UNIVERSITÄTS-
KINDERSPITAL
ZÜRICH

Pachlopnik Lab.

INTRODUCTION TO IMAGE ANALYSIS

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OBJECTIVES

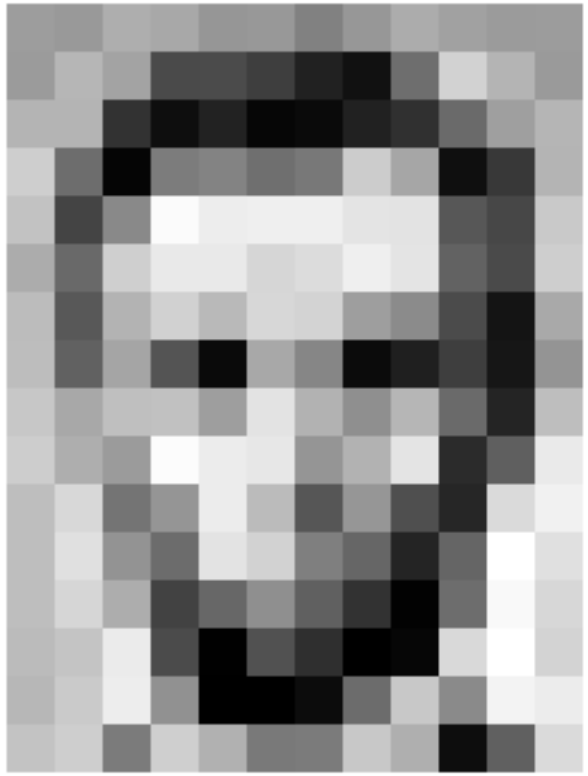
At the end of today, you will be able to:

1. Understand the use-case for image analysis
2. Use different Python-based tools to process your images
3. Process single cell measurements
4. Create plots and scaled images of your data using Python and FIJI

SCHEDULE

- **09:00 – 09:30** Introduction to Image Analysis
- **09:30 – 12:00** Image processing and data preparation
- **12:00 – 13:00** Lunch
- **13:00 – 15:00** Data Analysis
- **15:00 – 15:30** Lecture on Degranulation Assays
- **15:30 – 17:00** Finishing up, or if done: early weekend!

MOTIVATION



157	153	174	168	150	152	129	151	172	161	155	156
155	182	163	74	75	62	33	17	110	210	180	154
180	180	50	14	34	6	10	33	48	106	159	181
206	109	5	124	131	111	120	204	166	15	56	180
194	68	137	251	237	239	239	228	227	87	71	201
172	105	207	233	233	214	220	239	228	98	74	206
188	88	179	209	185	215	211	158	139	75	20	169
189	97	165	84	10	168	134	11	31	62	22	148
199	168	191	193	158	227	178	143	182	106	36	190
205	174	155	252	236	231	149	178	228	43	95	234
190	216	116	149	236	187	86	150	79	38	218	241
190	224	147	108	227	210	127	102	36	101	255	224
190	214	173	66	103	143	96	50	2	109	249	215
187	196	235	75	1	81	47	0	6	217	255	211
183	202	237	145	0	0	12	108	200	138	243	236
195	206	123	207	177	121	123	200	175	13	96	218

