Das Deutsche Zentrum für
Neurodegenerative Erkrankungen

Introduction to Bioimage analysis:
Retreat 2022 Tuebingen (Constance)

31/05/2022

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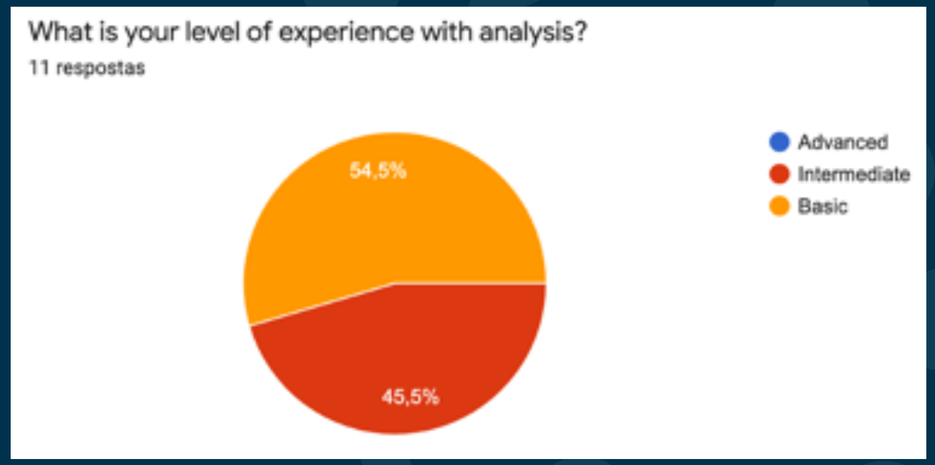
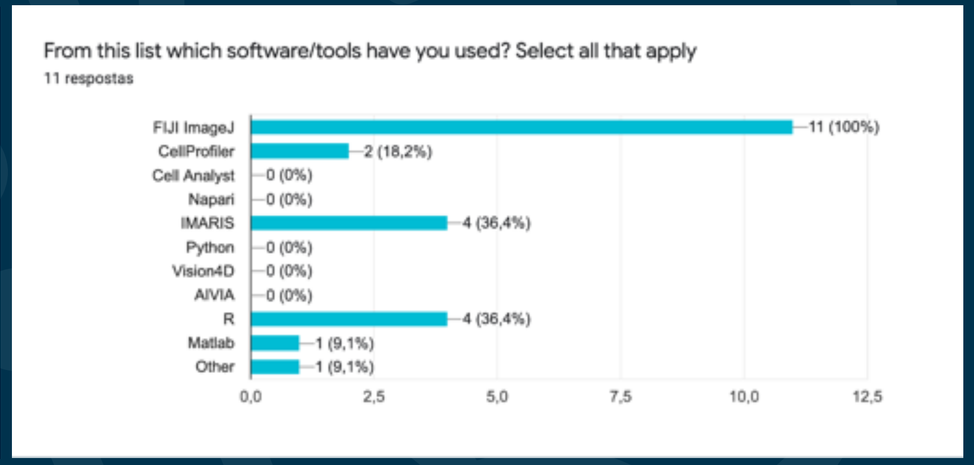
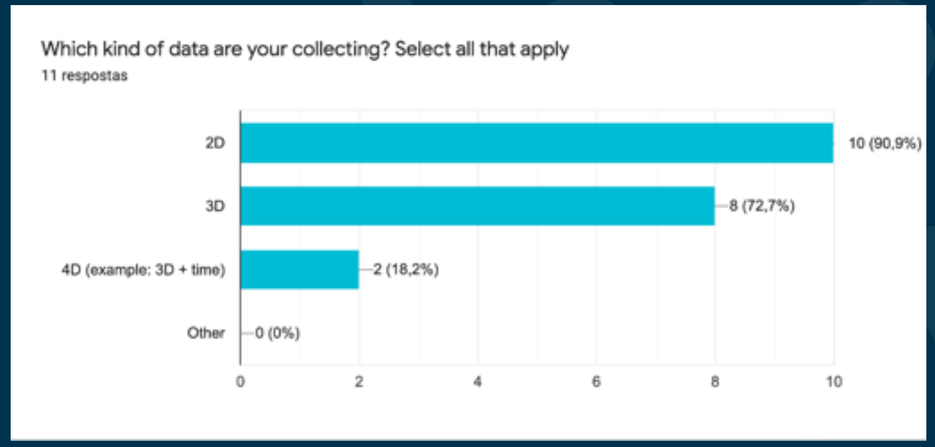
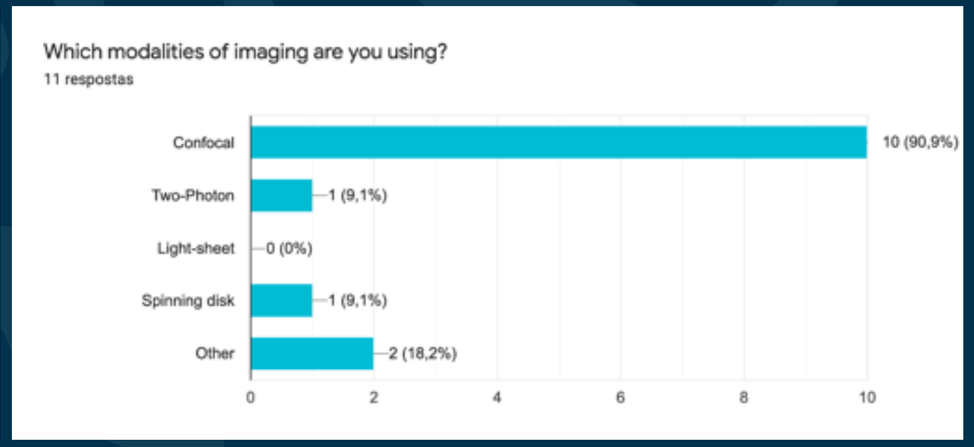
DZNE e. V. – Bonn / CRFS - Image and Data Analysis Facility (IDAF)

Survey


What is your level of experience with analysis?
11 respostas
54,5%
45,5%
Advanced
Intermediate
Basic


From this list which software/tools have you used? Select all that apply
11 respostas
FIJI ImageJ 11 (100%)
CellProfiler 2 (18,2%)
Cell Analyst 0 (0%)
Napari 0 (0%)
IMARIS 4 (36,4%)
Python 0 (0%)
Vision4D 0 (0%)
AIVIA 0 (0%)
R 4 (36,4%)
Matlab 1 (9,1%)
Other 1 (9,1%)
0,0 2,5 5,0 7,5 10,0 12,5


Which kind of data are your collecting? Select all that apply
11 respostas
2D 10 (90,9%)
3D 8 (72,7%)
4D (example: 3D + time) 2 (18,2%)
Other 0 (0%)
0 2 4 6 8 10


Which modalities of imaging are you using?
11 respostas
Confocal 10 (90,9%)
Two-Photon 1 (9,1%)
Light-sheet 0 (0%)
Spinning disk 1 (9,1%)
Other 2 (18,2%)
0 2 4 6 8 10

Talk outline:

- What is bioimage analysis?
- Basics of image processing
- FIJI: macros/ Napari
- What can we improve?
    - GPU-accelerated image analysis (CLIJ2)
    - AI vs ML vs DL
        - Trainable Weka Segmentation
        - CellProfiler and Cell Analyst
    - Visualization of results and statistical analysis with Python
- Do it yourself with your data!

What is bioimage analysis?

One possible definition:

The extraction of relevant information from digital images, at large scale and high throughput in the context of biological research

- Precise
- Unbiased
- Reproducible
- Scalable

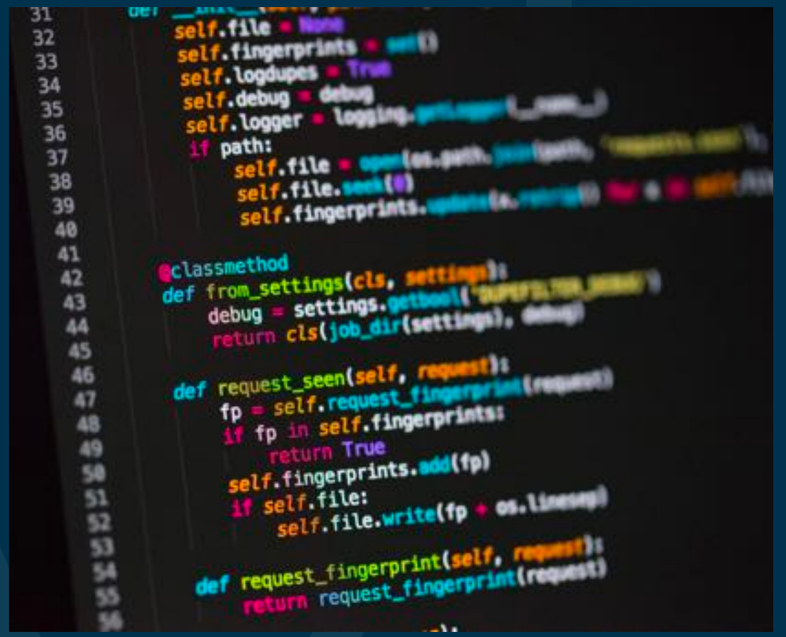
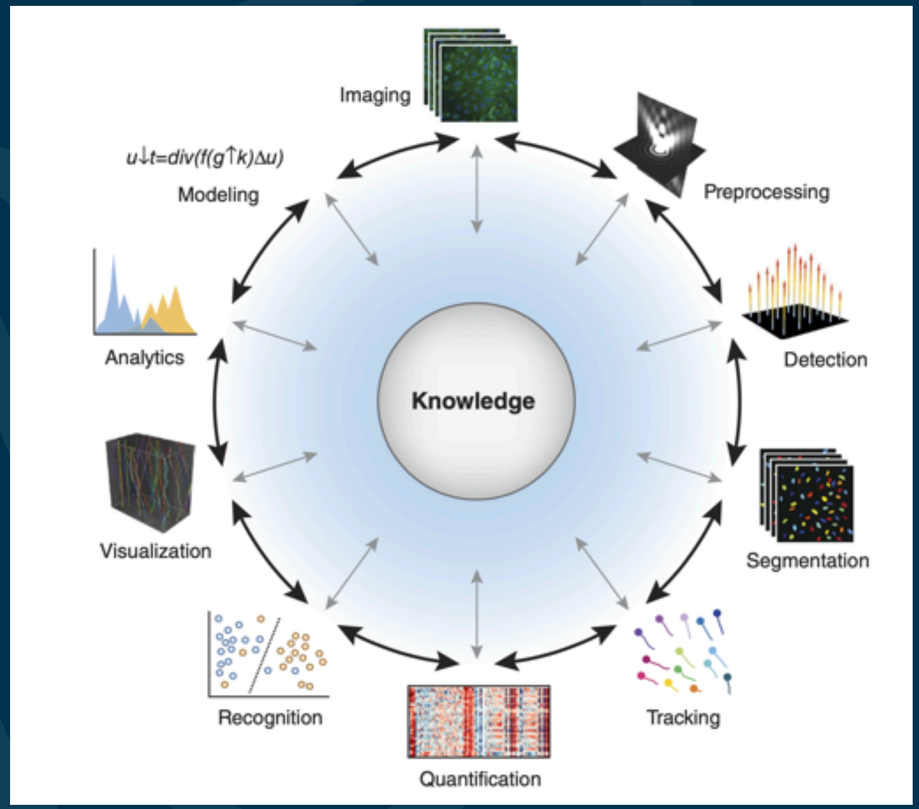


Image from Unsplashed

What is the goal of Bioimage analysis?

- *Obtain useful insights out of complicated and heterogeneous image and related metadata.*

See https://en.wikipedia.org/wiki/Bioimage_informatics for details)



From https://www.nature.com/articles/nbt.3722

Meijering, E. et al. “Imagining the future of bioimage analysis.”

