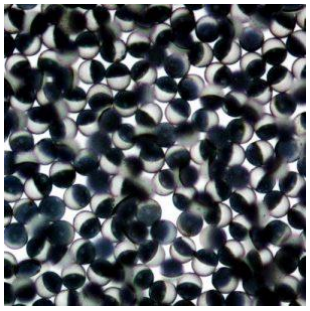
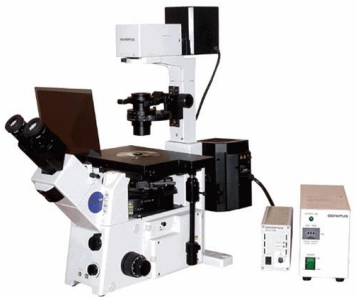
Technische Universität München

An Advanced Lab Course in

Motion Tracking of Janus Microswimmers

Yash Bogawat

Physics of Synthetic Biological Systems (E14)

yash.bogawat@tum.de

Contents

1. Introduction

2. Basics
   2.1. Active Matter and Self Propelling Micro Swimmers
   2.2. Brownian motion
   2.3. Diffusiophoresis
   2.4. Janus Particles
   2.5. Microscopy

3. Experiments
   3.1. Particle tracking
   3.2. Fiji and Matlab analysis

4. Quiz

5. References

1. Introduction

The word *'Janus'* is attributed to having two faces as the word comes from the name of a two-faced Roman god - Janus. These particles are a unique class of micro or nano particles which have two distinctive surfaces that account for different properties, the two surfaces can serve various functions while still being a single particle.

Breaking of symmetry allows for the motion of the Janus particle which can be activated by creating temperature, electrical or chemical gradients, resulting in phoretic phenomena such as thermophoresis, electrophoresis or diffusiophoresis, respectively, which cause them to swim around, i.e., self-propel in the system with higher velocities and cover larger distances.

An example for this would be a silica-gold Janus particle, wherein the silica side could be functionalized with enzymes (like catalase) and the gold side could be modified with DNA. Such a particle can be activated via a substrate for the enzyme present on the silica side while the gold side with the DNA can be used for sensing - and the entire system can be utilized as a self-propelling biosensor.

The motion of these particles can be tracked via microscopy and we can estimate their diffusion coefficients, velocities, mean squared displacements (MSD) and correlation to the stimuli causing the changes. These particles can be used to clean wastewater, deliver drugs and construct sensing systems.

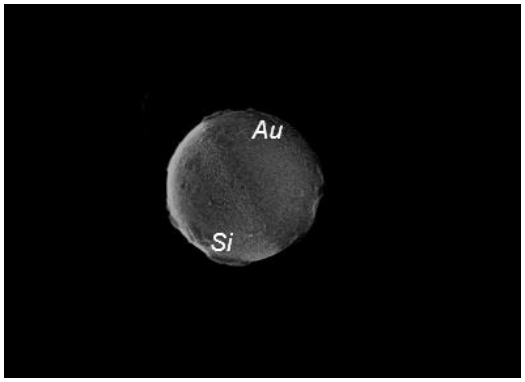


Figure 1 SEM image of an Au-Si Janus particle with a diameter of 1 micron

In this practical class, we will work with silica-platinum Janus particles which can be activated with hydrogen peroxide. We will record movies using an optical microscope (an Olympus IX71 inverted microscope), then process them via FIJI and Matlab. We will observe how the particles transition from Brownian motion to diffusiophoresis upon the addition of hydrogen peroxide and how its concentration affects the particles.

We will use a FIJI plugin called 'Mosaic' and a Matlab code called MSD Analyzer to generate the tracks, MSDs and diffusion coefficients at different concentrations and compare them. This course will be a quick introduction to active matter physics with a hands-on introduction to microscopy followed by some data analysis.

I will deliver a short presentation that will include videos and graphics to introduce you all to the theory and the experiments we have in line.

2. Basics

2.1. Active Matter and Self Propelling Micro Swimmers

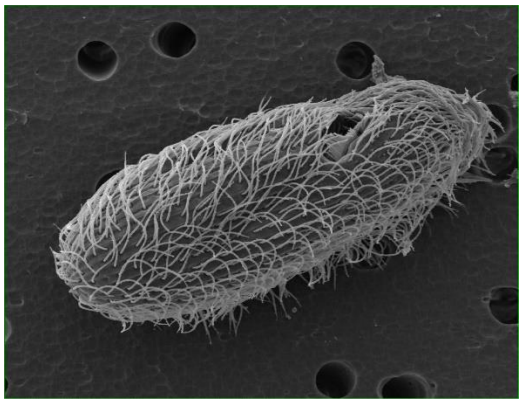
Active matter comprises of agents that produce mechanical motion upon energy consumption - as they consume energy these systems are not in thermal equilibrium. Naturally occurring examples of active matter are bacteria, schools of fish and flocks of birds. In flocks or swarms or schools, the movement of every individual agent is based on the movement of its neighbour and that is how the entire system coordinates and moves together.

