Homework

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Establish a dose-response curve between nuclear Ki-67 expression and FBS concentration in the medium

In another experiment performed in your lab, you ran a dose-response assay by growing U2OS cells in the presence of different concentrations of Fetal Bovine Serum (FBS). You labelled **Cell nuclei** with Hoechst and endogeneous **Ki-67** (through indirect immunofluorescence) with Alexa Fluor 647. Ki-67 is known to localize specifically in the cell nucleus.

The concentration of nuclear Ki-67 is expected to vary as the concentration of FBS is increased, and to reach a plateau at FBS concentrations that are good for cell viability.

Using a 96-well plate, you grew your U2OS cells in 5 replicates (rows C through G) of 10 different FBS concentrations (0.0, 0.5, 1.0, 3.0, 5.0, 10.0, 15.0, and 20.0%) as depicted in the scheme below. You used the **Testing** row B to optimize the acquisition protocol, but you did not save the images. Hence, your final `.ND2` file should contain exactly $5x8 = 40$ acquisitions.

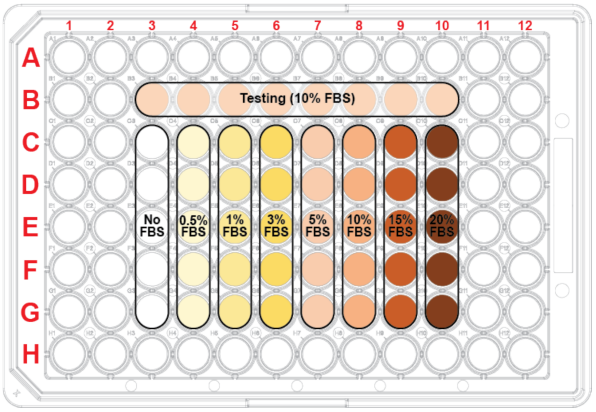

Figure 1: Acquisition scheme (testing wells were not imaged)

Please note that, in contrast to the file we used in the hands-on session, this time you acquired all rows from left to right.

Your tasks

You are expected to work in pairs (one trio is allowed).

- Download the `.nd2` file to work with from https://ia-res.ethz.ch/pc2023/U2OS.zip.
- In a first **Jupyter notebook**, write code to process all wells sequentially and **i)** segment the nuclei from the Hoechst channel, and **ii)** extract the mean Ki-67 signal over all segmented nuclei (*i.e.*, the mean intensity of each nucleus). Write the resulting dataframe to a `.csv` file. (Remember: Ki-67 localizes only in the nucleus.)
- In a separate **Jupyter notebook**, import the data from the `.csv` file and plot the intensities averaged over the 5 replicates with their standard errors of the mean.
- How would you describe the trend of the Ki-67 concentration? Does it plateau somewhere as expected, or does it keep growing/shrinking?
- Think of which model best fits your Ki-67 *vs.* FBS data. The `iaf.fit.models` package (https://ia-res.ethz.ch/docs/iaf/fit/models/index.html) implements 4 models that you can use. You can also have a look at the table at the beginning of chapter 7 of `Python.pdf` for guidance.
- Fit your selected model using the standard errors of the mean as weights.
- Plot the predictions `y_hat` along with your data and show the model (and its optimal parameters) in the legend.

- Based on the data and your model, do you think it is legitimate to say that 10% FBS is a good concentration for growing U20S cells?

Your submission

Please upload a **zip archive** with your **family names as part of the file name** to https://u.ethz.ch/5KC0z containing the following files:

- a **Jupyter notebook** with the code that extracts the relevant numeric data from the `.ND2` file,
- the resulting dataframe as a `.csv` file,
- the **Jupyter notebook** with the analysis of the `.csv` file,
- the answers to the questions above (they can be part of the analysis notebook).

Deadline: In the past, it has been rare for students to submit a flawless solution on their first attempt. Therefore, you may be required to resubmit your work for corrections or completion, possibly more than once. **To successfully complete the course, your final solution must be approved no later than Friday, October 27th.** I strongly encourage early submissions!

Have fun!