Computational analysis is becoming the bottleneck

Seeing Is Believing: Quantifying Is Convincing: Computational Image Analysis in Biology

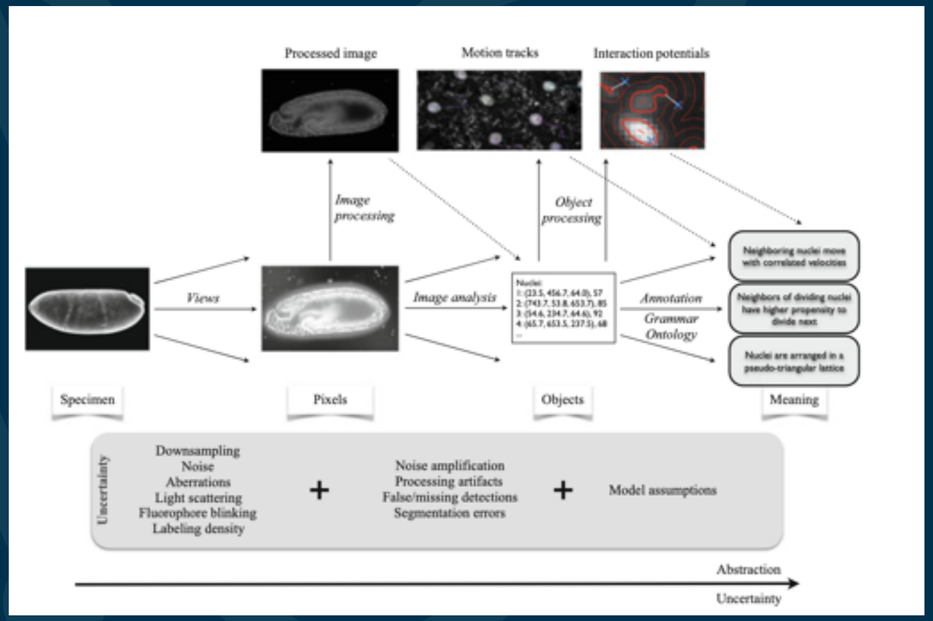
Ivo F Sbalzarini [1 2 3]

Affiliations + expand

PMID: 27207361 DOI: 10.1007/978-3-319-28549-8_1

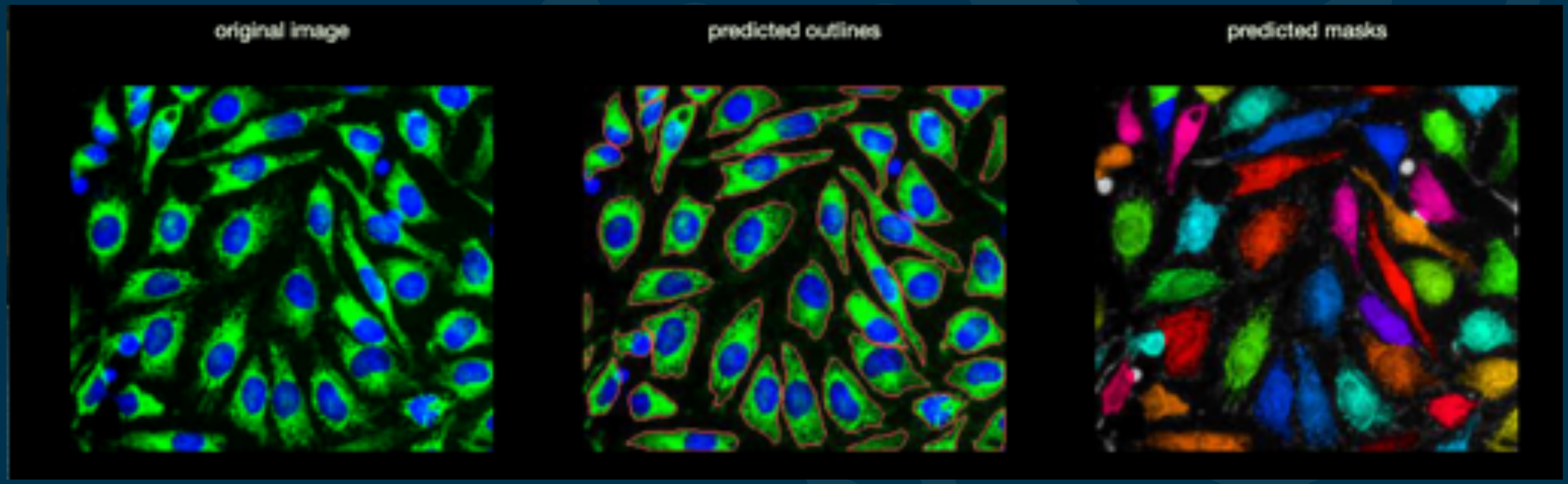
Abstract

Imaging is center stage in biology. Advances in microscopy and labeling techniques have enabled unprecedented observations and continue to inspire new developments. Efficient and accurate quantification and computational analysis of the acquired images, however, are becoming the bottleneck. We review different paradigms of computational image analysis for intracellular, single-cell, and tissue-level imaging, providing pointers to the specialized literature and listing available software tools. We place particular emphasis on clear categorization of image-analysis frameworks and on identifying current trends and challenges in the field. We further outline some of the methodological advances that are required in order to use images as quantitative scientific measurements.



https://pubmed.ncbi.nlm.nih.gov/27207361/

Example of data analysis



Example Cellpose workflow

Many different tools and steps

How to approach?

Many resources freely available:

https://bioimagebook.github.io/

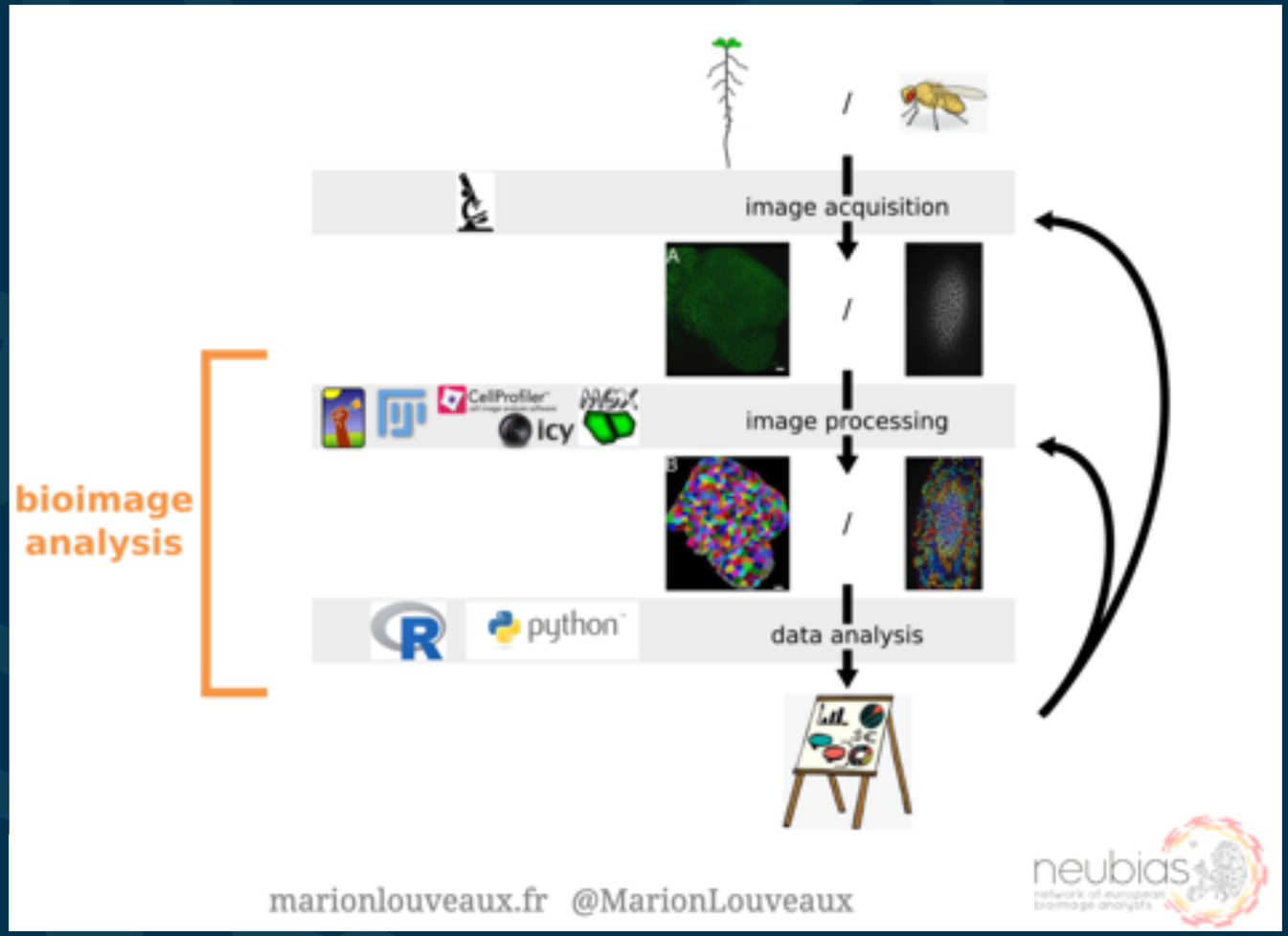
https://forum.image.sc/

http://eubias.org/NEUBIAS/

http://biii.eu/

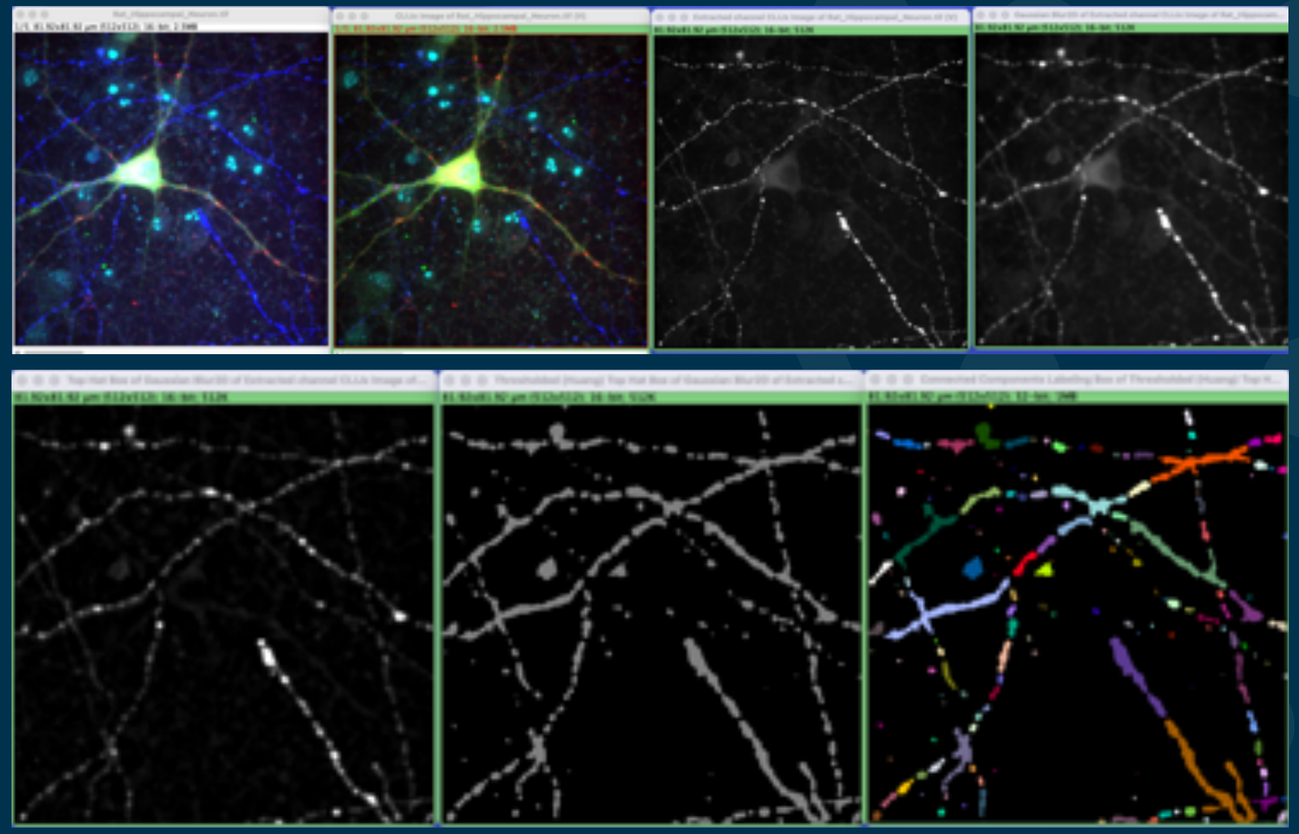
https://www.youtube.com/c/haesleinhuepf/videos