Figure 2a. A school of fish

3b. A flock of birds

Inspired by the principles of natural active matter, artificial active matter particles have been created which work in the same way as they harvest energy from the system and convert it into mechanical motion. These particles are self-propelling Janus particles or nanomotors.
Artificial microswimmers are colloids typically sized between 1-10 microns that can propel themselves in liquid media their sizes are pretty comparable to the size of natural microswimmers i.e. bacteria.
Unlike natural microswimmers which move by utilizing their external appendages like flagella or cilia (Check figures 4a and 4b), artificial microswimmers do not have any moving parts, they instead generate propulsive forces via chemical reactions.

3a. JSTOR Daily, How do fish schools work?
3b. Nature, The Physics of Life, Gabriel Popkin



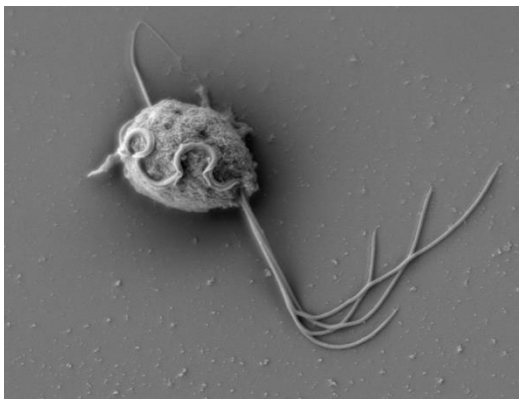


Figure 4a. A freshwater Ciliate *4b. Pentatrichomonas hominis- a flagellate*

2.2. Brownian motion

When microscopic particles are suspended in a liquid they tend to move randomly in the system, the movement is caused due to collisions with fast-moving molecules of the liquid this phenomenon is termed as Brownian motion. These collisions generate forces that are thermal in nature as their amplitudes are proportional to the temperature of the liquid molecules. This motion was extensively studied by a botanist named Robert Brown in 1827 who initially came across it while studying pollen grains, and it was validated by Einstein in 1905. In his theory, Einstein derived an expression to calculate the diffusion coefficient of these Brownian particles and went on to indirectly confirm the existence of molecules and atoms.
The diffusion constant of a spherical particle is given by the well-known Stokes-Einstein relation:

$D= k_bT/(6\pi\eta R)$ (i)

Where,
k_b is the Boltzman's Constant
T is temperature, k_bT is the thermal energy
ŋ is the dynamic viscosity of water
And R is the radius.

4a. Smith College, CMI

4b.Brložnik, Maja, et al. "Pentatrichomonas hominis coinfection in a puppy from a Slovenian animal shelter." Slovenian veterinary research 53 (2016): 229-235

The mean-squared displacement (MSD) of a particle in one dimension in time *t* can be calculated as -

$<x^2> = 2Dt$ (ii)

Quick watch -
https://www.britannica.com/science/Brownian-motion/media/1/81815/203883

A Simulation for Brownian motion -
http://web.mit.edu/8.334/www/grades/projects/projects17/OscarMickelin/brownian.html

2.3. Diffusiophoresis

When an object generates phoretic motion in response to a chemical gradient it is termed as diffusiophoresis and when the microswimmer creates the gradient itself, it is termed as self-diffusiophoresis.

In self-diffusiophoresis, the microswimmer reacts with the solute through asymmetric surface reactions and thus creates an imbalanced concentration of it, i.e., a gradient. When the motion of these micro-swimmers is compared to that of the same particles performing Brownian motion a systematic enhancement of a directional component can be observed. Naturally, their diffusion coefficients and MSDs are also larger than those of particles performing simple Brownian motion, which can be calculated using the equations i and ii.

In figure 5a, the particle with 0% H_2O_2 is performing typical Brownian motion which is random and the trajectory is limited to a short distance, as the concentration of H_2O_2in the subsequent particles increases we see an increasing directionality and the trajectory now spans a larger area, the motion also transcends from being randomly Brownian to being more ordered. These are the kind of trajectories we will generate in our experiments too.