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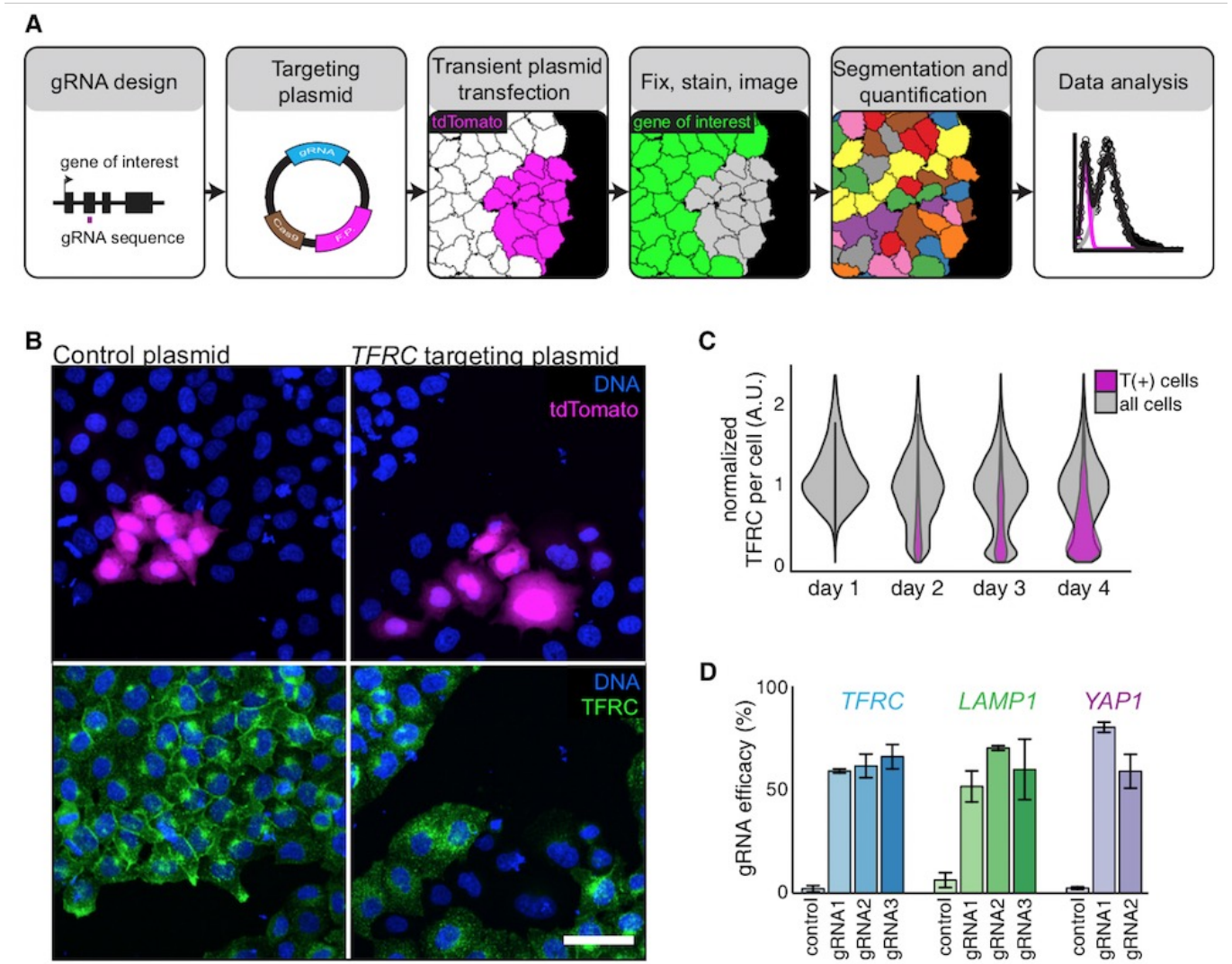
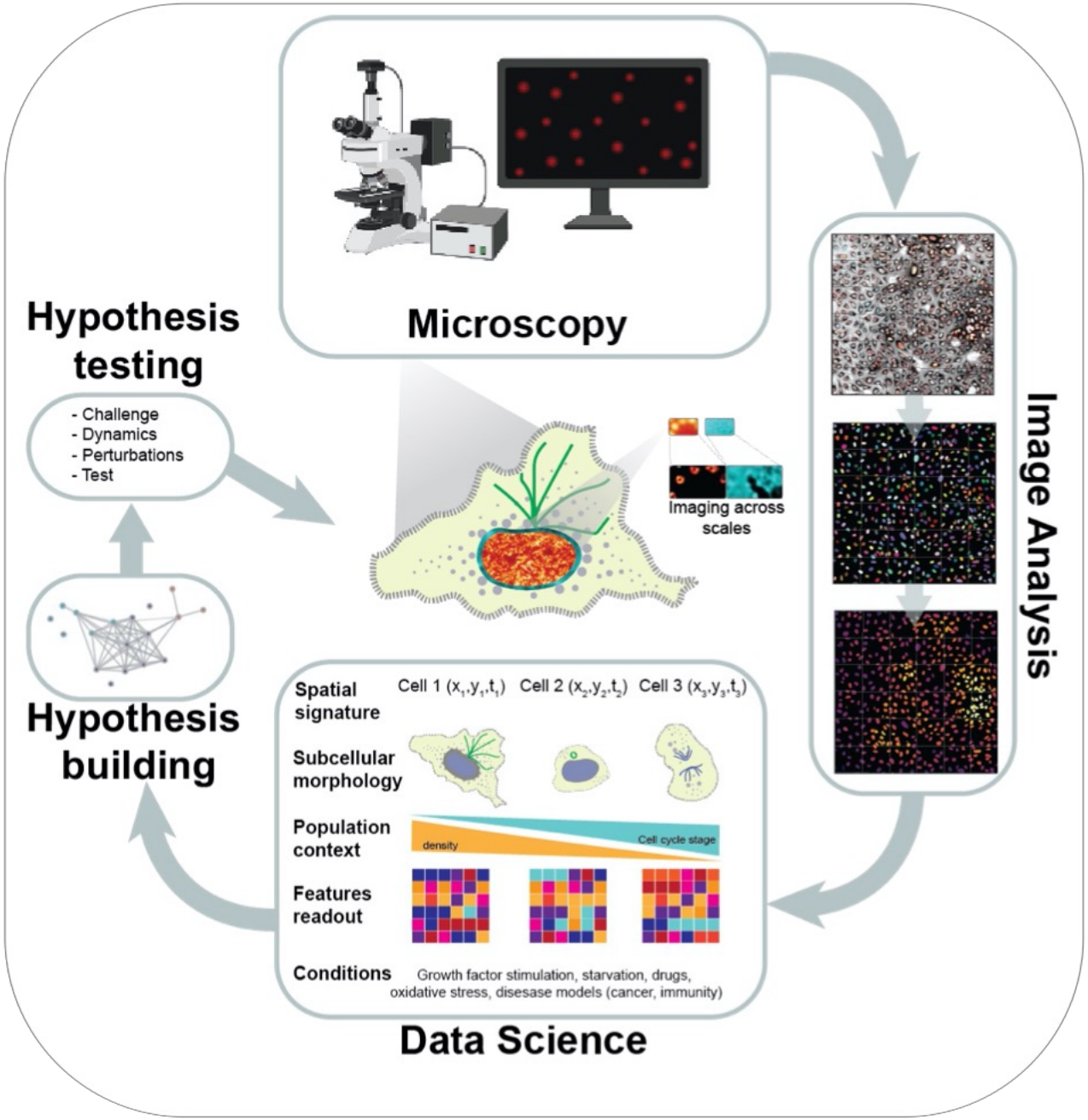
An image is essentially a matrix of pixel values. We can use each of these pixels as a data point.