… and more



Modified from https://github.com/marionlouveaux/NEUBIAS_TS15_DataViz

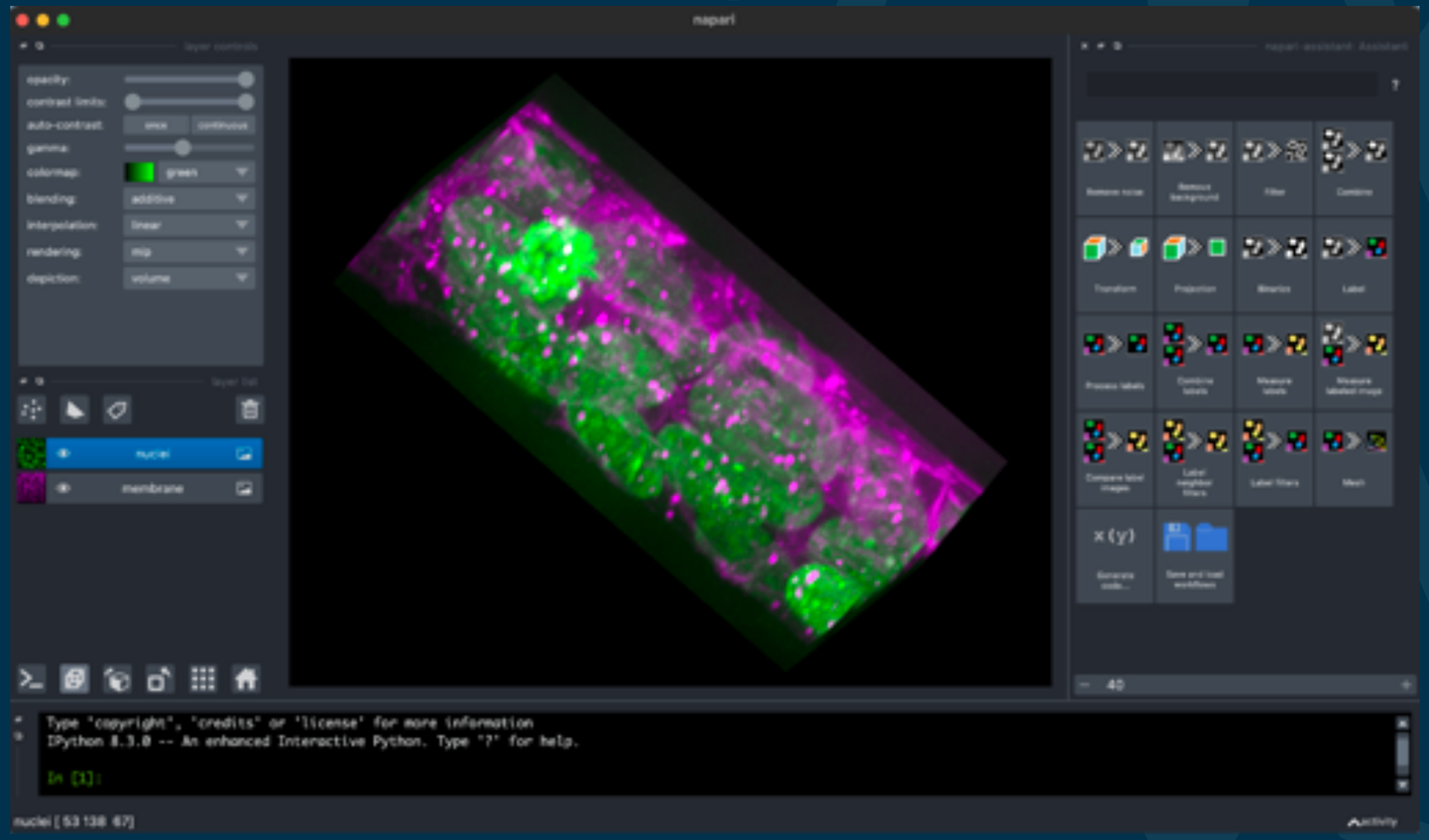
Example today: Image J / FIJI



Fiji (Fiji Is Just ImageJ):
distribution of ImageJ2, bundling a lot of plugins which facilitate scientific image analysis

Example CLIJ2 analysis workflow
https://github.com/clij/clij2

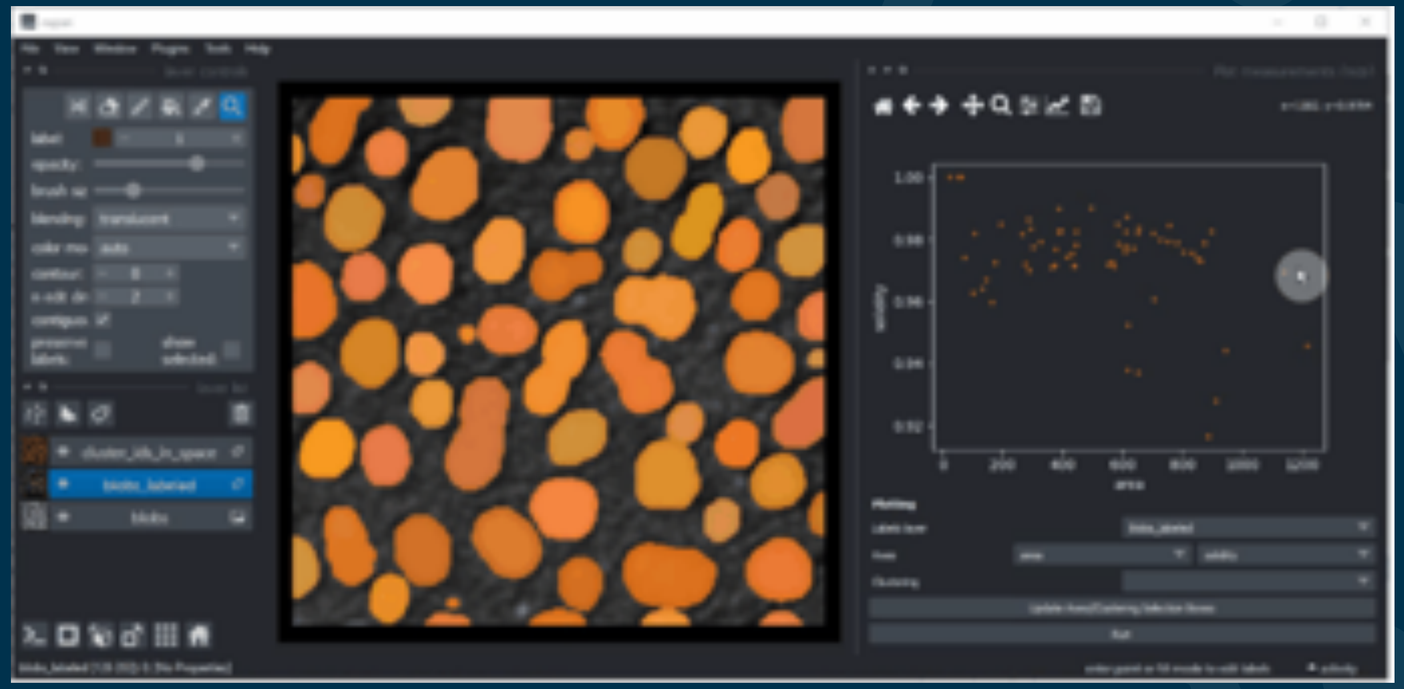
Alternative to FIJI: Napari (Python)



napari is a fast, interactive, multi-dimensional image viewer for Python

https://napari.org/

Napari (Python)



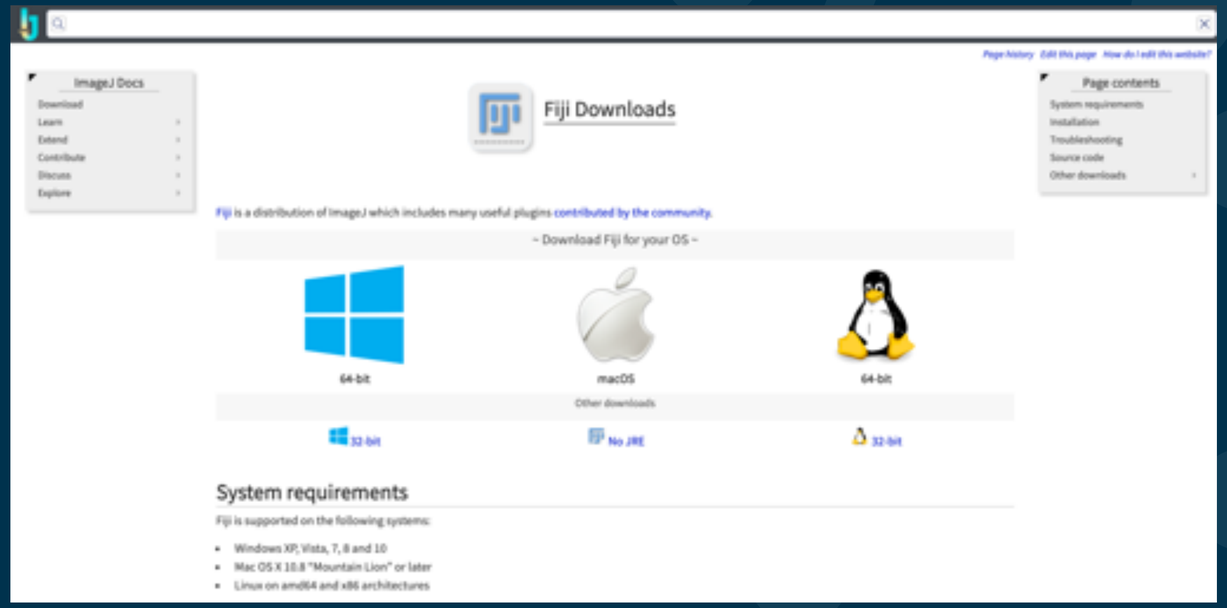
You can write scripts as well for automation

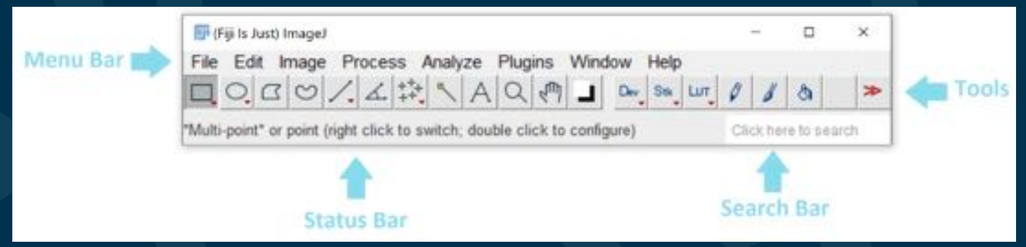
```
from skimage.io import imread
import pyclesperanto_prototype as cle
import napari_simpleitk_image_processing as nsitk
import napari
if 'viewer' not in globals():
    viewer = napari.Viewer()
image0_m = viewer.layers['membrane'].data
# gaussian blur
image1_gb = cle.gaussian_blur(image0_m, None, 1.0, 1.0, 0.0)
viewer.add_image(image1_gb, name='Result of gaussian_blur
(clesperanto)')
```