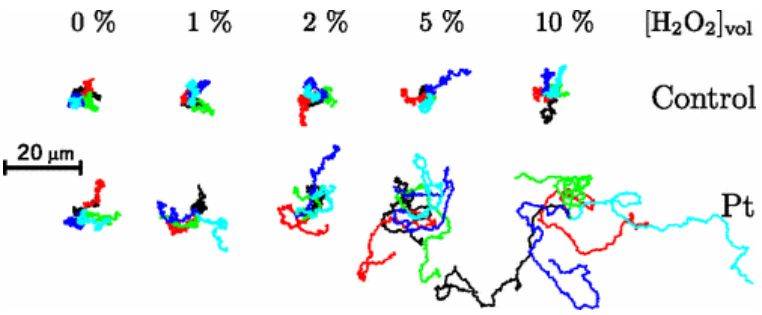


Figure 5a. Trajectories for particles at different concentrations of H_2O_2

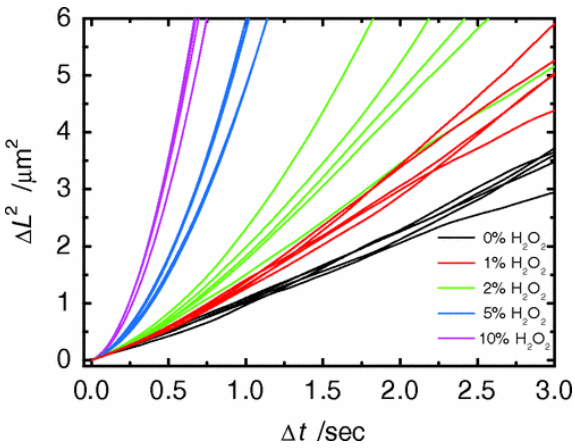


Figure 5b. MSDs plotted as a function of time for different concentrations of H_2O_2

In figure 5b, for 0% H_2O_2 the curve is linear, as the particle displays simple Brownian motion, whereas the other curves show an increasing parabolic shift with an increasing H_2O_2 concentration, implying larger displacement owing to the gradient created by the asymmetric, surface-catalysed reactions.

2.4. Janus Particles

Now that we know what Janus particles are, let's look at how to synthesize them. Si-Pt Janus particles are synthesized using silica particles with a diameter of 1-micron. A monolayer of these particles is prepared on a sterile glass slide, then, we use an E-beam evaporator to deposit firstly, a 3 nm layer of chromium followed by a 10 nm layer of platinum. The chromium layer is deposited to facilitate the adhesion of platinum onto the surface.

Fig 5a and 5b Howse, Jonathan R., et al. "Self-motile colloidal particles: from directed propulsion to random walk." Physical review letters99.4 (2007): 048102.

The particles are harvested by swiping a lens tissue dipped in water across the slide, the tissue is sonicated for 10 minutes causing the trapped particles to be released in solution, the solution then can be centrifuged and the particles can be concentrated to a stock.

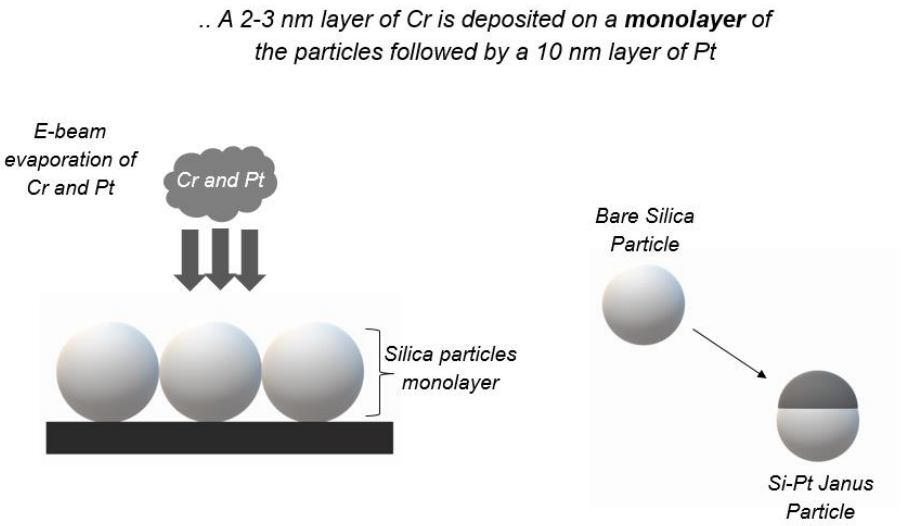


Figure 3 A schematic for the synthesis of Si-Pt Janus Particles

These Janus particles can self-propel upon the addition of hydrogen peroxide, the Pt surface reacts with the H_2O_2 molecules to produce oxygen and water, and the particles propel by performing diffusiophoresis owing to the concentration gradient of hydrogen peroxide.

