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DRESDEN LEIPZIG
CENTER FOR SCALABLE DATA ANALYTICS
AND ARTIFICIAL INTELLIGENCE

Bio-Image Data Science

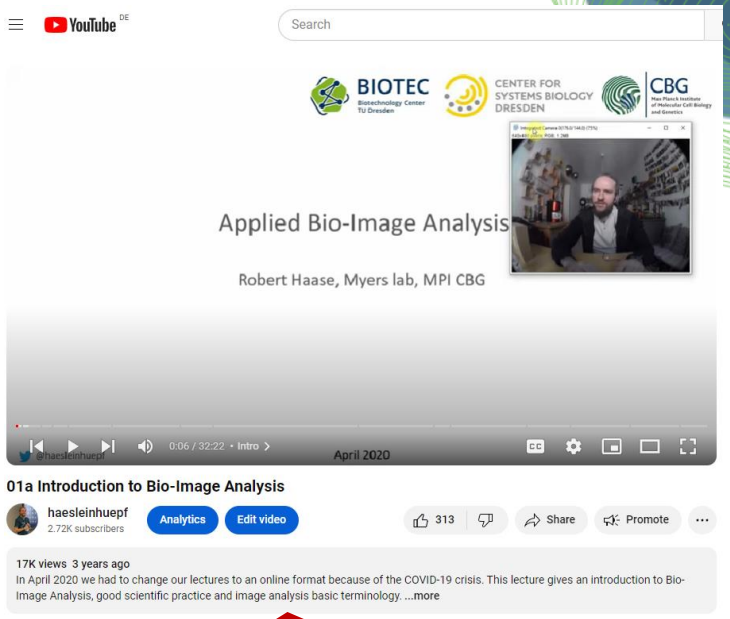
Robert Haase

GEFÖRDERT VOM
Bundesministerium
für Bildung
und Forschung

SACHSEN
Diese Maßnahme wird gefördert durch die Bundesregierung aufgrund eines Beschlusses des Deutschen Bundestages.
Diese Maßnahme wird mitfinanziert durch Steuermittel auf der Grundlage des von den Abgeordneten des Sächsischen Landtags beschlossenen Haushaltes.

Hello my name is…

- Robert Haase
- Applied image data scientist, research software engineer
- Trained computer scientist (2010 Dipl-Inf(FH))
  with a medical PhD (2016 Dr. rer. medic.)
- Worked 15 years on the biomedical research campus in Dresden-Johannstadt (Hospital, MPI-CBG, TU-Dresden)
- Teaching bio-image analysis to life scientists 2019-2023 @ TU Dresden
- I maintain about 50 Python and Java packages other people use to analyze microscopy image data (GPU-acceleration, machine learning, large language models)
- New at Leipzig University



Large parts of this online lecture are outdated!

Survey

Think about the FAIR principles for data sharing, which one is wrong?

Findable

Accessible

Interoperable

Reproducible

Survey

Which open-source license might be the least popular in companies?

GPL

BSD

MIT

Apache

Survey

Which topic is typically not covered in a Research Data Management Plan?

- Backup
- Publishing
- Acquisition
- Career development

Survey

Which git command does not exist?

fetch

pull

add

submit

Survey

You typically install Python packages using…

pip

conda

mamba

(other / not)

Survey

Your favorite Python IDE is…

VS Code

Jupyter

Pycharm

(other / none)

Survey

What does this Python code spit out?

```
test = "ScaDS.AI"
print(test[-3:])
```

.AI	AI	ScaDS	(Error message)

Survey

Which is a background-removal filter?

Laplace

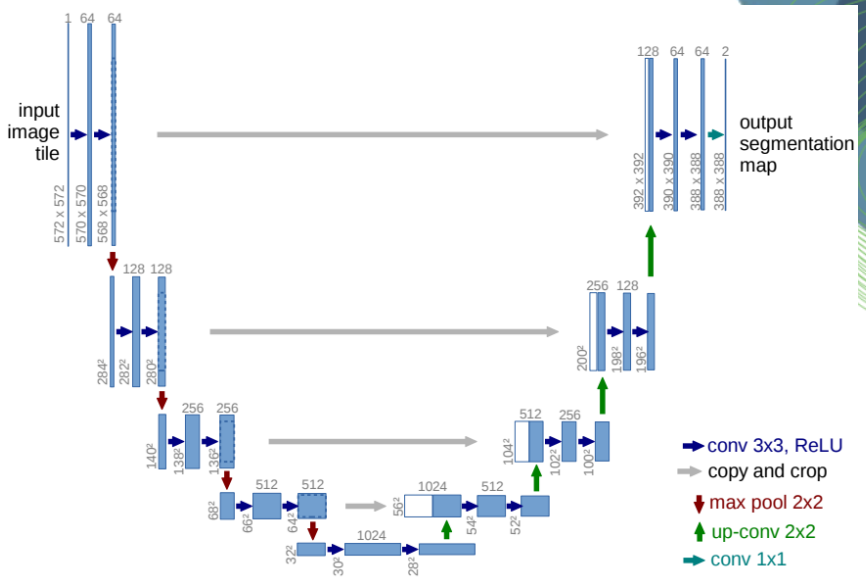
Gaussian

Top-hat

Sobel

Exercise: Survey

How is this neural network architecture called?