MOTIVATION



De Groot et al. (2018)

WORKFLOW


Microscopy
Image Analysis
Hypothesis testing
- Challenge
- Dynamics
- Perturbations
- Test
Imaging across scales
Hypothesis building
Spatial signature
Cell 1 (x1,y1,t1)
Cell 2 (x2,y2,t2)
Cell 3 (x3,y3,t3)
Subcellular morphology
Population context
density
Cell cycle stage
Features readout
Conditions
Growth factor stimulation, starvation, drugs, oxidative stress, disesase models (cancer, immunity)
Data Science

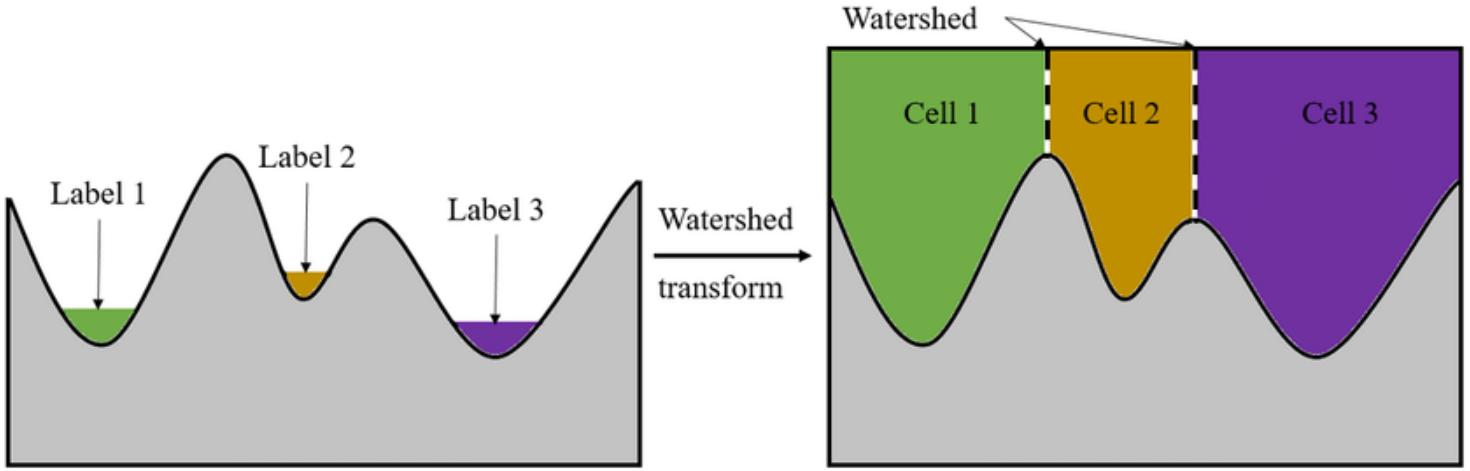
SEGMENTATION

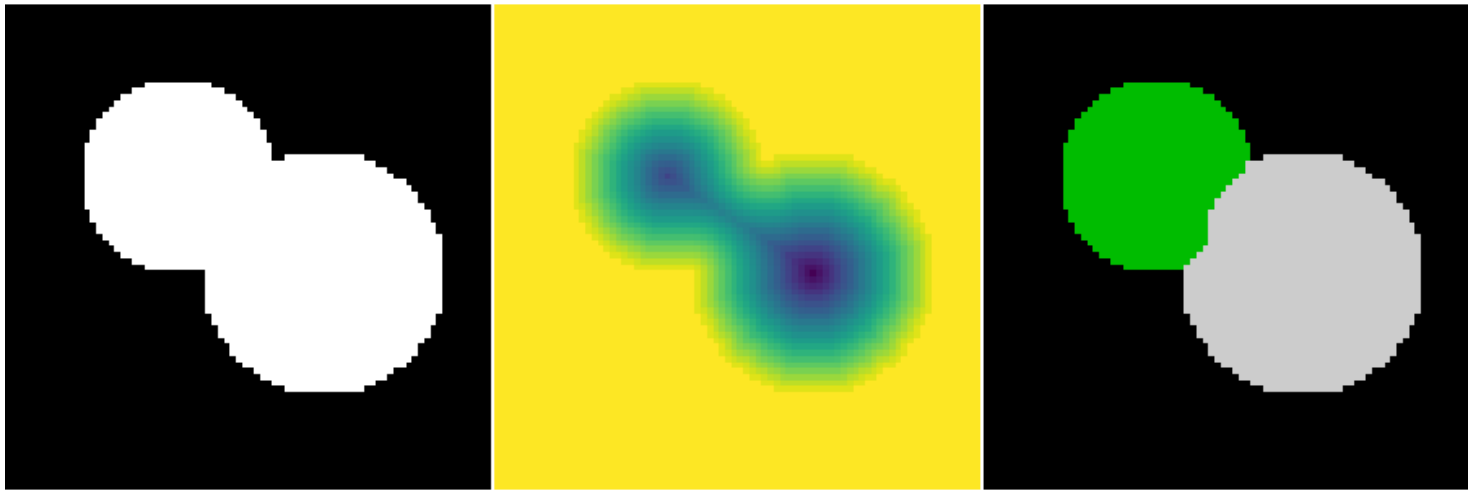
After segmentation we have:

1) An image with **signal**
2) A mask image with **unique labels** for each cell

So now we can see that the **signal at coordinates (X,Y)** corresponds to a **label at (X,Y)** and assign this measurement to that cell! Et voilà, we have a way of extracting data from our image.

