An Innovative Tracking Technique…in 3D

Aaron Reisman, SAS 2024  Mentors: Arnold J.T.M Mathijssen, Dept. of Physics & Astronomy; Erçag Pinçe, Postdoctoral Fellow

Introduction

Bacteria display unique motility patterns when traveling in complex three-dimensional (3D) environments. Unfortunately, two-dimensional microscopy fails to detail critical features of 3D motile behavior. Today, there exists an easy and effective high-throughput 3D bacterial tracking method applicable in standard phase-contrast microscopy. We can localize bacteria (i.e., E. coli) at a micron-scale resolution by maximizing image cross-correlations amongst their phase rings and a reference library. Ultimately, we can create 3D trajectories of different microorganisms over a specific time span.

Methods

- Bacteria culturing
- Bacterial sample chamber preparation
- Microscopy and data acquisition
- Image cross-correlation
- Reference stack generation
- Tracking algorithm

Progress

- This project is still in its fledgling stages of development, so research with this innovative technique has yet to begin.
- Building the reference library → silica particles of 1 micron in diameter were stuck inside 5% Polyacrylamide gel.
- The colloids at different heights help construct an aggregated set of images that constitute a reference library.
- Once complete, image cross-correlation between reference library frames with known z-distances and out-of-focus diffraction patterns will establish a trajectory from which we can analyze specific motility patterns.

Looking Ahead

1) To solve technical issues on the reference library data. There is a drift along XY on some of the colloidal samples. Also, some images show uneven illumination over the field of view.
2) Begin tracking E. coli as a reference in the motility buffer.
3) Analyze the tracking performance and retrieve physical observables and statistical quantities related to bacterial motility (e.g., instantaneous speed distribution, turning angle distribution, etc).
4) If the data verifies a fully functional tracking, we can start experimenting on the wild-isolates.

Citations


Acknowledgements

This research was supported by a grant for faculty mentoring undergraduate research and sponsored by the University of Pennsylvania’s Center for Undergraduate Research and Fellowships (CURF).

Figure #1: 3D bacterial tracking by comparing out-of-focus diffraction patterns to a reference library (Reference #1)

Image #1: Single colonies of *E. coli* in LB agar (left). Cultured falcon tubes with single colonies and a negative control (right).

Image #2: Phase-contrasted images of swimming *E. coli* with noticeable phase rings