Auto-encoder

U-Net

Alex-Net

(Don't know)

Source: Ronneberger et al.
https://arxiv.org/pdf/1505.04597.pdf

Survey

Which of the following is no language model?

chatGPT

claude

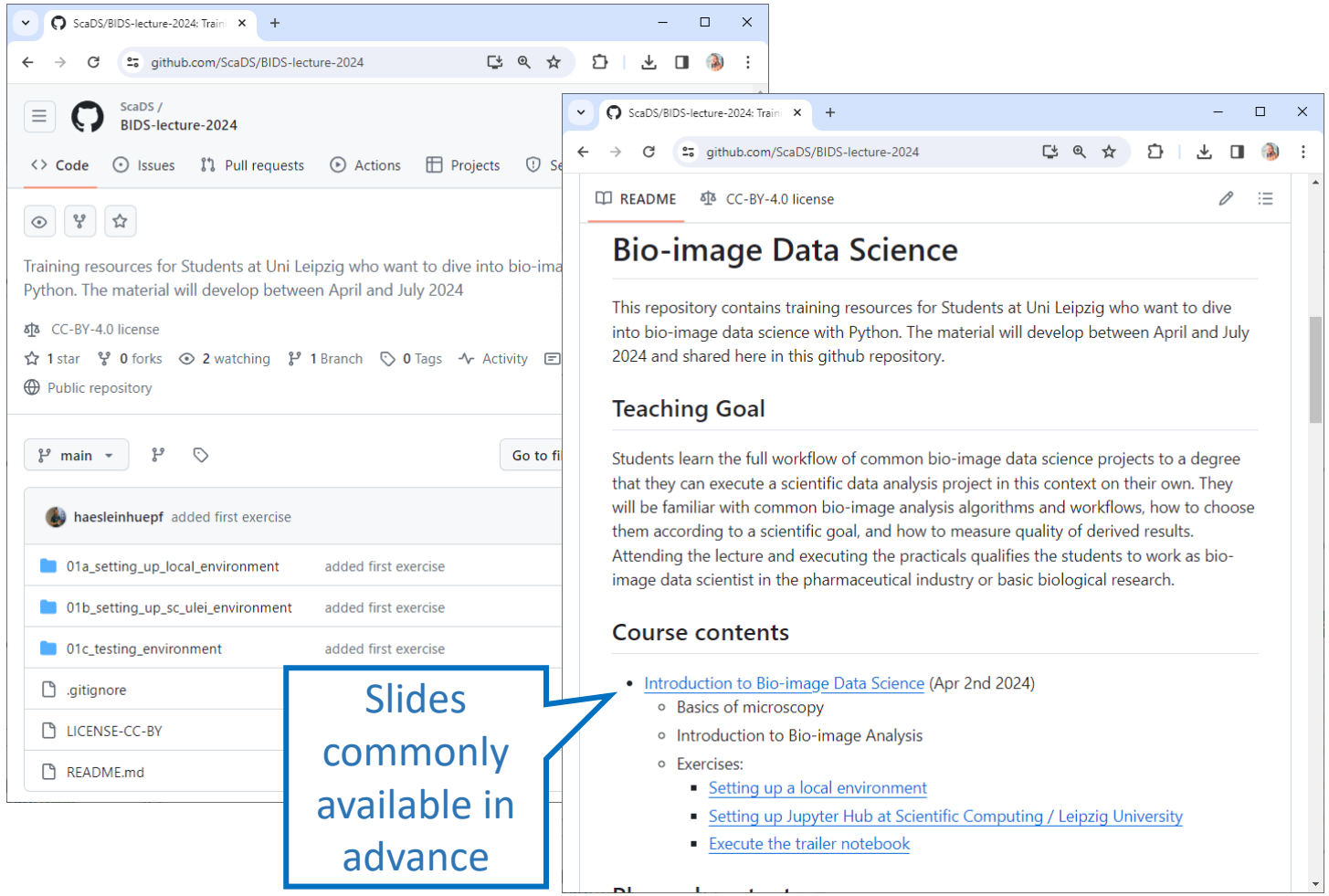
mistral

llama

Lecture overview

- Every week will follow the same rough scheme
  - 15:15 : 90 min lecture
  - 17:15 : 90 min exercises (programming intense)
  - when you're done, enjoy the sun!
- Exam will cover the semester content accordingly
  - Bio-image Analysis / Microscopy
  - Machine/Deep Learning
  - Generative Artificial Intelligence
  - "closed book exam"

Lecture materials



Slides commonly available in advance

Preliminary schedule

- April 2nd 2024 – Introduction Microscopy & Bioimage Analysis
- April 9th 2024 – Research Data Management
- April 15th 2024 - Microscopy Image Processing
- April 23rd 2024 – Segmentation of cells and nuclei
- April 30th 2024 - Quality Assurance for image segmentation