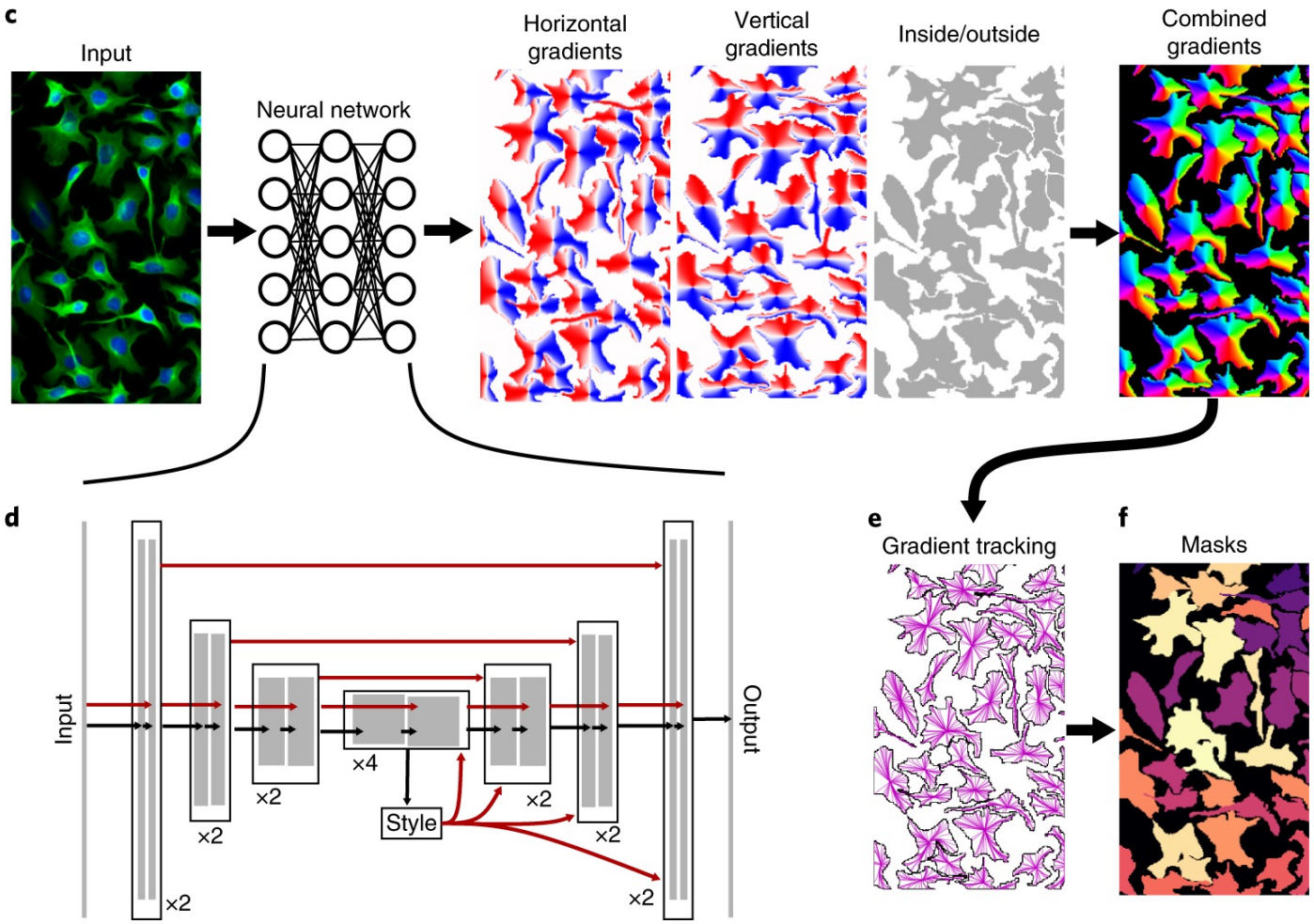
SEGMENTATION





A simple watershed segmentation in theory (above) and in praxis (below).