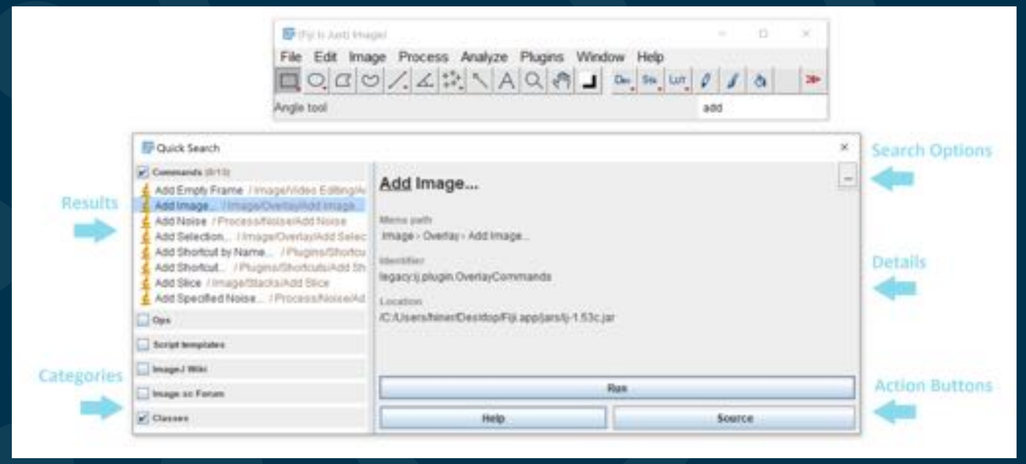
https://github.com/BiAPoL/napari-clusters-plotter

https://napari.org/

Installing FIJI





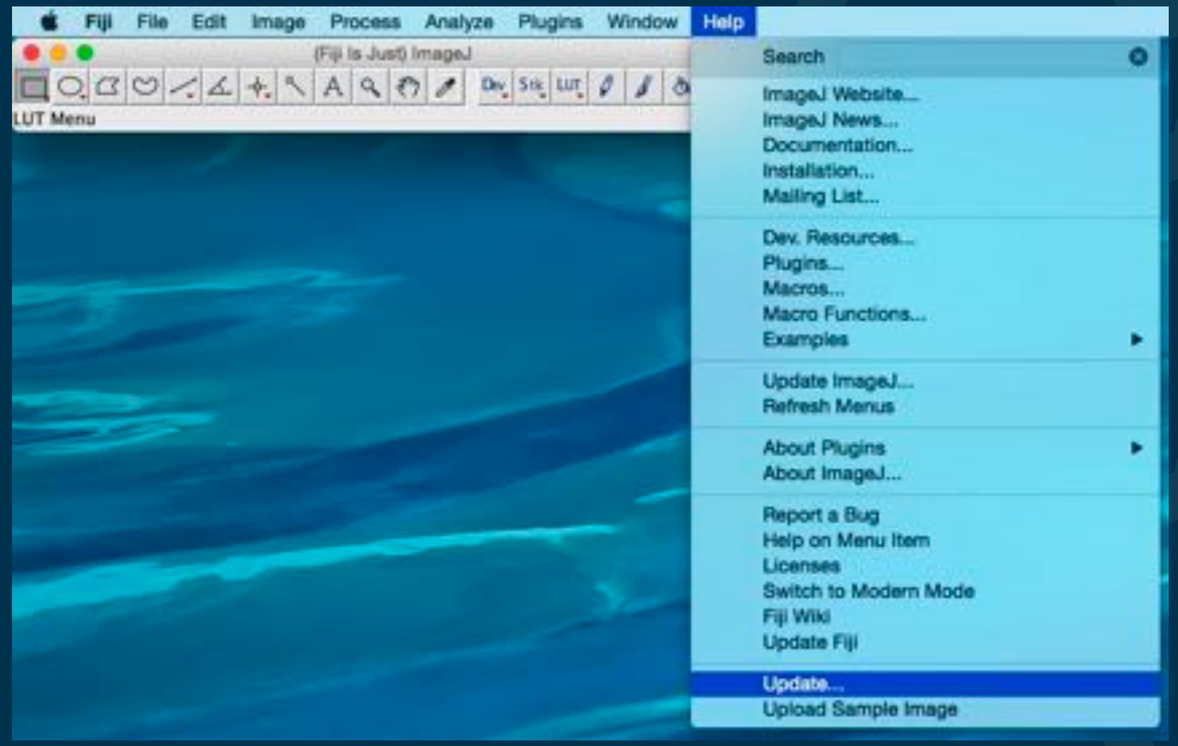


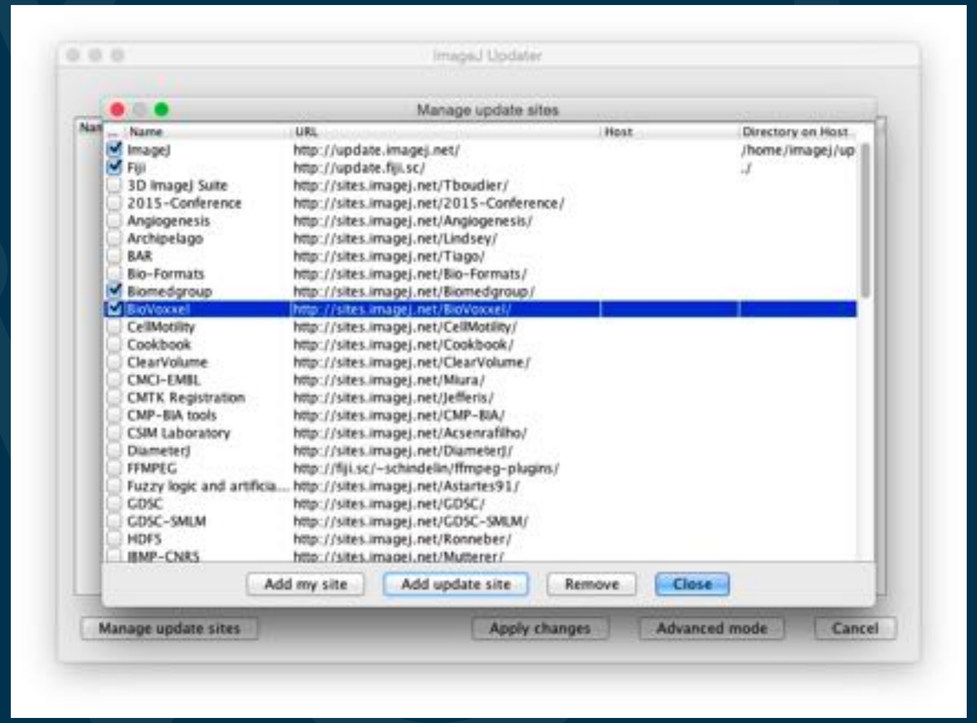
You can even run ImageJ on your browser

https://ij.imjoy.io/

From https://imagej.net

Updating FIJI and installing plugins, scripts and macros





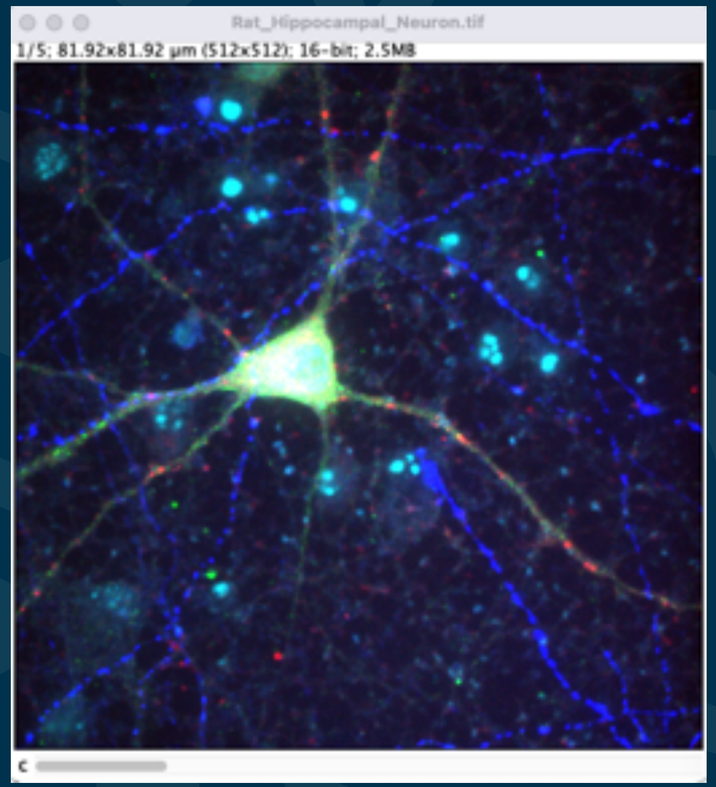
From https://imagej.net

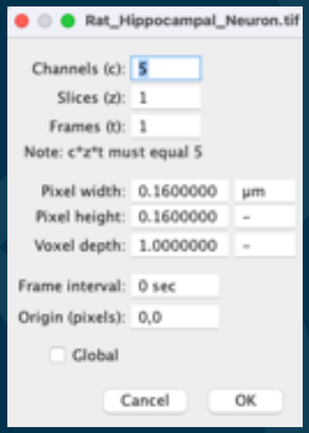
Check properties of the image using FIJI

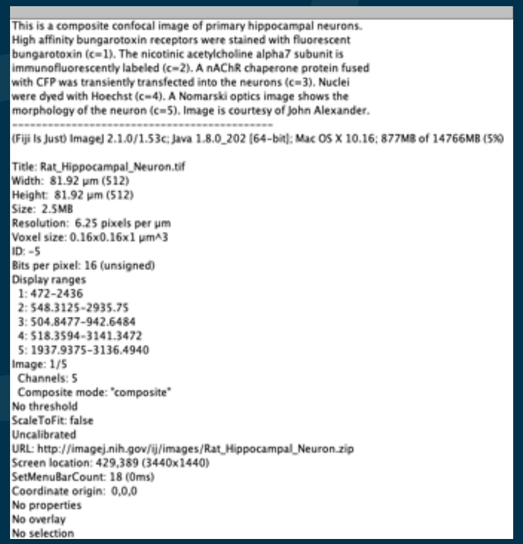
Image > Properties

and

Image > Show Information







Basics of image processing

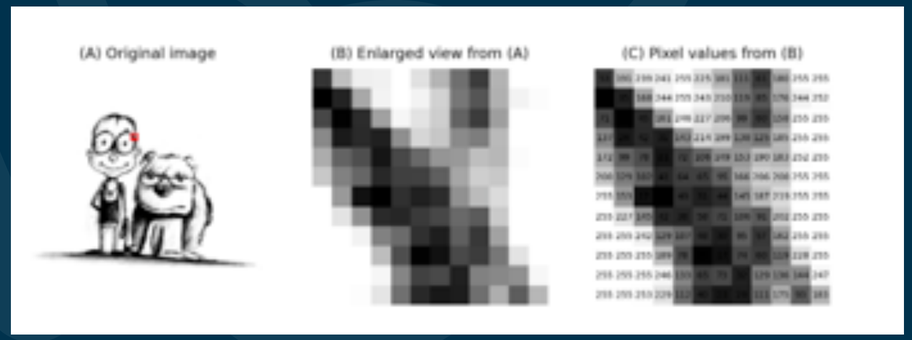
What is a pixel?

A pixel (“picture element”) is the smallest unit of a digital image or graphic that can be displayed and represented on a digital display device.

Pixels are combined to form a complete image, video, text, or any visible thing on a computer display.

A Pixel Is Not A Little Square!!!

