ME480 project 2 – Deformability cytometry

Due date: December 31st at midnight

The group of Prof. Jochen GUCK introduced a technique called Deformability Cytometry (check the references provided at the end). The basic idea is as follow. 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, with questions to guide you through the process.

Format. The grade will be based on the report. You are expected to organize the report as a research article using the provided Latex template (present in TemplateReportME480.zip). Do not simply answer the questions; use the questions to organize your thought into a coherent story. The data you provide should be comprehensive, show all the material that you base your conclusions on. 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. Remember that the introduction and conclusion sections will be graded as well. Feel free to mention your expectations, deductions and surprises. The bibliography provides some resources on scientific writing that may help you for with this assignment and beyond. 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.

As a final advice, start working on the project before November 22nd. You will have the opportunity to ask questions about the assignment during the class.

Description of the datasets and experimental parameters:

All data sets are accessible here : https://drive.switch.ch/index.php/s/XRdZv2UkKTZUHkM
Password : M1CROBS

- Flow cytometry parameters:
  - Cell sample flow rate: 4 µL/min
  - Sheath flow rate: 12 µL/min
  - Channel dimensions (length x width x depth): 300x30x30 µm
- 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_noDoxycycline.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 research assistants working at a mechanobiology laboratory. One of your colleagues used deformability cytometry technique to study the effects of the expression of a gene, ME480, that she believes has an impact on the mechanics of cells. To this end, she chose to work with a particular cell type, cell line A, that endogenously expresses ME480. As a first test, your colleague transfected the cells with a gene that, once activated, will produce a protein that silences the expression of ME480. This transfected cell line is labelled CellA_ShME480. The expression of the regulator gene is activated via exposure to doxycycline. She exposed the CellA_ShME480 cells to doxycycline for 6 days prior to the deformability cytometry measurements. The data is given in “Dataset_PartB.xlsx”.

The duration displayed in each table corresponds to the time passed from the detachment of the cells from the petri dish to the image acquisition. She did the segmentation and feature extraction properly. She is puzzled with this data. We would like to figure out why.

- What do you notice when comparing the data recorded at different time points?
- What would be the implications of these results for the general use of deformability cytometry and pharmacological tests?

Part C – Cells segmentation and measurement

We would like to study further the impact of ME480. This time, there is a second cell type, cell line B, that does not endogenously express ME480. The experiment involves four different conditions:

- cellA_ShME480 (CellA_ShME480.avi): the same cell line that was used in part B.
- CellA_GFP (CellA_GFP.avi): cellA cells are transfected with a gene that produces a green fluorescent protein when the cells are exposed to doxycycline.
- CellB_ME480 (CellB_ME480.avi): cellB cells that are transfected with ME480 gene which gets expressed when the cells are exposed to doxycycline.
- CellB_GFP (CellB_GFP.avi): cellB cells are transfected with a gene that produces a green fluorescent protein when the cells are exposed to doxycycline.

All cell lines were exposed to doxycycline for 6 days before the experiment. They are sequentially characterized in the same deformability cytometry chip while ensuring that cells do not remain in the chip between conditions. The movies were recorded as soon as the cells were harvested, which corresponds to 15 minutes after detachment. The movies were processed to remove redundant frames (see Part A).

- 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?

Bibliography on deformability cytometry (not exhaustive):

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Suggested read:

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