of this thesis are primarily based on the following previously published works:


Other works to which the author has contributed in graduate school:


Other publications relating to the work discussed in this thesis are in preparation.
Acknowledgments

*When you are a Bear of Very Little Brain, and you Think of Things, you find sometimes that a Thing which seemed very Thingish inside you is quite different when it gets out into the open and has other people looking at it.*

– Винни-Пух

It is with hesitation that I write these acknowledgements – partly because I’m in denial about my time here being over, and partly knowing that my command of words (in any language) is not expressive enough.

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So the little prince tamed the fox. And when the hour of his departure drew near –

“Ah,” said the fox, “I shall cry.”

“It is your own fault,” said the little prince. “I never wished you any sort of harm; but you wanted me to tame you...”

“Yes, that is so,” said the fox.

“But now you are going to cry!” said the little prince.

“Yes, that is so,” said the fox.

“Then it has done you no good at all!”

“It has done me good,” said the fox, “because of the color of the wheat fields.”

– Antoine de Saint-Exupéry, Le Petit Prince
When you see someone putting on his Big Boots, you can be pretty sure that an Adventure is going to happen.

– Винни-Пух

1

Introduction

1.1 Particles at interfaces

Oil and water don’t mix. Yet dispersing one inside the other to create emulsions, whether it be for vinaigrettes, sunscreens, cosmetics or washing dishes, is hugely desirable. Ordinarily, these immiscible liquids quickly separate back into the bulk after mixing because the large amount of interfacial area is energetically costly to maintain. Reducing the energy per area of interface, or the interfacial tension, is therefore key to creating stable emulsions. There are two main ways to do that.

On the smallest scales, there are surfactants – molecules with a hydrophilic head that stays immersed in water, and a hydrophobic tail that can immerse itself in oil. These molecules can line oil-water interfaces and reduce the interfacial tension of the system. Examples of such molecules include soap, which helps to mix the oil from dirty dishes with running water, and mustard, which helps sta-
Figure 1.1: A particle sits at an oil-water interface. Its height is defined by its equilibrium contact angle, where all interfacial tensions are balanced.

A larger amphiphilic particle can also fulfill this purpose—for instance, a colloidal particle, which is 100 nm – 10 μm in size. Very few materials are completely hydrophilic or hydrophobic, so a typical colloidal particle such as a polystyrene latex bead will straddle the interface at a height determined by the oil-water (σ_{ow}), particle-oil (σ_{po}), and particle-water (σ_{pw}) interfacial tensions. The relation between the interfacial tensions and the equilibrium contact angle is known as the Young-Dupré law and is illustrated in Figure 1.1. By balancing the force tangent to the particle at each point along the three-phase contact line, we find the equilibrium contact angle θ_{E}:

\[
σ_{po} = σ_{ow} \cos θ_{E} + σ_{pw}
\] (1.1)

Using this relation, we can compute the energy required to remove such a particle from the interface, \( E = σπr^2(1 - |\cos θ_{E}|)^2 \). For a \( r = 1 \) μm particle sitting exactly halfway between oil and water (\( θ_{E} = 90° \)), this energy cost is over \( 10^7 \) times the thermal energy. Thus, unlike surfactant molecules, which are constantly attaching to and leaving interfaces owing to thermal fluctuations, a colloidal particle cannot detach from the interface after it breaches it.
The strong binding of colloidal particles to interfaces is exploited in a range of fields. Particles can stabilise oil-water interfaces in Pickering emulsions, which can be used in food, oil recovery, pharmaceuticals, and cosmetics. Also, oil-water interfaces can be used to scaffold the arrangement of particles in the fabrication of materials such as colloidosomes, Janus particles, particle monolayers, and photolithography masks.

Because the interfacial tension provides a strong driving force to equilibrium, it is widely assumed that the particles reach equilibrium rapidly when they adsorb to the fluid-fluid interface. Recently, Kaz et al. found that a variety of functionalised latex microspheres breaching an aqueous phase-oil interface relaxed logarithmically with time toward equilibrium. The relaxation is so slow that the time projected for the particles to reach the equilibrium contact angle of $110^\circ$ is months – far longer than typical experimental timescales. Other researchers such as Coertjens et al. have since also observed that the contact angle of polymer particles changes over long periods of time.

The direct consequence of this slow relaxation is that a collection of identical particles at an interface, such as the surface of a Pickering emulsion droplet, have different contact angles that change over time. The contact angle of a particle determines the length of the three-phase contact line and how much of the particle is exposed to the aqueous or oil phases. It therefore affects the capillary interactions between particles and the electrostatic interactions. Contact angles that change over time might be responsible for heterogeneous pair-interactions and long-ranged attractions between identically charged particles. For emulsions, the emulsion type (water-in-oil, or oil-in-water) also depends on contact angle, and so a changing contact angle might change the emulsion type and stability over time.

The complexity that arises from the unexpectedly slow relaxation of particles necessitates better understanding of how particles breach and interact at an interface.
1.2 Overview

In this thesis, we seek to understand the out-of-equilibrium behaviour of particles near interfaces. Because Kaz et al.\textsuperscript{11} found that contact line pinning is likely an extra source of dissipation at interfaces, we start with experiments to elucidate the origins of contact line pinning (Chapter 2). We then extend our digital holography capabilities to track non-spherical particles (Chapter 3). This new function enables us to image ellipsoidal particles as they breach an interface (Chapter 4). Because both the centre-of-mass and the orientation of the ellipsoids can be tracked, we are able to investigate how contact-line pinning affects not just the timescales of breaching, but also the pathway to equilibrium.

A particle’s path to equilibrium can in fact have complications even before the particle breaches the interface. A particle can be attracted to and remain within a few nanometers of an interface without ever breaching. We refer to this binding-mode as ‘non-capillary binding’, and we investigate when such binding occurs (Chapter 5) and how non-capillary bound particles interact differently from breached ones.

The last few chapters in this thesis are extensions of ideas developed in the first part. We track the run and tumble of \textit{E.coli} to demonstrate the potential of digital holographic microscopy as an imaging tool for active particles (Chapter 6). Finally in Chapters 7 and 8, we outline some simple design principles for making particle-stabilised Pickering emulsions.

1.3 Digital holographic microscopy

The main imaging tool we use is the Digital Holographic Microscope (DHM). For a more comprehensive overview of the technique in the Manoharan lab, I refer you to the theses of David M. Kaz\textsuperscript{19}, Ryan McGorty\textsuperscript{20} and Jerome Fung\textsuperscript{21}. Here I will describe the technique in brief.

Colloquially, the word ‘hologram’ evokes shiny stickers, Tupac, and Princess Leia. It is common knowledge that holograms involve something that is three-dimensional (3D), yet strictly speaking, the
holograms themselves are not 3D.

To understand what holograms are, let us first consider light waves. Like radio waves, they can transmit information. The signal that is measured is usually the intensity, as recorded on a sensor or film, that is inherently two dimensional. The other source of information – the phase – is lost because there’s no simple way to detect it.

Dennis Gabor realised that one way to capture phase information from light waves is to interfere the electric field with a reference wave that has a known phase and amplitude. This interference pattern (reference + signal) is a hologram, which contains more information than a photograph owing to the presence of phase information in the interference fringes. More formally, we write the intensity as

\[
I_{\text{holo}} = |E_r + E_s|^2 = |E_r|^2 + E_s E_r^* + E_r E_s^* + |E_s|^2
\]  

where \(E_r\) and \(E_s\) are the reference and scattered electric fields, and \(I_{\text{holo}}\) is the hologram. The reference field might be the undiffracted light if the sample is dilute; in this case, the scattered and unscattered fields propagate in the direction of light transmission, and the technique is known as in-line transmission holographic microscopy. Other holographic methods include reflection-mode holography, which uses the backscattered light for \(E_s\) and a reflection from a coverslip surface for \(E_r\), and Mach-Zehnder holography, where the reference beam is split off from the source beam before being recombined with the scattered field. An excellent source of information on these and other holographic imaging techniques is the *Handbook of Holographic Interferometry* by Thomas Kreis.

If you shine light back through a hologram, the hologram can, in effect, focus the light. For holograms recorded on a digital camera, light can be ‘shined back through’ the hologram numerically. For a point scatterer, the light will refocus to a point at the distance where the original object was if the wavelength of light is the same as that used to record the hologram. For colloidal particles, there exists a plane in which the light is refocused to an image resembling a bright-field microscopy image. Other
focal planes can also be chosen that resemble slightly blurred, defocused bright-field images. These refocused slices are called reconstructed images, or reconstructions, and in principle making a stack of reconstructions is all that is needed in order to reproduce the original three-dimensional object. Holographic stickers are shiny because the ambient room light needs to reflect off the shiny surface and back-illuminate the hologram to give us the reconstructed three-dimensional object. Princess Leia, as Ryan McGorty pointed out to me, is not the hologram. She is the three-dimensional reconstruction.

We use an in-line holography setup, because our samples scatter weakly enough that the undiffracted incident beam can be used the reference beam. We illuminate the colloidal particles or other microscopic objects with a laser beam and ensure that (a) the laser beam is aligned with the imaging axis of the microscope and that (b) the part of the beam illuminating the sample is approximately a plane wave. We accomplish (b) by first filtering the beam through a single-mode optical fibre. Some of the light will be scattered by the particle, and some will continue onwards undiffracted. We collect the downstream interference pattern of the scattered and undiffracted light, which changes with the scat-
Figure 1.2 shows simulated holograms of a sphere in different positions. They are calculated with the Lorenz-Mie solution to Maxwell’s equations, which is part of our Python-based open-source software HoloPy (http://manoharan.seas.harvard.edu/holopy/). As the particle moves from side to side, so does the center of the hologram. And as it moves further from the detector, the fringes get wider. We capture these holograms through a microscope objective, and we save them digitally for post-processing.

There are two advantages of this imaging technique over bright-field or confocal microscopy. First, the laser is bright enough that we can take a hologram every 30 microseconds or less and make movies at frame rates over 30,000 frames per second. This is a much faster way to acquire 3D data than by using confocal microscopy. Second, and perhaps more importantly, we don’t have to focus on the object to see it. Information about the scatterer can be captured by the hologram even if the scatterer is out of focus. In principle, we can image all objects that scatter light within an approximately 100 × 100 × 100 μm³ volume, rather than just the objects in the focal plane. This makes holography an ideal technique for imaging 3D distributions of scatterers, or scatterers that move axially.

If we were to reconstruct the images in Figure 1.2, we would get reconstructions that resemble bright-field images. Cheong and coworkers used this method to track the 3D translation and rotation of a high-aspect-ratio (1:25) copper oxide nanoparticle. However, for wavelength-scale particles the precision of reconstructions is degraded by distortions in the reconstructed volume.

We therefore use a different approach to extract the information from a hologram. By fitting holograms calculated from Maxwell’s equations to the data, we determine the parameters that describe the scatterer’s position, orientation and optical properties. The hologram fitting routine performed with HoloPy is outlined in Figure 1.3. It consists of the following steps:

- Divide the recorded hologram by a background image which contains no particles in the field of view. This step accounts for scattering from objects outside the sample.
Figure 1.3: We extract information from holograms by fitting a scattering solution to them (see text for details).

- Use a model to calculate the scattered electric field $E_s$ based on initial guesses for the scatterer's size, refractive index, shape, orientation, position, and a scaling parameter $\alpha$.

- Numerically interfere $E_s$ with a reference field to yield the calculated hologram.

- Calculate the pixel-by-pixel squared difference ($\chi^2$) between the recorded and calculated holograms.

- Perturb the parameters using the Levenberg-Marquardt algorithm to minimise the cost function $\chi^2$.

The result of this fitting procedure gives the best-fit position, orientation, size, and refractive index of the scatterer.

This inverse-scattering analysis follows the work of Ovryn and Izen$^{27}$ and of Lee and coworkers$^{28}$, who showed that fitting a Lorenz-Mie scattering solution to a measured hologram of a spherical particle can yield nanometer-scale-precision measurements of its position. Researchers from our group, including Jerome Fung, Rebecca Perry, and Thomas Dimiduk$^{29,30,31}$ later showed that the translational, rotational, vibrational, and nonequilibrium dynamics of several interacting wavelength-scale
spheres could be measured with high precision by fitting Mackowski and Mishchenko’s multi-sphere
scattering solution\textsuperscript{32} to holograms of multiple spherical colloidal particles.

1.4 Colloidal motion

To determine whether we have appropriately aligned the laser beam and are correctly extracting data
from holograms, we use holography to measure a known physical quantity. One such quantity for
colloidal particles is their diffusion coefficient. As Robert Brown observed in 1827 and Albert Ein-
stein theoretically described in 1905, micrometer-sized particles in water jiggle owing to the stochastic
impacts of water molecules.

We can characterise a sphere’s diffusion by computing its mean squared displacement, or average
squared distance it travels for a given lag time $\tau$. In one dimension, the relation between the mean
squared displacement and diffusion is:

$$\langle (x(t+\tau) - x(t))^2 \rangle = 2(D\tau + \epsilon^2), \quad (1.3)$$

where

$$D = \frac{k_B T}{2\pi \eta r} \quad (1.4)$$

is the diffusion coefficient (Stokes-Einstein relation) for a sphere of radius $r$ in a fluid with dynamic
viscosity $\eta$, and $\epsilon$ is the tracking precision. The relationship between the mean squared displacement
and lag time is linear, and the slope $2D$ can be used to determine $D$. The intercept $2\epsilon^2$ can be used to
determine the precision of the instrument $\epsilon$.

The reason we interpret the intercept term as the tracking error is as follows. Say that when we
measure the absolute position in one dimension we measure its actual position $x$, plus an error, such
that $x_m = x + \delta$. The errors $\delta$ are normally distributed about 0; that is, $\langle \delta \rangle = 0$. When we calculate
the mean squared displacement with all the measured data points, we are actually incorporating the error:

\[
\text{MSD} = \langle (x_{m,1} - x_{m,2})^2 \rangle = \langle ((x_1 + \delta_1) - (x_2 + \delta_2))^2 \rangle \\
= \langle (x_1 - x_2)^2 \rangle + 2\langle x_1 - x_2 \rangle \langle \delta_1 - \delta_2 \rangle + \langle (\delta_1 - \delta_2)^2 \rangle = 2D\tau + 2\langle \delta^2 \rangle \tag{1.5}
\]

which, when compared to Equation 1.3, shows that \(\epsilon\) is the standard deviation of \(\delta\).

Using this method, we find that we can determine the positions of spherical particles to nanometre-scale precision\(^\text{11}\), and non-spherical particles to a precision of 35 nm. Further details can be found in Chapter 3. Thus, not only can we track particles over a wide range of timescales, but we can also measure fluctuations on nanometre scales.
I now have over 3000 different [pencil] sharpeners, with no duplicates [...] it keeps my mind sharp.

– Paul A. Johnson

Particles at interfaces

To determine how ubiquitous slow relaxation is, as well as the underlying mechanism for it, we study the breaching behavior of spherical particles with different surface properties. We use digital holographic microscopy, a fast three-dimensional imaging technique, to image particles as they breach. We show that a variety of different particles, including charge-stabilized polymer spheres, surfactant-stabilized polymer spheres, and silica spheres, relax logarithmically to equilibrium. By contrast, silica particles and oil-dispersed hard-sphere PMMA particles are able to reach equilibrium on experimental timescales. By fitting models to the data, we are able to extract details about the pinning sites on the particle surfaces that lead to slow relaxation of the contact line. The pinning features on aqueous-dispersed polymer particles pin the contact-line with an order of magnitude more energy than the features on other particles, resulting in a longer logarithmic regime. We conclude that the likely pinning sites are nanoscale topographical features such polymer ‘hairs’.
2.1 Background

The logarithmic relaxation of particles at interfaces can be explained by considering the motion of the contact line as a dynamic wetting problem within the context of molecular kinetic theory (MKT)\textsuperscript{33}. As the contact line moves across the surface of the particle, it gets pinned to defects and requires thermal kicks to keep moving toward equilibrium. Because the driving force decreases as the particles approach equilibrium, a particle slows down as it progresses through the interface (Figure 2.1). The activated hopping of the contact line results in much more dissipation than that predicted from hydrodynamics. If hydrodynamics were the only relevant effect, we would expect the particle to follow an exponential path to equilibrium with a timescale $T_D \approx \eta r / \sigma_{ow}$, where $\eta$ is a weighted average of the viscosities of the two fluids, $r$ the radius of the particle, and $\sigma_{ow}$ the interfacial tension between oil and water\textsuperscript{10}. For a 1-μm-radius particle at a water-alkane interface, $T_D$ is approximately 0.1 μs, which is several orders of magnitude smaller than the times observed in experiments\textsuperscript{11}.

A model based on MKT, presented in the supplementary information section of Kaz et al.\textsuperscript{11}, captured the experimentally observed dynamics from $10^{-2}$ s to $10^{2}$ s after the particle breaches. By fitting the model to the data, the authors inferred that the pinning defects were nanometer-sized or larger. We note that although the distances between defects in MKT are presumed to be on the molecular length scale, there are many other successful applications of MKT to surfaces with defects larger than 1 nm\textsuperscript{34,35}.

Here we present a brief derivation of the model from Kaz et al.\textsuperscript{11}. We model the activated hopping process using an Arrhenius equation for the velocity of the contact line\textsuperscript{33}. In the regime when forward hopping dominates, the the velocity of the contact line tangent to the particle is given by:

$$V = V_0 \exp \left( -\frac{\Delta U}{kT} + \frac{F_{cl}(t)A}{2kT} \right)$$  \hspace{1cm} (2.1)
where $V_0$ is a molecular velocity scale, $\Delta U$ is the pinning energy from defects of area $A$, and $kT$ is the thermal energy. The force per unit length on the contact line, $F_{cl}$, is given by the unbalanced oil-water ($\sigma_{ow}$), particle-oil ($\sigma_{po}$), and particle-water ($\sigma_{pw}$) interfacial tensions prior to equilibrium (Figure 2.1):

$$F_{cl} = \sigma_{ow} \cos \theta_D(t) + \sigma_{pw} - \sigma_{po}$$

$$= \sigma_{ow} \left( \cos \theta_D(t) - \cos \theta_E \right)$$

where $\theta_D$ is the dynamic contact angle. We assume the interface remains flat at all times.

Substituting Equation 2.2 into Equation 2.1 and rewriting the resulting equation of motion in terms of the observable axial coordinate $z$, we obtain

$$\dot{z} = \nu r \sin(\theta_D) \exp \left( \frac{A \sigma_{ow} z^2}{2kT} \right) = \nu \sqrt{z(2r-z)} \exp \left( \frac{A \sigma_{ow} z^2}{2kT} \right)$$

(2.3)

where $r$ is the radius of the particle and

$$\nu = \left( \frac{V_0}{r} \right) \exp \left( -\Delta U/kT + [1 - \cos \theta_E] A \sigma_{ow} / 2kT \right).$$

When the particle is close to equilibrium, we can expand around the equilibrium contact angle to reveal the trajectory’s logarithmic form:

$$z \approx \frac{2r kT}{A \sigma_{ow}} \log \left( \frac{A \sigma_{ow}}{2r kT} \nu r \sin(\theta_E) t \right)$$

(2.4)

which we can rewrite as

$$\frac{z}{r} \approx \frac{2kT}{A \sigma_{ow}} \log \left( \frac{t}{t_0} \right) + C; \quad C = \frac{2kT}{A \sigma_{ow}} \log \left( \nu r \sin(\theta_E) t_0 \frac{A \sigma_{ow}}{2r kT} \right)$$

(2.5)

to make it clear how the area per defect affects the approach to equilibrium. We can use this model to
fit particle trajectories that we measure with two fitting parameters, \( \nu \) and \( A \). We choose an arbitrary \( t_0 \) \((t_0=1)\) because the fits are not sensitive to \( \nu \). We are therefore unable to recover \( \nu \) or the pinning energy using these fits. The fits are, however, highly sensitive to \( A \), which determines the slope of the logarithmic trajectory. By fitting this model to the data, Kaz et al.\(^{11}\) found that the area per pinning defect for latex microspheres was on the order of the area per charged group (as reported by the particle manufacturer) and varied with particle surface functionalization but not particle size (for a given functionalization).

The pinning energy of each defect \( \Delta U \) can be determined from experimental data using Kramer's theory\(^{36}\), as shown by Colosqui et al.\(^{10}\). They found that particles having heterogeneous surface defects (which result in metastable minima in the free-energy) have an initial exponential relaxation regime followed by logarithmic relaxation at longer times. This model also captures the experimental results from Kaz et al. well; indeed, the models from Kaz et al. and Colosqui et al. are mathematically equivalent\(^{10}\) when the dynamic contact angle \( \theta_D \) is approximately \( \pi/2 \). The area per defect \( A \) from Kaz et al. is related to the length scale \( l \) from Colosqui et al. by \( A \sim 2\pi R^*l \) where \( R^* \) is the radius of the contact line when the particle is at \( z_C \) and \( z_C \) is the height at which the relaxation changes from exponential to logarithmic. The crossover point between exponential and logarithmic regimes can be used to infer \( \Delta U \):

\[
z_E - z_C = \frac{\Delta U \pi R^*}{2\sigma_{ow}A}.
\]
We can therefore fit Equation 2.5 to the logarithmic regime in experimental data to obtain $A$, then use Equation 2.6 to determine the defect energy $\Delta U$.

2.2 Materials and methods

Though colloidal particles may appear smooth under bright-field microscopy, the particle surfaces are heterogeneous because of charges, asperities and, in the case of polymer particles, polymer ‘hairs’$^{37,38}$. To determine which of the surface features can affect a particle’s relaxation to equilibrium, we track particles with a variety of different surface properties as they breach an aqueous-oil interface (Table 2.1). The types of particles we examine are 1.9-μm-diameter charge-stabilized sulfate- and carboxyl-functionalized latex microspheres (Invitrogen), 2.48-μm-diameter sulfate-functionalized PMMA microspheres (Bangs Laboratories, synthesized by emulsion polymerization), 1.7-μm-diameter polyvinylalcohol-stabilized polystyrene (synthesized according to the procedure in Paine et al.$^{39}$, courtesy of Dr. Jin-Gyu Park) and 3.7-μm-diameter polyvinylpyrrolidone-stabilized PMMA (synthesized according to the procedure in Cao et al.$^{40}$, courtesy of Dr. Jin-Gyu Park), and 1.0-μm-diameter non-functionalized silica microspheres with SiOH surface groups (Bangs Laboratories). We centrifuge and wash them ten times in deionized water (Elix, EMD Millipore, resistivity = 18.2 MΩ·cm) to remove contaminants and surface-active compounds, then dilute them for use in experiments.

We also examine several different types of oil-dispersible particles: 1.0-μm-diameter (Bangs Laboratories) and 4.0-μm-diameter (AngstromSphere) silica microspheres, both with SiOH surface groups, 1.1-μm-diameter polydimethylsiloxane-stabilized PMMA particles (synthesized according to the procedure in Klein et al.$^{41}$, courtesy of Dr. Guangnan Meng), and 1.6-μm-diameter poly(12-hydroxystearic acid)-stabilized PMMA particles (synthesized according to the procedure in Elsesser et al.$^{42}$, courtesy of Dr. Andrew D. Hollingsworth). These are listed in Table 2.1.
Table 2.1: Different particles used in breaching experiments.

<table>
<thead>
<tr>
<th>Particle</th>
<th>Diameter (μm)</th>
<th>Shorthand</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulfate-functionalized PMMA spheres (Bangs Laboratories)</td>
<td>2.48</td>
<td>PMMA</td>
</tr>
<tr>
<td>Polyvinylpyrrolidone-stabilized PMMA spheres</td>
<td>3.67</td>
<td>PVP-PMMA</td>
</tr>
<tr>
<td>Sulfate-functionalized polystyrene (Invitrogen)</td>
<td>1.88</td>
<td>PS</td>
</tr>
<tr>
<td>Polyvinylalcohol-stabilized polystyrene</td>
<td>1.65</td>
<td>PVA-PS</td>
</tr>
<tr>
<td>PDMS-PMMA (oil-dispersed)</td>
<td>1.1</td>
<td>PDMS-PMMA</td>
</tr>
<tr>
<td>PHSA-PMMA (oil-dispersed)</td>
<td>1.6</td>
<td>PHSA-PMMA</td>
</tr>
<tr>
<td>Silica (oil- and water-dispersed)</td>
<td>1.0</td>
<td>silica</td>
</tr>
<tr>
<td>Silica (oil-dispersed)</td>
<td>4.0</td>
<td>large silica</td>
</tr>
</tbody>
</table>

Table 2.2: The different aqueous-decane interfaces used.

