ME480 project 2 – Deformability cytometry

Due date: November 30th at midnight

In 2015, the group of Prof. Jochen GUCK introduced a technique called Deformability Cytometry (10.1038/nmeth.3281). The basic idea is as follows. A suspension of cells is injected into a narrow microfluidic channel. The flow profile in the channel imposes predictable stresses on the cells that are high enough to deform them. The deformation profile reveals certain mechanical properties of the cells. Compared to alternative techniques, deformability cytometry is quite fast. Thousands of cells could be mechanically loaded within seconds. Thus, the technique allows the study of the mechanics of a whole population of cells dissociated from a tissue or treated with a certain drug. In this project, your goal is to analyze microscopy data recorded during deformability cytometry experiments and propose conclusions based on this analysis. The assignment is divided into 3 parts.

Format. The grade will be based on the report. Do not simply answer the questions; use the questions to organize your analysis into a thought into a coherent story. Discuss your results, notably identified limitations and ways of improvement. The data you provide should be comprehensive, show all the material that you base your conclusions on. Finish your report by putting results in perspective. Do not forget to provide the code used for the analysis in the form of JupyterLab notebook, MATLAB live scripts, imageJ Macros, etc.

Advice. Put some effort into preparing decent figures. Effective communication of results is as important as the quality of the data. Feel free to mention your expectations, deductions and surprises. Spend a bit of time looking at the images before diving into the process. Contemplate on the visual changes that you observe, geometric features that could be quantified, potential challenges on image processing, and how to reduce the processing time. The best way to make progress in image analysis is trial and error. We will not penalize you for trying alternative approaches, and we are open to non-conventional methods. Build your image analysis pipeline brick by brick from file handling and data input, preprocessing (every step to improve the signal to noise ratio), measurement per se, data exportation. This simplifies the distribution of tasks, the debugging steps, the readability of your code, and its modularity.

Description of the datasets and experimental parameters:

All data sets are accessible here: https://drive.switch.ch/index.php/s/OR7wJVykTFNIk6q

Password: RTDC2024

- Flow cytometry parameters:
  - Cell sample flow rate: 4 µL/min
  - Sheath flow rate: 12 µL/min
  - Channel dimensions (length x width x depth): 500x30x30 µm
  - Carier fluid: PBS containing 0.59% w/v of methyl cellulose
- Image acquisition parameters:
  - Camera: Phantom VE 640 L
  - Image type: phase contrast
  - Exposure time: 1µs
  - Frame rate: 10,000 fps
  - Number of frames per movie: 356,953
  - Pixel size: 10x10 µm
  - Magnification: 30x
  - Image dimensions: 256x128 pixels

Part A – Size reduction

This part is to get you familiarized with the raw data and introduce a particular data analysis trick. You are given a movie (CellA_PartA.avi) recorded by a high-speed camera during the experiment. You will see that the size is quite large (11GB).

- How would you process this movie so that images that do not contribute to the deformability cytometry analysis could be removed? How much size reduction do you expect as a result?
- Write a script that automatically removes redundant frames and generates a new movie with only relevant images. How many frames does the new movie have?

Part B – Data analysis

Imagine that you are a research assistant working on image analysis of deformability cytometry experiments at an organoid laboratory. One of your colleagues want to analyze the composition of her gut organoids after a few days of culture, and in particular identify the relative proportion of different cell types in the organoids. To this end, she dissociates chemically her organoids, filter the resulting solution from cell debris and extracellular matrix, and sort the cell population into 3 subpopulations using a fluorescence-activated cell sorting (FACS) machine. Each population of cells are run in a deformability chip and the automated pipelines output the 3 CSV files (CellPopulationA.csv, CellPopulationB.csv, and CellPopulationC.csv) containing the measured properties. The pipeline uses the measurement function of FIJI to measure the different values of each segmented cells and generate the CSV files (distance units are in pixels). You both collaborate together to analyze the data.

- Why would one want to use deformability cytometry specifically to solve this problem?
- Using the CSV files, can you derive a pipeline to distinguish the different cell types from a mix population? What classifier would you suggest and what parameters? How reliable the discrimination between the populations would be?
- Bonus questions:
    - If you were to repeat the experiment, what would you do?
    - Could we use a RTDC based method to sort the cells instead of using FACS?

Part C – Cells segmentation and measurement

A collaborating team reached out the lab to ask if you can help them study the effect of a particular gene, the gene ME480, on the population of cancer cell they are interested in. They provide you with 3 cell populations, one endogenously expressing the gene ME480 (native cells), one overexpressing it (OE cells), one with the gene knocked down (KD cells). A postdoc in the lab performed the experiments and provide you with the movies (Native_cells.avi, OE_cells.avi, and KD_cells.avi) where he deleted the images not containing cells.

- Write a code (in your language of preference) that segments the cells. You need to 1) segment all cells and 2) segment them properly. The output should be a binary movie with the same resolution and frame number as the input movie.
- Process the segmented images to measure cell deformation. The output should be a table identifying each cell, the corresponding frame number, and the amount of deformation.
- Can you effectively distinguish the different cell populations from one another? Present the data in the best way possible to highlight trends.
- What did you learn about the effect of gene ME480 on cell mechanics?

- Comment on the capabilities and limitations of your algorithms for segmentation and feature extraction by highlighting in which context they do perform well and not so well.
- Can you extract other features of cells from the data to allow further classification? If you extract more than 2 features, how can you combine them to improve population separation? Which features are more relevant?
- How can you improve the runtime of your code?
- Can you calculate the actual Young's modulus of the cells? What do you observe?

Bibliography on deformability cytometry

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