$$H_2O_2 \xrightarrow{Pt.} H_2O + ½\ O_2$$

2.5. Microscopy

The microscope that we will employ for our experiment will be an Olympus IX71 which is an *inverted* optical microscope. An inverted microscope is different from an *upright* microscope in the sense that-inverted microscopes have the objectives placed below the sample and the light source lays above the sample, as seen in figure 6.

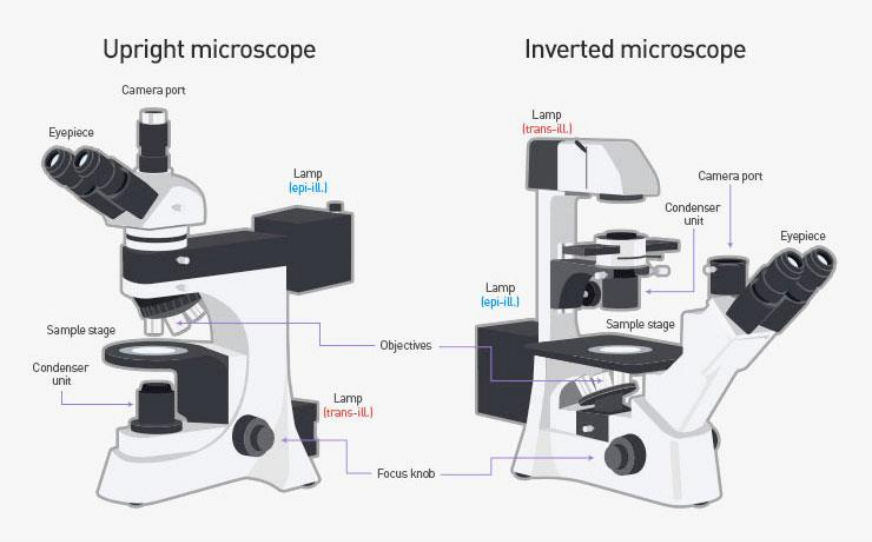


Figure 6. Upright microscope vs an Inverted microscope

The microscope is equipped with 4 objectives with powers of 4X, 10X, 40X and 100X, we will use the 100X oil immersion objective to track the particles.

A few good to know microscopy terms –

i. Magnification power – the degree to which a specimen can be visually enlarged by the microscope. In order to find the total magnification while viewing an image, multiply the power of the objective lens (4X, 10X..) with the power of the eyepiece (usually 10X).

ii. Resolution power – is the ability of the microscope to distinguish between two closely located specimens. It is given by the Abbe diffraction limit

Resolution $r = \lambda/(2NA)$

where λ is the imaging wavelength, NA is the numerical aperture.

iii. Aperture – is a part of the microscope's optical system which regulates the amount of light reaching the specimen.

Fig 6. Epifluorescence Microscope Basics – ThermoFisher Scientific

The numerical aperture value indicates the light acceptance angle, which in turn determines the light-gathering power, the resolving power, and depth of field of the objective. NA = n sin (Θ) n is the refractive index of air i.e. 1, Θ is the half angular aperture.

iv. Working Distance – is the distance between the objective front lens and the top of the cover glass when the specimen is in focus. In most instances, the working distance of an objective decreases as magnification increases.

v. Coarse and fine adjustment – the coarse adjustment allows the user to bring the specimen into general focus, while the fine adjustment allows for fine-tuning and detailing of the specimen.

vi. Objective lenses – one of the most important parts of a microscope, as they are the lenses closest to the specimen. A standard microscope has three, four, or five objective lenses that range in power from 4X to 100X.