<table>
<thead>
<tr>
<th>Shorthand</th>
<th>Aqueous phase (index)</th>
<th>Oil (index)</th>
</tr>
</thead>
<tbody>
<tr>
<td>water/glycerol</td>
<td>59% w/w glycerol in water (1.411)</td>
<td>decane (1.411)</td>
</tr>
<tr>
<td>water</td>
<td>Water (1.333)</td>
<td>decane (1.411)</td>
</tr>
<tr>
<td>water/ethanol</td>
<td>10 % v/v ethanol in water (1.380)</td>
<td>decane (1.411)</td>
</tr>
</tbody>
</table>

The aqueous solvents used are deionized water (Elix, EMD Millipore, resistivity = 18.2 MΩ·cm), anhydrous glycerol (≥99% Sigma-Aldrich), pure ethanol (100%, KOPTEC), and hydrochloric acid (Fluka). For the oil phase we use decane (≥99% anhydrous, Sigma-Aldrich) which is first filtered through a PTFE membrane filter (Acrodisc). The different liquid-liquid interfaces we use in experiments are summarized in Table 2.2.

We measure the interfacial tension between water/glycerol and decane using the pendant drop method.43,44 A 1 mL syringe (Sigma-Aldrich) with a blunt end syringe needle (18 gauge, Kimble) is filled with the aqueous phase. The needle is then submerged into a disposable cuvette (VWR) filled with decane. A droplet of the aqueous phase is slowly injected into decane while images are recorded. The profile of the droplet is analyzed from the images to determine the interfacial tension.

Our custom-made polyether ether ketone (PEEK) sample cells are glued to a glass coverslip with UV-cured epoxy (Norland 60). Details on their fabrication can be found in the Supplemental Mate-
rials of Kaz et al.\textsuperscript{11}. Using these cells, we can create a stable oil-water interface consisting of a 30–80 μm-thick aqueous phase and a 2–3 mm-thick decane superphase. We use No.1 coverslips (VWR) so that the interface is within the working distance of a NA=1.4 oil-immersion objective (Nikon CFI Plan Apo VC 100X) or a NA=1.2 water-immersion objective (Nikon CFI Plan Apo VC 60XWI). We bake all glassware used to handle the colloidal particles and fluids in a pyrolysis oven (Pyro-Clean Tempyrox) to incinerate organics, then sonicate and wash the glassware with deionized water. This protocol is designed to eliminate interfacially-active contaminants.

We place the sample cell on a Nikon TE-2000 inverted microscope. We focus 5–15 μm below the interface to capture holograms of individual particles as they breach. If we start with particles that are suspended in the aqueous phase, we push them toward the interface using radiation pressure (force less than 1 pN) from out-of-focus optical tweezers, as shown in Figure 2.2. If the particles are suspended in oil, we simply allow them to sediment toward the interface.

2.2.1 Tracking particles with Digital Holographic Microscopy

We modify a Nikon TE-2000 inverted microscope into an in-line digital holographic microscope to track the particles with high temporal and spatial resolution in all three dimensions (Figure 2.2). We illuminate samples with a 660 nm imaging laser (Opnext HL6545MG) that is spatially filtered through a single-mode optical fiber (OzOptics SMJ-3U3U-633-4/125-3-5). We use a counterpropagating 830 nm trap laser (Sanyo DL8142-201), which is spatially filtered through a single-mode optical fiber (OzOptics SMJ-3U3U-780-5/125-3-5), to push particles toward the interface.

The imaging beam scatters from the sample and interferes with undiffracted light to produce an interference pattern, or hologram. After passing through the objective, holograms are recorded on a monochrome CMOS camera (Photon Focus MVD-1024E-160-CL-12), captured with a frame grabber (EPIX PIXCI E4), and then saved to disk for further processing. A background image, taken in a part of the sample with no particles, is also recorded and divided from each time-series of holograms to
Figure 2.2: Experimental setup. A sample sits on an inverted microscope and is illuminated from above with a 660 nm laser. The hologram formed by the interference of the scattered light from the sample with the undiffracted beam is then captured on a camera. An 830 nm laser is used to push spheres from the aqueous phase toward the interface. To observe the breaching and relaxation of a particle from the aqueous phase, we push it gently toward the interface using optical tweezers, and measure its trajectory using holographic microscopy. To observe particles breaching from the oil phase, we simply let the particles fall to the interface.

remove artifacts arising from scattering from imperfections on the camera, lenses, and mirrors. We typically work at 50 mW power for the 660 nm illumination beam. We use a short camera exposure time, 20 μs, to minimize motion blur. We capture holograms at up to 2000 frames per second, giving us sub-millisecond time resolution.

The background-divided holograms are analyzed using the open-source software package HoloPy (http://manoharan.seas.harvard.edu/holopy), developed in our research group. We extract particle positions in each hologram frame by fitting the Lorenz-Mie scattering model to the background-divided holograms, as described in Fung et al. 45, following the work of Ovryn and Izen 27.
and of Lee and coworkers\textsuperscript{28}.

The accuracy of the Lorenz-Mie model depends on the refractive index mismatch between the two liquid phases. The Lorenz-Mie scattering solution used to analyze the holograms is only exact for particles in an optically homogeneous medium. Exact light scattering solutions for particles straddling an optically discontinuous boundary do not exist. Therefore, to determine the position of bound particles with maximum accuracy, we index-match the aqueous phase to decane ($n = 1.41$) by mixing anhydrous glycerol ($\geq 99\%$ Sigma-Aldrich) with water (59\% w/w glycerol) so that the system is optically continuous. This index-matching also prevents reflections from the fluid-fluid interface, which would produce additional interference.

For some of the experiments, we cannot index match the aqueous medium to the oil phase. Because silica particles typically have a refractive index of 1.42 at our imaging wavelength (660 nm) and a high density compared to water\textsuperscript{46,47}, we cannot obtain sufficient radiation pressure to push them toward the interface if they are submerged in an aqueous medium with $n = 1.41$. Instead we disperse them in water ($n = 1.33$), so that the refractive index contrast between the particles and medium is large enough for us to manipulate them with the trapping laser. In our analysis, we allow the particle’s refractive index relative to the medium to vary during the fit, which helps compensate for the particle moving through fluids of different refractive indices. Although the index mismatch across the interface sacrifices some of the accuracy of measurements, omitting glycerol allows us to measure the relaxation of silica spheres.

The refractive index of the aqueous phase raises another imaging problem. Microscope objectives and their immersion fluids are designed to image objects in two dimensions. Having an immersion oil with a refractive index different from that of the medium in which the particle is submerged results in spherical aberration, which distorts distances in the axial direction\textsuperscript{48}. This effect compromises the positioning accuracy in the direction along the imaging axis. To reduce spherical aberration, we use an immersion oil with $n=1.4140$ (Series AA, Cargille) in conjunction with our $100\times$ oil-immersion
Figure 2.3: Typical trajectories of different particles as they breach various aqueous-oil interfaces, as listed in Table 2.2. The distance between the top surface of the particle and the interface is shown as a function of the time after the breach. All the trajectories show logarithmic relaxation. We define \( z = 0 \) µm as the height at which the particle and interface first touch.

2.3 Results

2.3.1 Slow relaxation is not particular to a water/glycerol-decane interface

We begin by showing that the slow relaxation of particles at an interface is not particular to the decane-water/glycerol system of Kaz et al.\(^{11}\). In that work, the aqueous phase was chosen to match the refractive index of decane yet still have an interfacial tension and Debye screening length similar to water. Here we track 1.9 µm sulfate-functionalized polystyrene particles (Invitrogen) as they approach the interfaces from several different aqueous solutions (Table 2.2). All aqueous solutions contain 100 mM NaCl to screen electrostatic repulsion between the particle and the interface.
The motion of the polystyrene particles through the interface is approximately logarithmic with time in all of the systems, as shown in Figure 2.3. Although some error is introduced in our analysis by the assumption of an index-matched interface, we do not expect the error to change the functional form of the relationship between height and time. Therefore, we conclude that the slow dynamics are not unique to the water/glycerol and decane system studied in Kaz et al.¹¹ and are potentially relevant to a variety of liquid interfaces.

2.3.2 Polymer hairs can pin the three-phase contact line

We now return to the index-matched water/glycerol and decane system to quantitatively measure how different particles breach the interface. The area per defect, \( A \), can be determined by fitting Equation 2.5 to the logarithmic regime of the measured trajectories. Kaz and coworkers determined that for polystyrene latex particles, the fitted \( A \) did not vary for different particle sizes but did change with the surface functionalization. They found that \( A \) was on the order of the area per charge group for sulfate- \( (A \approx 5 \text{ nm}^2) \), carboxyl- \( (A \approx 3 \text{ nm}^2) \), amidine- \( (A \approx 15 \text{ nm}^2) \), and carboxylate-modified-latex \( (A \approx 25 \text{ nm}^2) \) spheres. These results suggest that the charges themselves, or some surface features associated with the charges, could be the pinning sites.

To understand how the charges influence the pinning, we do experiments on 1.9-\( \mu \text{m} \)-diameter carboxyl latex spheres suspended in a 59% glycerol in water solution containing 100 mM NaCl. We work with carboxyl-functionalized spheres because the pKa is higher than that of sulfate-functionalized spheres.⁴⁹ We add acid to the suspension to alter the particles’ zeta potentials (measured on a DelsaNano C zeta potentiometer (Beckman Coulter)) as shown in Table 2.3. By increasing the amount of water in the aqueous phase by about 1% w/w to introduce a slight index mismatch with the oil phase, we are able to measure the interfacial tension using the pendant drop method. We find no variation in the interfacial tension with acid concentration.

We find that at any given time after the particles breach, particles submerged in higher acid con-
Table 2.3: Zeta potentials, measured on a DelsaNano C zeta potentiometer (Beckman Coulter.)

<table>
<thead>
<tr>
<th>Concentration of HCl (M)</th>
<th>Zeta potential (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>-95 ± 10</td>
</tr>
<tr>
<td>10⁻⁵</td>
<td>-68 ± 2</td>
</tr>
<tr>
<td>10⁻⁴</td>
<td>-60 ± 2</td>
</tr>
<tr>
<td>10⁻³</td>
<td>-24 ± 1</td>
</tr>
</tbody>
</table>

Figure 2.4: Trajectories of carboxyl-functionalized latex particles in solutions of varying acid concentration. Lines are the average of several particle trajectories at each acid concentration.

Concentrations are at larger heights (Figure 2.4). According to Equation 2.6, there are two possible interpretations of this observation: either the energy of the defect decreases with acid concentration, or the equilibrium contact angle increases with acid concentration. These two quantities cannot be determined independently using either of the two dynamic models\textsuperscript{10,11}; however, it stands to reason that a smaller surface charge should increase the hydrophobicity of the particles and thus their equilibrium contact angle.

To better understand the nature of the pinning sites, we fit Equation 2.5 to the logarithmic regime in our data (Figure 2.4). The area per defect, which influences the slope of the trajectory, is between 4 nm\(^2\) and 6 nm\(^2\) for each of the four samples. Because the zeta potentials (and hence the areas per charge) change markedly with the acid concentration, the nearly constant fitted areas per
defect suggest that the pinning sites are probably not the charges themselves. The contact line may instead be pinning to the surface features associated with the charged groups.

One possible candidate for the surface feature that pins the contact line is polymer ‘hairs’, which include the polymer chains that carry the functional groups. The presence of flexible polymer chains on the surfaces of polymer particles has been inferred from electrophoretic mobility measurements\textsuperscript{38,50} and optical measurements of the interaction between a polymer particle and a surface\textsuperscript{51}. Jensenius and Zocchi\textsuperscript{51} found that polymer particles can become attached to surfaces, and that the attachment tether has a spring constant expected of a 50-nm-long polymer chain. Rosen and Saville\textsuperscript{38,50} found that both ‘hairy’ latex particles (with chains grafted onto their surface) and ‘bare’ latex particles had much lower electrophoretic mobilities than those predicted by classical electrokinetic theory. The discrepancy between experiment and theory was similar for both types of particles, suggesting that even ‘bare’ particles have ‘hairs’. For both types of particles, the agreement between experiment and theory improved dramatically after the particles were heated past their glass transition temperature to allow the ‘hairs’ to anneal to the surfaces of the particles. These experiments suggest that polymer hairs may be a common feature of polymer particles, whether there are chains deliberately grafted onto the surface or not.

We probe the breaching behavior of a range of other polymer particles to investigate the role of the type of polymer on the pinning (Table 2.1). The aqueous-dispersed particles we examine are charge-stabilized polystyrene, charge-stabilized PMMA, PVA-stabilized polystyrene synthesized by dispersion polymerization, and PVP-stabilized PMMA synthesized by dispersion polymerization. In the case of the polymer-stabilized particles, the polymer is adsorbed onto the surface rather than grafted, so we expect much of the PVP and PVA to wash off during our cleaning protocol.

All of these polymer particles relax logarithmically after breaching, as shown in Figure 2.5. We fit Equation 2.5 to the data to yield $A = 4.6 - 11$ nm$^2$ for the particles. Using Equation 2.6, we calculate the pinning energies assuming $\sigma_{ow} = 37$ mN/m, $T = 295$ K, $z_C/r = 0.3$ for PS and 0.2 for the other
particles, and equilibrium contact angles of 110° for PS and 90±10° for the other particles to obtain \(\Delta U = 50–85 \, kT\). These results are shown in Table 2.4.

Table 2.4: Fitted \(A\) and \(\Delta U\) for various polymer particles. The errors account for uncertainties in the values for \(z_C\) and \(z_E\).

<table>
<thead>
<tr>
<th>Particle type</th>
<th>(A) (nm(^2))</th>
<th>(\Delta U) (kT)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PMMA</td>
<td>7.1</td>
<td>55 ± 10</td>
</tr>
<tr>
<td>PVP-PMMA</td>
<td>6.3</td>
<td>50 ± 10</td>
</tr>
<tr>
<td>PS</td>
<td>4.6</td>
<td>50 ± 5</td>
</tr>
<tr>
<td>PVA-PS</td>
<td>11</td>
<td>85 ± 10</td>
</tr>
</tbody>
</table>

We also examine the relaxation of 1.1-μm-diameter polydimethylsiloxane-stabilized PMMA particles (PDMS-PMMA)\(^{41}\) and 1.6-μm-diameter poly(12-hydroxystearic acid)-stabilized PMMA particles (PHSA-PMMA)\(^{42}\) dispersed in the oil phase. We can determine the contact angle of the particles (see Figure 2.6) from the heights before and after they sink into the aqueous phase. Both PHSA- and PDMS-stabilized particles reach a steady-state contact angle of 130–150° within a second of breaching. These values are close to the measured equilibrium contact angles for PMMA particles\(^{52}\).

Compared to the relaxation of aqueous-dispersed polymer spheres, that of hard-sphere PMMA
particles is much faster. This may be because the surfaces of the PDMS- and PHSA- stabilized PMMA particles, which contain grafted hydrophobic PHSA- and PDMS- groups, pin the interface with lower energy. We fit Equation 2.5 to the first second of the breaching trajectory for the PDMS-PMMA particle (Figure 2.6c) and obtain $A = 8 \text{ nm}^2$. Using Equation 2.6, we calculate the pinning energy with $z_E - z_C = 110 \text{ nm}$, $\sigma_{ow} = 37 \text{ mN/m}$, $T = 295 \text{ K}$ and $R^* = 330 \text{ nm}$. We obtain $\Delta U = 4kT$, an order of magnitude smaller than the pinning energy found for aqueous-dispersed polymer particles (Table 2.4).

![Image of Figure 2.6](https://example.com/figure26.png)

Figure 2.6: a) A PHSA-stabilized PMMA sphere reaches a steady-state contact angle of 150°. b) Fluctuations of a 1.1-μm-diameter PDMS-PMMA particle decrease after the particle ‘sinks’ into the interface. The sphere reaches a steady-state contact angle of 130°. c) The same data from b) plotted on semilogarithmic axes.

2.3.3 Inorganic particles can also pin contact lines

We now determine whether inorganic particles also have surface features that can pin the contact line. We track 1 μm bare silica spheres (Bangs Laboratories) approaching a water-decane interface from both phases, and 4 μm bare silica spheres (AngstromSphere) approaching an index-matched glycerol-water/decane interface from the decane phase.

The 4 μm silica spheres relax logarithmically at the interface before reaching a steady-state height after less than 1 s (Figure 2.7). Holographic snapshots of the particle during the breach are shown in Figure 2.7; these silica particles are large enough that the slow evolution of the fringe pattern is obvious even by eye. The first hologram in Figure 2.7 shows the particle prior to breaching, and the rest of the...
holograms show the fringe pattern change from having a dark center to a bright center, reflecting a change in the axial-position of the sphere. Fitting Equation 2.5 to the logarithmic regime yields \( A = 1 \text{ nm}^2 \). We calculate \( \Delta U \) using Equation 2.6 with \( z_C/r \) between 0 and 0.84 and find that the pinning energy is 5–10 \( kT \). This value is low compared to the pinning energies found for aqueous-dispersed polymer spheres. The result is consistent with the notion that particles that reach a steady-state contact angles on experimental timescales pin the contact-line with smaller energies.

If we assume the surface asperities are roughly hemispherical caps, then we can compare our fitted \( A \) directly with the root-mean-squared surface roughness measurements from the literature. Ruiz and coworkers\(^{53} \) measured the RMS roughness of 5.2-\( \mu \text{m} \)-diameter silica particles from Bangs Laboratories to be 1.4 nm. Our holographic measurement of asperity size for large silica spheres, \( A = 1 \text{ nm}^2 \), is in good agreement with this RMS roughness measurement.

The smaller silica spheres reach a steady-state position within 20 ms when approaching from either phase, as shown in Figure 2.7. The lack of any observable logarithmic relaxation suggests that either the crossover between the fast and logarithmic relaxation regimes is at later times than what we can observe, or that the difference between \( z_E \) and \( z_C \) is small. According to Equation 2.6, a small dif-
ference between $z_E$ and $z_C$ means that $\Delta U/A$ is small. Using AFM, Chen and coworkers\textsuperscript{54} measured the RMS roughness to be about 0.36 nm for similar-sized silica spheres (0.74 μm-diameter particles from Duke), which is smaller than the RMS roughness value for larger silica spheres (1.4 nm)\textsuperscript{53}. It is therefore likely that the asperities on smaller particles pin the contact line with less energy than the asperities on larger silica particles, resulting in a more rapid path to equilibrium for smaller silica particles, as we observe.

2.3.4 \textbf{Logarithmic relaxation is expected in vigorously mixed systems}

We conclude with a discussion of whether slow relaxation is likely to affect systems that are mixed more vigorously than ours – for example, Pickering emulsions prepared by shearing. For a 1.9-μm-diameter polystyrene sphere with an equilibrium contact angle of 110°, Equation 4.1 reveals that the force on the particle integrated along the contact line is 10–100 nN for dynamic contact angles between 2° and 107°. The force on a 1.9-μm-diameter particle in a suspension that is mixed at 11000 rpm in an Ultra Turrax homogenizer is 1 nN\textsuperscript{55} – orders of magnitude smaller than the capillary driving force. Thus the logarithmic relaxation we observe will likely be unaffected by mixing, and the effect may be important to take into account in the preparation of Pickering emulsions.

2.4 \textbf{Conclusions}

The relaxation of colloidal particles at interfaces appears to be logarithmic with time in a variety of systems. When interpreted in the context of molecular kinetic theory, the slow relaxation is consistent with contact line pinning onto surface defects. Because the trajectories of particles at interfaces are dictated by the presence of surface features, measuring their adsorption trajectories emerges as a method for probing surface features that are otherwise difficult to image or measure.

We ruled out whether the ‘defects’ are in fact surface charge groups. Instead, we believe nanoscale
surface roughness (in the case of silica particles) and polymer ‘hairs’ (in the case of polymer particles) are likely responsible for pinning. We also showed that silica particles and oil-dispersed PMMA hard-spheres all reach steady-state heights within one second, likely because their surfaces pin the contact-line with an order of magnitude less energy than the aqueous-dispersed polymer particles. For aqueous-dispersed polymer particles, it is likely that the polymer chains on the surface are polyelectrolytes. To move a polyelectrolyte from the aqueous phase into the oil phase may require a large amount of energy, because all of the charges need to first be neutralized. This may explain why aqueous dispersed polymer particles have the strongest pinning sites, and the most prolonged logarithmic relaxation regimes.
So far, we have studied how spherical particles breach interfaces. In this section, we show that the discrete dipole approximation (DDA) can be used to fit holograms of non-spherical particles and track them with high precision. In Chapter 4, we demonstrate a T-matrix method for tracking axisymmetric scatterers.

3.1 Introduction

Measurements of the dynamics of colloidal particles are key to understanding the mechanisms of colloidal aggregation\(^{56}\) and self-assembly\(^{57}\). Furthermore, microscopic measurements of the diffusion of individual particles can be used to infer interactions between particles\(^{58}\) as well as the local rheological properties of the medium in which the particles are suspended\(^{59}\). Most microscopic measurements are constrained to two dimensions because of the limited depth of field of wide-field microscopy\(^{60}\).
Confocal microscopy can be used to capture three-dimensional (3D) dynamics of spherical and nonspherical particles, but the time required to scan the beam through a 3D sample limits these measurements to large particles or particles in a viscous fluid.

Holographic microscopy is an alternative technique that can in principle capture 3D colloidal dynamics with acquisition times orders of magnitude smaller than those of confocal microscopy. In a holographic microscope, light from a coherent source scatters from the sample and interferes with a reference wave, which can simply be the transmitted, undiffracted beam. The interference pattern, or hologram, contains phase information about the scattered wave and can be used to determine the 3D position of the particle. Because holograms can be captured as fast as a camera allows and then processed offline, the technique can be used to probe millisecond or even microsecond dynamics. The 3D information is typically recovered through optical or numerical reconstruction. Cheong and coworkers used this method to track the 3D translation and rotation of a high-aspect-ratio (1:25) copper oxide nanoparticle through numerical reconstruction and a skeletonization procedure. However, for wavelength-scale particles the precision of reconstructions is limited owing to distortions in the reconstructed volume.

We demonstrate a precise way to measure the dynamics of wavelength-scale, non-spherical colloidal particles from holographic measurements. Our approach, which uses an inverse-scattering analysis rather than reconstruction, follows the work of Ovryn and of Lee and coworkers, who showed that fitting a Lorenz-Mie scattering solution to a measured hologram yields nanometer-scale-precision measurements of the positions of spherical particles. Researchers from our group, including Fung and coworkers and Perry and coworkers later showed that the translational, rotational, vibrational, and nonequilibrium dynamics of several interacting wavelength-scale spheres could be measured with high precision by fitting Mackowski and Mishchenko’s multisphere scattering solution to holograms of multiple colloidal particles. All of these measurements were limited to spheres, or collections of spheres, because the fitting technique requires a solution for the scattered field, and exact
solutions are known for very few particle morphologies.

One can, however, perform approximate numerical scattering calculations for a wide variety of particles using the Discrete Dipole Approximation (DDA) of Purcell and Pennypacker. We show that numerical scattering calculations using the DDA can be fit to holograms of non-spherical particles, allowing us to track 3D translational and rotational dynamics at high precision, even for particles with subtle asymmetries. We use these measurements to determine translational and rotational diffusion coefficients and show that the measured values agree well with theoretical calculations, validating the technique. Although DDA calculations are orders of magnitude slower than calculations of exact scattering solutions, parallelization of the scattering calculations and the fitting algorithm can significantly reduce the analysis time.

Figure 3.1: The non-spherical particles used in our experiments. Top row, silica rods; bottom row, Janus particles consisting of a polystyrene sphere coated with a 50-nm-thick layer of TiO$_2$. The orientation of both types of particles is defined by the orientational unit vector $u$ and its projection onto the $x-y$ plane, $p$. The z-axis coincides with the direction of the incident plane wave illumination. (a) Scanning electron micrograph of the rods. Scale bar is 1 µm. (b) The rod is modeled as a spherocylinder with semi-minor-axis length $a$ (which is the radius of both the cylinder and the hemispherical caps) and semi-major-axis length $b$. (c) Measured hologram of a single rod. (d) Scanning electron micrograph of the Janus particles. The dotted line outlines the TiO$_2$ cap. Scale bar is 300 nm. (e) The Janus particle is modeled as a plain polystyrene sphere with a hemispherical cap of TiO$_2$ (orange). A cross section perpendicular to the equator is also shown. (f) Measured hologram of a Janus particle, shown in false color to highlight asymmetry.
3.2 Experimental methods

We use two types of anisotropic particles in this study: silica rods and polystyrene/TiO$_2$ Janus particles suspended in water (Figure 3.1). The silica rods are synthesized using a modified one-pot method recently reported by Kuijk and coworkers$^{64,65}$. In the initial growth step, we make silica rods with a length of $1.45 \pm 0.06$ $\mu$m and diameter of $0.29 \pm 0.02$ $\mu$m, which are then used as seeds in subsequent growth steps$^{66}$ to produce the final silica rods. The size of the rods, as determined by scanning electron microscopy (SEM), is $1 \pm 0.2$ $\mu$m (minor axis) by $2 \pm 0.2$ $\mu$m (major axis) (Figure 3.1a). We fabricate the Janus particles by depositing $50$ nm of TiO$_2$ onto sulfate-terminated polystyrene (PS) particles (Invitrogen), as described by Song and coworkers$^{67}$. The polystyrene particle diameter, as determined by SEM, is $900 \pm 100$ nm (neglecting anomalously large particles). The TiO$_2$ cap covers approximately one hemisphere of the particle, as shown in Figure 3.1d.