SEGMENTATION



Cellpose, a neural network based segmentation algorithm by Stringer et al. (2021).

SEGMENTATION

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MOTIVATION

- “What you see is what you get”
- Lots of data (1 pixel = 1 measurement), lots of statistical power
- Images offer high resolution in space -> Single-cell resolution, environment context
- With multiplexing, we can measure information on multiple RNAs and/or proteins for single cells

TODAY'S WORKFLOW

1. Setup
2. Select nice images from your experiment
3. Nuclear segmentation (DAPI)
4. Cell outline segmentation (CellMask)
5. Measurements (feature extraction)
6. Data Analysis

SETUP

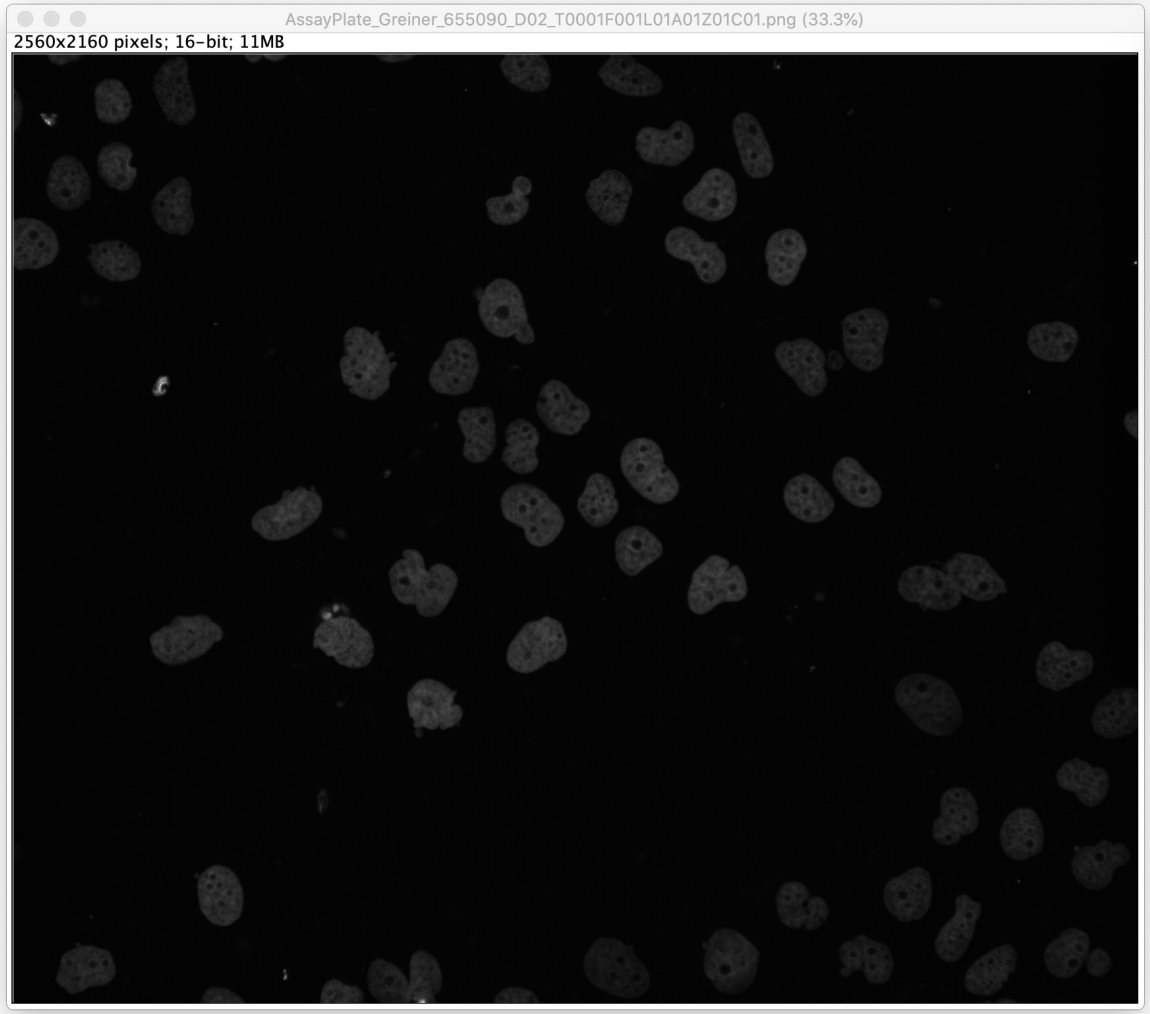
1. **Install FIJI / ImageJ from https://imagej.net/software/fiji/**
2. **Go to https://github.com/sevwal/BME362_image_analysis and open the Google Colab Notebook**

