An Innovative Tracking Technique…in 3D

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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.

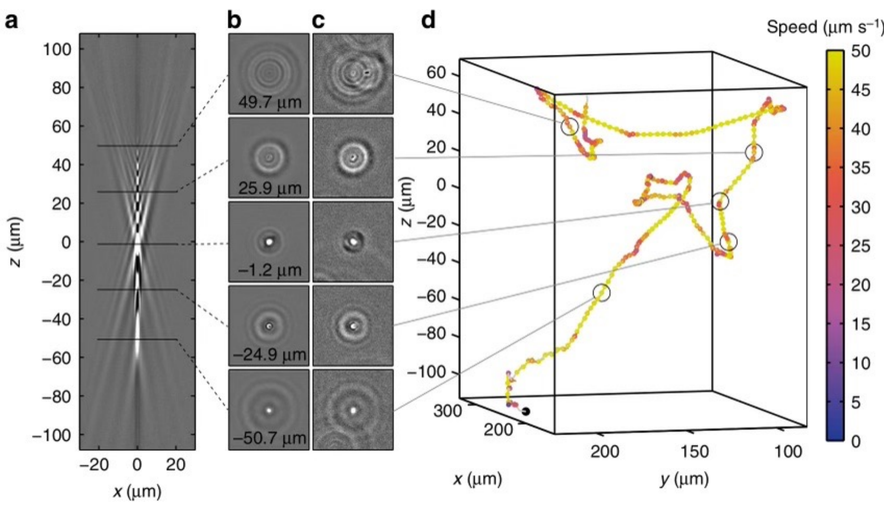

Figure #1: 3D bacterial tracking by comparing out-of-focus diffraction patterns to a reference library (Reference #1)

Citations

Reference #1: Taute, K., Gude, S., Tans, S. *et al.* High-throughput 3D tracking of bacteria on a standard phase contrast microscope. *Nat Commun* **6**, 8776 (2015). https://doi.org/10.1038/ncomms9776

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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

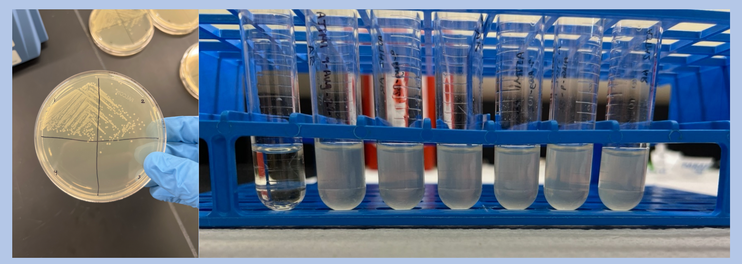

Image #1: Single colonies of *E. coli* in LB agar (left). Cultured falcon tubes with single colonies and a negative control (right).

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.

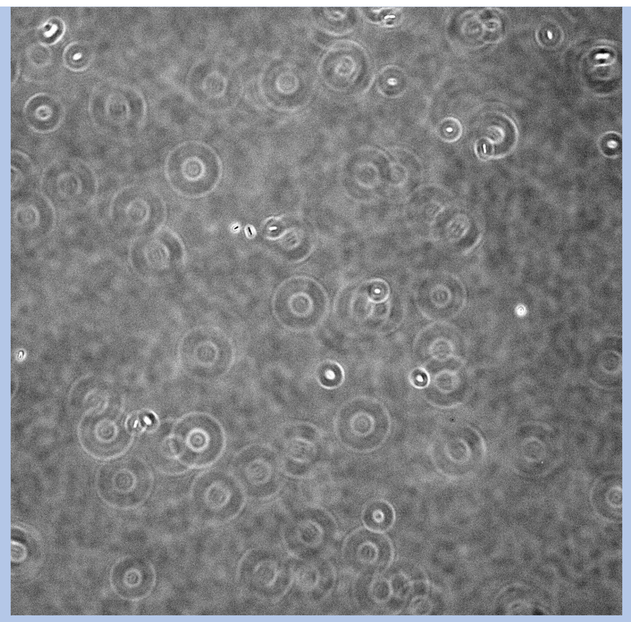

Image #2: Phase-contrasted images of swimming *E. coli* with noticeable phase rings

QR Code: 3D tracking method paper