For holographic imaging, we suspend the particles in deionized water at approximately $10^{-5}$ volume fraction and place them in sample cells consisting of two No. 1 glass coverslips (VWR) separated and sealed by vacuum grease (Dow Corning). To validate the DDA method, we also image a $0.95$-$\mu$m-radius polystyrene sphere (Invitrogen) diffusing in a $54\%$ v/v glycerol solution.

We capture holograms with a digital holographic microscope built on a Nikon (TE2000-E) inverted microscope, as shown in Figure 3.2 and described in detail by Kaz and coworkers$^{11}$ and Fung and coworkers$^{29}$. When imaging the Janus particles we use a $100 \times$ oil-immersion objective (Nikon) with a high numerical aperture (1.40) that allows us to maximize detail in the holograms. When imaging the silica rods, which sediment rapidly owing to their density, we use a $60 \times$ water-immersion objective (Nikon), which has a lower numerical aperture (1.20) but a larger working distance that allows us to capture longer trajectories. To obtain true bulk-diffusion measurements, we retain data only from particles that remain at least $20$ $\mu$m away from the sample cell boundaries throughout the trajectory.
Figure 3.2: Schematic of our digital in-line holographic microscope. A series of lenses, including the condenser, shapes light from a 660 nm laser diode (Opnext HL6545MG) into a plane wave to illuminate the sample. An objective collects the transmitted light and light scattered from the sample, and a high-speed camera (Photon Focus) captures the hologram formed by the interference between scattered and transmitted waves.

In a typical experiment we use 50 mW of laser power and a 0.015 ms exposure time per camera frame for the 60× objective or 0.05 ms for the 100× objective. These exposure times are short enough to minimize blurring due to Brownian motion. We capture holograms with a Photon Focus MVD-1024E-160 camera at 100 frames per second, store them in RAM using a frame grabber (EPIX PIXCI E4), and then save to disk for further processing. For each trajectory we also record a background image from the same region of the sample cell before or after the particle is present to account for scattering and illumination defects in the optical train.

3.3 Fitting holograms using the DDA

In contrast to holograms of spheres, holograms of non-spherical particles do not have azimuthal symmetry about the central maximum (see Figure 3.1c, f). The azimuthal asymmetry encodes information about the particle shape and orientation, while the radial spacing of the interference fringes encodes the particle position. To quantitatively extract this information, we fit a scattering model to the holo-
grams.

In our fitting procedure, we compute holograms from a model, compare the computed holograms to experimentally recorded ones, and iterate until the model matches the data. To compute the hologram, we model the physical process of hologram formation: the interference of scattered and reference fields. In an inline hologram, the reference field is approximately equal to the incident field $E_i$, as long as the density of scatterers is low. In our experiments, we work at low concentrations of particles, so that we can assume $E_i$ is a constant plane wave. The observed intensity is therefore

$$I_{\text{holo}} = |E_i + E_s|^2,$$  \hspace{1cm} (3.1)

where $E_s$ is the scattered field, which we must compute to simulate the hologram. Previous work$^{28,29,11,31}$ used numerical implementations of the Lorenz-Mie solution or a multi-sphere superposition solution$^{32}$ to compute $E_s$. However, these exact solutions are limited to spheres or collections of spheres. For the non-spherical particles used here, we must compute $E_s$ approximately. We do this using the discrete dipole approximation, as implemented by the open-source scattering code ADDA$^{68}$.

Figure 3.3: Voxelations of the particles. White represents vacuum, light gray is silica, medium gray is polystyrene, and dark gray is TiO$_2$. Each dipole is represented by a square. (a) Side view of a rod with a dipole size of 44 nm, a semi-minor-axis length of 500 nm and a semi-major-axis length of 1 \textmu m. (b) Side view of a Janus particle with a dipole size of 24 nm, inner particle radius of 439 nm and a maximum cap thickness of 50 nm. (c) Cross section of the same Janus particle.

In the DDA, a scatterer is represented as an array of point dipoles. Therefore the first step of
our data analysis is to discretize the particle, dividing it into an array of voxels (volumetric pixels). To ensure validity of the DDA, we discretize the scatterer using at least 10 dipoles per wavelength in the scattering medium, as recommended by the ADDA documentation. The voxelations of both particles are shown in Figure 3.3. For the Janus particle, the dipole size is comparable to the thickness of the TiO₂ layer, but because the shape and size of the cap varies from particle to particle, a smaller dipole size would not necessarily increase accuracy.

To compute a hologram, we calculate the scattering angles for each pixel in the detector, then invoke ADDA to compute the scattering matrix of the voxelated particle at each scattering angle. We obtain the electric field \( E_s(i, j) \) at each pixel \((i, j)\) on the detector from the scattering matrices, angles, and distances. Finally, we numerically interfere the computed \( E_s(i, j) \) with \( E_l(i, j) \), a plane wave, to obtain the hologram. All of these steps are implemented in our open-source hologram processing code, HoloPy (http://manoharan.seas.harvard.edu/holopy/).

Once we have created a DDA scattering model for a particle, we use nonlinear minimization to fit the model to measured, background-divided, normalized holograms, thereby obtaining measurements of the positions, refractive index, and geometrical parameters of individual particles. Formally, we use the Levenberg-Marquardt algorithm to minimize the objective function

\[
f(p) = \sum_{i,j} |I_{\text{measured}}(i, j) - I_{\text{computed}}(i, j; \{ p \})|^2 = \chi^2
\]

where

\[
I_{\text{measured}} = \frac{I_{\text{data}}}{I_{\text{background}}} \frac{I_{\text{background}}}{I_{\text{data}}},
\]

and \( \{ p \} \) is the set or a subset of the parameters used by the DDA model to calculate the scattering from the particle. The minimization typically requires several iterations to converge. We then use the fitted position and orientations obtained from a time-series of holograms to compute the diffusion coefficients.
Because the two types of particles have different geometries, we must use different sets of parameters to model them. We model the rod, which has rounded ends as shown in Figure 3.1a, as a dielectric spherocylinder with refractive index \( n \), hemisphere radius \( a \) (equal to the length of the semi-minor-axis), and semi-major-axis length \( b \). The full set of parameters is

\[
\{p_{\text{rod}}\} = \{r, n, a, b, \theta, \psi, \alpha\},
\]

where \( r \) is the center of mass position, \( \theta \) and \( \psi \) are Euler angles describing particle orientation (see Figure 3.1b), and \( \alpha \) is a normalization constant\(^2\). We model the Janus particle (Figure 3.1d) as a dielectric sphere with refractive index \( n_{\text{PS}} \) and radius \( a_{\text{Janus}} \) capped by a hemispherical dielectric shell with index \( n_{\text{TiO}_2} \) and thickness \( t \). Here the set of parameters is

\[
\{p_{\text{Janus}}\} = \{r, n_{\text{PS}}, n_{\text{TiO}_2}, a_{\text{Janus}}, t, \theta, \psi, \alpha\},
\]

where \( r \) is the center of the sphere, \( \theta \) and \( \psi \) are the Euler angles shown in Figure 3.1e, and \( \alpha \) is again a normalization constant.

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**Figure 3.4**: Procedure for fitting DDA models to experimental holograms of non-spherical particles to extract position and orientation. The numbers refer to the steps in the text.

To assess the validity of the fits, we examine the coefficient of determination, \( R^2 \), which measures
how much of the variation from the mean value of one hologram is captured in another:

\[ R^2 = 1 - \frac{\sum_{i,j} |I_1(i,j) - I_2(i,j)|^2}{\sum_{i,j} |I_1(i,j) - \bar{I}_1|^2}, \]  

(3.5)

where \( \bar{I}_1 = 1 \) is the mean value of the normalized hologram. \( R^2 \) is a useful quantity because unlike \( \chi^2 \), which is sensitive to the amplitude of the hologram fringes, the \( R^2 \) values for good fits do not vary markedly across experimental systems with different scattering cross sections.\(^{69} \) We use \( R^2 \) to assess how well DDA reproduces the results of exact Lorenz-Mie calculations for holograms of spheres and to assess how well the holograms from our model match data from experiments.

Because DDA calculations take at least an order of magnitude longer than Lorenz-Mie calculations for scatterers of the same size, we aim to fit all the frames in parallel. This goal is complicated by convergence requirements. In practice, we have found that the minimization does not converge unless the initial guess for \( \{p\} \) is close to the global minimum of the objective function. Therefore our usual procedure\(^ {29,30} \) is to fit the frames sequentially: we use reconstruction to manually obtain an initial guess for the first frame of the trajectory and then use the best-fit result for each frame as the initial guess for the next. Here such a process would take too long. A DDA calculation takes on the order of 30 seconds on a single CPU; since about 200 such calculations are required for each minimization to converge, it would take 20 weeks to sequentially fit a 2000-frame trajectory. We therefore use the procedure described below and illustrated in Figure 3.4 to obtain the initial guesses for each frame and fit the frames in parallel:

1. **Select the most asymmetric region**: The particle orientation is primarily encoded in the rotational asymmetry of a hologram. We maximize sensitivity to the particle orientation by cropping the hologram to leave only the most asymmetric region, the first fringe or two around the central maximum. We use a Hough transform-based algorithm\(^ {70} \) to locate the center of the hologram. For our experiments the first fringe is located within a 140 × 140 pixel area.
2. **Find approximate position with Lorenz-Mie fit:** We obtain an estimate of the particle’s position by approximating it as a sphere with parameters \( \{ p \} = \{ r_{\text{Mie}}, n, a_{\text{Mie}}, \alpha \} \) and fitting the Lorenz-Mie solution to the hologram. This fit is very fast (order of 10 seconds per frame) and although \( \chi^2 \) is large, \( r_{\text{Mie}} \) provides a good initial guess for the position of the particle’s center.

3. **Make rotation library:** We use the DDA to calculate holograms of the non-spherical particle over a range of Euler angles \( \theta, \psi \), assuming a center position \( r_{\text{Mie}} \).

4. **Use library to guess angles:** We compare this library to the experimental hologram and determine the Euler angles \( \theta, \psi \) that yield the highest \( R^2 \) value.

5. **Initial DDA fit:** We fit the DDA model to the measured hologram using the guess for \( r_{\text{Mie}} \) from step 2, \( \theta \) and \( \psi \) from step 4, bulk values for the index or indices, and an estimate of the geometrical parameters \( a \) and \( b \) or \( a_{\text{PS}}, t \) from SEM images. We allow all parameters to vary during the fit. In this step, we fit the first 500 frames of a trajectory, discard the fits that fail to converge, then calculate the average best-fit refractive indices and radii.

6. **Final DDA fit:** Because the particle’s physical properties should not change across a trajectory, we fix the refractive indices and radii to the average values from step 5 and fit the cropped holograms for an entire trajectory to obtain the position and orientation as a function of time.

This procedure removes the serial dependency between the frames in a time series, allowing us to send each frame in the initial (step 5) and final (step 6) DDA fits to an individual CPU on a computing cluster. However, removing the serial dependency potentially introduces artifacts in the trajectories of the particles, since the results from each frame are not used to constrain the results of the next. We must therefore detect and correct spurious fit results.

There are two kinds of errors. The first arises because the rod particles are symmetric, having two degenerate orientational unit vectors pointing in opposite directions. In a trajectory constructed
from a series of parallel fits, the fitted orientational unit vector may therefore flip almost 180° between
frames. Using the probability density function for rotational displacements described by Fung and
coworkers\(^3^0\) and an estimated rotational diffusion coefficient \(D_{\text{est}} = 0.3 \text{ s}^{-1}\), we estimate that the
probability of a 90° or greater change in angle between frames is zero, to within machine precision.
Therefore we can correct artificial flips by examining pairs of frames in the trajectory and flipping the
vector in the second frame by 180° if the angle changes by more than 90° between frames.

The second type of spurious fit result arises because the Levenberg-Marquardt algorithm is sensi-
tive to noise. Depending on the initial guess, a fit can converge to a secondary minimum in which the
orientational unit vector \(\mathbf{u}\) points in a direction mirrored about the x-y plane from that of the previous
frame. A small number of these bad fits systematically increases the apparent diffusion coefficient.

We therefore reject contributions to the mean-squared displacement where the probability of the
measured angular displacement is less than 0.0001. Again, we calculate the probability of observing
a given displacement using the probability density function for rotational displacements described
by Fung and coworkers\(^3^0\) and an estimated rotational diffusion coefficient \(D_{\text{est}} = 0.3 \text{ s}^{-1}\). Using
\(D_{\text{est}} = 0.2 \text{ s}^{-1}\) or \(D_{\text{est}} = 0.4 \text{ s}^{-1}\) changes the coefficients extracted from the data by less than 7%, an
effect which we include in the error on the measured diffusion coefficients. This procedure eliminates
poor fit results while minimizing the bias of results toward our estimated diffusion coefficient.

3.4 Results and discussion

We demonstrate the validity and usefulness of the DDA as a tool for holography and particle tracking
through a series of experimental and computational tests. First we compare the results of DDA and
Lorenz-Mie calculations for scattering from spherical particles, which the Lorenz-Mie solution mod-
els exactly. Next we fit the DDA model to holograms of a rod and a Janus particle, and we obtain
translational and rotational diffusion coefficients from the mean-squared displacements measured
across time-series of holograms. We compare the measured diffusion coefficients to predicted values to demonstrate that we accurately and precisely capture the particle motion.

3.4.1 Validation of DDA calculations and fits

We first examine how the approximations involved in our DDA implementation affect the quality of the fits and calculated holograms. To do this, we compare holograms of spheres calculated with our DDA model to those calculated from the Lorenz-Mie solution. We are interested in two effects: first, how aliasing introduced by the voxelation affects the precision of fitting holograms and, second, how the absence of a near-field calculation in ADDA affects the accuracy of the holograms. By “near field” we mean the part of the scattered field that does not follow an asymptotic $1/r$ decay. ADDA calculates only the asymptotic portion of the scattered field. In previous work we found that it was necessary to include the full radial dependence of $E_s$ to accurately fit holograms of particles close to the focal plane.

We test the validity of the scattering model, including our voxelation scheme, by fitting the DDA model and the Lorenz-Mie solution to 893 measured holograms of a 0.95-μm-radius polystyrene sphere (Invitrogen) freely diffusing in a 54% v/v glycerol solution. We fix both the refractive index and radius of the particle and allow only the center position of the sphere to vary. Throughout the trajectory, the particle remains 15 μm to 18 μm from the focal plane.

We find that the best-fit $x$- and $y$-coordinates obtained by fitting the DDA model differ negligibly (0.3±0.1 nm) from those obtained from Lorenz-Mie, while the best-fit $z$-coordinates differ by 194±1 nm, a significant offset. This difference in apparent axial position might arise from voxelation errors or the asymptotic approximation for the radial dependence of the scattered field. The Lorenz-Mie model includes non-asymptotic corrections for the scattered field, while the DDA model does not.

The offset in the $z$-coordinate does not affect our dynamical measurements, which depend only on the displacement of the particle, not its absolute position. We find that the total displacements
\|\mathbf{r}(t) - \mathbf{r}(0)\| \text{ for the DDA and Lorenz-Mie models, as calculated from the best-fit coordinates for the entire trajectory, agree to within 0.5 nm (Figure 3.5a) for most of the frames analyzed. The maximum deviation in displacement is 2 nm, which is within the reported accuracy of holographic measurements based on inverse-scattering analysis.}^{28} \text{ Thus the systematic error in the absolute particle position appears to cancel when calculating the displacement, and the resulting displacement measurements are nearly as precise as those obtained by fitting the data to the exact Lorenz-Mie solution.}

![Figure 3.5: Comparison of the DDA model and Lorenz-Mie solution for fitting holograms of a single sphere. (a) Histogram of differences between displacements of a 950-nm-radius diffusing sphere measured by fitting the DDA and Lorenz-Mie models to measured holograms. (b) } R^2 \text{ for pairs of holograms calculated from the exact Lorenz-Mie solution and the DDA model of a 450-nm-radius sphere at various distances from the imaging plane. Inset: voxelation of the 450-nm-radius sphere used in the DDA model. The voxel size is 34 nm. The particle refractive index is 1.585 (gray).}

To further examine the impact of the asymptotic approximation for the scattered field, we compare holograms computed from the DDA model and Lorenz-Mie solutions for a 450-nm-radius sphere as a function of distance from the focal plane. The agreement between the two models, as measured by the } R^2 \text{ value, increases with distance from the imaging plane, as shown in Figure 3.5b. These data suggest that near-field effects start to vary significantly with distance when the particle is 5 \mu m or less from the focal plane. To ensure the validity of our DDA fits, we take all measurements with the particle at least 5 \mu m from the imaging plane.

As another check on the validity of the technique, we examine the average of fitted values for the optical properties and sizes of our non-spherical particles. We find } n = 1.495 \pm 0.012 \text{ for the rod,}
$n_{PS} = 1.581 \pm 0.046$ for the polystyrene particle and $n_{TiO_2} = 2.74 \pm 0.23$ for the shell of the Janus particle, all of which are close to bulk values for the materials. The best-fit dimensions of the rod are $2a = 1.002 \pm 0.037 \ \mu m$ and $2b = 2.158 \pm 0.143 \ \mu m$, in agreement with the measurements from SEM. The fitted dimensions of a Janus particle are $a_{Janus} = 443 \pm 20 \ \text{nm}$, in excellent agreement with the SEM images, and $t = 47 \pm 8 \ \text{nm}$, in good agreement with the expected value based on the TiO$_2$ deposition process.

Figure 3.6: Trajectories of particles as determined from DDA fits. (a) Rod trajectory captured at 100 frames per second for 20 seconds. (b) Janus particle trajectory captured at 100 frames per second for 30 seconds.

Qualitatively, the trajectories of the particles we obtain by fitting the DDA model to time-series of holograms are consistent with stochastic Brownian motion (Figure 3.6). Furthermore, the rod appears to sediment at 0.9 $\mu m/s$ whereas the Janus particle diffuses about a steady height. This behavior agrees qualitatively with our expectations for the two systems: the rod should sediment more rapidly because the density difference between the rod and solvent is about three times larger than that of the Janus particle, and the rod has about three times the volume of the Janus particle.

Finally, to quantitatively investigate the validity of the DDA fits, we determine the rotational and translational diffusion coefficients from the data and compare them to theoretically predicted values, as described in the following sections.
3.4.2 Rods

When viewed in the laboratory frame, the motion of a rod is complicated because the viscous drag along any laboratory axis depends on the orientation of the particle. But in the frame of the particle, translational diffusion can be broken down into two components, one parallel to the long axis of the particle (∥) and the other along either of the two principal axes perpendicular to the long axis (∥). Due to the optical symmetry of the particle, we cannot observe rotations about the major axis. We can therefore measure only one rotational diffusion coefficient, corresponding to rotation about either of the two degenerate principal axes that are coincident with the minor axis.

To measure these diffusion coefficients, we use the best-fit positions obtained from the holograms to calculate the translational and angular mean-squared displacements in the particle frame as a function of lag time. We average these displacements over time and calculate the uncertainties associated with these averages using a block decorrelation method. Finally, we fit the mean-squared displacements using the calculated uncertainties as weights. The uncertainties are represented as error bars in the figures below. In the following sections we report the formulas used to calculate and fit the displacements, as well as our measured and predicted values for the diffusion coefficients.

Theoretical predictions

For clarity, we denote all predicted values of diffusion coefficients with a prime symbol ('). The diffusion coefficients of a spherocylinder can be expressed as power series in the aspect ratio $\omega = b/a$, as discussed in the appendix of Martchenko et al., which is adapted from Yoshizaki et al. and
Norisuye et al.\textsuperscript{74}:

\begin{equation}
D_t' \approx \frac{k_B T}{6 \pi \eta b} \left( \ln \omega + 0.3863 + \frac{0.6863}{\omega} - 0.0625/\omega^2 - \right.
\left. 0.01042/\omega^3 - 0.000651/\omega^4 + 0.0005859/\omega^5 \right) \tag{3.6}
\end{equation}

where \( D_t = (2D_\perp + D_\parallel)/3 \). We compare our results to the predicted value for \( D_t \), which is a linear combination of the measured diffusion coefficients. From the same reference, we obtain the rotational diffusion coefficient:

\begin{equation}
D_r' \approx \frac{3k_B T}{8 \pi \eta b} \left( \ln \omega + 2 \ln 2 - \frac{11}{6} + \frac{\ln 2}{\ln(1 + \omega)} \left[ \frac{1}{3} - 2 \ln 2 + \frac{11}{6} - |a| \right] + a \cdot \Omega \right) \tag{3.7}
\end{equation}

where

\[ a = [13.04468, -62.6084, 174.0921, -218.8365, 140.26992, -33.27076], \]

and

\[ \Omega = [\omega^{-1/4}, \omega^{-2/4}, \omega^{-3/4}, \omega^{-4/4}, \omega^{-5/4}, \omega^{-6/4}]. \]

Because the rods are slightly asymmetric—flatter on one end of the rod than the other (see Figure 3.1a)—we also compare our results for the translational and rotational diffusion coefficients to theoretical predictions for a cylinder with flat ends\textsuperscript{75}:

\begin{equation}
D_\perp' \approx \frac{k_B T}{8 \pi \eta b} \left( \ln \omega + 0.839 + 0.185/\omega + 0.233/\omega^2 \right) \tag{3.8}
\end{equation}

\begin{equation}
D_\parallel' \approx \frac{k_B T}{4 \pi \eta b} \left( \ln \omega - 0.207 + 0.980/\omega - 0.133/\omega^2 \right) \tag{3.9}
\end{equation}
\[ D' \approx \frac{3k_BT}{8\pi \eta b^3} \left( \ln \omega - 0.662 + 0.917/\omega - 0.050/\omega^2 \right). \]  

(3.10)

We use the laboratory temperature and uncertainty, \(21 \pm 2^\circ C\), the corresponding viscosity for water, and the dimensions of the particle from the hologram fits to calculate the predicted diffusion coefficients and their uncertainties. Because the values of rod’s fitted dimensions are not normally distributed, we do not include them when propagating the errors.

**Translational diffusion**

To extract translational diffusion coefficients from the data, we first calculate the mean-squared displacements parallel and perpendicular to the major axis. For short lag times \(\tau\) we calculate

\[ \Delta r^2_\parallel (\tau) = \langle |(r(t+\tau) - r(t)) \cdot u(t)|^2 \rangle = 2D_\parallel \tau + 2\epsilon_\parallel^2 \]  

\(3.11\)

\[ \Delta r^2_\perp (\tau) = \langle |(r(t+\tau) - r(t)) \times u(t)|^2 \rangle = 4D_\perp \tau + 4\epsilon_\perp^2 \]  

\(3.12\)

where the angle brackets denote a time average over all contributing pairs from a single trajectory. We then determine the diffusion coefficients \(D_\parallel\) and \(D_\perp\) and measurement errors \(\epsilon_\parallel\) and \(\epsilon_\perp\) by fitting a linear model to the measured mean-squared displacements.

Our measured results agree with those predicted by the spherocylinder model. We obtain \(D_\parallel = 0.307 \pm 0.028 \mu m^2/s\), \(D_\perp = 0.293 \pm 0.025 \mu m^2/s\) and \(D_t = 0.298 \pm 0.026 \mu m^2/s\) (see Figure 3.7 and Table 3.1.). These values are larger than the predictions for a cylinder with flat ends \((D'_\parallel = 0.301 \pm 0.013 \mu m^2/s\) and \(D'_\perp = 0.265 \pm 0.012 \mu m^2/s\)) but agree quantitatively with the spherocylinder model \((D'_t = 0.297 \pm 0.016 \mu m^2/s)\), suggesting that the rods are better modeled as spherocylinders.