A pixel is a data point, a point sample. A pixel does not need to be rendered as a small square.



https://en.wikipedia.org/wiki/Pixel

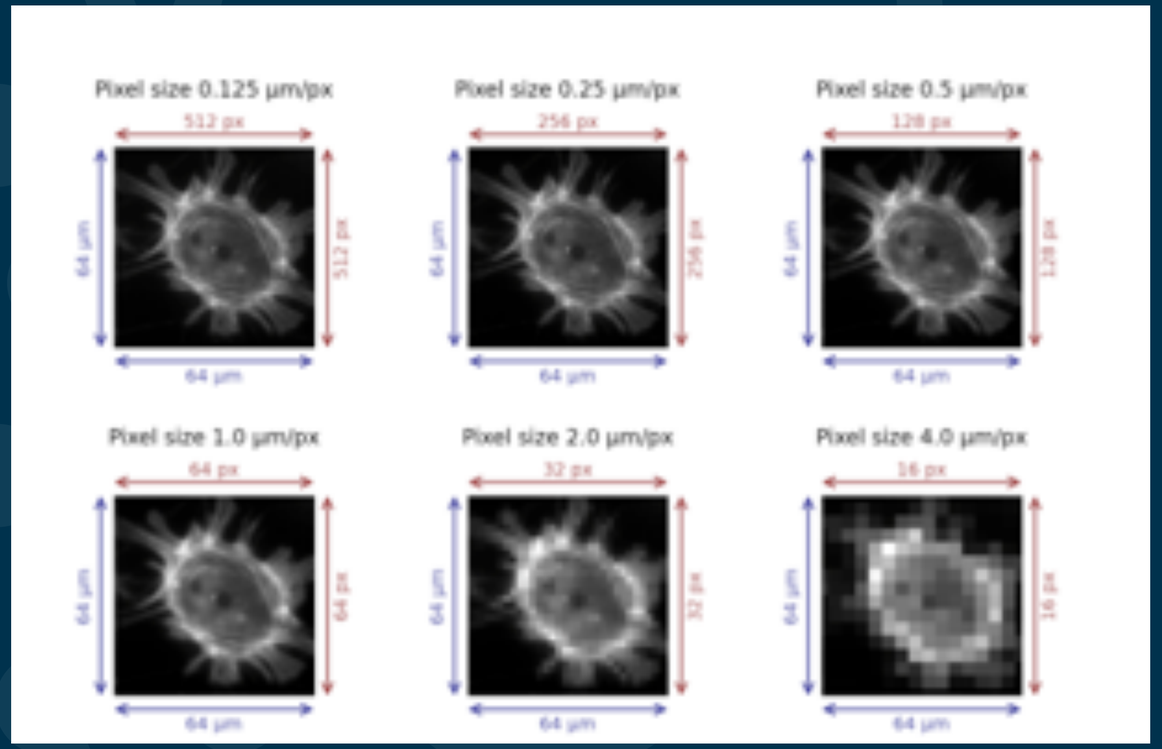
https://bioimagebook.github.io/

Pixel size vs resolution

Pixel size is a property you configure during imaging at the microscope

Resolution is a property of your imaging system.

Spatial resolution: measure of how closely lines can be located in an image while still being differentiable



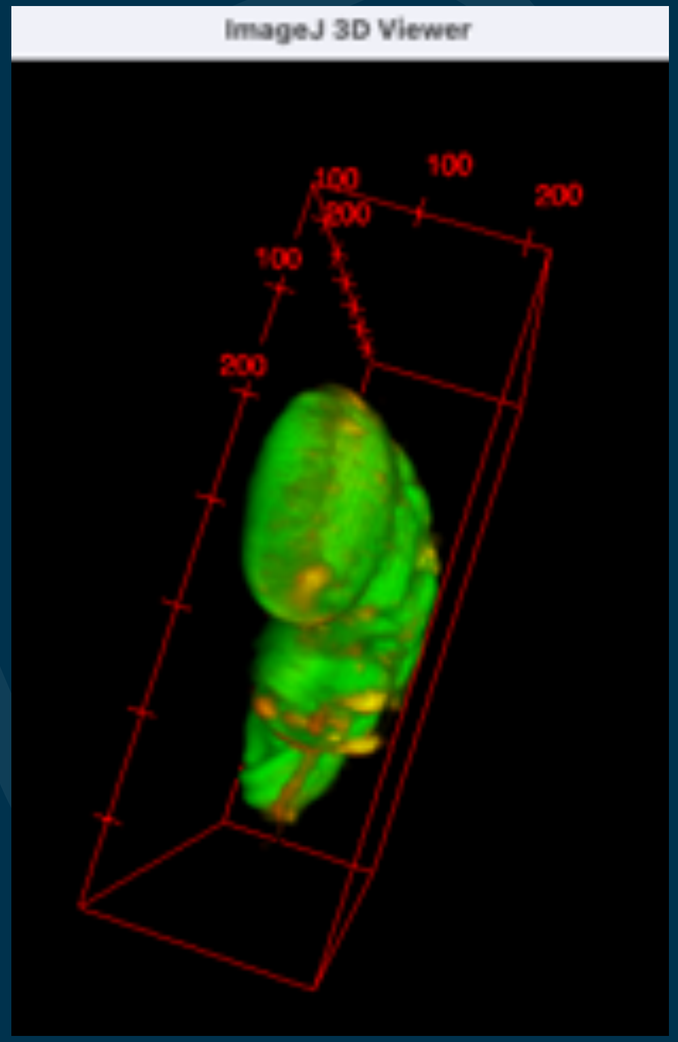
https://bioimagebook.github.io

Voxels (3D data)

Voxel: “Volume element”, the smallest unit of the sampled 3D volume

Usually anisotropic: not the same in all directions

The section thickness together with the xy-pixel dimension defines the voxel size.



Bit depth

The bit-depth n enumerates how many different intensity values are present in an image: 2^n grey values

In microscopy, images are usually stored as 8, 12 or 16-bit images.

Higher bit depths corresponding to a greater range of useful image information available

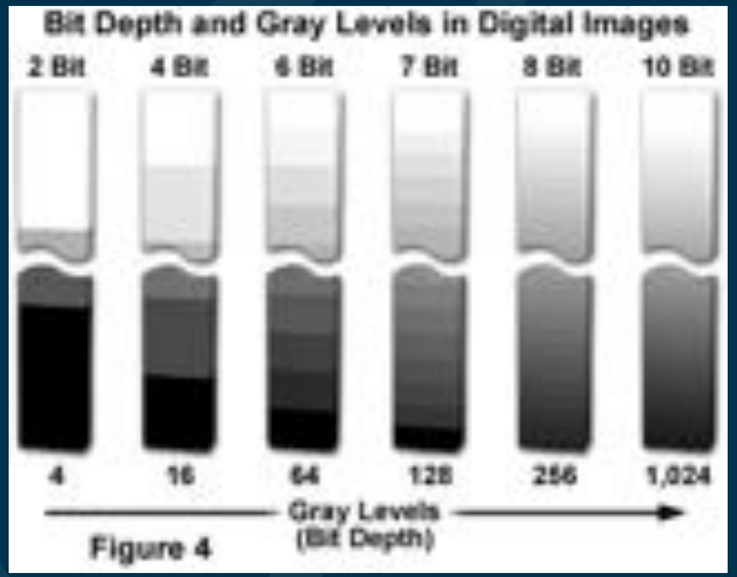
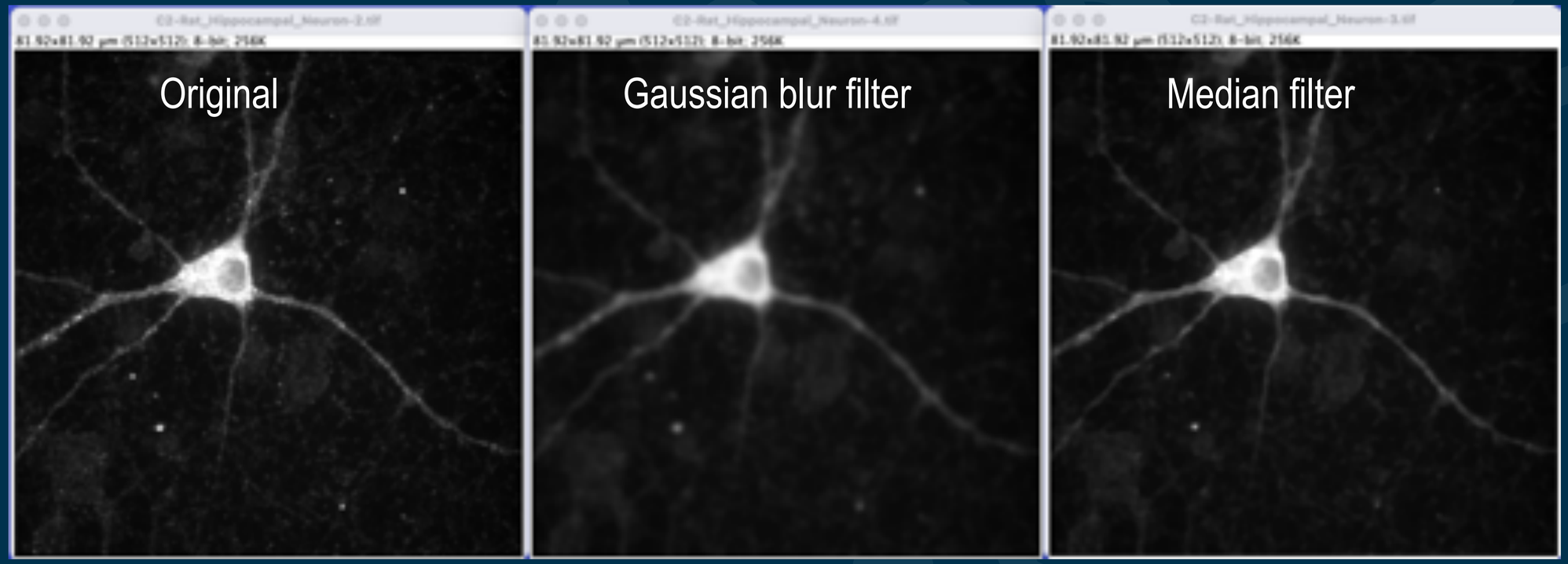


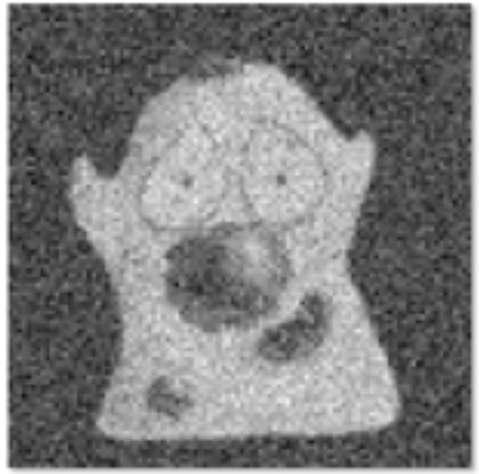
Image from http://zeiss-campus.magnet.fsu.edu/articles/basics/digitalimaging.html