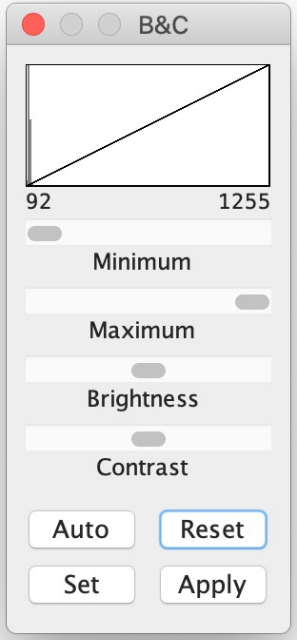
SETUP: FIJI

Simply drag & drop any files onto this bar to open

Various draw and measure tools

Zoom and Move





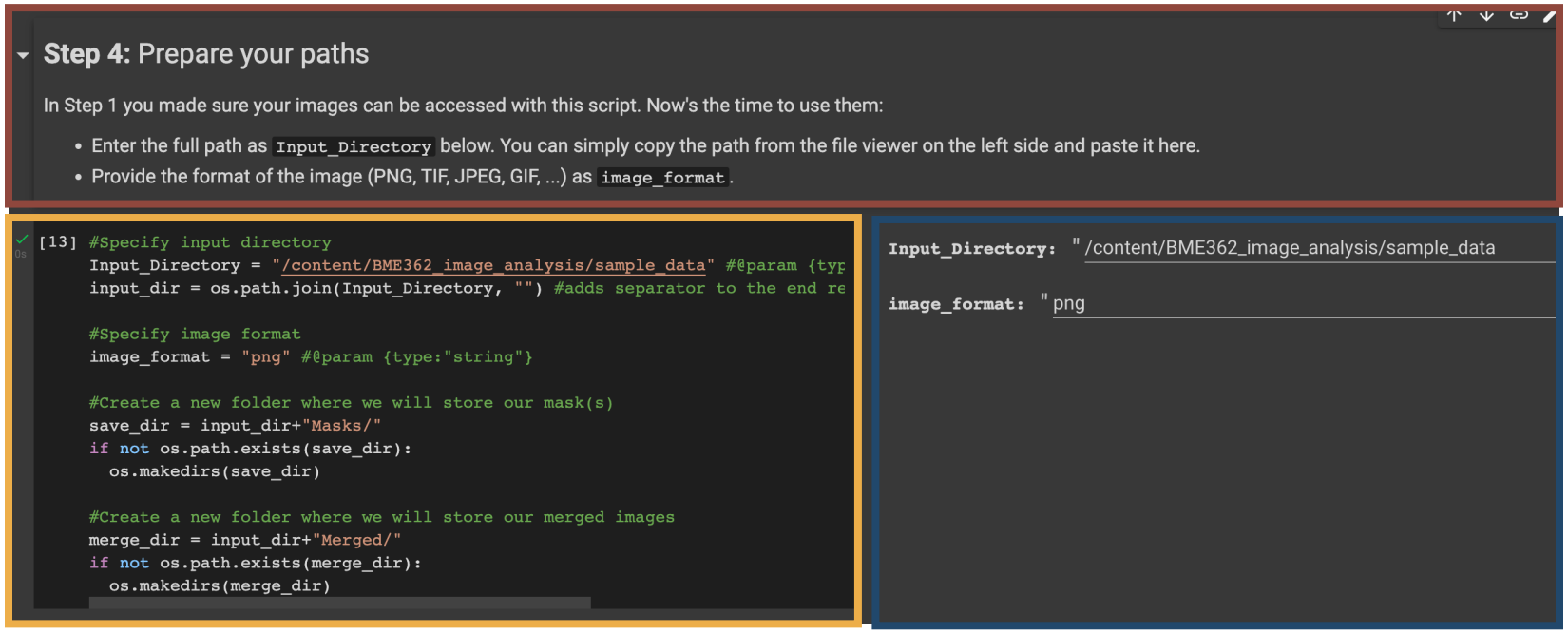
Adjust Brightness and Contrast
(`Image > Adjust > B&C`)