The fit, which is constrained by the small uncertainties at shorter lag times, falls outside the error range for the perpendicular direction at larger lag times (Figure 3.7). However, the fit for the paral-
Figure 3.7: Measured mean-squared displacements for the silica rods. (a) mean-squared displacements parallel and perpendicular to the major axis. The solid lines are fits to $2D_\parallel \tau + 2\epsilon_\parallel^2$ and $4D_\perp \tau + 4\epsilon_\perp^2$. (b) mean-squared displacement of the orientational unit vector $\mathbf{u}$. The solid line is a fit to an exponential decay $2 - 2\exp(-2D_c \tau) + 2\epsilon_\tau^2$.

lel component is well within the error bars throughout the entire range of lag times. The apparent augmented motion in the perpendicular direction may be due to radiation pressure from the incident beam or coupling between sedimentation and diffusion.\(^76\)

Table 3.1: Measured and expected values of parameters for silica rods. Theoretical predictions for the spherocylinder model are based on relations in Martchenko et al.\(^72\), and those for the cylinder model on relations in Tirado et al.\(^75\).

<table>
<thead>
<tr>
<th>Quantity</th>
<th>Experiment</th>
<th>Expected</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>$a$ (nm)</td>
<td>501 ± 19</td>
<td>500 ± 50</td>
<td>SEM</td>
</tr>
<tr>
<td>$b$ (nm)</td>
<td>1079 ± 72</td>
<td>1000 ± 100</td>
<td>SEM</td>
</tr>
<tr>
<td>$n$</td>
<td>1.495 ± 0.012</td>
<td>≤ 1.54</td>
<td>bulk value</td>
</tr>
<tr>
<td>$D_t \times 10^{-13}$ m$^2$s$^{-1}$</td>
<td>0.298 ± 0.026</td>
<td>0.297 ± 0.016</td>
<td>spherocylinder model</td>
</tr>
<tr>
<td>$D_r$ (s$^{-1}$)</td>
<td>0.311 ± 0.034</td>
<td>0.311 ± 0.017</td>
<td>spherocylinder model</td>
</tr>
<tr>
<td>$D_\parallel / D_\perp$</td>
<td>1.04 ± 0.05</td>
<td>1.13</td>
<td>cylinder model</td>
</tr>
</tbody>
</table>

The measured ratio $D_\parallel / D_\perp = 1.05 ± 0.04$ depends only on the aspect ratio of the particle. Our value is smaller than that predicted by the cylinder model ($D'_\parallel / D'_\perp = 1.13$). The small discrepancy in the measured and predicted values of $D_\parallel / D_\perp$ is not surprising, given how much the measured $D_\parallel$ and $D_\perp$ values differ individually from those predicted by the cylinder model. We are not able to calculate $D_\parallel / D_\perp$ for the spherocylinder model because we do not have explicit expressions for the two individual components. In general, however, we expect $D_\parallel / D_\perp$ to be of order unity for particles...
that have an aspect ratio of 1:2. Therefore our measured value is physically reasonable.

From the linear fit to the mean-squared displacement, we find that the precision to which we track the rod’s center of mass is 35 nm or better ($\epsilon_\parallel = 28.3$ nm and $\epsilon_\perp = 35.4$ nm), which is around the same size as one voxel (approximately 35 nm). Though about an order of magnitude lower than the precision to which single spheres can be tracked, this precision compares favorably with that of tracking more complex scatterers, such as spheres in clusters.39,31

### Rotational diffusion

To determine the rotational diffusion coefficient, we first calculate the mean-squared displacement of the orientational unit vector $u$:

$$\Delta u^2(\tau) = \left\langle (u(t + \tau) - u(t))^2 \right\rangle = 2 \left( 1 - e^{-2D_r\tau} \right) + 2\epsilon_r^2. \quad (3.13)$$

We then fit $\Delta u^2(\tau)$ to find the diffusion coefficient $D_r$ and its angular uncertainty $\epsilon_r$.

We find $D_r = 0.311 \pm 0.034$ rad$^2$/s (Figure 3.7), which is larger than that predicted by the cylinder model ($D'_r = 0.205 \pm 0.008$ rad$^2$/s) but in quantitative agreement with the spherocylinder model ($D'_r = 0.311 \pm 0.017$ rad$^2$/s). The error obtained from the fits is $\epsilon_r = 0.0265$ rad, yielding an angular tracking precision of 1.5°. Like the translation results, these results suggest that the rods are modeled well, both hydrodynamically and optically, as spherocylinders. Furthermore the tracking precision is better than that reported for nanorods using reconstructions (approximately 3°) and dimers using multisphere superposition solutions (3.4°).24,29

#### 3.4.3 Janus particles

We find that it is more difficult to fit our scattering model to the Janus particle holograms because they are only weakly asymmetric. In particular, we find that the best-fit polar angle $\theta$ is often spurious.
There are two local minima in the objective function, corresponding to two polar angles reflected about the x-y plane, and two different z-coordinates: if we calculate a hologram of Janus particle with $\theta = 0$ and $z = 8.0 \, \mu m$, and we fit to it using an initial guess of $\theta \geq \pi/2$, the fit converges to $\theta = \pi$ and $z = 7.811 \, \mu m$. We find an $R^2 = 0.9997$ and a per-pixel $\chi^2$ of $3.8 \times 10^{-6}$, showing that the best-fit and original holograms are essentially identical. These calculations suggest that errors in the best-fit polar angle are correlated with those of the z-coordinate. Noise in the hologram could therefore cause the fitting algorithm to converge to either local minimum. Indeed, we estimate our noise floor for the 8-bit images to be at least an order of magnitude larger at $(1/255)^2 = 1.5 \times 10^{-5}$. Increasing the asymmetry of the hologram, for example by increasing the aspect ratio or using a metal-coated Janus particle as in Anthony et al., should eliminate this problem. But for the results shown below, we determine dynamical data only from the best-fit $x$- and $y$-coordinates and the azimuthal angle $\psi$.

**Theoretical predictions**

We use the Stokes-Einstein and Stokes-Einstein-Debye relations to model the translational and rotational diffusion of the Janus particles, which are approximately spherical:

$$D'_t = k_B T / 6\pi \eta a$$  \hspace{1cm} (3.14)

$$D'_r = k_B T / 8\pi \eta a^3$$  \hspace{1cm} (3.15)

Because the hydrodynamic radii of polymer particles—like the PS core in our Janus particle—tend to be larger than the radii measured optically or with electron microscopy due to charged or hairy surfaces, we do not directly compare the measured diffusion coefficients to values predicted from the theory. Instead, we calculate the effective radius $a_{\text{eff}}$ from both the measured translation and rotational diffusion coefficients using Equations (3.14) and (3.15). We then compare these values to
the best-fit diameter from the holograms and to each other.

**Translational diffusion**

To determine the translational diffusion coefficients for the Janus particle, we ignore the translational motion in the direction parallel to the imaging axis ($z$-axis) because the polar angle $\theta$ has an uncertainty that affects the best-fit $z$-position, as discussed previously. We treat the Janus particle as a sphere and calculate the translational diffusion coefficient from the mean-squared displacement projected onto the $x-y$ plane:

\[
\langle (\Delta x^2(\tau) + \Delta y^2(\tau)) \rangle = 4D_{\perp,\text{Janus}} \tau + 4\epsilon^2
\]  

(3.16)

where $\epsilon$ is the tracking precision.

![Figure 3.8](image)

Figure 3.8: (a) Measured mean-square displacement of the Janus particle in the axes perpendicular to the imaging axis. The solid line is a fit to $4D\tau + 4\epsilon^2$. (b) Measured autocorrelation of the cylinder’s projected orientational unit vector $p$. The solid line is a fit to an multi-exponential decay.

By fitting the mean-squared displacement to a linear model, we obtain $D_{\perp,\text{Janus}} = 0.419 \pm 0.003 \ \mu m^2/s$ (see Figure 3.8). We use then use Equation 3.14 at $21 \pm 2^{\circ}C$ to obtain an effective particle radius $a_{\text{eff}} = 524 \pm 15$ nm, which is larger than the optical radius from holography ($443 \pm 20$ nm for bare polystyrene, $490 \pm 20$ nm with the TiO$_2$ layer). As discussed above, the larger hydrodynamic radius is expected for polymer particles\textsuperscript{79,80}. These results are summarized in Table 3.2.

We obtain a tracking precision of $\epsilon = 21.8$ nm from the fits for the directions perpendicular to the
Table 3.2: Measured and expected values of parameters for Janus particles.

<table>
<thead>
<tr>
<th>Quantity</th>
<th>Experiment</th>
<th>Expected</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>$a_{\text{Janus}}$ (nm)</td>
<td>443 ± 20</td>
<td>450 ± 50</td>
<td>SEM</td>
</tr>
<tr>
<td>$t$ (nm)</td>
<td>47 ± 8</td>
<td>50</td>
<td>deposition parameters</td>
</tr>
<tr>
<td>$n_{\text{TiO}_2}$</td>
<td>2.74 ± 0.23</td>
<td>2.3–2.8</td>
<td>bulk value</td>
</tr>
<tr>
<td>$n_{\text{PS}}$</td>
<td>1.581 ± 0.046</td>
<td>≤ 1.585</td>
<td>bulk value</td>
</tr>
<tr>
<td>Translational $a_{\text{eff}}$ (nm)</td>
<td>524 ± 15</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Rotational $a_{\text{eff}}$ (nm)</td>
<td>523 ± 6</td>
<td>–</td>
<td></td>
</tr>
</tbody>
</table>

Imaging axis, which is smaller than the size of one voxel (approximately 35 nm) and again on par with the tracking precision of complex scatterers such as spheres in clusters\textsuperscript{29,31}.

**Rotational diffusion**

Although the best-fit polar angle $\theta$ is unreliable, we can extract the rotational diffusion coefficients from the data by considering only the azimuthal angle $\psi$. To do this, we derive an expression for the autocorrelation of the projection of the orientational unit vector onto the $x-y$ plane $p(t) = \cos \psi(t) \hat{x} + \sin \psi(t) \hat{y}$:

$$
\langle p(t) \cdot p(t + \tau) \rangle = \frac{1}{4} \sum_{\ell=1}^{\infty} \frac{2\ell + 1}{\ell(\ell + 1)} \left( S_1^\ell \right)^2 \exp \left[ -\ell(\ell + 1)D_r \tau \right]. \quad (3.17)
$$

where $S_1^\ell \equiv \int_{-1}^{1} P_1^\ell(x) \, dx$ and $P_1^\ell(x)$ are associated Legendre polynomials of order 1 (see Section 3.5 for further details). This expression is a multiexponential that depends on $D_r$ and can be evaluated numerically.

To determine the rotational diffusion coefficient we fit Equation 3.17 to the data and obtain $D_{r,\text{Janus}} = 1.15 \pm 0.05 \, s^{-1}$ (see Figure 3.8). From Equation 3.15 and a temperature of $21 \pm 2^\circ C$, we calculate an effective particle radius of $a_{\text{eff}} = 523 \pm 6 \, nm$, in excellent agreement with the $a_{\text{eff}} = 524 \pm 15 \, nm$ obtained from translational motion. These results are summarized in Table 3.2.
The quantitative agreement between the hydrodynamic radii calculated from the translational and rotational diffusion coefficients demonstrates the internal consistency of our fitting method, showing that the technique can effectively track the rotation and translation of Janus particles in water, despite the small asymmetry of the particles and holograms.

Although we do not use the $z$-coordinate or the polar angle $\theta$ to obtain these results, they would nonetheless be difficult to obtain with a traditional optical microscope. The particles diffuse rapidly, moving more than 5 $\mu$m in $z$ throughout the trajectory, which might take them too far out of focus during a 2D microscopic measurement. Furthermore, the fit to the scattering model allows a quantitative determination of the azimuthal angle with no calibration required. Tracking this angle could be useful in other studies, for example to determine how quickly a particle orients in response to an external field or another object.

3.5 Conclusions and future work

We have shown a new technique to measure the 3D translational and rotational dynamics of colloidal particles. Our results show that holographic microscopy can capture the 3D position and orientation of non-spherical colloidal particles, and that these variables can be tracked precisely by fitting scattering models based on the discrete dipole approximation to the measured holograms. The technique has high precision and temporal resolution, as evidenced by the measured rotational diffusion coefficient for the Janus particle, which is an order of magnitude larger than previously measured 3D diffusion coefficients of a freely-diffusing particle.

We can address many of the limitations of our approach by improving our DDA implementation and fitting procedure. We intend to improve the accuracy of the measurements by antialiasing the voxelation and incorporating near-field corrections to the scattering calculations, as described in Dagostino et al.\textsuperscript{81}. Spurious fits might be suppressed with an additional fitting pass that enforces a
physically plausible trajectory, perhaps assisted by a Kalman filter.

Because the DDA is applicable to scatterers with arbitrary size, shape, and refractive index profiles, our technique could be used to measure the dynamics of a wide variety of particles. This makes it suitable for a number of different applications, including microrheology, measurements of interactions between non-spherical particles, and fundamental studies of colloidal self-assembly and bacterial motion.

**Appendix: Projection correlation function** \( \langle \mathbf{p}(t) \cdot \mathbf{p}(t + \tau) \rangle \)

In this Appendix, we derive Eq. 3.17, with which we measure the rotational diffusion coefficient \( D_r \) of a Janus particle in Sec. 3.4.3.

The isotropic rotational diffusion of a particle can be quantified by studying the trajectory on the unit sphere of a unit vector \( \mathbf{u} \) fixed to the particle. Computing the autocorrelation \( \langle \mathbf{u}(t) \cdot \mathbf{u}(t + \tau) \rangle \), where \( \tau \) is a lag time, from experimental data allows the measurement of the rotational diffusion coefficient \( D_r \). This requires tracking the entire 3D orientation of the particle. Here we consider how \( D_r \) can be measured when only a two-dimensional azimuthal projection of \( \mathbf{u} \) is observed, as is the case for the Janus particles.

In our experiments, we observe the normalized projection of \( \mathbf{u} \) onto the laboratory \( x - y \) plane, which is perpendicular to the optical axis. In spherical polar coordinates, where the tip of \( \mathbf{u}(t) \) has coordinates \( \theta(t) \) and \( \psi(t) \) on the unit sphere, this projection is given by \( \mathbf{p}(t) = \cos \psi(t) \hat{x} + \sin \psi(t) \hat{y} \). From the data, we can then compute the autocorrelation of \( \mathbf{p}(t) \):

\[
\langle \mathbf{p}(t + \tau) \cdot \mathbf{p}(t) \rangle = \langle \cos(\psi(t + \tau)) \cos(\psi(t)) + \sin(\psi(t + \tau)) \sin(\psi(t)) \rangle. \tag{3.18}
\]

To simplify the notation, we will use primes to denote angles at time \( t + \tau \); the unprimed angles \( \theta \) and
ψ are at time $t$. Thus,

$$\langle p(t + \tau) \cdot p(t) \rangle = \langle \cos \psi' \cos \psi + \sin \psi' \sin \psi \rangle. \quad (3.19)$$

We show that $D_r$ can be determined from experimental measurements of $\langle p(t + \tau) \cdot p(t) \rangle$ by calculating this autocorrelation for a particle undergoing isotropic rotational diffusion characterized by $D_r$. We neglect translation-rotation coupling and therefore ignore the translational diffusion of the particle. Let $f(\theta, \psi; t) d\Omega$ be the probability of finding $u$ in the solid angle $d\Omega$ near $(\theta, \psi)$ at time $t$. The probability density $f$ is governed by a rotational Fick’s law:

$$\frac{\partial f}{\partial t} = D_r \left( \frac{1}{\sin \theta} \frac{\partial}{\partial \theta} \left( \sin \theta \frac{\partial f}{\partial \theta} \right) + \frac{1}{\sin^2 \theta} \frac{\partial^2 f}{\partial \psi^2} \right). \quad (3.20)$$

The operator on the right is the Laplacian on the unit sphere. Computing $\langle p(t + \tau) \cdot p(t) \rangle$ requires knowing the transition probability density $K(\theta, \psi, \theta', \psi'; \tau)$ for $u$ to move from $(\theta, \psi)$ to $(\theta', \psi')$ after a lag time $\tau$. If we assume that the distribution of initial orientations $(\theta, \psi)$ is uniform, such that $f = 1/(4\pi)$, then using Eq. 3.19 the autocorrelation of $p$ will be given by

$$\langle p(t) \cdot p(t + \tau) \rangle = \int \int (\cos \psi \cos \psi' + \sin \psi \sin \psi') \frac{K(\theta, \psi, \theta', \psi'; \tau)}{4\pi} d\Omega d\Omega'. \quad (3.21)$$

The transition probability $K$ is given by the probability density $f(\theta', \psi'; \tau)$, governed by Eq. 3.20, with the following initial condition:

$$f(\theta', \psi'; 0) = \frac{\delta(\theta' - \theta)\delta(\psi' - \psi)}{\sin \theta'} \quad (3.22)$$

where $\delta$ denotes the Dirac delta function. Separation of variables leads to the following solution for
\( K(\theta, \psi, \theta', \psi'; \tau) = \sum_{\ell=0}^{\infty} C_{\ell 0} P_\ell (\cos \theta') \exp [-\ell(\ell + 1)D_r \tau] \)

\[ + \sum_{\ell=1}^{\infty} \sum_{m=1}^{2} \sum_{p=1}^{2} C^{(p)}_{\ell m} Y^{(p)}_{\ell m} (\theta', \psi') \exp [-\ell(\ell + 1)D_r \tau]. \tag{3.23} \]

Here, \( P_\ell (\cos \theta') \) is a Legendre polynomial, and the \( Y^{(p)}_{\ell m} \) are real spherical harmonics\(^{84} \):

\[
Y^{(p)}_{\ell m} (\theta', \psi') \equiv \begin{cases} 
P_{\ell m} (\cos \theta') \cos m\psi' & \text{if } p = 1 \\
P_{\ell m} (\cos \theta') \sin m\psi' & \text{if } p = 2. \end{cases} \tag{3.24} \]

The initial condition in Eq. 3.22 results in the expansion coefficients being

\[
C_{\ell 0} = \frac{2\ell + 1}{4\pi} P_\ell (\cos \theta) \tag{3.25} \]

for the azimuthally symmetric \((m = 0)\) terms and

\[
C^{(p)}_{\ell m} = \frac{2\ell + 1}{2\pi} \frac{(\ell - m)!}{(\ell + m)!} Y^{(p)}_{\ell m} (\theta, \psi) \tag{3.26} \]

for the remaining terms.

Consider the first term on the right side of Eq. 3.21 which contains \( \cos \psi \cos \psi' \). By orthogonality of \( \cos \psi \) and \( \cos \psi' \), only terms in \( K \) with \( m = 1 \) and \( p = 1 \) contribute to the integral. Integration
over $\psi$ and $\psi'$ contributes two factors of $\pi$, and so we obtain

$$\int\int \cos \psi \cos \psi' \frac{K(\theta, \psi, \theta', \psi'; \tau)}{4\pi} d\Omega d\Omega' =$$

$$\sum_{\ell=1}^{\infty} \left( \frac{2\ell + 1}{\ell(\ell + 1)} \frac{1}{8\pi^2} \exp[-\ell(\ell + 1)D_r\tau] \pi^2 \right. \times$$

$$\left. \int P_{\ell}^1(\cos \theta) \sin \theta d\theta \int P_{\ell}^1(\cos \theta') \sin \theta' d\theta' \right).$$

(3.27)

Integrating the remaining term of Eq. 3.21 over $\psi$ and $\psi'$ gives exactly the same result. Defining

$$S_{\ell}^1 \equiv \int_{-1}^{1} P_{\ell}^1(x) dx,$$

(3.28)

we obtain Eq. 3.17 in the manuscript:

$$\langle \mathbf{p}(t) \cdot \mathbf{p}(t + \tau) \rangle = \frac{1}{4} \sum_{\ell=1}^{\infty} \frac{2\ell + 1}{\ell(\ell + 1)} (S_{\ell}^1)^2 \exp[-\ell(\ell + 1)D_r\tau].$$

(3.29)

Note that $S_{\ell}^1 = 0$ for even $\ell$ due to the parity of $P_{\ell}^1(x)$. To evaluate our result numerically, we use DiDonato's recursion relation for $S_{\ell}^1$:

$$S_{\ell+2} = \frac{\ell(\ell + 2)}{(\ell + 1)(\ell + 3)} S_{\ell}^1.$$

(3.30)

Unlike the results obtained by prior workers on this problem, our solution can be easily computed and used to measure $D_r$ from experimental data. Saragosti et al. obtain a series expression equivalent to Eq. 3.29, but their solution contains complicated angular integrals that are left unevaluated. They therefore determine $D_r$ from a $\ell = 1$ approximation of Eq. 3.29. While the $\ell = 1$ term indeed dominates when $D_r\tau$ is large, the autocorrelation can be measured most precisely near $\tau = 0$, at which the number of independent angular displacements is largest. In this regime, the single-exponential
approximation of Saragosti et al. fails, and our full solution is necessary.

We verified our result, Eq. 3.29, by computing \( \langle \mathbf{p}(t) \cdot \mathbf{p}(t+\tau) \rangle \) for simulated rotational trajectories of particles undergoing rotational diffusion. We simulated rotational diffusion using the algorithm of Beard and Schlick\(^8^7\). Figure 3.9 shows the autocorrelations computed from simulated trajectories with two different \( D_r \) along with fits to Eq. 3.29. We find excellent agreement between the simulated autocorrelations and best fits to Eq. 3.29; the values of \( D_r \) determined from the best fits agree with the simulation input values to 0.5% or better.
4

Non-spherical particles at the interface

In the previous chapters we examined the behaviour of spherical particles near liquid interfaces. In this chapter we examine how non-spherical particles breach interfaces to learn how contact-line pinning affects their path towards equilibrium.

4.1 Adsorption of colloidal ellipsoids to interfaces

Slow relaxations towards equilibrium are found in a variety of systems, including molecular, spin, and colloidal glasses\textsuperscript{88,89}, granular systems\textsuperscript{90}, wetted surfaces\textsuperscript{35}, and viscoelastic complex fluids\textsuperscript{91,92}. In most of these cases, probing the microscopic dynamics is key to attaining a full understanding of the relaxation behavior. For instance, the rearrangement of molecules over time dictates how molecular glasses age\textsuperscript{88}; the entanglements between polymer chains determines the relaxation of viscoelastic polymers\textsuperscript{91}; and the motion of a contact-line over nanoscale surface defects determines the spreading.
of a droplet on a substrate.  

The nanoscale process we study here is contact-line pinning, which has been identified as a possible cause of slow relaxation of colloidal particles at liquid-liquid interfaces. Experiments by Boniello et al. reveal that particles straddling an air-water interface have slower in-plane diffusion than particles in bulk water. This behavior is the inverse of what is expected from hydrodynamic arguments. Boniello and coworkers propose that extra dissipation results from the hopping of the contact-line over nanoscale defects on the surface of the particle. They also show that slow diffusion (and hence contact-line pinning) is a feature common to all the colloidal particles they examined.  

Contact-line pinning also has ramifications for particles moving perpendicular to the interface. Kaz and coworkers showed that spherical colloidal particles breaching liquid-liquid interfaces relax towards equilibrium at a rate orders of magnitude slower than that predicted by viscous dissipation alone. Dynamic wetting models based on molecular kinetic theory and Kramer’s theory fit data well at times long after the particles breach, which indicates that both viscous and inertial effects can be eclipsed by nanoscale thermally activated processes.  

However, we still do not know whether contact-line pinning affects the pathway to equilibrium and not just the time required to get there. Spherical particles have only one observable degree of freedom that determines their equilibrium position — the height of the particle relative to the interface. Thus there is only one pathway to equilibrium, regardless of whether or not the contact-line becomes pinned along the way: the height increases monotonically with time. For non-spherical particles, the pathway can be more complex, owing to the rotational degrees of freedom. Simulations that model the path toward equilibrium based on interfacial energy minimization predict that the center-of-mass of a micrometer-sized ellipsoidal particle breaching an interface will follow a trajectory that is not necessarily monotonic with time: the particle will first rotate more perpendicularly to the interface before reaching the equilibrium configuration, where it lies parallel to the interface (Figure 4.1). Furthermore, these simulations, which do not model contact-line pinning, predict an equilibration
time of 10 µs based on the viscous dissipation.

Figure 4.1: Schematic showing the cross-section through the long axis of a particle breaching an interface. Simulations predict trajectories (solid lines) that are not monotonic in center-of-mass position or polar angle. Our experimental results (shaded ellipses) show a monotonic increase in both height and the polar angle.

Previous experimental studies of prolate spheroids at interfaces suggest that the equilibration time is much longer, hinting at pinning effects. Coertjens et al. used a freeze-fracture technique to image and measure the contact angles of polystyrene ellipsoids when they breach the interface and 60 mins afterward. They found that the average contact angle had changed during this time. Mihiretie measured the cross-sectional area of 5-µm by 15-µm ellipsoids at an air-water interface with bright-field microscopy, and showed that the area increased approximately logarithmically with time. However, neither of these studies measured the height and orientation of the particles during adsorption.