Examples of preprocessing the images before further analysis



Example mean filter

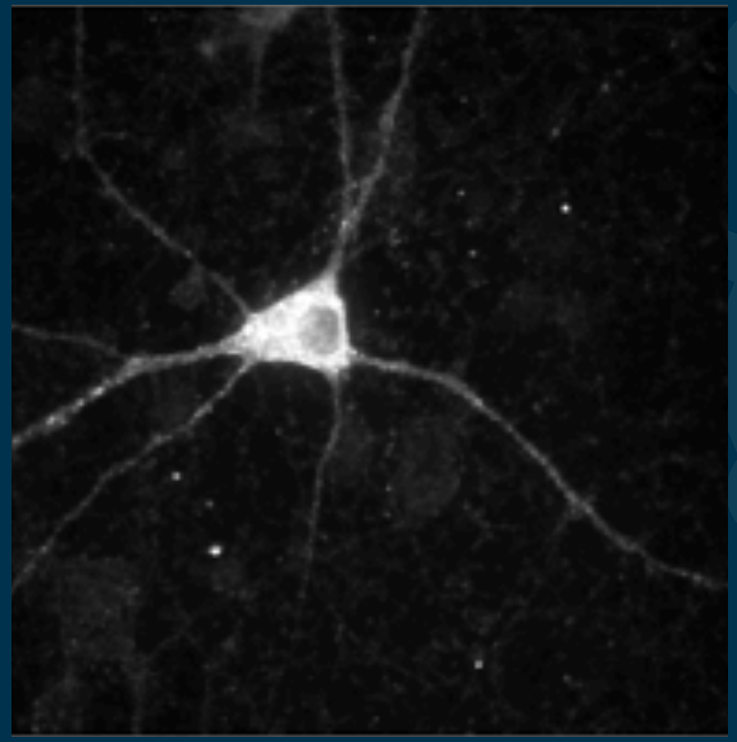
Implementing a 3x3 mean filter

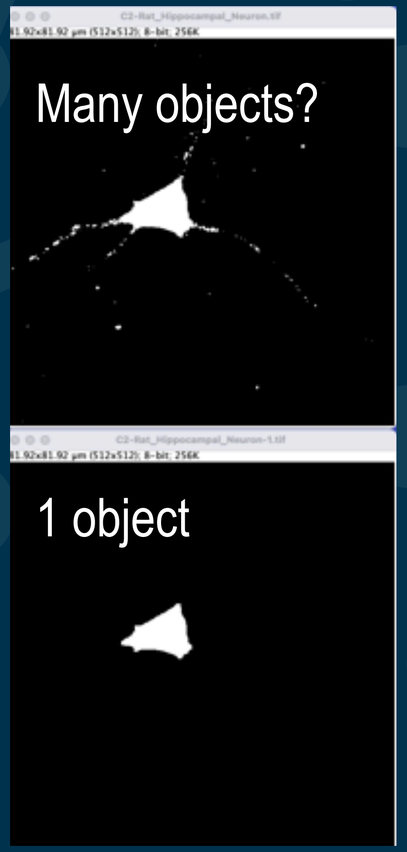


Original image

https://bioimagebook.github.io/

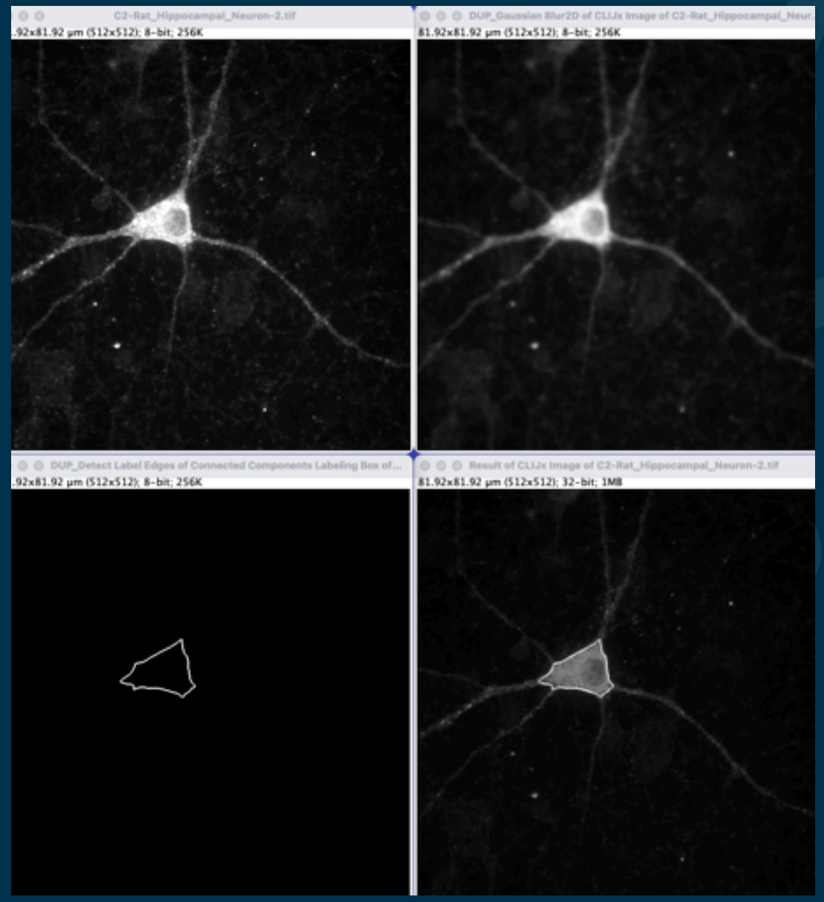
Removing noise / preprocessing the data before segmentation is important

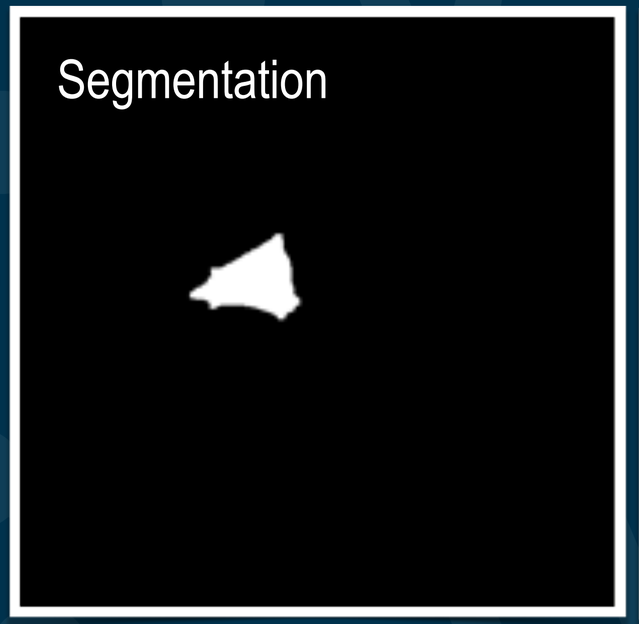


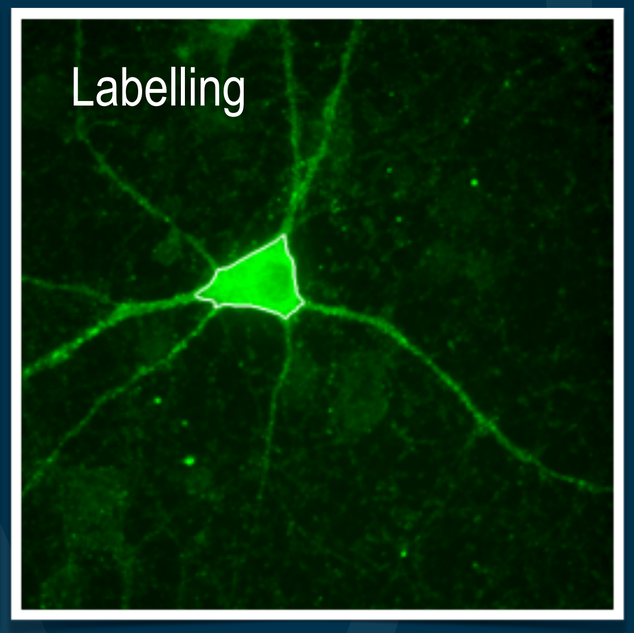


Today we are going to have a hands-on on segmentation and labelling

Segmentation and labelling





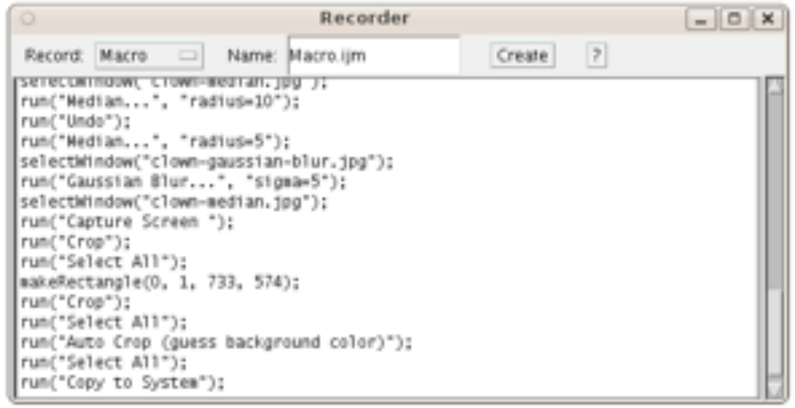


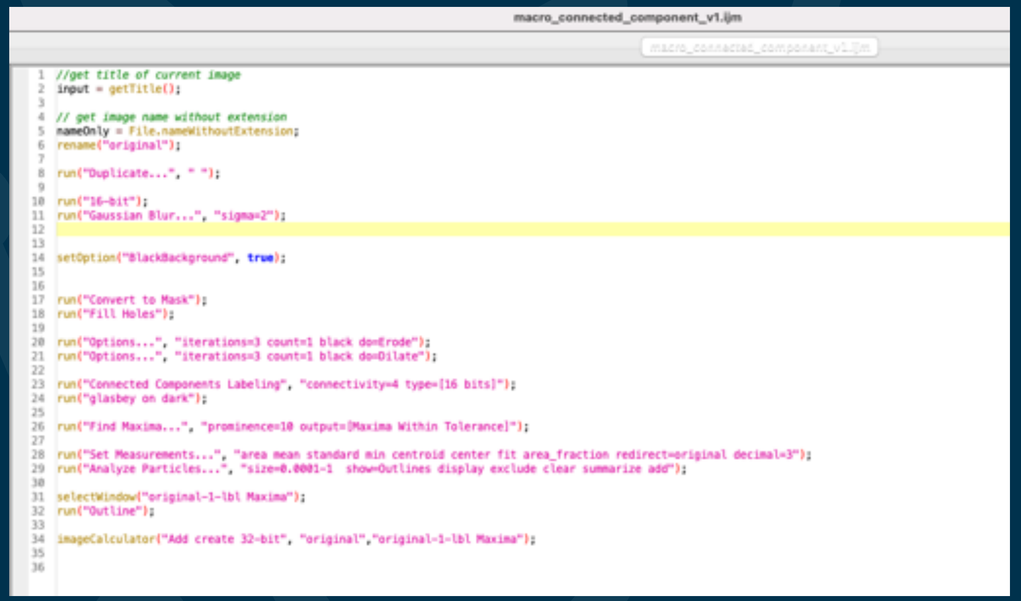
Macro recorder

Create our first macro

The recorder

Typically, macros are not written from scratch, but recorded using the Macro Recorder: Just click on *Plugins › Macros › Record…* and perform some actions. These actions will be recorded in the recorder window, and you can click **Create** to open the recorded instructions in an editor:





From https://imagej.net

Example script is provided
(macro_connected_component_v1.ijm)

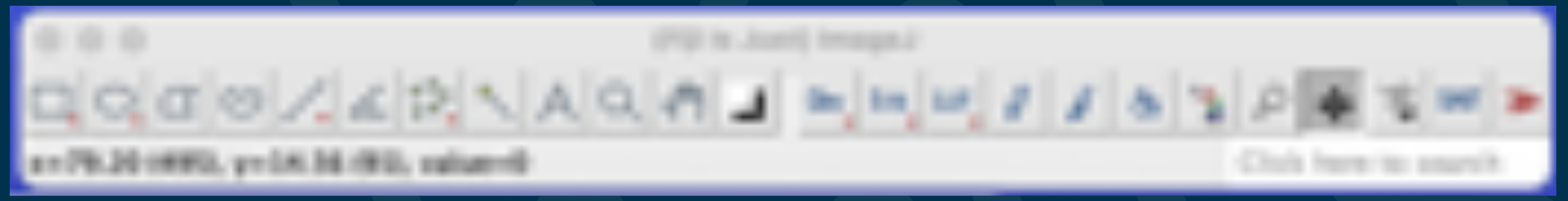
What if you want to apply these operations to all images in a given directory?

```
/* This macro counts the number of objects
 *  in a folder with multiple 2D images
 *
Author: Miguel Fernandes (IDAF)
Date: 16 May 2022
*/
/*
Usage:
There should not be any " " in your directory or file names
At the moment working for 2D image data

Only one channel is expected to perform analysis
Keep it consistent across experiments!

Run this macro and select a folder containing imaging files
(inputFolder)

Select type of input stacks with the format defined in suffix
(example .tif files)

Select output folder to save results for each individual file and summary across all images
(outputFolder)

At the moment only for 2D but in principle could be extended to 3D

TODO: improve documentation for each step

*/


#@ File (label="Select a folder to process", style="directory") inputFolder
#@ String (label = "File suffix input folder", value = ".tif") suffix
#@ File (label="Select a folder to save results", style="directory") outputFolder


dir=File.getDirectory(inputFolder);


print("User selected input folder: " + inputFolder);
print("User selected save folder: " + outputFolder);


setBatchMode(true); //batch mode on
```

Example script is provided (*macro_connected_component_v1_BATCH.ijm*)

Now is time to perform the analysis

FIJI time!!!



