

ME480 project - Bacterial mechanotaxis: tracking group behaviors

Tentative due date: **Oct 18 at midnight.**

The paper by [Kühn, et al. \(PNAS 2021\)](#) describes how single bacterial cells from the pathogen *Pseudomonas aeruginosa* can use a mechanosensory system to direct its surface-specific twitching motility. The paper described in detail the mechanisms by which single cells take input from their type IV pili to direct the deployment of new pili at the appropriate pole, thereby driving forward motility of individual cells. This process was therefore termed mechanotaxis.

The paper only hinted at a function for mechanotaxis: due to control of reversals upon collisions, the process may help cells coordinate the collective movements of single cells in groups. This was qualitatively observed from microscopy images showing motility of *P. aeruginosa* at high density. However, description of this process was very limited due to the lack of quantitative information extracted from these movies. In this project, your goal consists in analyzing microscopy data of motile cells to measure single cell and collective parameters highlighting the functions of mechanosensing in group behavior.

Format – One part of the report is expected to be written as a *JupyterLab* notebook. In addition, provide the output files of segmentation and tracking (images and tables of coordinates) which will be accompanied by a brief report to explain the key methodologies, analysis steps, results and a final discussion.

Advice – Be fearless and creative. We will never penalize you for trying alternative approaches, and we are open to non-conventional methods.

Data - We provide you with microscopy images of *P. aeruginosa* cells at the interface between an agarose gel and a glass coverslip as in the paper, except this time the cells are left to grow a bit denser. In order to extract useful features of these movies, we must first segment individual cells, then track them.

The data can be accessed on the following switch drive folder:

<https://drive.switch.ch/index.php/s/GhAC3ZzOvAja28F>

password: mechanosensing

Here is a description of the movies:

WT_Dense_mix2pc_PC.tif

- *Pseudomonas aeruginosa* PAO1 $\Delta fliC$
- wild type (WT) deleted in *fliC*. These cells do not produce flagella that are required for swimming
- high cell density (“dense”)
- WT bacteria fluorescently labelled in the cytoplasm are mixed at 2% with non-fluorescent ones (“mix2pc”). The fluorescent cells should behave like the non-fluorescent cells.

WT_Medium_mix10pc_PC.tif

- same strain as WT dense
- medium cell density (“medium”)
- WT bacteria fluorescently labelled in the cytoplasm are mixed at 10% with non-fluorescent ones (“mix10pc”). The fluorescent cells should behave like the non-fluorescent cells.

WT_Dilute_mix10pc_PC.tif

- same strain as WT dense
- lower cell density, mostly single cell twitching (“dilute”)
- ratio fluorescent vs. non-fluorescent bacteria around 10% (“mix10pc”). The fluorescent cells should behave like the non-fluorescent cells.

pilH_Dense_mix10pc_PC.tif

- *Pseudomonas aeruginosa* PAO1 $\Delta fliC \Delta pilH$
- high cell density
- ratio fluorescent vs. non-fluorescent bacteria around 10% (“mix10pc”). The fluorescent cells should behave like the non-fluorescent cells.

pilH_Medium_mix10pc_PC.tif

- same strain as *pilH* dense
- medium cell density
- ratio fluorescent vs. non-fluorescent bacteria around 10% (“mix10pc”). The fluorescent cells should behave like the non-fluorescent cells.

pilH_Dilute_mix10pc_PC.tif

- same strain as *pilH* dense
- lower cell density, mostly single cell twitching
- ratio fluorescent vs. non-fluorescent bacteria around 10% (“mix10pc”). The fluorescent cells should behave like the non-fluorescent cells.

pilGcpdA_Dense_mix2pc_PC.tif

- *Pseudomonas aeruginosa* PAO1 $\Delta fliC \Delta pilG \Delta cpdA$
- high cell density
- ratio fluorescent vs. non-fluorescent bacteria around 2% (“mix2pc”). The fluorescent cells should behave like the non-fluorescent cells.

pilGcpdA_Medium_mix10pc_PC.tif

- same strain as *pilG cpdA* dense
- medium cell density
- ratio fluorescent vs. non-fluorescent bacteria around 10% (“mix10pc”). The fluorescent cells should behave like the non-fluorescent cells.

pilGcpdA_Dilute_mix10pc_PC.tif

- same strain as *pilG cpdA* dense
- lower cell density, mostly single cell twitching
- ratio fluorescent vs. non-fluorescent bacteria around 10% (“mix10pc”). The fluorescent cells should behave like the non-fluorescent cells.