We use a fast 3D imaging technique, digital holographic microscopy, to observe ellipsoidal colloidal particles adsorbing to an oil–water interface. We are able to measure the center-of-mass position and the azimuthal and polar angles of the particles as a function of time. We find that not only is the timescale of equilibration long, but also that the pathway differs from that predicted from simulations based on energy minimization. In particular, the rotational and translational degrees are coupled. We argue that these effects are due to contact-line pinning.

To make ellipsoidal particles, we heat 1.0-µm-diameter sulfate-functionalized polystyrene particles (Invitrogen) above their glass transition temperature and stretch them using the apparatus described in Appendix A. We capture holograms of the ellipsoids at 100 frames per second. The holograms encode the three-dimensional position and orientation of the particle in the spacing and shape of the interference fringes. We fit a T-matrix model of the scattering from axisymmetric particles
to our data to extract the particle’s size, refractive index, three-dimensional position, and orientation. More details of the particle fabrication and hologram fitting procedure are given in Appendices A and B.

![Experimental setup schematic](image)

Figure 4.2: Schematic of experimental setup. a) The sample is illuminated with a red laser beam. A counterpropagating infrared beam is used to gently push the particles against gravity towards an interface. In the coordinate system used here, the imaging axis is the $z$-axis, and the interface is at $z=0$. The center-of-mass position and the polar angle $\theta$ of the particle are defined relative to the laboratory frame by a unit vector $u$ that points along the long axis of the ellipsoid. b) A typical hologram of an ellipsoid at an interface.

We observe that the particles relax to equilibrium slowly, despite some abrupt motion along the way. After 1 s, or five orders of magnitude longer than the equilibrium time observed in simulations\textsuperscript{95}, the particles are not equilibrated. Furthermore, as with spheres, their height scales roughly linearly with the logarithm of the elapsed time after the breach. Such slow dynamics point to a source of dissipation other than viscous drag. Because the particles are stretched versions of the same spheres used by Kaz \textit{et al.}\textsuperscript{11}, and the relaxation scales with the logarithm of the elapsed time after the breach as in Kaz \textit{et al.}\textsuperscript{11}, the most likely source of the dissipation is contact-line pinning and depinning, the same mechanism observed in spherical particles.

More surprisingly, we find that the abrupt changes in height (Figure 4.3a) correlate with the abrupt changes in $\theta$ (Figure 4.3b). Indeed, when we examine $\theta$ as a function of $z$ we find that the relationship is approximately linear (Figure 4.3c). Furthermore, although the particles approach the interface from a variety of different polar angles, the $\theta - z$ plots all collapse.

The relationship between $\theta$ and $z$ is reminiscent of rolling, where translation and rotation are
Figure 4.3: The height (a) and polar angle $\theta$ (b) of ellipsoids after they breach show abrupt changes, but both variables change slower than expected from simulations. To compare data from particles of different aspect ratios on the same plot, we normalize the center-of-mass height relative to the interface (per de Graaf et al.\textsuperscript{95}) to get $z_{\text{COM}} = z/\sqrt{2a^2 + b^2}$, where $a$ and $b$ are the semi-minor and semi-major axes of the spheroid. c) The polar angle varies with the height. d) Our model, based on contact-line hopping over defects, produces almost linear relationships between $z$ and $\theta$ for the particles from the experiments.

coupled by friction. The particles ‘pivot’ into the interface, as shown by the rendering of individual points along the observed trajectories (Figure 4.1). In contrast, the simulations by Günther et al.\textsuperscript{94} and de Graaf et al.\textsuperscript{95} predict that $\theta$ and $z$ should vary non-monotonically in time (Figure 4.1).

We therefore seek a different model to explain the observed dynamics, one that takes into account contact-line pinning. We adopt the viewpoint of molecular kinetic theory\textsuperscript{33} and assume that the contact-line pins to defects on the surface of the particle and hops between pinning sites with the aid of thermal kicks. We calculate the velocity of the contact-line using an Arrhenius equation coupled to a model for the force, determined by the dynamic contact angle $\alpha_D(t)$. Our model is similar to that of Kaz et al.\textsuperscript{11}, except that we allow $\alpha_D(t)$ to vary as a function of position on the particle (we assume the interface remains flat). The force on each segment of the contact-line is determined from the
imbalance between the three interfacial tensions (oil–water $\sigma_{ow}$, particle–water $\sigma_{pw}$, and particle–oil $\sigma_{po}$):

$$F_{cl} = \sigma_{ow} \cos \alpha_D(t) + \sigma_{pw} - \sigma_{po}$$

$$= \sigma_{ow} (\cos \alpha_D(t) - \cos \alpha_E)$$

(4.1)

We relate $F_{cl}$ to the velocity of the contact-line using an Arrhenius equation \textsuperscript{33,11}

$$V = V_0 \exp \left( -\frac{U}{kT} + \frac{F_{cl}(t)A}{2kT} \right)$$

(4.2)

where $V$ is the velocity of the contact-line tangent to the particle, $V_0$ is a molecular velocity scale, $A$ is the area per defect on the surface of the particle, and $U$ is the energy with which each defect pins the three phase contact-line. Because the particle is non-spherical, the force per unit length along the contact-line is asymmetric about the short-axis of the ellipsoid unless the particle breaches the interface at exactly $\theta = \pi/2$. This is because some parts of the contact-line are nearer the tip than others, and the dynamic contact angle is greater near the tips than it is near the center of the particle ($\alpha_D,\text{tip} > \alpha_D,\text{center}$), as illustrated by the cross-section through the particle shown in Figure 4.4. The part of the contact-line nearest the tip travels more slowly than the part nearest the center ($V_{\text{tip}} < V_{\text{center}}$ according to Equations 4.1 and 4.2), leading to the observed ‘pivoting’ motion.

Figure 4.4: The dynamic contact angles at the parts of the contact-line closest to the center and to the tip are shown. The dotted lines show where the contact-line moves in the particle’s frame of reference.
To calculate the trajectory, we work in the frame of reference of the particle starting from an initial position in which the particle just touches the interface at a given polar angle \( \theta_{\text{init}} \). When we model trajectories for comparison with experimental data, we choose \( \theta_{\text{init}} \) to be the polar angle of the particle just before it breaches. At each point along the contact-line, we use Equation 4.2 to find \( V \) in the direction tangent to the particle. We then multiply each velocity by a discrete time-step to find the displacement of the contact-line. We use this displacement to update the positions of the points along the contact-line, and we fit a plane to the updated positions to define the new location of the interface. As stated above, we assume that the interface does not deform, a simplification that we justify based on the energetic cost of bending the interface. The height \( z \) and polar angle \( \theta \) are then determined by the angle and position of the plane relative to the center of the ellipsoid. For further details of the model, please see Appendix A.

Before comparing the model to the data, we first determine which parameters the trajectories are most sensitive to. Changing \( U \) and \( \alpha_E \) alters how \( \theta \) and \( z \) depend on time, but not how \( \theta \) and \( z \) evolve with each other. The only parameters that change the \( \theta-z \) relationship are the dynamic contact angles \( \alpha_D \) and the area per defect \( A \). We can understand this dependence by looking at the velocity of the contact-line at the ‘tip’ and the ‘center’, as defined in Figure 4.4. Considering only these two points, the ratio \( V_{\text{center}}/V_{\text{tip}} = \exp(\sigma_{\text{ow}} A (\cos \alpha_{D, \text{center}} - \cos \alpha_{D, \text{tip}})/2kT) \) approximates the rate at which \( z \) and \( \theta \) change relative to each other. We therefore expect the aspect ratio of the particle, which determines the values of \( \alpha_D \) for a given \( \theta \) and \( z \), and the area per defect \( A \) to control the shape of the \( \theta-z \) curve.

The model produces trajectories that agree with experimental observations. In Figure 4.3d, we plot modeled \( \theta-z \) trajectories for each of the particles in Figure 4.3c using the measured aspect ratio determined from fitting the holograms and a defect area equal to that measured in Kaz et al.\(^{11} \), \( A = 4 \text{ nm}^2 \). The modeled \( \theta \) and \( z \) are both monotonic with time, in contrast to the predictions from energy minimization. Moreover, the \( \theta-z \) plots are straight lines, reflecting the translational-rotational
coupling that is evident in our experimental results. The only discrepancy is that the modeled trajectories almost overlap onto a single line, whereas the lines in our data have slightly different slopes. This discrepancy may be due to deviations in shape from perfect prolate spheroids (see Figure 4.2), which would affect the curvature of the particle and hence $\alpha_D$. The area per defect $A$ may also vary between particles. However, the slope predicted by the model for particles that do not breach at an angle of $\pi/2$ (-1.75 ± 0.16 rad) is close to the average slope in our experimental results (-1.64 ± 0.76 rad). Particles that breach at an angle of $\pi/2$ have predicted and expected slopes of 0 rad.

![Diagram of spherocylinder breach angles](image)

Figure 4.5: a) A spherocylinder can breach the interface at a range of angles that are all energetically equivalent. The hemispherical cap of the spherocylinders is drawn with dotted lines. b) Experimentally, a hydrophilic silica spherocylinder is free to rotate in the plane of the interface once it has attached to the interface (shaded region) but not to move out of plane.

These results suggest that the velocity of the contact-line dictates the adsorption trajectories of the particles. In the case of breaching spheres, our model predicts no net torque on the particle because of the particle’s symmetry. Thus, one way to test the model is to confirm that the contact-line does not exert a torque on particles that present spherical features. Consider, for example, a spherocylinder (depicted in Figure 4.5), which consists of a cylinder with hemispherical caps. If the spherocylinder is hydrophilic, such that a sphere of the same material has an equilibrium contact angle smaller than
\(\pi/4\) (Figure 4.5a), it can attach to the interface at a range of polar angles all having the same energy (Figure 4.5a). Due to the spherical symmetry, we expect that the contact-line will not exert a torque on the particle. Therefore, the particle should remain at the same polar angle as its height increases.

Indeed, when we send hydrophilic spherocylinders to the interface, we do see them attach and become constrained at a particular polar angle while they remain free to rotate in-plane (Figure 4.1b). The absence of equilibration is surprising because the minimum-energy configuration for spherocylinders is \(\theta = \pi/2\). It is thus conceivable that a pill-shaped bacterium, such as \(E.\ coli\), might get ‘stuck’ at a particular angle once it attaches to an interface and never equilibrate. These results suggest that accounting for contact-line pinning, and not just interfacial energy, is necessary to understand the dynamics of non-spherical particles.

We therefore conclude that for particles adsorbing to interfaces, slow relaxation implies more than just that the road to equilibrium is long. It is a sign that contact-line pinning is present, which can alter the pathway to equilibrium and determine whether thermodynamic equilibrium will ever be reached. These results have implications for practical considerations of assembling particles at an interface and for our fundamental understanding of the role of interfaces in particle assemblies.

In terms of practical considerations, the unexpectedly long adsorption time we find in our experiments might significantly affect emulsion ageing. Günther and coworkers\(^94\) simulated how ellipsoidal particles interact hydrodynamically at a spherical interface and found that though single particle adsorption takes 0.1 \(\mu\)s, the particles require 10 ms to equilibrate in response to each other\(^94\). Because our particles require at least 1 s to equilibrate, we estimate that in emulsions made with ellipsoids, it could take hours for the particles to rearrange and re-equilibrate.

In terms of fundamental understanding, the ability of our model to recreate the nearly linear \(\theta-z\) adsorption trajectories for ellipsoids validates the idea that contact-line pinning can couple orientational and translational degrees of freedom. The role that contact-line pinning plays is akin to the role of friction in rolling, but instead of two hard surfaces interacting via microscopic features, there are
nanoscale defects on a surface interacting with a deformable liquid–liquid interface. Though these interactions are weak enough to be disrupted by thermal fluctuations, and the capillary driving forces are large, contact-line pinning can nonetheless drive observably different $\theta-z$ trajectories from what is expected from energy minimization alone.

**Appendix A**

**Making the particles**

We prepare ellipsoidal particles by following a previously published protocol\(^98\) with slight modifications. We embed spherical particles in a 10% w/w poly(vinylalcohol) matrix (average $M_w$ 146,000-186,000, 87-89% hydrolyzed from Sigma-Aldrich). We then allow the film to dry until brittle over at least one week at room temperature. Afterward, we clamp the film in a custom-made apparatus consisting of two clamps on rails and heat the polymer film with a heat gun (Master Appliance, set to $1000 \, ^\circ \text{F}$) held approximately 5 cm over the film. After heating the film for 30 s, we stretch the film by pulling apart the two clamps\(^98,99\). We dissolve the film in a mixture of isopropyl alcohol ($\geq 99\%$ purity, BDH chemicals) and deionized water (Elix, EMD Millipore, resistivity 18.2 M$\Omega\cdot$cm), then wash the freed particles in fresh isopropanol/water and then pure deionized water using a ‘double cleaning’ protocol as described in Coertjens et al.\(^12\). The particles are sulfate-functionalized polystyrene spheres ($d = 1.0 \, \mu$m) from Invitrogen.

The silica spherocylinders are synthesized using a modified one-pot method\(^64,65\), and their size (as determined by scanning electron microscopy) is $1 \pm 0.2 \, \mu$m by $2 \pm 0.2 \, \mu$m.

We dilute the particles to a volume fraction of less than $10^{-5}$ in an aqueous solution of glycerol (59% w/w) in water so that in the experiments, there is no more than one particle in the $120 \times 120 \times 100 \, \mu$m imaging volume. We place approximately 5 $\mu$L of suspension in a custom sample holder and add approximately 300 $\mu$L of anhydrous decane ($\geq 99\%$ purity, Sigma) to form the interface.
aqueous solution is refractive-index matched to the decane \((n = 1.41)\) to minimize reflections from the interface.

**Taking and analyzing holograms**

We capture the dynamics of the ellipsoidal particles with a digital holographic microscope. Our experimental apparatus is described in detail by Kaz et al.\(^\text{11}\). In brief, we capture holograms with an in-line digital holographic microscope built on a Nikon TE2000-E inverted microscope. We push particles towards the interface with an out of focus optical trap \((\lambda = 785 \text{ nm}, \text{Thorlabs L785P090})\), while a laser diode \((\lambda = 660 \text{ nm}, \text{Thorlabs HL6545MG})\) illuminates them. The scattered and unscattered light interfere to form a hologram, which is collected with a \(100 \times, \text{NA} = 1.4\), oil-immersion objective (Nikon) with a \(n = 1.414\)-index oil to minimize spherical aberration in the hologram\(^\text{11,27}\). The holograms are then recorded with a Photon Focus MVD-1024E-160 camera at 50 \(\mu\)s exposure time.

We analyze the recorded holograms by fitting a light-scattering model to the data using the HoloPy package (http://manoharan.seas.harvard.edu/holopy). We have previously shown that we can use holography and an implementation of the discrete dipole approximation (A-DDA\(^\text{68}\)) to track non-spherical particles diffusing in water with high temporal resolution and spatial precision\(^\text{100}\). We use the same technique to analyze holograms from the spherocylinder. For the ellipsoids, we calculate the scattered field with a T-matrix solution\(^\text{97}\), which is faster than the discrete dipole approximation: calculating a 200 by 200 pixel hologram of a 0.5- by 2.0-\(\mu\)m ellipsoid takes 1 s using the T-matrix solution compared to 10 s with A-DDA. We use the fit results from one frame, obtained using the Levenberg-Marquardt algorithm, as the initial guess for the following frame in the time series.

To fit a time-series of holograms, we must determine good initial guesses for the parameters in the first frame, including the refractive index of the particle, its size, orientation, and position. For the refractive index we use that of bulk polystyrene \((n = 1.59 \text{ at } 660 \text{ nm})\). To estimate the orientation and size of the particles, we calculate reconstructions of the hologram by Rayleigh-Kirchoff
back-propagation. We estimate the height from the \( z \) position where the reconstruction resembles an in-focus bright-field image of the particles. We then use this reconstruction slice to estimate the particle’s size and orientation. We estimate the particle’s \( x-y \) position using a Hough transform based algorithm in HoloPy. These estimates are then refined in the fit.

**Modeling contact-line motion on the surface of a particle**

To model the motion of the contact-line along the surface of a spheroid, we work in the particle’s frame of reference. The velocity of the contact-line along the surface of the particle is given by Equation 4.2, which we write again for convenience:

\[
V = V_0 \exp \left( -\frac{U}{kT} + \frac{F_{cl}(t)A}{2kT} \right). \tag{4.3}
\]

We define the contact-line as the intersection between a plane (the interface) and the surface of a rotated prolate spheroid. For an interface at \( z=0 \), the contact-line is given by

\[
y = \pm \sqrt{a^2 - \frac{a^2}{b^2}(-x \sin \theta + k \cos \theta)^2 - (x \cos \theta + k \sin \theta)^2}
\]

where \( a \) and \( b \) are the minor and major semi-axes of the prolate spheroid, \( \theta \) is the polar angle, and \( k \) is the height of the particle. In our calculations, we use 5000 points to define the contact-line.

For each of the points along the contact-line, we calculate the velocity from Equation 4.3 using \( T = 295 \) K, \( V_0 = 1 \) \( \mu \)m/s, \( U = 20 \) \( kT \), \( \sigma_{ow} = 37 \) mN/m, and \( A = 4 \) nm\(^2\). We take the value for \( U \) from Colosqui et al.\(^\text{10}\), and the values for \( \sigma_{ow} \) and \( A \) from Kaz et al.\(^\text{11}\). We then multiply each velocity by a time-step \( \delta t \) to find the displacements \( \delta l \). The \( \delta l \) at each of the 5000 points are in the direction tangent to the particle. The time-steps we use are smaller in the beginning of the trajectory than at the end (\( \delta t_i = e^{-2}, e^{-1.98}, e^{-1.96}, ..., e^0, ..., e^{1.98}, e^{2} \)) because the driving force \( F_{cl} \) is larger in the beginning of
the trajectory. Using uniformly small time-steps for the whole integration does not change the $\theta-z$ trajectories, only the speed of the integration. After each time-step, we update each point along the contact-line with the respective value of $\delta l$, and we fit the updated points to a plane to define the new position of the interface.

**Appendix B**

**Using T-matrix based scattering solutions to model scattered electric fields**

We have previously demonstrated that A-DDA is a powerful tool because the scattering from any object can in principle be calculated\textsuperscript{68}. The trade-off is its relatively slow speed. In an effort to speed up the calculations for axisymmetric particles, we use Michael Mishchenko’s LAPACK-based double- and extended-precision T-matrix codes for single scatterers in a fixed orientation\textsuperscript{97}. The scatterers that can be handled are spheroids, cylinders, and Chebyshev particles. For further details, see Mishchenko’s amp1q.ipf file.

To calculate the scattered electric field, we pass the scattering code details of the scatterer and the positions at which to calculate the scattered electric field. These positions are the locations of the pixels in our detector, defined by angles $\theta$ and $\phi$, and a distance $k$. The scattering code then returns the scattering matrix $S$ for each of these locations. However, the scattering conventions used in HoloPy are those of Bohren and Huffman\textsuperscript{101}, and Mishchenko uses different conventions. Notably, the scattered and incident fields are represented with different basis vectors. The main modifications to the scattering matrices are therefore a scaling factor of $-ki$ and a coordinate transform for $S$:

$$S \rightarrow \begin{pmatrix} 1 & 0 \\ 0 & -1 \end{pmatrix} S \begin{pmatrix} \cos \phi & \sin \phi \\ -\sin \phi & \cos \phi \end{pmatrix}$$
The Fortran scattering code itself was modified in the following ways:

- A temporary directory is created to store the input and output files. This directory is deleted once the data is passed to HoloPy.
- Input data is taken from a file written by HoloPy, `tmatrix_tmp.inp`.
- Output data is given to HoloPy in `tmatrix_tmp.out`.
- Incident light is set to normal incidence.
- Incident light is set to [1,0] polarization.
- The phase matrix calculation is removed.

Further details can be found in the HoloPy:holopy/scattering/theory/tmatrix.py code, and the HoloPy:holopy/scattering/theory/tmatrix_*/S.lp.f Fortran (* denotes double and extended precision) files.
The right understanding of any matter and a misunderstanding of the same matter do not wholly exclude each other.

– Franz Kafka, *The Trial*

5

Particles near interfaces

All the experiments in the previous chapters involved particles spontaneously breaching interfaces at NaCl concentrations of approximately 100 mM. At lower salt concentrations, particles approaching the interface from the aqueous phase should be repelled, owing to image charges. This chapter examines what happens to particles near interfaces as a function of salt concentration. The initial experiments were done by David M. Kaz and Ryan McGorty in the Manoharan lab.

5.1 Introduction

The reduction in interfacial energy when a colloidal particle adsorbs to a liquid interface can be $10^2 – 10^6$ times the thermal energy $k_B T$. The resulting irreversibly adsorbed particles are used to stabilize emulsions, assemble materials, and create model two-dimensional systems for studying phase transitions. Although the interactions between adsorbed particles have been exten-
sively studied, their interactions at the interface can be highly heterogeneous and our understanding is far from complete. Moreover, the interactions between particles and the interface prior to breaching are usually ignored. In this chapter we look precisely at this neglected window of time and describe a previously unreported mode of binding which we term ‘non-capillary binding’. We find that particles are attracted to and bind to an oil-water interface but do not actually penetrate the oil phase.

This non-wetting binding is different from that discussed by Leunissen et al. and Kelleher et al., who showed that very hydrophobic, charged PMMA colloidal particles in oil can bind to a oil/cyclohexyl bromide-water interface without wetting the aqueous phase. Their particles were suspended in oil and attracted to the interface through an image-charge interaction, but did not wet the interface due to their extreme hydrophobicity. Thus, non-wetting binding was the lowest energy state for their particular system.

In our system, where particles are suspended in the aqueous phase, the image charge carries the same sign as the particle, and the interaction with the interface is repulsive. Atomic force microscopy (AFM) experiments have confirmed the presence of a repulsive barrier to capillary adsorption and shown that the barrier height decreases with increasing salt concentration, consistent with the screening effect of free electrolytes. From this basic electrostatic argument, one might predict that not all particles breach the interface when presented from the aqueous phase, and those that do not breach are repelled.

Indeed, a non-capillary mode of binding was not expected or reported in any of these studies. There are several possible reasons why our results differ. Most experiments on particles at interfaces use a spreading solvent to deposit particles at the interface, which creates a mixing layer between oil and water, preventing the particle from interacting with the pure oil-water interface. Also, the optical microscopy techniques used in most experiments do not make it possible to distinguish between particles that are adsorbed to the interface or lie just below it. Finally, in AFM experiments, the applied
forces are large enough to deform the interface\textsuperscript{116}, and the particles are immobilized on the cantilever tip, which artificially constrains their motion.

To circumvent these limitations, we use digital holographic microscopy to track particles with nanometer-scale precision in all three dimensions as they approach an oil-water interface. We push each particle gently towards the interface with weak radiation pressure from out-of-focus optical tweezers\textsuperscript{11}. In all of our experiments, the particle remains free to rotate, and the interface remains undeformed under the weak radiation force from imaging\textsuperscript{*}.

We find that non-capillary binding not only varies with time, but also non-monotonically with salt concentration. We also show that a seemingly disordered interface containing many particles actually contains a crystal consisting of breached particles. The other particles are bound in a non-capillary manner. As the interaction between the particles and the interface change over time, so do the interactions between particles.

Our experiments show that understanding the three-dimensional dynamics of particles at interfaces is crucial to the interpretation of their behavior. The existence of non-capillary binding indicates that interactions between particles and interfaces are more complex than previously thought, and requires new physics to explain it. Because the mode of binding also determines the interparticle interactions, monitoring the mode of binding – which is heterogeneous across the sample and over time – is necessary for furthering our understanding of particle-interface systems.

5.2 Experimental methods

Our materials and experimental methods are described in detail in a previous publication\textsuperscript{11}. All materials are carefully cleaned to minimize contamination and maintain a surfactant-free interface. In brief, we suspend colloidal particles in aqueous solutions with 1.0 mM to 1 M NaCl. In holographic

\textsuperscript{*}Given a maximum radiation force of $10^{-12}$ N and an interfacial tension of 37 mN/m, the deformation of the interface should be less than 0.1 nm
imaging experiments, glycerol (59% w/w) is added to match the refractive index of the aqueous phase to that of the oil, decane ($n = 1.411$). The index-matching eliminates reflections from the oil-water interface and maximizes the precision of our holographic measurements. A small volume (2–4 µL) of each suspension and a bath of filtered decane are added to a custom sample cell, and the two liquids are allowed to equilibrate for at least 30 minutes.

The particles are 1.4- and 1.9-µm-diameter sulfate-functionalized polystyrene particles (Invitrogen), and 2.0-µm-diameter carboxyl-funtionalized polystyrene particles (Invitrogen). We also silanize some 1-µm-diameter silica spheres (Bangs Laboratories) by suspending 6% w/w particles in ethanol, then reacting 3 mL of that colloidal suspension with 0.1 mL (3-aminopropyl)trimethoxysilane (97% Sigma-Aldrich) and 50 µL 30% ammonium hydroxide solution (30%, JT Baker) for 4 hours. We wash all particles by repeated centrifugation and resuspension in deionized water (Elix EMD Millipore).