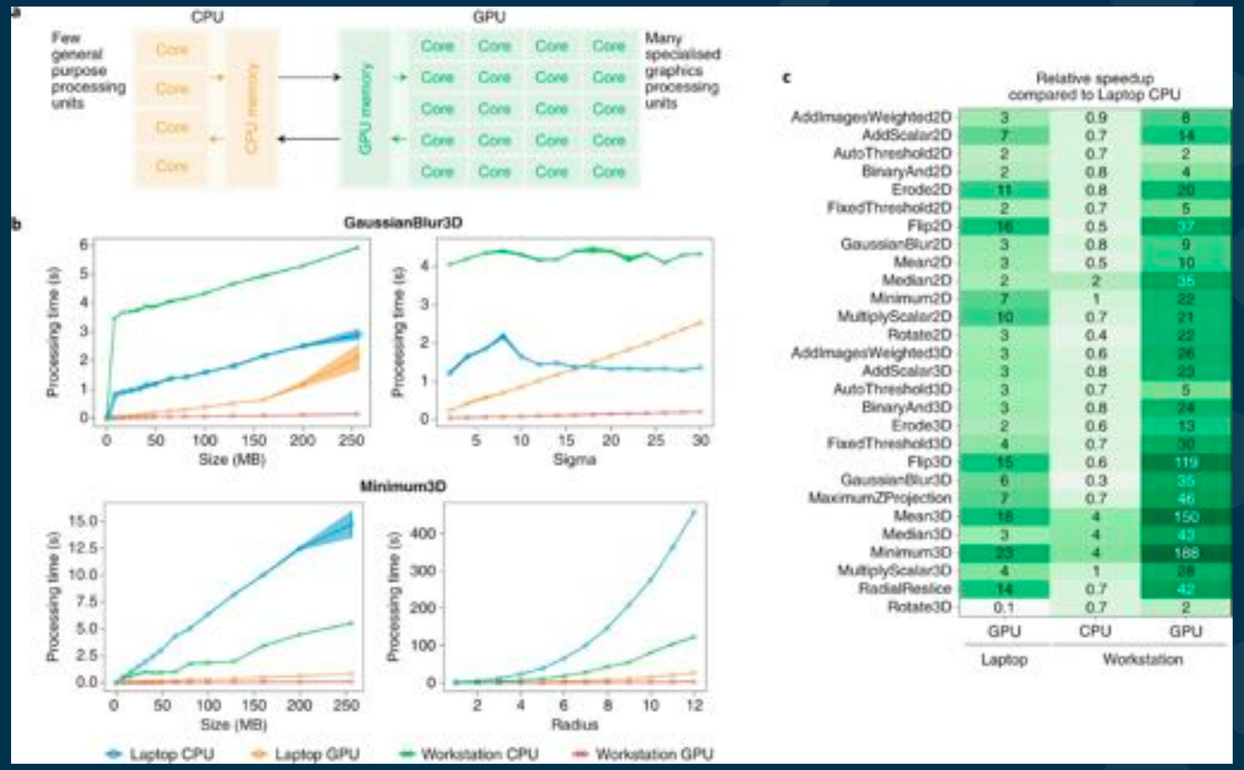
Your samples?

15 minutes. Lets try it out!

What needs to be improved? Ideas?

- Improve preprocessing and thresholds: what would you try?
- Accelerate imaging processing (e.g. GPU)
- Use Machine learning (ML) to classify our ROIs (Weka)
- Automated analysis for high-content assays (combine classical AI + ML). CellProfiler/Cell Analyst
- Plotting the results and perform statistical tests (Python, see Jupyter Notebook)

GPU Accelerates your image analysis



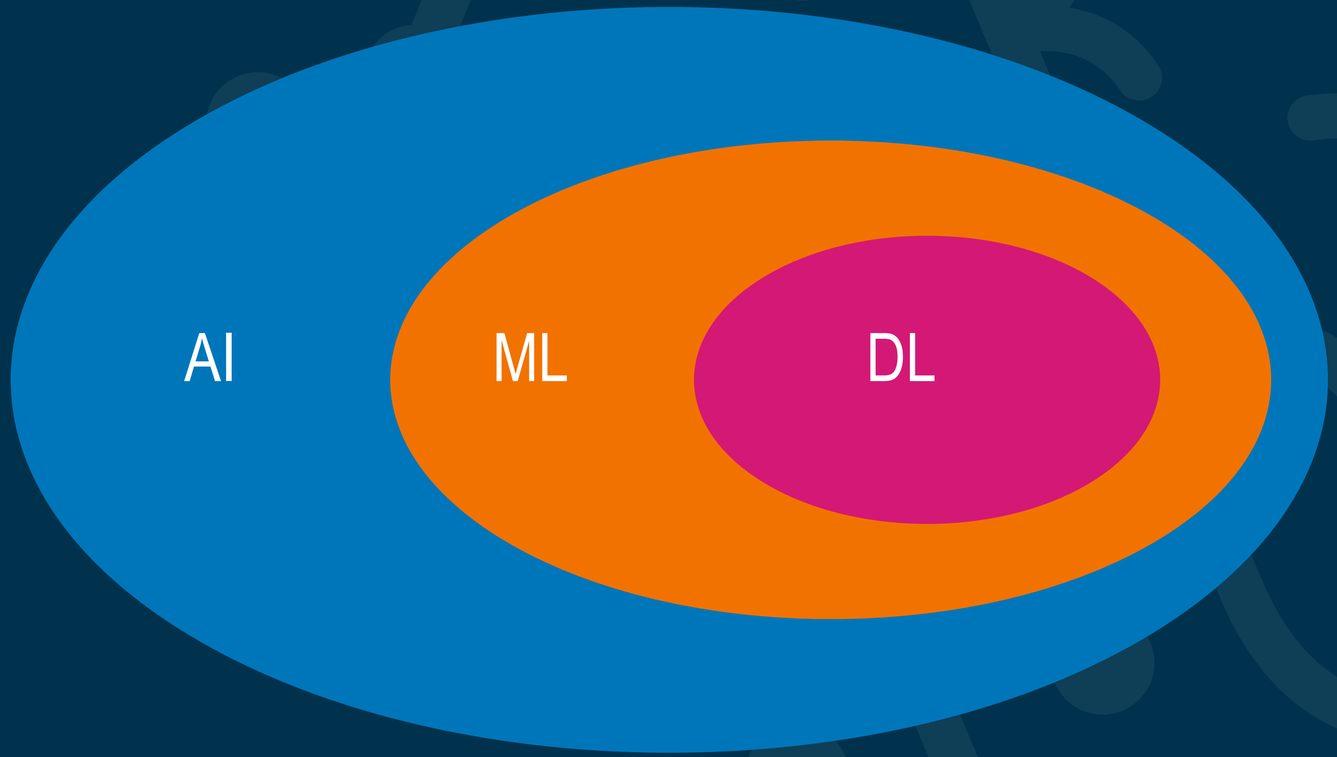
Haase et al 2020

For installation CLIJ2
https://clij.github.io/clij2-docs/installationInFiji

```
// To make this script run in Fiji, please activate
// the clij and clij2 update sites in your Fiji
// installation. Read more: https://clij.github.io

//Following an update site:
//https://imagej.net/update-sites/following#Add_update_sites


// Generator version: 2.5.1.1

// Init GPU
run("CLIJ2 Macro Extensions", "cl_device=");

// Load sample dataset Neuron
run("Neuron (5 channels)");

image_1 = getTitle();
Ext.CLIJ2_pushCurrentZStack(image_1);
// The following auto-generated workflow is made for processing a 2D or 3D dataset.
// For processing multiple channels or time points, you need to program a for-loop.
// You can learn how to do this online: https://www.youtube.com/watch?v=ulSq-x5_in4

// Copy
Ext.CLIJ2_copy(image_1, image_2);
Ext.CLIJ2_release(image_1);

Ext.CLIJ2_pull(image_2);
```

Example script is provided
(*macro_Neuron_demo_clij2.ijm*)

Artificial inteligence vs Machine learning vs Deep learning



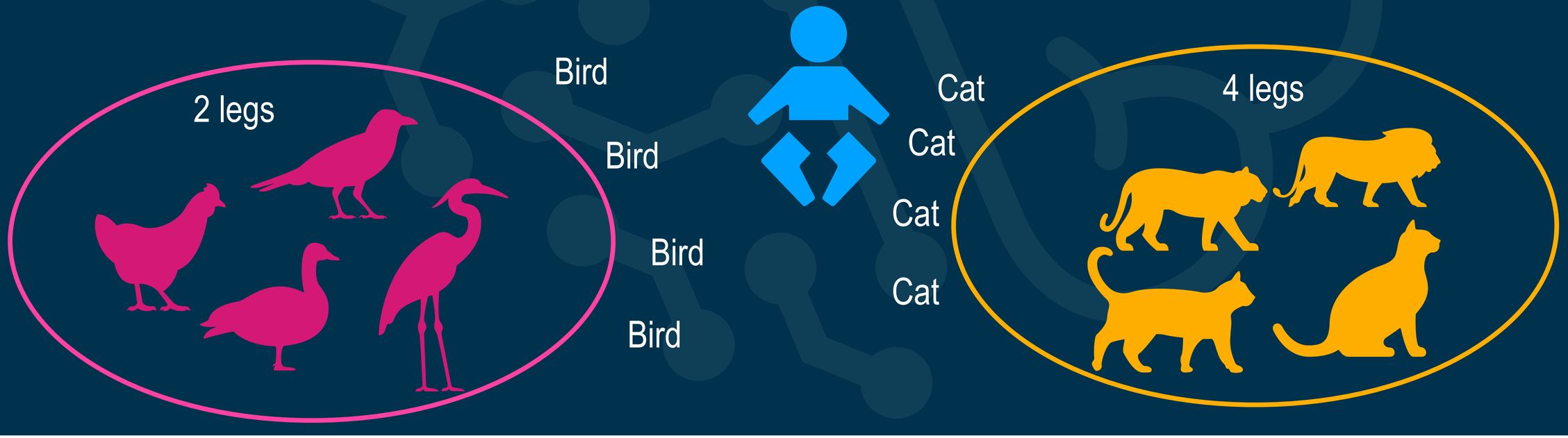
Classical AI vs ML

At the high-level:

