02-261 – Quantitative Cell and Molecular Biology Lab

High Content Screening

In microscopy experiments, we cannot simply rely on the appearance of images to make strong conclusions. We require computational methods to quantify the observed phenomena. In some situations, a single measurement is not sufficient to capture the variability of an observation. In this case, researchers may use machine learning methods to identify experimental outcomes. To do so, one must calculate features which are numerical descriptors of the images. Then a model can be learned to predict the labels of images based on the values of these features. In this Lab, you will, capture images of cells to enable machine learning based analyses.

In **High Content Screening (HCS)**, a technology used to collect and analyze large amounts of biological data from cells, the terms **plates**, **wells**, and **fields** refer to different levels of sample organization and imaging.

1. Plates

- **Plates** refer to the physical containers used to hold the biological samples during an experiment. Plates are usually made of plastic and have many small compartments (wells) arranged in a grid pattern.
- Each plate can hold multiple samples, making it an efficient way to conduct large-scale experiments.

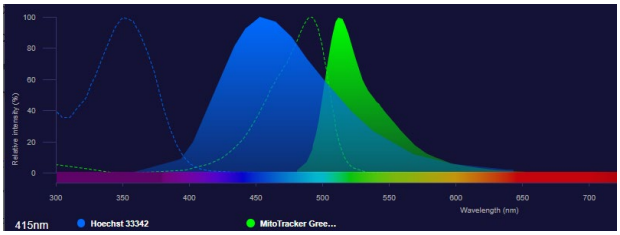
2. Wells

- **Wells** are the individual compartments within a plate. Each well holds a small volume of liquid, usually containing cells, drug compounds, or other reagents.
- In a 96-well plate, for example, there are 96 wells, arranged in rows and columns. Each well is like a small, isolated environment where an experiment can be carried out independently. Wells are identified by their row and column (A1, B2, etc.)
- Different treatments or conditions can be applied to different wells, allowing for comparisons across many samples in a single experiment.

3. Fields

- **Fields** refer to the specific areas of a well that are imaged or analyzed using a microscope. Since wells are often larger than the microscope's field of view, multiple **fields** may be captured from a single well to cover different areas within it.
- When imaging a single field, a separate image is taken for each channel. Each field is labeled numerically within a single well.

Fluorescent Stains:



The absorbance and emission spectra for our stains is shown above. They are clearly separable and can be viewed independently with an appropriate set of filters.

1. Hoechst

- **Function**: Hoechst dyes are fluorescent stains used to label DNA in cells, specifically binding to the minor groove of double-stranded DNA, particularly rich in adenine-thymine (A-T) base pairs.
- **Application**: These dyes are widely used to stain cell nuclei, allowing visualization of the nucleus and DNA in live or fixed cells under a fluorescence microscope.

2. MitoTracker Green FM

- **Function**: MitoTracker Green FM is a fluorescent dye that selectively labels mitochondria in live cells. Unlike some other mitochondrial dyes, it does not depend on mitochondrial membrane potential, so it can label both healthy and damaged mitochondria.
- **Application**: It is primarily used to visualize and track mitochondria, enabling researchers to study their structure, distribution, and function within cells during live-cell imaging.

Protocol:

1. Pick a column of the plate you wish to use for imaging. Four will be for controls (untreated) and four will be for the treatment.
2. Add 25 uL of FCCP to four treatment wells you intend to image. Add 25 uL of PBS to the four wells you intend to image for controls.
3. Incubate for 30 minutes.
4. Remove media and/or FCCP treatment and replace with Mitotracker Green FM and Hoechst stain.
5. Incubate 30 minutes.
6. Remove dye solution and replace with PBS and image. Take images in the blue channel, green channel and DIC channel. Take as many image sets as possible.
7. After capturing your images, write the file names and field information in the shared data spreadsheet. Submit only your images in a zip file to Canvas.

Important advice:

- Use 20x magnification.
- Initially you should adjust your brightness in each channel such that the brightest wells do not overexpose pixels. Once set, **do not adjust your brightness** for the remainder of the experiment.
- For each field, capture an image in each channel without adjusting focus or XY position.
- Try not to allow fields to overlap one another.

Potentially Useful information:

Hoechst 33342

Mitotracker Green FM

FCCP

Mitochondrial depolarization with FCCP