The sample cells are placed on a Nikon TE-2000 inverted microscope equipped with two counter-propagating lasers, an 830 nm laser used as an optical trap and a 658 nm laser used to generate holograms. The apparatus is documented in Kaz et al.$^{11}$ Particles in the aqueous suspension are initially placed several micrometers below the liquid interface using the optical trap. The trap is then deactivated, positioned several microns below the particle, and reactivated, thus pushing the particle upward with nearly uniform radiation pressure. A high-speed camera (Photon Focus MVD-1024E-160-CL-12 or Phantom v7.3) captures digital holograms of the particle at frame rates between 50 and 4807.7 Hz through a 100 × NA=1.4 oil-immersion objective (Nikon CFI Plan Apo VC 100X). We use HoloPy
(http://manoharan.seas.harvard.edu/holopy) to fit the Lorenz-Mie scattering solution to the holograms\textsuperscript{28}. This procedure yields the center-of-mass position of the particle to a precision better than 2 nm in all three dimensions\textsuperscript{11} for the refractive-index-matched system.

We also look at collections of 1.9-µm-diameter sulfate polystyrene particles at an interface using bright field microscopy on a Nikon TE-2000 inverted microscope. For these experiments, we prepare sample cells with a subphase of 0.1–1% v/v particles in solutions of NaCl in water, then top the cells with decane. We omit glycerol, so that the particles sediment in the aqueous phase ($\rho_{\text{water}} = 1.0 \text{ g/cm}^3$ and $\rho_{\text{particle}} = 1.05 \text{ g/cm}^3$). We invert the sample cells to allow particles to sediment towards the oil-water interface for 1 hr, then turn them back upright to allow particles that did not attach to the interface to sediment to the bottom of the sample cell. We then image the flat interface with a 60× water-immersion (NA=1.2) objective (Nikon CFI Plan Apo VC 60XWI).

5.3 Results

5.3.1 Particle-interface interaction

We observe three distinct ways in which particles and interact with the interface, corresponding to different salt concentrations. For the polystyrene latex samples, all trajectories show a discontinuity (Figure 5.2c), the signature of capillary adsorption\textsuperscript{11}, at salt concentrations above 100 mM. Such particles are so strongly bound to the interface that we cannot remove them with the optical trap. At low salt concentrations, below 10 mM NaCl, the velocity of the particles decreases with height, as expected from hydrodynamics\textsuperscript{11,17}, but the trajectories show no discontinuities (Figure 5.2a). These particles are repelled from the interface, and we can easily remove them with the optical trap. Furthermore, we cannot force them to adsorb even by applying the maximum upward radiation force of about 10 pN.

At salt concentrations between these two bounds we observe a range of behaviors including repulsion, capillary adsorption, and an unexpected non-capillary, nonwetting attraction. Trajectories
in this last category appear almost identical to those of pure repulsion: there is no discontinuity, and the raw trajectories agree well with hydrodynamic calculations incorporating the effects of drag near a shear-free interface.\textsuperscript{11,117} However, the particles cannot be removed from the vicinity of the interface using the optical trap, indicating that they are caught in an attractive well.

To confirm whether the non-breached particles are in an attractive well, we characterize each particle's fluctuations $\Delta z^2$, defined as the square of the particle displacement from the mean position. In Figure 5.2, the mean is a centered sliding average over a 1 second time interval. This high-pass filtering minimizes the effects of drift of the interface and mechanical vibrations of the microscope stage. In Figure 5.2b we find that the fluctuations are about 0.001 $\mu m^2$ when the particle first reaches the vicinity of the interface (at 5 s) but shrink dramatically to less than 0.0001 $\mu m^2$ (after 2 s), even though the particle does not breach. This decrease in fluctuations is significant, as breached particles that are bound with order $10^7 kT$ also have $\Delta z^2$ less than 0.0001 $\mu m^2$ (Figure 5.2c).

Figure 5.2: The height $z$ of 1.9-$\mu m$-diameter sulfate polystyrene particles is plotted versus time. The fluctuations $\Delta z^2$ from a 1 s centered sliding average are also shown. Different types of interactions with the aqueous-decane interface are observed. a) A particle reaches the interface with no noticeable attraction. Such particles can immediately be pulled away from the interface with an optical trap. b) A particle reaches the interface with no noticeable attraction, except the fluctuations reveal that the particle is bound. Particles like this usually stay at the interface unless the optical trap acts on them for an extended period of time. c) A particle penetrates the interface. A characteristic breach occurs at around 6 s.

A particle at the interface often does not interact solely in one of the manners described above. By looking at the fluctuations of a particle, such as in Figure 5.3, we infer that the particle behavior can be highly time-dependent. For instance, the 1.4-$\mu m$-diameter sulfate latex particle in Figure 5.3 is bound to the interface in a non-capillary manner just prior to breaching despite being in an extremely high
salt environment (1 M NaCl). In salt concentrations lower than 50 mM, we observe both polystyrene and silica particles (see Section 5.5.1) bind, unbind, then bind again in less than a minute.

![Figure 5.3: Trajectory of a 1.4-μm-diameter particle in a 1 M NaCl aqueous solution. The particle exhibits multiple types of interactions with the interface: at first it has no clear attraction to the interface (grey region). As seen from the reduction in the particle’s displacement from its position averaged over a period of 0.2 seconds, the particle appears to be attracted to the interface after $t = 1.5$ s (blue region). Then, at around $t = 2.5$ s, a sudden jump in the particle’s position indicates that it has adsorbed (red region).](image)

From these results, we conclude that not all particles bound by a non-capillary attraction end up breaching: they might remain bound, or they might fall off the interface and diffuse away or sediment. We note that at higher salt conditions, where all particles ultimately breach, not all breached particles first pass through a non-capillary binding regime. The particle in Figure 5.3 does, but the particle in Figure 5.2c does not.

To determine when non-capillary binding is a precursor to breaching, we holographically track sulfate- and carboxyl-functionalized latex spheres at range of salt concentrations in the glycerol/water mixture. We find the same salt-dependent breaching behavior for sulfate and carboxyl latex particles: particles that breach in less than 50 mM and greater than 250 mM NaCl solutions first bind in a non-capillary way. Particles in NaCl solutions between 50 mM and 250 mM breach immediately.
fluctuations are shown in Figure 5.4 for 2.0-μm-diameter carboxyl latex particles. The fluctuations of the particles at 50 mM and 250 mM NaCl indicate that they are in an attractive well before breaching. In contrast, the particles at intermediate salt concentrations breach from an unbound state. This non-monotonic behavior with salt concentration hints that electrostatic effects more complicated than image-charge repulsion are at play.

The high frame data reveal an additional interesting phenomenon, highlighted in Figure 5.4a: particles at low and high salt concentration seem to breach with a sharp upturn, but particles at intermediate salt concentrations have a softer ‘knee’ in the trajectory just before breaching. A particle that is bound in a non-capillary manner is therefore closer to the interface than an unbound particle when it breaches: Figure 5.4 indicates the difference is approximately 10 nm, which we take to be the position of the potential well relative to the interface.

![Figure 5.4: Trajectories (a) and fluctuations (b) of 2.0-μm-diameter carboxyl latex particles in aqueous solutions of varying salt concentration. The fluctuations are calculated from a centered 0.2 s sliding average value in z. The particles at the highest and lowest salt concentrations breach abruptly and have small fluctuations before breaching. Particles at intermediate concentrations have larger fluctuations before breaching.](image)

5.3.2 Particle-particle interactions

Because the non-capillary binding regime has not been previously reported, we examine the interactions not only between the particles and the interface but also between particles that are bound. For
these experiments we use 1.9-μm-diameter sulfate polystyrene particles (Invitrogen). We prepare sample cells with particles in 25 mM, 37.5 mM, 50 mM, 75 mM, and 100 mM NaCl without glycerol, invert the sample cells to allow particles to sediment toward the oil-water interface for 1 hr, then turn them back upright.

![Image](image_url)

Figure 5.5: We prepare sample cells with either a) 25 mM or b) 37.5 mM NaCl in the water phase. Breached particles in a) and b) (circled) form a crystal. Particles bound in a non-capillary manner are uncircled. The unbound particles that settle to the bottom of the sample are shown in the bottom images. The scale bar is 10 μm.

We find that at salt concentrations 50–100 mM, almost all particles bind to the interface by capillary adsorption and form long-ranged repulsive crystals, similar to those reported in other studies. At lower salt concentrations (Figure 5.5), the interfaces look disordered, with fewer particles attaching to the interface. Increasing the salt concentration from 25 mM (Debye screening length $\kappa^{-1} = 1.92$ nm) to 37.5 mM ($\kappa^{-1} = 1.57$ nm) increases the fraction of particles that attach to the interface from about 35% to about 95%. The particles that do not attach to the interface are found at the bottom of the sample cell, as shown in Figure 5.5. We note that in the glycerol-free system, the critical salt concentration at which all particles breach is 50 mM. For the glycerol/water system, the critical salt
The interfaces at lower salt concentrations are not as disordered as they first seem. Because breached particles are partially wetted by decane, we are able to distinguish particles which have breached from those which have not. Breached particles have a lower relative refractive index \( m_{\text{decane}} = \frac{n_{\text{particle}}}{n_{\text{decane}}} = 1.12 \) and appear paler than particles which are non-capillary bound \( (m_{\text{water}}=1.19) \). We circle the breached particles in Figure 5.5a and b, and it becomes evident that the breached particles still form repulsive crystals. The disorder at the interface arises from the presence of non-capillary bound particles. This suggests that non-capillary bound particles have only a weak, short-ranged interaction with other particles.

To determine the fate of the non-capillary bound particles over time, we image the 37.5 mM NaCl sample over a period of a day, and we capture how the bound particles transition from disordered to crystalline (Figure 5.6). Although the particles appear disordered at the one-hour mark, the Fourier
transform (Figure 5.6a) shows distinct peaks, indicating crystalline order. This order arises from the fraction of particles that are breached and form a repulsive crystal\textsuperscript{104}. At longer times, the Fourier peaks become brighter and the spacing between peaks increases, reflecting a decrease in the lattice spacing (Figure 5.6b–c). This observation suggests that the number of particles in the repulsive-crystal is increasing over time. The most likely explanation is that non-capillary bound particles are breaching.

5.4 Discussions

Here we examine the possible origin of non-capillary binding. We estimate the strength of the interaction between the particle and interface by comparing it to the strength of our optical trap, which is between 1 and 10 pN/\(\mu\)m in the axial direction\textsuperscript{118}. This strength is insufficient to detach a breached particle, which is held to the interface in a deep well (about \(10^7 k_B T\)) and with a spring constant comparable to the interfacial tension, 35–50 mN/m. This tension, which can be written as 35–50 nN/\(\mu\)m, is 3–4 orders of magnitude larger than our trap strength. However, pulling on non-capillary bound particles with our optical trap usually leads to detachment within seconds to minutes. The strength of the attractive interaction is therefore at least the force exerted by our trap divided by the size of a particle, 10 pN/\(\mu\)m.

We also extract a lower bound for the strength of the non-capillary binding potential from the axial fluctuations of the particle at the interface. The measured fluctuations of a non-capillary bound particle are similar in magnitude to those of an adsorbed (breached) particle, as shown in Figure 5.2. We determine the binding stiffness from the Boltzmann distribution (see Section 5.5.2). For the least noisy trajectories, we obtain stiffness values of at least 1.8 mN/m. We expect, however, that the actual stiffness for the breached particles should be approximately 37 mN/m, the magnitude of the interfacial tension. Therefore the value of 1.8 mN/m represents the upper bound of what we can measure given the noise in the instrument (which sets a lower bound on the size of the fluctuations that can

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be measured). Because non-capillary bound particles have fluctuations of similar magnitude to that of breached particles, we conclude that they are bound with a strength of at least 1.8 mN/m. This estimate is consistent with the estimate based on the strength of the optical trap.

The strength of the non-capillary interaction suggests that it might actually be capillary in origin. One could imagine that a small protrusion on the particle’s surface penetrates the interface and, owing perhaps to contact-line pinning, hinders further motion into the interface. However, this argument does not explain why the pinning would depend on the salt concentration of the aqueous phase or how such particles could unbind. Another possibility is that the particles become tethered to the interface by a polymer chain. Jensenius and Zocchi\textsuperscript{51} found that polystyrene spheres have a few surface-bound polymer chains approximately 50 nm long that can tether the particles to a surface. They found that the chains have an effective stiffness of 1.5 mN/m, similar to our estimate for the strength of non-capillary binding. Again, however, this hypothesis does not account for the salt-dependence of the interaction.

We therefore believe it is more likely that the interaction has an electrostatic origin. Electrostatics could account for the salt-dependence of the interaction, as well as the dependence of the critical salt concentrations on the composition of the aqueous phase: the critical salt concentration is lower in the pure water-decane system than in the glycerol/water-decane system, perhaps owing to the different dielectric constants of the media. The transitions in the interactions with the interface (Figure 5.3, Figure 5.6, and Figure 5.8) could be due to rotational diffusion of heterogeneously charged particles. As the particle rotates, patches with varying amounts of charge are presented to the interface, changing the close-range electrostatic interaction of the particle with the interface.

Simulations based on liquid state theory for primitive model electrolytes do predict two non-capillary binding regimes for our experimental system. Following the work by Zwanikken and coworkers\textsuperscript{120}, Zwanikken solves the Ornstein-Zernike equation with the Anisotropic Hypernetted-Chain closure. This method incorporates non-linear screening, steric, and electrostatic correlations
Zwanikken finds that for 1.9-μm-diameter sulfate polystyrene beads near a water/glycerol-decane interface, there is an attractive potential well of at least $10 \ k_B T$ about 5 nm from the interface for NaCl concentrations between 20–55 mM\textsuperscript{119}. A potential well of at least $10 \ k_B T$ emerges again at NaCl concentrations greater than 400 mM. These results are shown in Figure 5.7. The potential well at lower salt concentrations arises from the competition between a van der Waals attraction and a screened Coulomb interaction. At higher salt concentrations, the structure of the ion cloud, which is affected by the finite size of ions, becomes important. The ions pack to maximize entropy and minimize electrostatic energy, resulting in repulsion that is strong enough at some distances to create a potential well.
The agreement between simulations and our data supports the hypothesis that if a particle is charged enough, ionic effects and a van der Waals attraction are sufficient to give rise to a non-capillary mode of binding. This potential well is many times the thermal energy, and thus the particle can remain bound for minutes or hours before breaching, as we observe. At higher salt concentrations, the simulations again predict a non-capillary mode of binding, though at higher salt concentrations than that observed in experiments. The discrepancy may be because the location of the potential well at high salt concentrations is highly sensitive to the parameters of the system, such as the Hamaker constant of the particle. More importantly, the liquid state theory approach predicts the observed re-entrant non-capillary binding.

In conclusion, we have observed a range of interactions between a colloidal particle and an oil-water interface. The mechanism leading to particles binding to an interface is not capillary in nature, nor is such a mechanism necessarily constant in time. As particles transition from non-binding to binding in various modes, interparticle interactions and ultimately the arrangement of large numbers of particles at the interface are affected. It has already been noted that heterogeneities in charge and roughness of a particle at an interface strongly affect particle-particle interactions. We now add to the heterogeneities that ought to be considered: the nature of the particle-interface interaction.

5.5 Appendix

5.5.1 Binding and unbinding of particles

We find that some particles can bind to an interface, unbind, then bind again (as shown in Figure 5.8) indicating that the non-capillary binding interaction is heterogeneous with time. When we use a dense particle such as silica, we can tell whether a particle is bound to the interface from the trajectory upon approach from the aqueous phase. Figure 5.9 shows the trajectory of a 1 μm-diameter amino-propyltrimethoxysilanized silica particle that we push with radiation pressure, against gravity, towards a
water-decane interface. The fluctuations in $z$ show that the particle binds to the water-decane inter-
face at 2.5 s, falls off at 6 s, then binds again at 11 s. The detachment between 6 s and 11 s indicates
that it is unlikely to be bound by capillary forces: the energy required to remove a 1 $\mu$m particle from
the interface is on the order of the thermal energy $k_B T$ when the contact angle $\theta$ is less than 1.5° and
rises quickly to 10 $k_B T$ for $\theta = 2.5^\circ$ and 100 $k_B T$ for $\theta = 4.5^\circ$. A more likely explanation for the time-
dependence is that the particle experiences a non-capillary attraction similar to that seen in polystyrene
spheres.

Figure 5.8: Trajectory of a 1.9-$\mu$m-diameter sulfate polystyrene particle that is pushed to a glycerol/water-decane
interface with radiation pressure. The particle binds to the interface at 5 s, unbinds at 9 s, and binds again at 14 s.

Figure 5.9: Trajectory of an APTMS-silica particle that is pushed to a water-decane interface with radiation
pressure. The particle binds to the interface at 2.5 s, falls off at 6 s, then is pushed back to the interface and
re-binds at 11 s.
5.5.2 Boltzmann inversion

To determine the potential between capillary- and non-capillary-bound particles from the measured fluctuations, we use the Boltzmann distribution. In equilibrium, the probability of finding a particle at position $z$ given a potential well $U(z)$ is

$$P(z) = \frac{1}{Z} \exp \left( - \frac{U(z)}{k_B T} \right) \quad (5.1)$$

where $Z$ is the partition function. We invert the distribution to find the potential well $U(z)$:

$$U(z) = -k_B T (\ln P(z) + \ln Z). \quad (5.2)$$

To determine $P(z)$ we bin the measured displacements in $z$. We assume a harmonic potential with binding stiffness $K$ and an additive constant $C$. Because $\ln Z$ is also an additive constant, we write

$$-k_B T \ln P(z) = \frac{1}{2} K z^2 + C. \quad (5.3)$$

We use a least-squares fit to find the $K$ and $C$ that minimize the error in Equation 5.3 for our $z$ bins and measured $P(z)$. The best-fit $K$ is what we report for the binding stiffness.
Non-spherical swimming particles

So far we have studied the fine motion of particles near interfaces. Here we image active, non-spherical particles—bacteria. We show that the large depth of field and fast image acquisition time of holographic microscopy combined with fitting exact scattering solutions allows us to precisely image the motion of the organisms.

6.1 Introduction

‘Run and tumble’ is the canonical example of how microorganisms navigate their complex physical and chemical environments. This movement modality consists of straight ‘runs’ to cover distance, and ‘tumbles’ to randomly reorient. The statistics of both the length of the runs and the directional changes from tumbles of *Escherichia coli* (*E.coli*) can be influenced by the presence of surfaces, viscosity changes, and chemical gradients. These same variations in environment should also
affect individual runs and tumbles.

Understanding how runs and tumbles are affected by the environment can shed light on their mechanism and provide insights into swimming strategies at low Reynolds numbers. More specifically, our understanding of both runs and tumbles can benefit from knowing the orientation of the cell during the motion. Tumbling is often modeled as rotational diffusion; although this viewpoint may capture the statistics of tumbles, it neglects the mechanism by which cells actually change direction. Tracking individual tumbles might help improve models of how cells tumble. Because hydrodynamics are important to the motion, an understanding of microorganism motility is incomplete without knowledge of how the cell body is oriented as a function of time.

However, the orientation of the cell body is usually not tracked in microorganism motility studies. This is because most tracking methods do not have a way to quantitatively extract the orientation of the cell during motion. Berg et al. pioneered the use of a tracking microscope with a moving stage that keeps the cell in focus and centered as it swims. The motion of the stage is recorded to yield a three-dimensional (3D) trajectory. Though the orientation of the cells can be determined by eye in this experiment, it is laborious to track the orientation for the whole trajectory. More recently, Liu et al. used a similar technique to that of Berg et al. but used digital image processing to determine the orientation of Caulobacter crescentus. The crescent shape of the bacterium was used to determine its orientation, but it is unclear whether this method for determining cell orientation is applicable for other cell types. The moving-stage technique is also limited because only one cell can be tracked at any time. Taute et al. also used digital image processing to track micrometer-sized bacteria from diffraction patterns taken on a modified phase contrast microscope. By digitally comparing the diffraction patterns to a reference library of how spheres scatter light, Taute and coworkers could track the cells in 3D without a moving stage. However, the orientation of the cells was not measured, likely because there was no reliable way to extract the orientation information from the diffraction patterns.

We use holographic microscopy combined with light scattering solutions to track the orientation
and translation of freely swimming *E. coli*. In most holographic microscopy studies, images that resemble bright-field microscopy images are obtained by numerically back-propagating light through the holograms. Using this method, researchers have tracked the center-of-mass trajectories of *E. coli* and sperm. It is, however, difficult to recover the orientational information from reconstructions, owing to distortions in the axial direction. We therefore avoid numerical back-propagation and instead directly fit a scattering solution for spherocylinders to the holograms. With this method, we are able to track the orientation of *E. coli* cells as they freely swim in 3D.

### 6.2 Materials and methods

![Diagram](image.png)

Figure 6.1: a) Holograms are captured on an in-line holography setup. Our sample cells consist of two square coverslips glued with epoxy to a larger coverslip. A droplet of tryptone broth (blue) is placed in between the smaller coverslips. A smaller droplet of cells (pink) is placed on the smaller coverslip. A top coverslip is sealed in place with vacuum grease (gray). Right: We define the orientation and position of the *E. coli* cell relative to the laboratory frame. A unit vector $\mathbf{u}$ points along the long axis of the cell in the direction of travel. The angle between $\mathbf{u}$ and the imaging axis ($z$-axis) is defined to be the polar angle $\theta$. We define another unit vector $\mathbf{p}$ that is a projection of $\mathbf{u}$ onto the $x$-$y$ plane. The angle that $\mathbf{p}$ makes with the laboratory $y$-axis is the azimuthal angle $\phi$.

#### 6.2.1 *E. coli* samples

To prepare *E. coli* samples, we first prepare the growth media for the bacteria. We make Luria broth (LB) by mixing 5 g tryptone (Difco Laboratories, USA), 2.5 g Yeast Extract (Difco Laboratories, USA), 2.5 g NaCl (final concentration 86 mM, Sigma-Aldrich), and enough deionized water (Elix, EMD Millipore) to make 500 mL of broth. We filter the broth through a 0.22 μm filter (500 mL PES vacuum
filter, Corning USA), and store the broth at room temperature (21 ± 2 °C). We make Tryptone broth (TB) using the LB recipe but omit the Yeast Extract.

We then follow a two-day procedure to grow motile E. coli from a frozen stock solution of wild-type AW405 cells. We prepare 5–10 mL of LB in a sterile culture tube (VWR International, USA), scrape a pipette-tip against the frozen stock, and add the scraped cells to the LB solution. The inoculated LB is kept overnight at 37°C on a incubator shaker (New Brunswick Scientific, C24) that shakes at 200 rpm.

The next morning, we warm 50 mL of TB to 33°C in a small Erlenmeyer flask that has been cleaned in a pyrolysis oven (Pyro-Clean TempyroX). We add 500 µL of the overnight solution to the flask and incubate the flask at 33°C while shaking at 200 rpm. We check the cell density in the flask every hour on a CO8000 Cell Density Meter (WPA Biowave). Once the cell density reaches an optical density (OD<sub>600</sub>) of 0.5, we remove the flask from the incubator. We dilute the solution of cells 1:10 by volume with TB just prior to imaging.

Our sample chambers consist of a series of glass coverslips (No. 1 VWR), as shown in Figure 6.1. Two 18 × 18 mm coverslips are affixed to a 24 × 60 mm coverslip with UV-curable epoxy (Norland 60) to create two ‘shelves’ and a central chamber. A 100 µL drop of tryptone broth is deposited in the central chamber. We place polydimethylsiloxane grease (Dow Corning high vacuum grease) around the chamber to form walls, then add 10 µL of diluted E. coli broth onto one ‘shelf’. All pipetting of the E. coli is performed with with a cut pipette tip to minimize shear damage to the flagella<sup>129</sup>. A final 22 × 22 mm coverslip is placed on top of the vacuum grease to seal the chamber. Because E. coli tumble less and become easily trapped near surfaces<sup>122</sup>, we expect many cells to be trapped on the ‘shelf’ on which they are first deposited. We therefore image only the central chamber, which consists of cells that are not trapped on the ‘shelf’ and can swim in the bulk.