SETUP: FIJI

- Don't overwrite your original images! Create Duplicates using `Image > Duplicate`
- If you rescale your image using `Brightness & Contrast`, you need to save it by clicking `Apply`.
- To create nice figures, you can also add a Scale Bar using `Analyze > Set Scale` and then going to `Analyze > Tools > Scale Bar` (don't bother with the scale unit, pixels is fine for now)

SETUP: COLAB

- A convenient way of interacting with Python is through the Jupyter notebooks. In this course, we will use **Google Colab** to run such a notebook.
- Notebooks are made of "cells", which come in two flavors:
  - `Documentation cells`, containing text formatted according to the Markdown conventions;
  - `Code cells`, containing Python code
  - `Form cells`, containing input options for you (dropdown, checkboxes, ...)



SETUP: COLAB

BME_362_Image_Analysis.ipynb

File Edit View Insert Runtime Tools Help Cannot save changes

Share

Files

..
BME362_image_analysis
drive
sample_data
cyto_segmentation_overview.png

+ Code + Text Copy to Drive

RAM
Disk

Editing

BME362: Image Processing

We will be running a relatively simple image processing pipeline. As I'm not aware of your skill levels in coding, this script is meant to be fancy enough to excite you but simple enough to follow through easily - at least I hope so. Of course, I will be here to help you throughout the course should you have any questions!

We will make use of the open source functionalities of Python. One powerful aspect of Python is the easy install of the abundant packages and dependencies that suit your needs. And of course: the large community that codes all of this and makes it accessible to you for free :D We will mainly make use of the following packages:

- `OpenCV` (Open Source Computer Vision Library) is a tool for computer vision and machine learning. It provides us with some powerful algorithms to process our images.
- `Cellpose` is a tool for image segmentation of cells and nuclei. It provides us with the ability to train a neural network for image segmentation. Don't worry, we won't go that deep in this short training and will make use of the pre-trained models cellpose provides to us. For more information, check out their paper.
- `scikit-image` an open-source image processing library for Python.

Remarks: This script was inspired by the documentation on Cellpose and their example notebooks, workflows developed by Joel Lüthi (Twitter, Github), as well as the notebook Running cellpose 2.0 in colab with a GPU.

Preliminaries

[ ] ↳ 16 cells hidden

SETUP: COLAB

- To run code in your notebook:
  - Type your code in a cell
  - Press the `play button` at the top left of the cell
  - Press `Ctrl+Enter/Cmd+Enter` to evaluate the cell
  - Press `Ctrl+Enter/Cmd+Enter` to evaluate the current line or selection
  - When the Python kernel has done computing, the result appears below the code cell
  - The line where an error occurs will be marked by a red bar on the right side of your code.
  - If everything goes well, a green tick mark will appear in the top left of the cell.

NOW IT'S YOUR TURN!