Imaging parameters:

- cells were grown at the agarose-glass interface for 1h30 (“dilute”), 2h10 (“medium”) or 2h55 (“dense”) starting from high cell density
- phase contrast microscopy and epifluorescence
- 5 s frame interval
- 133 μm x 133 μm
- pixel size 0.065 μm

Part A - Segmentation

Goal

Write a code in Python that segments the cells in the microscopy data using the phase contrast images. Segmenting all cells in the movies is your ultimate goal. The output should be a binary movie with the same resolution and frame number as the input movie.

Technical considerations

While you are allowed to code from scratch, we suggest explore existing packages for segmentation of microscopy images, including ones that have been optimized for bacteria, such as Omnipose. Note that Fiji (also known as ImageJ) is the software of choice for getting started with visualizing and processing images. You can use Fiji at any step in the process but it is not a coding language. It is possible to use ImageJ within Python. To save time, you can proceed with these steps separately and explain them in your *JupyterLab* notebook or report.

To optimize your segmentation algorithm, it is best to first focus on a single image, or even a section of a single image. This can save you a lot of computational time and allow you to better inspect results. Explore the performance of your segmentation by optimizing quality, including the ability to segment as many cells as possible in a given image. Apply your segmentation to a full frame of each of the movies and make necessary adjustments.

Part B - Tracking

Goal

The next step is to extract the trajectories of single cells within movies. One software of choice for this task is TrackMate. Ideally, you want to track all cells within each movie. However, this is a difficult task which strongly depends on the nature of the image data. Therefore, we advise you to focus on the fluorescently labelled bacteria to assess the quality of your tracking pipeline. Quantify and comment the performance of the tracking algorithm you choose and show how you made modifications to improve its performance.

The outputs should be in the form of tables (e.g. *.xls file) with coordinates of each cell as a function of time. In addition, provide a graphical representation of the tracks overlapped with the original movies.

Technical considerations:

To achieve this, first apply your segmentation to full movies, since tracking algorithms work best on binary images. Again, we suggest you first work on tracking with regions of interest of your movie for optimization in order to save computational time. You can reduce the number of frames to save time and report how it impacts the tracking performance (e.g. in ImageJ > Image > Stacks > Tools > Reduce).

To assess the quality of your tracking, there will be more or less manual/visual steps including overlapping the trajectories with the movie. For this, we advise you to use TrackScheme in TrackMate.

Part B concerns with tracking individual cells, but there are other ways to explore how objects like cells move in an image. We here simply ask you to apply another method, including PIV or optical flow, to come up with a bacterial “flow field” in the denser parts of the colony. Qualitatively and quantitatively compare the results of these alternative approaches to the tracking methods used before.

Part C – Interpretation of the results

1. For each mutant, extract the relative orientation of single cells to compute a parameter known as the nematic order (see <https://elifesciences.org/articles/72187> or <https://www.pnas.org/doi/10.1073/pnas.2107107118#fig03> as examples). For each movie, graph the nematic order as a function of time. Compare the overall nematic order for the different movies. Is there a difference between these conditions? Can you explain why? Note that this only requires the results of the segmentation.
2. After visualizing the tracks, interpret the differences in speed and trajectories between mutants. To achieve this, quantify parameters which you think will highlight these differences. You can include parameters quantified in the original Kühn *et al.* paper (reversals), but also others (e.g., velocities). However, we would like you to particularly focus your discussion on dense groups of cells. One way to do this is to try to categorize cells based on local density.
3. Conclude by discussing quantitative differences in the motility patterns of the three different strains. Discuss potential enhancement of your methodology and potential additional experiments.