*AW405 cells courtesy of Karen Fahrner, from Professor Howard Berg’s laboratory
6.2.2 Taking holograms

![Figure 6.2: We capture holograms of freely swimming *E. coli* in a time series. Two frames are shown in the left column, where the asymmetry in the fringes is noticeably different between the frames. The best-fit holograms are shown in the middle, and three-dimensional renderings from the best-fit holograms are shown on the right.](image)

We use an in-line digital holographic microscope as described by Kaz *et al.* We use an in-line digital holographic microscope as described by Kaz *et al.* Laser light (λ = 660 nm, Opnext HL.6545MG) is spatially filtered with a single mode optical fiber (OzOptics SMJ-3U3U-633-4/125-3-5), then passes through a 10× objective (Newport) and a condenser (LWD 0.52, Nikon) to provide even, approximately plane-wave illumination on the sample. We use a 60×, NA = 1.2, water-immersion objective (Nikon CFI Plan Apo VC 60XWI) to capture the interference pattern, or hologram, formed by the scattered and undiffracted beams. We capture holograms (Figure 6.2) at 100 fps with a Photon Focus MVD-1024E-160 camera, store them in RAM using a frame grabber (EPIX PIXCI E4), and then transfer them to disk for analysis. The exposure time is 0.05 ms, which is short enough to minimize blurring due to bacterial motion.
6.2.3 Analyzing holograms

Because *E. coli* are small and have little refractive index contrast with water (*n*_{E. coli} = 1.36–1.39 vs *n*_{water} = 1.33), they are difficult to see in raw holograms. To enhance contrast and to remove imaging artifacts from uneven illumination and dust in the optical train, we divide each raw hologram by a background frame taken at a region that contains no cells. Background division yields holograms with good fringe contrast, as shown in Figure 6.2.

We then follow the protocol in Wang *et al.*\(^{100}\) to fit the holograms. In that work, we demonstrated that we could track silica spherocylinders of a similar size to *E. coli* to a precision of 35 nm in the centre-of-mass and 2° in the orientation. We expect the tracking precision to vary from cell to cell, depending on how much the cell’s shape deviates from a spherocylinder. We fit a discrete-dipole based light-scattering model to the recorded holograms using the software package HoloPy (http://manoharan.seas.harvard.edu/holopy/) and the A-DDA program.\(^6\) The adjustable parameters in the fit are the spherocylinder’s refractive index, radius, length, three-dimensional center-of-mass coordinates, and orientation relative to the lab frame (see Fig. 6.1b). We initialize the Levenberg-Marquardt fitting algorithm with a guess of all of these parameters. We use the fit results from one frame as the initial guess for the following frame in the time-series.

6.3 Results and discussions

We are able to fit a spherocylinder scattering model to holograms of *E. coli*. Examples of best-fit holograms and three-dimensional rendering of the fits are shown in Figure 6.2. To quantify how well the light scattering model fits our data, we compare the best-fit holograms to the data. We evaluate the coefficient of determination \(R^2 = 1 - \frac{\sum I_{\text{data}} - I_{\text{fit}}}{\sum I_{\text{data}} - 1}\) where \(I\) is the background-divided and normalized hologram, and the sum is over all the pixels. A perfect fit results in \(R^2 = 1\). When we previously fit a DDA model to holograms of silica spherocylinders in Wang *et al.*,\(^{100}\) we obtained \(R^2 \approx 0.9\). Here
we find $R^2 \approx 0.8$.

To determine whether this $R^2$ value is sufficient to track motion, we examine a captured run in detail (Figure 6.3). This cell travels 78 μm in 3.4 seconds (velocity = 23.3 μm/s). This speed is within the expected range for $AW405$ (see Table 6.1). We define a unit vector $u$ that points along the long axis of the cell in the direction of travel (Figure 6.1b). We find that $u$ rotates about the direction of the travel. This type of rotation is known as a ‘wobble’. We represent how $u$ changes over time on a unit sphere, as shown in Figure 6.3b, and find that the wobble is 50°. This value is also within the expected range for $AW405$ (see Table 6.1). Because this cell is more than one flagella-length away from any glass surface during the whole trajectory, we expect the average direction of $u$ to be aligned with the direction the cell is swimming. The average direction for $u$ is $\theta = 99^\circ$, $\phi = 14^\circ$, and the direction the cell is swimming is $\theta = 97^\circ$, $\phi = 13^\circ$. These two values agree within the measuring precision of 2°. These results show that the dynamics of the cell are captured accurately.

Because we can track the body rotation of the cells, we can track the tumbling behavior. The bacterium shown in Figure 6.4 performs two tumbles, the second of which is marked in red in Figure 6.4a. Again, we track $u$ and plot its orientation on a sphere (Figure 6.4b). We see that $u$ appears to rotate clockwise when viewed from the center of the sphere before the tumble (green square), then coun-

Figure 6.3: a) We capture holograms of freely swimming $E. coli$ in a time series and fit a scattering model to them to recover the trajectory. The blue square represents the position at the start of the time series. The circle marks the position at the end. b) We plot the direction $u$ points on a unit sphere for the duration of the trajectory in a) to show how $u$ precesses as the cell swims.
ter clockwise after the tumble (green circle). Because *E. coli* always rotate clockwise\textsuperscript{131}, this apparent change in rotation from clockwise to counterclockwise indicates that the leading end of the cell has become the trailing end after the tumble. This switching of ends is also known as a pole reversal.

![Figure 6.4](image)

Figure 6.4: a) Center-of-mass trajectory of a cell’s runs and tumbles. The blue square represents the position at the start of the time series. The circle marks the position at the end. The red square marks a tumble event. b) We represent the tumble by plotting where \( \mathbf{u} \) points on a sphere. The green square represents the start of the tumble. The circle marks the end.

We compare values of the parameters that we measure (Table 6.1) to values from the literature. We measure the refractive index, size, run speed, wobble frequency, and wobble angle for the cells and find that the agreement is good, further validating our technique.

Table 6.1: Properties of *AW405* cells determined from fitting holograms of ten cells, compared to values from Darnton *et al.*\textsuperscript{131} unless otherwise specified. The errors are standard deviations from the mean.

<table>
<thead>
<tr>
<th></th>
<th>Length (( \mu \text{m} ))</th>
<th>Width (( \mu \text{m} ))</th>
<th>( n )</th>
<th>Run speed (( \mu \text{m/s} ))</th>
<th>Wobble freq. (Hz)</th>
<th>Wobble angle ((^\circ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Our values</td>
<td>2.4 ± 0.6</td>
<td>0.95 ± 0.06</td>
<td>1.41 ± 0.01</td>
<td>25 ± 5</td>
<td>26 ± 9</td>
<td>68 ± 42</td>
</tr>
<tr>
<td>Other work</td>
<td>2.5 ± 0.6</td>
<td>0.88 ± 0.09</td>
<td>1.388 ± 0.005\textsuperscript{132}</td>
<td>25 ± 8</td>
<td>24 ± 12</td>
<td>46 ± 24</td>
</tr>
</tbody>
</table>

### 6.4 Conclusions

We have shown that by fitting a light scattering model to digital holograms, we can quantitatively track the 3D orientation and motion of freely swimming *E. coli*. We can track the cells in enough detail to resolve their tumbles and pole reversals. Unlike other techniques for tracking microorganisms, our
technique does not require a moving stage, and we are able to track the orientation of the cells for the entire trajectory. This technique can be used for studying bacteria as they freely traverse chemical and physical landscapes, providing insights into how runs and tumbles are altered by the environment. Acquiring more data with this method can also help improve models for the hydrodynamics of runs and tumbles.
Pickering stabilisers for fluid foundation

So far, we have been studying the interaction of colloidal particles with oil-water interfaces using laboratory-grade fluids and particles. The vast majority of Pickering emulsions seen in the literature also use laboratory-grade oils and particles. Many papers report the successful formation of ‘emulsions’ if some droplets form, when in fact there might not be many droplets at all, the droplets might be large, or they might not be very stable.

Here we aim to go beyond this definition of a ‘successful emulsion’. We attempt to define what additional properties are required to make ‘successful’ Pickering emulsions, while working with industrial-grade particles and fluids.
7.1 **Background**

Solid particles can bind to fluid-fluid interfaces to stabilize droplets against coalescence. Such solid-stabilized emulsions are known as Pickering emulsions and are an active area of research because of the tunable properties of particles and their potential to be more environmentally friendly than surfactant-stabilized emulsions. Moreover, emulsions stabilized by solid particles are also of great interest to cosmetics and personal care industries since they can be made without surfactants, which can be irritating to the skin. Since particles are already present in many cosmetic formulations, it may be possible to use the same particles to both stabilize the emulsion and control the appearance. This would reduce the overall number of ingredients in formulations.

We focus on using the same particles to both stabilize the emulsions and control their appearance. Specifically, by making Pickering emulsions with a range of particles we aim to discover how the properties of these particles, such as composition, shape, and surface characteristics, affect the type and stability of Pickering emulsions.

7.2 **Materials and methods**

Particles were obtained from Kobo Products, Inc. and used without modification. The oils used are Cetiol CC and isododecane (BASF), Xiameter PMX-200 and TMF 1.5 (Dow Corning) and Miglyol 812 (IOI Oleo). The phospholipid used is Emulmetik 320 (Lucas Meyer Cosmetics), which is mostly hydrogenated lecithin.

7.2.1 **Emulsion preparation**

Lab-scale quantities of Pickering emulsions were prepared using the following protocol:

1. Disperse 2% (w/w) of particles in the appropriate phase by vortexing and sonication.
2. Emulsify equal parts of the particle suspension and the other phase (oil or water) with a T25 Ultra Turrax (IKA) for 1 min at 6500 rpm and then 1 min at 13500 rpm.

Emulsions were stored in 15 mL polystyrene centrifuge tubes (VWR, Batch No. 8495) or 2.5 mL glass vials (VWR), both with screw caps, at laboratory temperature (21 ± 2°C).

7.2.2 Characterisation of emulsion stability

The emulsion stability was assessed by centrifugation, a fast and quantitative method available in our laboratory. During centrifugation, the particle-covered emulsion droplets either rose to the top or sedimented to the bottom of the centrifuge tube. The greater the acceleration in the centrifuge, the more the creamed/sedimented droplets pushed against each other. Centrifugation therefore tested how well particle-covered droplets resisted coalescence.

After preparing the emulsions and determining their type, 0.5 mL of each emulsion was centrifuged using a Galaxy 14D microcentrifuge (VWR) for 4 minutes at increasing acceleration until the emulsion broke. The acceleration at which the emulsion broke was called the critical centrifugal acceleration, $A_C$. Higher accelerations imply better stability.

7.3 Results

7.3.1 Effect of particle coating and phospholipid on emulsion type

We made emulsions with hydrophobic and hydrophilic titanium dioxide and iron oxide particles, with and without Emulmetik 320, and found that both particle surface treatment and the presence or absence of Emulmetik 320 influenced the emulsion type. In the absence of Emulmetik 320, the particle surface treatment determined the emulsion type. Particles with hydrophobic coatings produced water-in-oil (W/O) Pickering emulsions; particles with hydrophilic coatings produced oil-in-water
(O/W) emulsions. This observation is consistent with the Bancroft rule, which states that “A hydrophilic colloid will tend to make water the dispersing phase while a hydrophobe colloid will tend to make water the disperse phase.”\textsuperscript{133,134} In the presence of Emulmetik 320, the surfactant appeared to override the influence of the particle coating. All formulations with Emulmetik 320, regardless of pigment surface treatment, produced O/W emulsions (Figure 7.1).

![Figure 7.1: The type of Pickering emulsion formed depends on the particle coating and the presence or absence of Emulmetik 320.](image)

### 7.3.2 Influence of Surface Coating on Emulsions Stability

Because titanium dioxide (TiO\textsubscript{2}) is an abundant pigment in many industrial formulations, we first focused on Pickering emulsions stabilized by TiO\textsubscript{2} alone. We considered particles with five surface treatments available from Kobo Products Inc., four that are hydrophobic [coated with sodium dilauramidoglutamide lysine (ASL-1), dimethicone (RBTD-DS4), isopropyl titanium triisostearate (BTD-401), isopropyl titanium triisostearate and sodium lauroyl aspartate (ASI)] and one that is hydrophilic [coated with PEG-8 methyl ether triethoxysilane (RBTD-SW)]. The relative stabilities of the five emulsions are summarized in Figure 7.2.
Figure 7.2: The relative stability of titanium-dioxide-stabilized Pickering emulsions depended on the TiO$_2$ surface treatment. Particles coated with isopropyl titanium triisostearate produced the most stable W/O Pickering emulsion; particles coated with PEG-8 methyl ether triethoxysilane yielded the most stable O/W emulsion and the most stable emulsion. The ASL-1 coating produced the least stable TiO$_2$-stabilized Pickering emulsion. All particles were provided by Kobo Products Inc.

We found that the surface treatment determined the stability of titanium-dioxide-stabilized Pickering emulsions. Of the hydrophobic surface treatments, particles coated with isopropyl titanium triisostearate produced the most stable W/O Pickering emulsions, with critical centrifugal accelerations roughly twice as large as the weakest Pickering emulsions (those stabilized by ASL-1- or RBTD-DS4-coated TiO$_2$). Water-dispersible particles coated with PEG-8 methyl ether triethoxysilane produced the most stable O/W Pickering emulsions, about twice as stable as the best W/O-type emulsion.

We found similar trends in Pickering emulsions stabilized by yellow iron oxide particles (Figure 7.3). Namely, ASL-1 coated particles produced marginally stable W/O Pickering emulsions, while PEG-coated (SW2 series) particles yielded very stable O/W emulsions. The Pickering emulsions prepared from PEG-coated yellow iron oxide (BGYO-SW2) particles were stable not only in our centrifugation tests, but also at ambient conditions for longer than six months.

We also tested a number of other particles, but none produced stable Pickering emulsions. We found that neither oil-dispersible nor water-dispersible boron nitride particles were able to stabilize Pickering emulsions to centrifugal accelerations above 2000 $g$. 

100
7.3.3 Influence of particle shape

Comparing Figures 7.2 and 7.3, we saw that Pickering emulsions stabilized by PEG-coated yellow iron oxide particles were much more stable than those stabilized by PEG-coated titanium dioxide particles, even though the particles have the same surface chemistry and are roughly the same size. Scanning electron microscopy (SEM) revealed that the primary difference between the particles was their shape. The titanium dioxide particles are roughly spherical; the yellow iron oxide particles are more rodlike (Figure 7.4). We hypothesize that the enhancement in emulsion stability resulted from these differences in particle shape.

Cryo-SEM images of our yellow-iron-oxide-stabilized emulsions (Figure 7.4) showed that the O/W emulsion consisted of roughly 10-μm-diameter droplets that were covered with a dense monolayer of yellow iron oxide particles. The particles appeared to be close packed, as expected, but did not seem to adopt any preferential orientation with respect to the interface or one another.

7.3.4 Influence of multiple particle types

Thus far we have only considered Pickering emulsions consisting of a single particle type. However, many industrial formulations contain more than one type of particle. These particles are present for
optical or rheological purposes and cannot be removed without compromising the product, so ideally they could also be used to aid emulsion formation. Because there have been few studies on the effect of multiple particle types on emulsion stability, we consider how two or more particles can be used to create Pickering emulsions.

Here we define “surface-active” as the ability of a particle to stabilize a Pickering emulsion. The basic requirement for making a Pickering emulsion is that the particles adhere to the oil-water interfaces of droplets and prevent the droplets from coalescing. For the two-particle systems, the requirement is the same.

**Results when all particles are surface active**

To determine whether adding multiple particles to a stable formulation would compromise its stability, we made two samples with all SW2-coated pigments present: (1) all SW2-stabilized particles dispersed in the aqueous phase and (2) all SW2-stabilized particles dispersed in the oil phase. Quantities of the different particles are given in Table 7.1.

We found that adding other SW2-coated particles did not compromise the stability of the BGYO-
Table 7.1: Pigment powders used in formulations

<table>
<thead>
<tr>
<th>Colour</th>
<th>Trade Name</th>
<th>Weight percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>White (titania)</td>
<td>RBTD-SW</td>
<td>5.7</td>
</tr>
<tr>
<td>Yellow (iron oxide)</td>
<td>BGYO-SW2</td>
<td>1.4</td>
</tr>
<tr>
<td>Red (iron oxide)</td>
<td>BGRO-SW2</td>
<td>0.3</td>
</tr>
<tr>
<td>Black (iron oxide)</td>
<td>BGBO-SW2</td>
<td>0.2</td>
</tr>
</tbody>
</table>

SW2 (yellow) stabilized emulsion (Figure 7.5). The critical centrifugal accelerations were comparable to those of emulsions with BGYO-SW2 particles alone, indicating that the additional particles did not play a significant role in determining the emulsion stability. Bright-field microscopy images of the Pickering emulsions show that the yellow iron oxide particles were adsorbed at the droplet interfaces; the majority of other particles appear to be free in solution.

![Critical centrifugal acceleration graph](image)

Figure 7.5: Left: The addition of other PEG-coated particles did not change the stability of yellow-iron-oxide-stabilized Pickering emulsions, regardless of whether the particles were introduced into the aqueous or oil phase. Right: Bright-field microscopy image of the emulsion where all particles are introduced into the aqueous phase. Many free-floating black particles can be seen in solution.

**RESULTS WHEN ONE OR MORE PARTICLES ARE NOT SURFACE ACTIVE**

Particles that are not surface active have extreme contact angles, which might arise because because the particle is porous or has a coating to help it disperse well in either oil or water, or both.

To understand how to utilize non-surface-active particles, we investigated a new method for making Pickering emulsions, as described by Saha, John and Bose. Pickering emulsions usually require
the stabilizing particle to have a contact angle intermediate between fully hydrophobic and fully hy-
drophilic. Saha, John and Bose propose that hydrophilic and hydrophilic particles can actually attract
each other across an oil-water interface through a van der Waals interaction. The particles then form
amphiphilic complexes, which are able to coat the interface and stabilize droplets. Even particles with
extreme contact angles can be used.

Saha, John and Bose calculated the interaction energy as a function of the oil, water, and particle
properties. They found that the interaction is attractive if the interaction energy is negative and repul-
sive if the interaction energy is positive. The critical property that determines the interaction is the
Hamaker constant of each material with itself across vacuum, which is a measure of its polarizability.

If one particle is surface active, it will straddle both fluids. Particles in either phase are able to
interact with the surface-active particle within one medium. Unlike particles interacting across an
interface, particles interacting across a single medium usually aggregate if electrostatic repulsion and
steric effects do not prevent attractive van der Waals interactions from occurring.

We did not find cases where two non-surface-active particles could be utilized together to stabilize
an emulsion, so instead we focused on using a surface-active particle with a non-surface-active one.
All formulations contained 50% water and 50% oil phase by weight. The results are summarized in
Table 7.2.

We encountered several situations where the model of Saha, John and Bose predict an attractive
interaction – whether the particles are interacting in water, oil, or across an interface – but where no
successful emulsion was made. In these cases, all of the particles were similarly sized. One example is
hydrophilic titania (TiO₂ TA-100 Kobo, diameter 1-2 µm) and polystyrene (SX-130 Soken, diameter
= 1.3 µm) shown above. The rest are particle pairs where both particles are smaller than 500 nm. We
hypothesize that because the surface coatings are larger in comparison to the particle diameter if the
particles are smaller, they may contribute non-negligibly to the van der Waals interaction. It is also
possible that having two particles of opposite hydrophilicity but similar size that form a complex at
Table 7.2: Pigment powders used in formulations. \( A_C \) = critical centrifugation acceleration, \( F_o \) = fraction of dispersed phase emulsified

<table>
<thead>
<tr>
<th>Particle added (weight %)</th>
<th>Oil phase</th>
<th>Emulsion type</th>
<th>( A_C ) (g)</th>
<th>( F_o )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Covabead LH 70-3 Sensient (2%)</td>
<td>Cetiol CC/isododecane</td>
<td>W/O</td>
<td>2000</td>
<td>2/3</td>
</tr>
<tr>
<td>TiO(_2) TA-100 Kobo (2%)</td>
<td>Cetiol CC/isododecane</td>
<td>O/W</td>
<td>700</td>
<td>∼0</td>
</tr>
<tr>
<td>Combined (1%/1%)</td>
<td>Cetiol CC/isododecane</td>
<td>O/W</td>
<td>2000</td>
<td>1</td>
</tr>
<tr>
<td>Silica MSS-500/3 Kobo (2%)</td>
<td>TMF 1.5/Xiameter</td>
<td>None</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>Tres BN Kobo (2%)</td>
<td>TMF 1.5/Xiameter</td>
<td>W/O</td>
<td>2000</td>
<td>1</td>
</tr>
<tr>
<td>Combined (1%/1%)</td>
<td>TMF 1.5/Xiameter</td>
<td>O/W</td>
<td>2000</td>
<td>1</td>
</tr>
<tr>
<td>TiO(_2) Kobo RTBD-SW (2%)</td>
<td>Cetiol CC/isododecane</td>
<td>O/W</td>
<td>4000</td>
<td>1</td>
</tr>
<tr>
<td>TiO(_2) TA-100 Kobo (2%)</td>
<td>Cetiol CC/isododecane</td>
<td>O/W</td>
<td>700</td>
<td>∼0</td>
</tr>
<tr>
<td>Combined (1%/1%)</td>
<td>Cetiol CC/isododecane</td>
<td>O/W</td>
<td>4000</td>
<td>1</td>
</tr>
<tr>
<td>Soken PS SX-130 (2%)</td>
<td>Cetiol CC/isododecane</td>
<td>W/O</td>
<td>2000</td>
<td>1</td>
</tr>
<tr>
<td>TiO(_2) TA-100 Kobo (2%)</td>
<td>Cetiol CC/isododecane</td>
<td>O/W</td>
<td>700</td>
<td>∼0</td>
</tr>
<tr>
<td>Combined (1%/1%)</td>
<td>Cetiol CC/isododecane</td>
<td>O/W</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>TiO(_2) TA-100 Kobo (2%)</td>
<td>Cetiol CC/isododecane</td>
<td>O/W</td>
<td>700</td>
<td>∼0</td>
</tr>
<tr>
<td>Styrene-DVB (2%)</td>
<td>Cetiol CC/isododecane</td>
<td>W/O</td>
<td>2000</td>
<td>1/3</td>
</tr>
<tr>
<td>Combined (0.5% TiO(_2)/1.5% St)</td>
<td>Cetiol CC/isododecane</td>
<td>W/O</td>
<td>mm droplets</td>
<td>2/3</td>
</tr>
<tr>
<td>Combined (1%/1%)</td>
<td>Cetiol CC/isododecane</td>
<td>O/W</td>
<td>2000</td>
<td>1</td>
</tr>
</tbody>
</table>

the interface yields a structure that is too amphiphilic. This argument is consistent with the findings of Destribats et al., who suggest that particles with a contact angle of very close to 90° may not be able to form an emulsion\(^{336}\).

In summary, additional particles can be added to a formulation if there is already a surface-active particle in the formulation. When the total weight of each particle is the same, the smallest surface-active particle will determine the type of the stabilized emulsion (W/O or O/W), because small particles are more efficient Pickering stabilizers by weight.

### 7.4 Conclusions

We developed three simple design rules for making Pickering emulsions stabilized by industrial particles. First, the surface treatment determines the type of Pickering emulsion that forms. Particles with hydrophobic coatings produce water-in-oil emulsions; particles with hydrophilic coatings produce oil-in-water emulsions. Second, the stability of the Pickering emulsions depends on the nature of the
particle surface coating. Of the particles tested, PEG-coated particles (SW2) made much more stable emulsions than the others. Third, shape-anisotropic particles produce more stable Pickering emulsions than more spherical particles with the same surface chemistry. PEG-coated yellow iron oxide particles produced significantly more stable emulsions than PEG-coated titanium dioxide ones.
It is more fun to talk with someone who doesn't use long, difficult words but rather short, easy words like “What about lunch?”

– Винни-Пух

Simple design rules for Pickering emulsion formation

The previous chapter identified promising particles for specific applications in industrial formulations, and so we only experimented with metal oxide powders. These are usually irregularly shaped. Here we aim to find general rules for creating Pickering emulsions from spherical particles. Though the formation and stability of Pickering emulsions with spheres have been studied extensively over the last few decades, the results depend sensitively on the particular combination of particles and fluids. We seek to develop some simple design rules for making Pickering emulsions by relating the stability and quality of the emulsions to the size and surface functionalisation of the particles and the solvent conditions (type of oil and salt concentration in the aqueous phase).
8.1 Materials and methods

Particles were obtained from the suppliers listed in Table 8.1 and used without modification. Polystyrene microspheres were synthesized in-house by the procedure in Paine et al.\textsuperscript{39} by Dr. Jin-Gyu Park. The oils used are Cetiol CC and isododecane (BASF), Xiameter PMX-200 and TMF 1.5 (Dow Corning) and Miglyol 812 (IOI Oleo). The phospholipid used is Emulmetik 320 (Lucas Meyer Cosmetics), which is mostly hydrogenated lecithin.