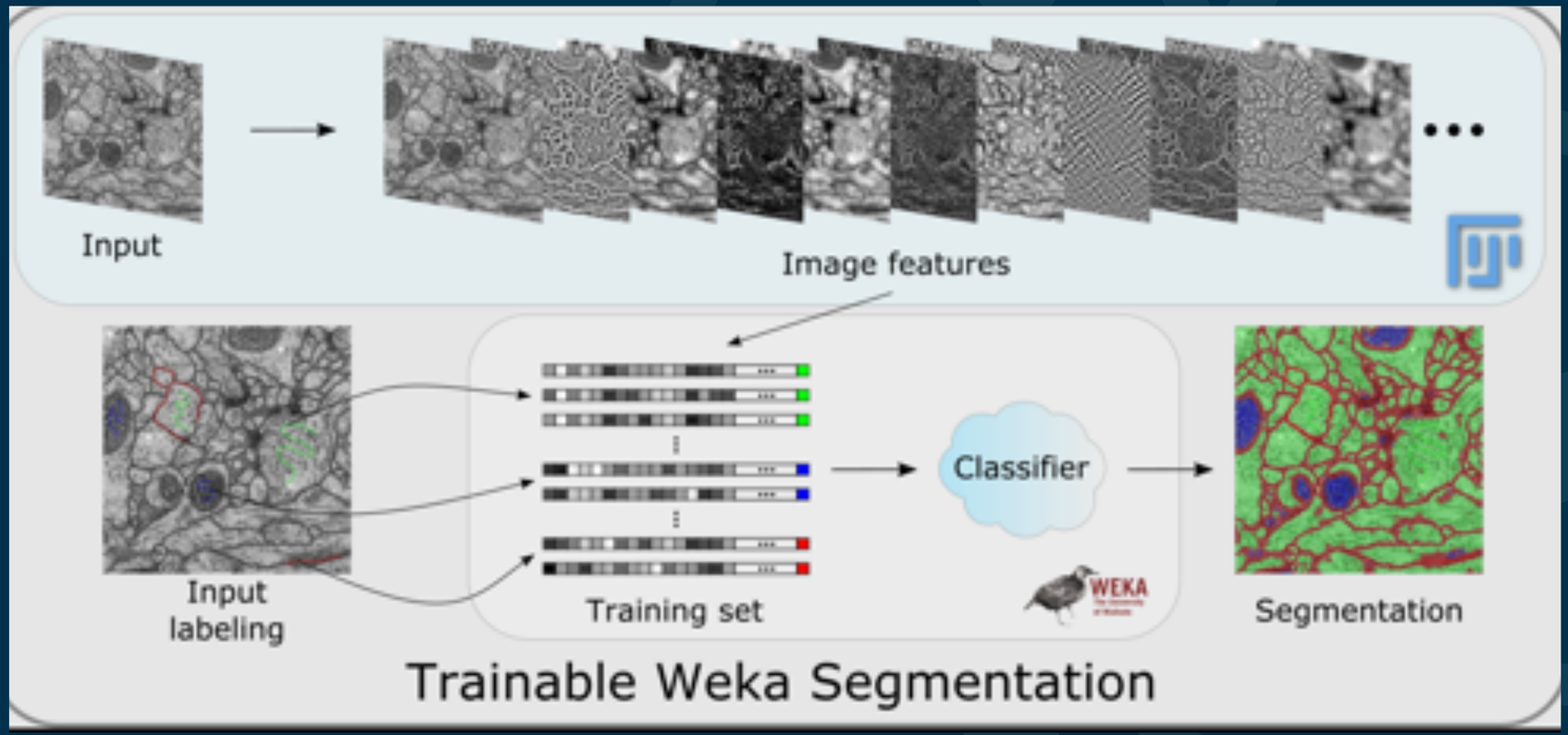
a) either you create rules (Classical AI)

or

b) you give examples with labels (Machine learning)



Trainable Weka Segmentation



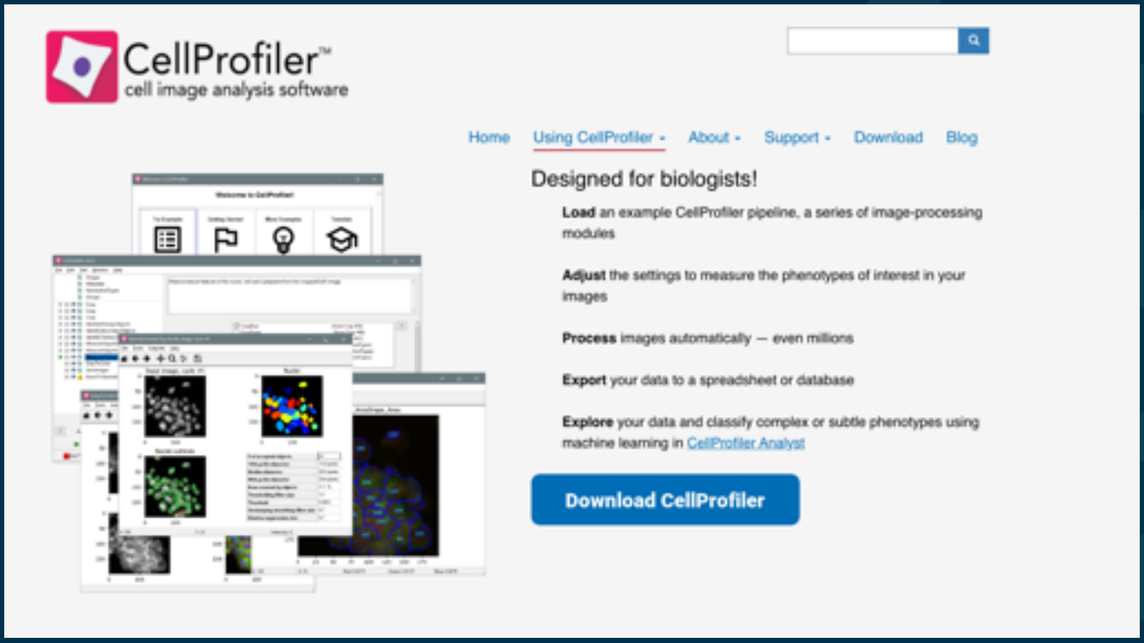
Lets try it out!

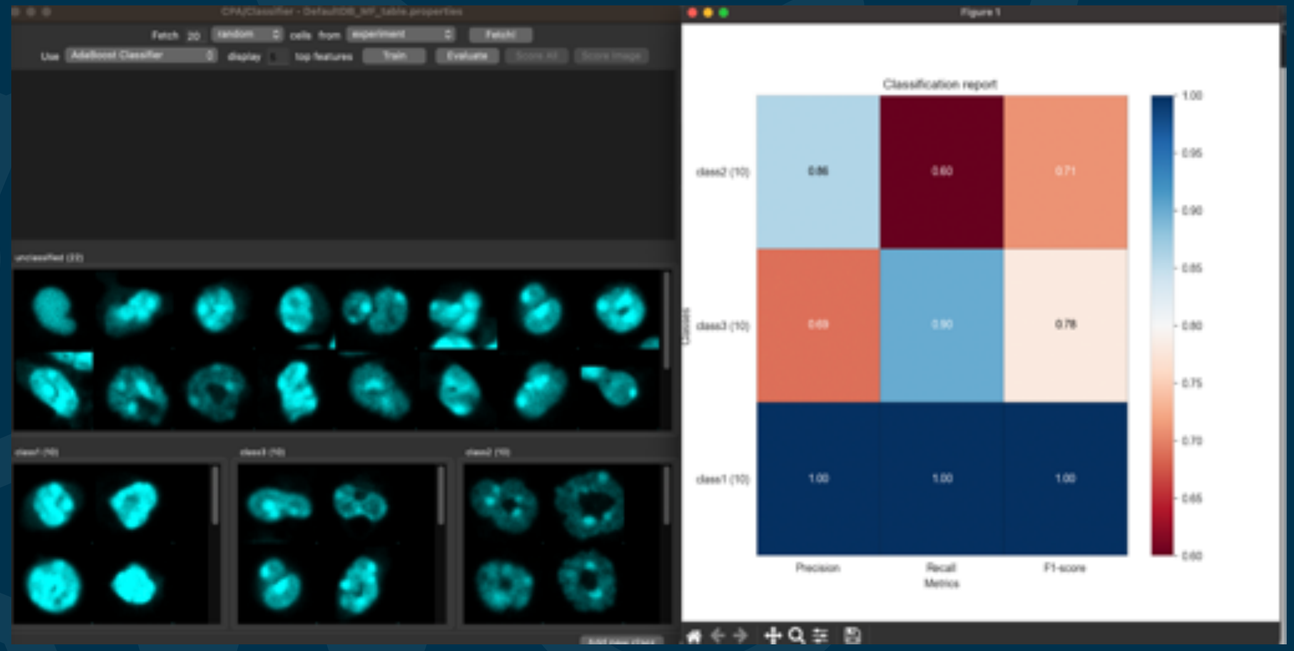
15 minutes

https://imagej.net/plugins/tws/

CellProfiler and CellProfiler Analyst

Lets try it out!
15 minutes





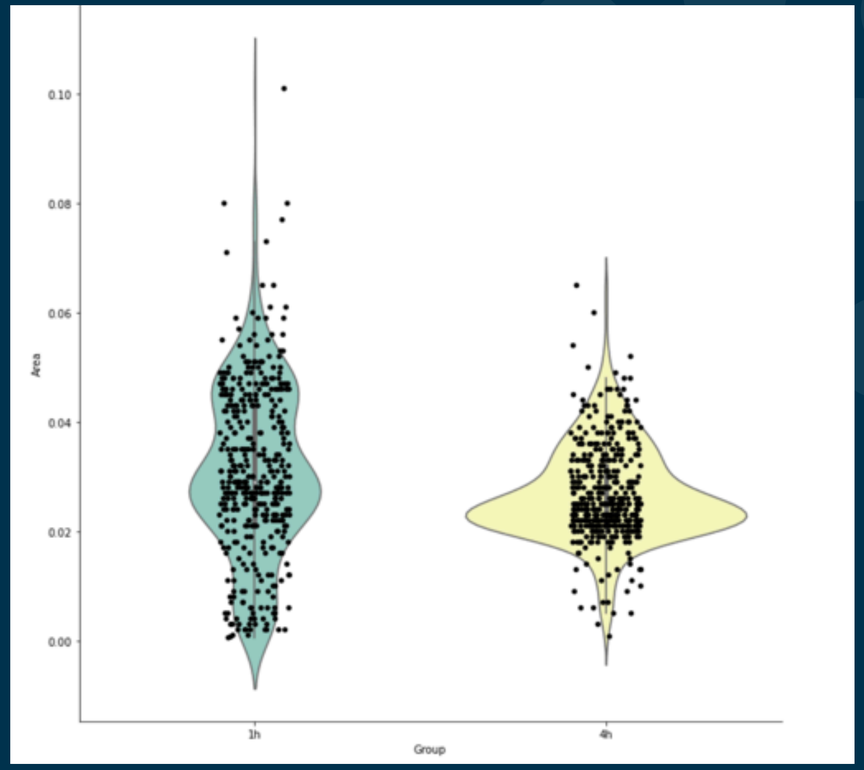
https://cellprofiler.org/

CP4_MF_workshop.cpproj and
DefaultDB_MF_workshop.workspace provided

https://cellprofileranalyst.org/

Visualization of results and statistical analysis (Python and Jupyter Notebook)

Pandas, Matplotlib, Seaborn



Pingouin is an open-source statistical package written in Python 3

https://pingouin-stats.org/#

```
pg.ttest(df_1h.Area, df_4h.Area)
```

	T	dof	tail	p-val	CI95%	cohen-d	BF10	power
T-test	4.995508	669.592392	two-sided	7.496529e-07	[0.0, 0.01]	0.341099	1.389e+04	0.998789

Is the data normal?

```
print(pg.normality(df_1h.Area))
print(pg.normality(df_4h.Area))
```

```
             W      pval  normal
Area  0.976816  0.000002   False
             W          pval  normal
Area  0.95573  4.555235e-10   False
```

Pingouin can be used for statistical analysis

10 minutes

Notebook with analysis is provided

Thank you!!!

Lets use the remaining time to perform analysis on your own datasets/discuss about what you could use for your project

Additional Resources

- https://git.mpi-cbg.de/rhaase/lecture_applied_bioimage_analysis_2020
- https://montpellierressourcesimagerie.github.io/mri-workshop-machine-learning/
- https://imagej.nih.gov/ij/docs/examples/index.html
- https://petebankhead.gitbooks.io/imagej-intro/content/
- Bioimage Data Analysis Workflows book: https://www.springer.com/gp/book/9783030223854
- See also Bioimage Analysis: Recommended Reading and Viewing: https://github.com/amgfernandes/Workshop_May_2022_Tuebingen/blob/main/README.md