ME480 project - Bacterial mechanotaxis: tracking group behaviors

Tentative due date: Nov. 5 at midnight.

In the paper by Kühn, et al. (PNAS 2021), you learned that 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 is termed mechanotaxis.

The paper only hinted at a function for mechanotaxis: this 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 is to analyze microscopy data of motile cells to measure single and collective motion, and speculate on 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/eg93qfMmgehdURN
password: mechanosensing

Here is a description of the movies:

WT_dense_PC.tif

- *Pseudomonas aeruginosa* PAO1 *ΔfliC*
- wild type (WT) deleted in *fliC*. These cells do not produce flagella that are required for swimming
- high cell density (“dense”)

WT_dilute_PC.tif

- same strain as WT dense
- lower cell density, mostly single cell twitching (“dilute”)

pilH_dense_PC.tif

- *Pseudomonas aeruginosa* PAO1 *ΔfliC ΔpilH*
- high cell density

pilH_dilute_PC.tif

- same strain as pilH dense
- lower cell density, mostly single cell twitching

pilG_dense_PC.tif

- *Pseudomonas aeruginosa* PAO1 *ΔfliC ΔpilG ΔcpdA*

pilG_dilute_PC.tif

- same strain as pilG dense
- lower cell density, mostly single cell twitching

Imaging parameters for "dense" files

- cells were grown at the agarose-glass interface for 2 h starting from high cell density
- phase contrast microscopy (except pilH_dense_mSc.tif)
- 3 s frame interval
- 374 µm x 374 µm
- pixel size 0.1625 µm

Imaging parameters for "dilute" files

- cells were grown at the agarose-glass interface for 2 h starting from low cell density
- phase contrast microscopy
- 1 s frame interval
- 333 µm x 333 µm
- pixel size 0.1625 µm

Additional file for Part B – not to be analyzed in part 1.

pilH_dense_mSc.tif

- same sample and imaging parameters as pilH dense
- spinning disk confocal fluorescence microscopy
- 2% fluorescent cells were mixed in → these cells appear dilute, however, they are mixed into the high density sample and should behave like the non-fluorescent cells
- images were processed to enhance contrast and binarized (several steps including denoising, thresholding, etc.)

Part A - Segmentation

Goal

Write a code (in your language of preference) that segments of the cells in the microscopy data (except pilH_dense_mSc.tif). Segmenting all cells in the movies is your ultimate goal. It is difficult to reach this goal. **Quantify, show and explain your progress towards better segmentation. Comment on the limitations of your segmentation by highlighting in which context it does well or not so well.** 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. 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. 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, quantify and comment the performance of your tracking algorithm, and show how you made modifications to improve its performance.

To help you in the process, we provide you with a movie (pilH_dense_mSc.tif) of a few single cells that have been segmented out of pilH_dense_PC.tif. Apply your tracking algorithm to this data and compare the results with the tracks of the same cells you generated from the entire dataset.

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. The dilute movies were recorded with a higher frame rate (1 s interval instead of 3 s). This can in theory improve tracking, however, it may not be required. You can reduce the number of frames to save time as 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 example in ImageJ.

Bonus question 💰🤑
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 two different mutants. Discuss potential enhancement of your methodology and potential additional experiments.