Some links –

For online tutorials and glossary

https://www.microscopyu.com/

For an introduction to fluorescence microscopy and imaging basics

https://www.thermofisher.com/de/de/home/life-science/cell-analysis/cell-analysis-learning-center/molecular-probes-school-of-fluorescence.html

3. Experiments

3.1. Particle Tracking

In this experiment, we will first observe the Janus particles and record a movie without hydrogen peroxide and then do the same with 15% hydrogen peroxide, when Pt reacts with the H_2O_2 we will see some bubbles as Pt will split H_2O_2 into water and oxygen. We will also observe a distinctive change in motion and speed. Further, we will record a movie at a 10% concentration followed by another one at 5% concentration and observe the effects of these concentrations on the particles.

Materials –

a. Si-Pt Janus particles stock
b. 30% Hydrogen peroxide stock
c. 2% Hellmanex
d. Ethanol
e. Microwells (can be handmade)
f. Microwell covers
g. Glass slides

Instruments –

a. Plasma cleaner
b. Olympus IX71 Microscope

Protocol –

i. Clean the slides by sonicating them with 2% Hellmannex for 5 minutes followed by sonication with ethanol and finally distilled water.
ii. Dry the slides and plasma etch them using oxygen plasma for 2-3 minutes.
iii. Stick perforated wells on the clean slides.
iv. Sonicate the Janus colloidal solution for 5-7 minutes.
v. Prepare stocks of H_2O_2 at 15%, 10% and 5%.

vi. Turn on the IX71, adjust the illumination and focussing to match experimental requirements. Move to the 100X oil immersion objective.
vii. Load the Janus particles and cover the well with microwell covers to avoid flow and evaporation of the sample.
viii. After focussing and adjusting, record a movie with desired frame rate and length.
ix. In an Eppendorf tube, take 10 ul 15% H_2O_2 and add an equal volume of a diluted Janus particle stock. Let the system settle for a few seconds and then load the sample into another well, look out for bubbles.
x. Record a movie with the same parameters as before, repeat the same for 10% and 5% of H_2O_2.
xi. Remove the slide, discard it safely and clean the objective with ethanol.

3.2. FIJI and Matlab Analysis

In this section of the practical, we will analyse the movies we recorded using FIJI and Matlab. We will need a FIJI plugin called Mosaic and a Matlab code for MSD analysis that I will share with you. In FIJI we will process the movie, track the particles and generate files for MSDs and trajectories. We will then go to Matlab where we will feed the trajectory file generated by Fiji to our code and then generate graphs for the MSD, calculate the diffusion coefficient and compare them together.

Software –

a. FIJI with Mosaic Suite
b. Matlab

Protocol –

i. Adjust the scale for the movie (for the 100X objective 1pixel = 80 nm).
ii. Convert the movie to an 8-bit version, adjust brightness and contrast to suit requirements.

iii. Threshold the movie to generate a binary version. Clear the noise to get rid of any outliers. Crop the movies if necessary using the ROI (Region of interest) manager, make sure all the movies are processed in the same way.
iv. Open the Mosaic plugin listed in the plugin menu (after installing), enter the analysis parameters and run the plugin.
v. The plugin will generate a window with all the trajectories and a menu to generate the files for MSDs and tracks.
vi. Save these files and move over to Matlab.
vii. Add the tracks file generated via FIJI to the Matlab path and run the code.
viii. The code will generate graphs for individual tracks, MSD calculations with standard deviation, mean MSD and a mean fitting curve, it will also read out a value for the diffusion coefficient.
ix. Next, we will construct a file with one representative track from all of the movies and feed this file to Matlab, this will generate comparative analysis graphs and help us compare the movies all at once.

4. Quiz

I. Calculate the diffusion coefficient for a particle with a diameter 1 X 10^{-6} m.
II. How would you differentiate between Brownian motion and diffusiophoresis?
III. What is active matter?
IV. Describe the Matlab graph for the comparative diffusion coefficient.
V. If you could fabricate your own Janus particle, what combinations of materials would you like to use?
VI. Summarize in your own words your understanding of the experiments, include definitions on Brownian motion and diffusiophoresis also add a conclusion.

5. References and Interesting Reads

i. The Physics of Life – Gabriel Popkin
ii. Moran, Jeffrey, and Jonathan Posner. "Microswimmers with no moving parts." *Physics Today* 72.5 (2019): 44-50.
iii. Howse, Jonathan R., et al. "Self-motile colloidal particles: from directed propulsion to random walk." *Physical review letters*99.4 (2007): 048102.
iv. Purcell, Edward M. "Life at low Reynolds number." *American journal of physics* 45.1 (1977): 3-11.
v. Mechanics of motor proteins and the cytoskeleton – Johnathon Howard
vi. microscopyu.com