8.1.1 Emulsion preparation

Lab-scale quantities of Pickering emulsions were prepared using the following protocol:

1. Disperse 2\% (w/w) of particles in the appropriate phase by vortexing and sonication.

2. Emulsify equal parts of the particle suspension and the other phase (oil or water) with a T25 Ultra Turrax (IKA) for 1 min at 13500 rpm.

Emulsions were stored in 3.6 mL glass vials (VWR) with screw caps, at laboratory temperature (21 ± 2°C). We characterised the stability as described in Section 7.2.2.

8.2 Results

8.2.1 Effect of particle contact angle

A particle's equilibrium contact angle at the oil-water interface is expected to predict the emulsion type\textsuperscript{18,136}. The equilibrium contact angle is defined with respect to the aqueous phase, so particles that have an equilibrium contact angle smaller than 90° are hydrophilic and are expected to make water-continuous phase emulsions, whereas particles that have a contact angle greater than 90° are hydrophobic and are expected to make oil-continuous phase emulsions.
The contact angle does not by itself predict whether a particle can be dispersed in oil or water. For instance, some charge-stabilized spheres are dispersible in water but are hydrophobic. Whether a particle can be dispersed in oil or water should be determined by how easily it disperses in the medium after mixing and thorough sonication.

To measure the contact angle of the particles, we first dispersed particles in whichever medium that they most easily disperse in. We then placed a droplet of water onto a glass slide some oil (Cetiol CC) around it. After allowing the system 10 minutes to equilibrate, we imaged the oil-water interface on the side of the water droplet to determine the particle position within the interface.

After making emulsions with the spherical particles listed in Table 8.1, we found that hydrophilic particles consistently made O/W emulsions, and hydrophobic particles consistently made W/O emulsions. We also found that particles with extreme contact angles (near 0° or 180°) did not stabilize emulsions. These results are consistent with others in the literature.18,136

Table 8.1: The contact angles (θE) of the particles at a Cetiol CC and water interface are listed, as are the types of emulsions formed with the particles. Emulsions marked with an asterisk (*) are instances where very little emulsion was made. In the last column O indicates an oil-continuous phase emulsion, and W indicates a water-continuous phase emulsion.

<table>
<thead>
<tr>
<th>INCI name</th>
<th>Particle name</th>
<th>Supplier</th>
<th>Size (μm)</th>
<th>θE (°)</th>
<th>Emulsion continuous phase: with/without salt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methyl Methacrylate Crosspolymer</td>
<td>Covabead LH 70-3</td>
<td>Sensient</td>
<td>3</td>
<td>135</td>
<td>O/O</td>
</tr>
<tr>
<td>Polymethylsilsequioxane</td>
<td>M250</td>
<td>Kobo</td>
<td>4 - 6</td>
<td>90</td>
<td>O/O</td>
</tr>
<tr>
<td>Nylon-12</td>
<td>IS-300</td>
<td>Kobo</td>
<td>4</td>
<td>70 - 100</td>
<td>O/O</td>
</tr>
<tr>
<td>Alumina MSP-A504 (smooth)</td>
<td>Creaspheres EL-WL5</td>
<td>Creations Céladons</td>
<td>7 - 9</td>
<td>&gt;180</td>
<td>O/O</td>
</tr>
<tr>
<td>Vinyl Dimethicone/Methicone Silsesquioxane Crosspolymer</td>
<td>DIM WL1</td>
<td>Creations Céladons</td>
<td>0.6-1</td>
<td>&gt;90</td>
<td>O/O</td>
</tr>
<tr>
<td>Polymethylsilsequioxane</td>
<td>Sterona MR05</td>
<td>Kobo</td>
<td>4</td>
<td>135</td>
<td>O/O</td>
</tr>
<tr>
<td>Methyl Methacrylate Crosspolymer (And) Polymethylsilsequioxane</td>
<td>Alumina MSP-AK06 (spiky)</td>
<td>Kobo</td>
<td>3 - 4.5</td>
<td>90 - 135</td>
<td>O/O</td>
</tr>
<tr>
<td>Styrene/MA copolymer (and) ethylene</td>
<td>BE-900</td>
<td>Kobo</td>
<td>6</td>
<td>135</td>
<td>O/O</td>
</tr>
<tr>
<td>Cellulose</td>
<td>Cellulobeads D-5</td>
<td>Kobo</td>
<td>3</td>
<td>0</td>
<td>W/W</td>
</tr>
<tr>
<td>Silica (porous)</td>
<td>Cellulobeads USF</td>
<td>Kobo</td>
<td>4</td>
<td>0</td>
<td>W/W</td>
</tr>
<tr>
<td>Zinc oxide</td>
<td>Microspheres H-12</td>
<td>Aozu glass</td>
<td>3</td>
<td>&lt;0</td>
<td>W/W</td>
</tr>
<tr>
<td>Titanium dioxide (And) Alumina (And) Silica</td>
<td>TZ-902 YA-150</td>
<td>Kobo</td>
<td>1 - 2</td>
<td>~&lt;0</td>
<td>W/W</td>
</tr>
<tr>
<td>Silica</td>
<td>MSS-5003N</td>
<td>Kobo</td>
<td>3 - 8</td>
<td>45</td>
<td>W/W</td>
</tr>
<tr>
<td>Silica (more porous)</td>
<td>MSS-5003S</td>
<td>Kobo</td>
<td>3</td>
<td>0</td>
<td>W/W</td>
</tr>
<tr>
<td>Titanium dioxide (And) Aluminum Hydroxide (And) Silica</td>
<td>TELL-100-1H10</td>
<td>Kobo</td>
<td>1 - 9</td>
<td>~&gt;90</td>
<td>O/O</td>
</tr>
<tr>
<td>Polystyrene</td>
<td>Microspheres 2505</td>
<td>Microspheres Inc.</td>
<td>2 - 4</td>
<td>&gt;90</td>
<td>O/O</td>
</tr>
<tr>
<td>Polyethylene</td>
<td>Soken XL-130</td>
<td>Soken</td>
<td>1.5</td>
<td>~90</td>
<td>O/O</td>
</tr>
</tbody>
</table>
8.2.2 Effect of particle size

Most literature on Pickering emulsions concerns particles that are 10 nm – 1 µm in size. This is much smaller than the typical size of industrial spherical particles, which range from 1 – 10 µm. We expect that larger particles will:

- bind to the interface with a higher energy, meaning that they bind more stably than smaller particles.
- tend to stabilize larger emulsion droplets better than smaller droplet.
- have more surface area and drag, and hence need higher shear to bring them to the interface.

Furthermore, many industrial formulations require a particular weight percent of particles. Say particle A is larger and particle B is smaller ($R_A > R_B$). For identical particles of different sizes:

$$W_A = W_B \Rightarrow V_A = V_B \Rightarrow \frac{A_A}{A_B} = \frac{R_B}{R_A}$$  \hspace{1cm} (8.1)

where $W_i, V_i$ and $A_i$ denote the total weight, total volume, and total surface areas of powders A and B.

Thus, for the same weight percent, the larger particles can stabilize less interfacial area than the smaller particles. This effect is significant enough to be seen experimentally: 2 wt% of polymethylsilsequioxane particles of two different sizes (∼5 µm vs ∼1 µm) both stabilize W/O emulsions as shown in Figure 8.1. However, the smaller particle emulsified all of the water whereas the larger particle only emulsified half of the water. The smaller particles are more efficient at stabilizing the emulsion droplet interface.

8.2.3 Effect of ionic strength and the particle zeta-potential

Many water-dispersible particles carry a surface charge that prevents the particles from aggregating in water. Though the electrostatic repulsion between particles is helpful for making stable suspensions
Figure 8.1: We homogenized 2% (w/w) particle, 50% oil phase (Cetiol CC/isododecane 1:1 by weight) and 50% water in an IKA Ultra Turrax mixer for 1 minute at 13500 rpm. a) Larger polymethylsilsequioxane particles (Kobo MST-547) formed a W/O emulsion, but only half the water was emulsified. b) Smaller polymethylsilsequioxane particles (Creation Couleurs Creaspheres DIM WL1) also stabilized a W/O emulsion, but all the water was emulsified.

of particles, it can prevent the particles from attaching to an oil-water interface. This is because a particle in the aqueous phase experiences an electrostatic repulsion from the interface due to image charge effects. Also, negatively charged particles are repelled from an oil-water interface because the interface itself carries a negative charge.

Both of these types of electrostatic repulsion can be screened by adding a small amount of salt to increase the ionic strength of the aqueous phase. The amount of salt needed is determined by the charge on the particle surface, which is related to its zeta potential. The zeta potential is the potential on the surface of the particle at the slip plane. A higher zeta potential corresponds to a higher surface charge density.

To investigate the role of electrostatics in Pickering emulsions, we prepared emulsions of water and decane stabilized by 290-nm-diameter, sulfate-modified polystyrene particles (Invitrogen) with and without 100 mM NaCl. We found that such particles were unable to form stable Pickering emulsions in the absence of salt; the decane/water emulsion completely phase separated immediately after homogenization with the Ultra Turrax. In contrast, we found that the same particles formed stable Pickering emulsions upon addition of only 100 mM NaCl (Figure 8.2).
consistent with earlier results\textsuperscript{11}, suggests that particles experience an electrostatic barrier preventing attachment to the droplet interface and that interfacial coverage can be improved through addition of salt. We hypothesize that the slight difference in stability between the two emulsions may be due to differences in the viscosity and density of the two oils.

![Graph showing critical centrifugal acceleration (g) for decane and miglyol with and without NaCl.](Image)

Figure 8.2: Polystyrene microspheres stabilize water/decane and water/miglyol Pickering emulsions. Neither decane (left) nor miglyol (right) are emulsified by 1 % (w/w) 290-nm-diameter polystyrene spheres in the absence of NaCl. With 100 mM NaCl, the same suspensions form very stable Pickering emulsions, with the water/decane emulsion being slightly more stable than the water/miglyol emulsion.

The amount of salt needed depends not only on the zeta potential of the particle, but also on the type of oil used (which changes the image charge and the charge on the interface) and the amount of shear used during emulsion formation. For industrial formulations, we recommend adding at least 25 mM NaCl when there are charged particles in the aqueous phase.

\subsection*{8.2.4 Effect of adding Emulmetik 320}

Many formulations contain Emulmetik 320 to improve the stability and rheology of products. It is a hydrogenated phospholipid complex containing amphiphilic molecules. We made emulsions with and without 1.5 wt\% Emulmetik 320 for a variety of different particles.

For oil-dispersible particles, adding Emulmetik 320 generally did not make better emulsions. For example, the polymethylsilsequioxane particles from Creations Couleurs aggregated in the presence
of Emulmetik 320. When it did improve the emulsion, it changed the type of emulsion from O/W to W/O.

For water-dispersible particles, adding Emulmetik 320 dramatically improved the stability and appearance of the emulsions. Microscopy revealed that the particles do not play a role in stabilizing the emulsions once Emulmetik 320 is added. We were also able to make an O/W emulsion using just Emulmetik 320 by itself, suggesting that Emulmetik 320 dominates emulsion stabilization when it is added to formulations.

8.2.5 Effect of using different oils

We examine three broad classes of oils: esters, silicones, and triglycerides. All of these oils are used in cosmetic formulations. We were particularly interested in understanding the effect of the polarity of the oil, which affects the particle’s affinity for the oil phase and hence the equilibrium contact angle $\theta_E$.

Here, we rank the polarity of some oils from least to most based on their dielectric constants: alkanes, for example isododecane; silicone oils, for example TMF 1.5 and Xiameter; triglycerides, for example Miglyol 812; esters, for example Cetiol B and Cetiol CC.

We made Pickering emulsions with a selection of different particles, with one candidate oil from each of the three categories. Details are listed in Table 8.2. The particles we used are listed in Table 8.3.

Table 8.2: Candidate oils from the categories: ‘silicone’, ‘esters’, ‘triglyceride’. The viscosity $\eta$ and relative permittivity $\epsilon$ were provided by the manufacturers, the surface tension $\sigma$ was measured using a ring tensiometer (KSV Sigma 700), and the density was calculated from masses measured on a balance (Sartorius Genius).

<table>
<thead>
<tr>
<th>Oil phase</th>
<th>$\eta$ (mPas)</th>
<th>$\sigma$ (mN/m)</th>
<th>Density (g/mL)</th>
<th>$\epsilon$</th>
</tr>
</thead>
<tbody>
<tr>
<td>50/50 w/w TMF 1.5/Dimethicone 5cP</td>
<td>4</td>
<td>35</td>
<td>0.88</td>
<td>2.6</td>
</tr>
<tr>
<td>50/50 w/w Isododecane/Cetiol CC</td>
<td>4</td>
<td>33</td>
<td>0.8</td>
<td>$\sim$3</td>
</tr>
<tr>
<td>Miglyol (triglyceride)</td>
<td>30</td>
<td>17</td>
<td>0.95</td>
<td>3.8</td>
</tr>
</tbody>
</table>

We found that for hydrophobic particles, the least polar oil made more stable emulsions (see Figure 8.3). We also found that for hydrophilic particles, the most polar oil made the best emulsions. We
Table 8.3: Both oil-dispersed (top 3) and water-dispersed (bottom 3) were used. The diameter $D$ of the particles was provided by the manufacturers and the equilibrium contact angle $\theta_E$ was measured at a Cetiol CC-water interface.

| Shorthand | Particle type | Particle name (Supplier) | $D$ ($\mu$m) | $\theta_E$
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>MMA$_X$</td>
<td>Methyl Methacrylate Crosspolymer</td>
<td>Covabead LH 70-3 (Sensient)</td>
<td>3</td>
<td>135</td>
</tr>
<tr>
<td>PMSQ</td>
<td>Polymethylsilsequioxane</td>
<td>Creaspheres DIM WL1 (Creations Couleurs)</td>
<td>0.6–1.0</td>
<td>90</td>
</tr>
<tr>
<td>St/DVB</td>
<td>Styrene/Divinylbenzene crosspolymer - squalene</td>
<td>BPB-500 (Kobo)</td>
<td>6</td>
<td>135</td>
</tr>
<tr>
<td>TiO$_2$-Al-Si</td>
<td>Titanium Dioxide (And) Alumina (And) Silica</td>
<td>TiO2 TA-100 (Kobo)</td>
<td>1–2</td>
<td>-</td>
</tr>
<tr>
<td>Si</td>
<td>Silica</td>
<td>MSS-500/3N (Kobo)</td>
<td>3–8</td>
<td>45</td>
</tr>
<tr>
<td>Si-porous</td>
<td>Silica</td>
<td>Mss-500/3 (Kobo)</td>
<td>3–8</td>
<td>0</td>
</tr>
</tbody>
</table>

believe this is because decreasing the polarity of the oil also decreases the contact angle. For hydrophobic particles this reduction is beneficial because the particle contact angle is shifted closer to $90^\circ$. The reverse is true for hydrophilic particles. We therefore recommend using more polar oils when hydrophilic particles dominate (and less polar oils when hydrophobic particles dominate) to maximize the emulsion stability and quantity.

8.3 Conclusions

After examining the emulsions made with a range of cosmetically relevant particles and oils, we propose the following list of design rules for making industrial emulsions:

- use hydrophilic particles to make O/W emulsions, and hydrophobic particles to make W/O emulsions
- if the weight of particle is restricted to a certain percentage, use smaller rather than larger particles
- use salt if the particle is charged to screen out any electrostatic repulsion between the particle and the interface
Figure 8.3: More polar oils made better emulsions when hydrophilic particles were used, whereas less polar oils made better emulsions when hydrophobic particles were used. The fraction of dispersed phase that was emulsified is written in black. The stability of the emulsions to the centrifugation test are written in white. An asterisk (*) indicates that the emulsion contained mm-sized droplets and was very coarse.

- use more polar oils when hydrophilic particles dominate the formulation, and less polar oils when hydrophobic particles dominate the formulation so that the particles have less extreme contact angles
What is [..] asking us to do? What are the consequences of this?

– former Goldman Sachs employee

Conclusions and future work

We used digital holographic microscopy to study the adsorption dynamics of particles to liquid-liquid interfaces with high temporal resolution and spatial precision, and we showed that nanoscopic surface roughness impedes the progress of particles toward equilibrium and causes orientational-translational coupling at the interface when the particles are asymmetric. We also demonstrated that the interaction between particles at an interface depend strongly on the interaction between the particles and the interface, which can only be measured with a sensitive technique such as holographic microscopy. It has been a pleasure researching these topics. Here I present some suggestions that have been inspired by my dabblings in this field.
9.1 Future work

9.1.1 Interparticle interactions

A natural extension of the work on particle relaxation would be to image two or more particles at once as they are breaching the interface, to discover how the slow relaxation affects their interaction. This was attempted one summer by James Callahan, a Harvard College student. However, it was difficult to determine whether the particle positions relative to each other were due to the interface not being flat, or differences in their progress to equilibrium. Of course if the interface is perfectly flat, then any difference in height can be attributed to the particles having different contact angles. But the interface position can change slowly over time, and sometimes the interface is not flat due to imperfections in the pinning lip or slight over- or under-filling the sample cell.

The most promising strategy for doing this experiment is to take holograms while the particles breach. The height at the start of the breach will then define the ‘true’ position of the interface, and the shape of the interface can be determined from these data points. In case the interface height fluctuates over time (due to objective nosepiece drift, for instance), the breaches should be performed in quick succession. This is possible with our current setup but requires moving the field of view. It can be more easily done with a time-shared or holographic optical trap, where the location of the optical trap can be moved. Using larger particles can also be of use, as larger particles will interact more strongly with each other.

For some reason we have never been able to image particles at an interface for longer than a few hundred seconds without seeing some large scale fluctuations. Perhaps these were due to the microscope being on the 5th floor, where it is subject to large vibrations. The holography setup is now in a basement laboratory, so it may be more stable. This will enable better measurements of particle fluctuations, isolated from sample or microscope fluctuations.
9.1.2 **Particles at ‘softer’ interfaces**

The interfacial tension between oil and water is high, 37 mN/m in our glycerol/water-decane system. There are systems with a lower or zero interfacial tension that might be interesting to study:

- Thomas E. Kodger suggested that putting particles in a phase-separated aqueous system (for instance a high- and low-molecular weight polyethylene glycol solution) could eliminate pinning but not the interface itself.

- Proteins and other moieties in biomembranes are critical for cellular function. These membrane proteins are nanometer sized, but studies of larger particles (such as colloidal particles) in phospholipid bilayers might yield insight into membrane dynamics, including how the particle disrupts the properties of the membrane and facilitates formation of structures such as lipid nanotubes\(^{138}\). One can also study how the presence of the membrane affects particle interactions.

9.1.3 **‘Softer’ particles at interfaces**

Another future direction is to track deformable particles as they reach an interface. In principle, we can model shape changes with DDA and obtain information on them with high time resolution.

A visiting PhD student from the University of New South Wales (Australia), Gholamreza Keshavarzi, did look at how microbubbles and droplets behave at an interface. The aim was to understand whether bubble traps in dialysis machines really remove bubbles or whether small daughter bubbles are created when larger ones ‘pop’ at an air-blood interface. Small bubbles or droplets were created by rapidly pushing liquid back and forth through two connected syringes, then loaded into the PEEK sample cells for imaging.

Interestingly, many bubbles stuck to the interface and showed no diffusion at all. It is possible that
this behaviour was due to a contaminated interface, but preliminary investigations involving adsorbed polystyrene particles showed that the interface was still fluid. This project could benefit from having a higher concentration of solid particle tracers at the interface to check for interfacial contamination.

Because HoloPy was not able to model the scattering of air bubbles in water correctly (no non-complex value of $\alpha$ would give the correct fringe contrast), we investigated the breaching of silicone oil droplets at a water-decane interface instead. We found that silicone oil droplets would spread and form a flat lens at the interface. We are still investigating the speed of spreading.

From these preliminary investigations there are several ways forward:

- Minimising interfacial contamination and finding a way to model bubble holograms better will make it possible to understand how bubbles burst.

- A better understanding of the equilibrium droplet shapes could provide a route to fabricate nonspherical particles: to do this, we might let droplets of monomer or uncrosslinked polymer reach an interface, then polymerise the droplets.

- Studying how soft microparticles deform at the interface due to interfacial tension could also be fruitful for understanding the elasticity of the microparticles.

9.1.4 Forcing particles against their will

Our experiments have been passive, so it would be interesting to place an external field onto our samples to exert an additional force on the particles. Once such way would be to use magnetic particles and a magnetic field to drive them. Questions that can be answered using such a technique include:

- How does the external force affect contact-line pinning?

- Is the contact-line really ageing? Ageing has a specific definition, which is not synonymous with slow or logarithmic relaxation. By perturbing the particle and allowing it to relax back,
we can determine whether the relaxation time depends on the amount of time the system has spent being perturbed\textsuperscript{139}.

- One can measure the advancing/receding contact angles of colloidal particles, akin to the measurements made on millimeter-sized particles in Prof. Tony Dinsmore’s group\textsuperscript{140}. The particle is pushed further into the oil or water phase by the external field while a non-index matched interface is imaged with reflection-mode holography. This can reveal what interfacial deformations occur in these experiments and what the advancing and receding contact angles are.

\section*{9.1.5 Untangling rotational and translational motion}

It is evident that asymmetry in the particles gives rise to rotational-translational coupling at the interface. It would be interesting to see whether pinning affects rotational and translation motion at an interface when the particle is a sphere. Using core-shell particle where the core is off-centre (‘eggs’, courtesy the lab of Prof. Gi-Ra Yi) could be ideal for this purpose. However, in preliminary holography experiments we have not been able to detect enough asymmetry in the holograms to discern the orientation. The materials are a TPM ($n \approx 1.5$) shell and a polystyrene ($n \approx 1.6$) core. The refractive index contrast can be increased by calcining the particles so that the shell is silica ($n \approx 1.5$) and the core is air ($n = 1$). Preliminary experiments on calcining the particles resulted in the surfaces of the particles having some debris, possibly due to incomplete combustion of polystyrene.

If we can track the orientation of these particles while maintaining their uniform surface properties, we can answer questions about rotational and translational motion in clusters of particles, and in single particles near hard surfaces and at interfaces.
9.1.6 Other anisotropic particles

We analysed many holograms of Janus particles breaching the interface. Because we obtain identical holograms when the caps are up or down, we were unable to tell which way the particles were oriented. Determining the orientation might reveal how the electrostatics at interfaces orient anisotropic particles prior to their breaching.

One neat result from these experiments was that the azimuthal angle could be determined by looking at the pixel in the hologram with the smallest value (the darkest pixel). We can use the location and intensity of the minimum pixel in the hologram to find the approximate azimuthal angle of a Janus particle to estimate its polar angle.

9.1.7 Elucidating E.coli tumbles

We can now track E.coli with holographic microscopy, albeit with great computational effort. Dr. Rees Garmann suggested that because we can see the tumbles, we can quantify their efficiency and determine what strategies the cells use to optimise their direction of travel. If we obtain a strain with fluorescently-labelled tails, we could image the tails fluorescently and simultaneously image the bacteria holographically to understand the mechanics behind the tumbles.

It would also be interesting to see the bacteria at an interface. Prof. Kathleen Stebe’s group at the University of Pennsylvania is currently researching such systems without holography. Holography could help in understanding how the cells wet the interface and how the interface constrains their motion.

9.1.8 Holography

Holography has been an information-rich imaging technique that I’ve had much pleasure in using. The Manoharan lab is currently finding a way to fit holograms using a Bayesian approach thanks to...
the efforts of Thomas G. Dimiduk and Prof. Vinothan Manoharan. The additional parameter $\alpha$, which is currently in the model, can then be marginalized.

DDA has been a great workhorse for calculating holograms of arbitrary scatterers, but our calculations could benefit greatly from using the parallelised version.

The microscopy setup in the basement lab is now capable of fluorescence and bright-field microscopy as well as dual-wavelength holography at 520 nm and 660 nm. Solomon Barkley is currently refining this dual-wavelength holography system and taking holograms of colloidal particles with both wavelengths. Once we verify that this setup gives reliable fits for both wavelengths, we can use the two wavelengths to provide additional insights into more complex samples such as living cells.

9.2 Final remarks

It’s been a great joy and privilege to use holographic microscopy. It’s allowed me to discover the intricacies of particle-interface systems, and watch *E. coli* run and tumble. Extracting information from how objects scatter light is like being let in on a little secret, and as a researcher there’s no better feeling than that.

**Forever Afters**

Served, as always, for the last.
The tail end of the menu.
The main course’s epitaph.
A pudding knows the meaning of waiting one’s turn in queue.
Patience is what the puddings know best.
And when all face the final test on that day of reckoning, puddings will array their glory down to the smallest gooseberry, for every pudding knows one truth – that the first shall be last and the last shall be first.
Yes, puddings shall have the last laugh When the sweet inherits the tooth.

– John Agard

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References