Handout exam pre-requisite complex exercise

- May 7th 2024 – Feature extraction, data visualization
- May 14th 2024 – Big data, parallel processing & distributed computing
- May 21st 2024 – Introduction to Machine Learning for bio-image analysis
- May 28th 2024 – Unsupervised Machine Learning
- June 4th 2024 – Deep Learning for image denoising + segmentation
- June 11th 2024 – Generative Artificial Intelligence (LLMs)
- June 18th 2024 – Image generation + vision models
- June 25th 2024 – Quality assurance

Flexible; I would like to focus as much as possible on LLMs

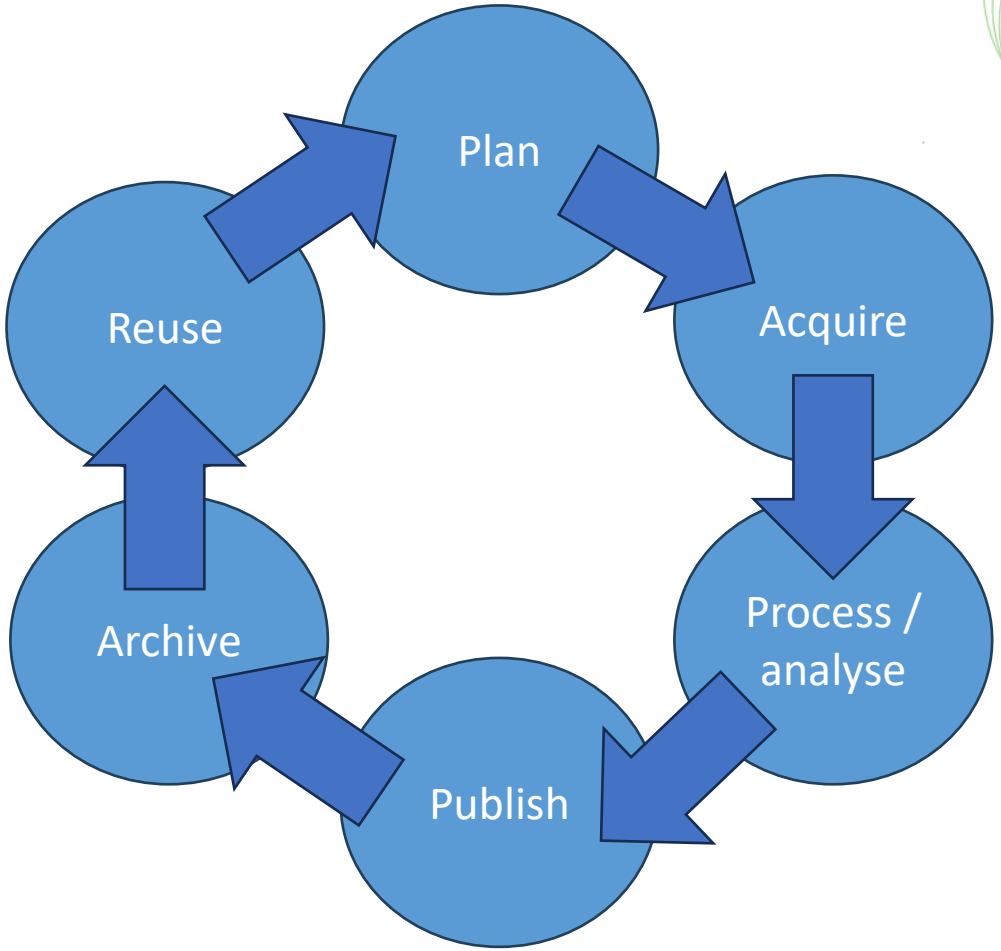
8 weeks

Submission deadline complex exercise

- July 2nd 2024 – Summary, exam preparation

Lecture overview: Research Data Management

- FAIR Principles
- Sharing / licensing
- Open Source code
- Data Management Plans
- Big-Data
- Distributed computing



Source: https://www.biorxiv.org/content/10.1101/2020.11.19.386565v1

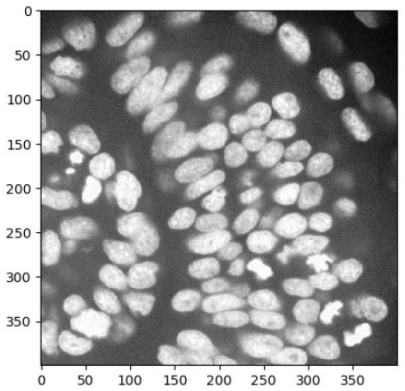
Lecture overview: Bio-image Analysis

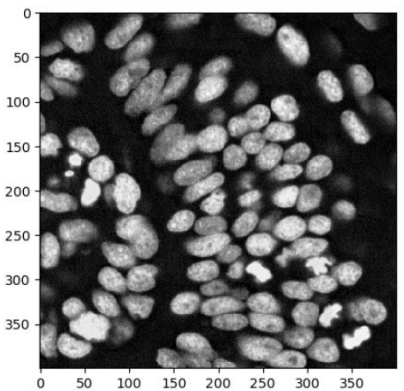
- Image Data Analysis workflows
- Goal: **Quantify observations, substantiate conclusions with numbers**

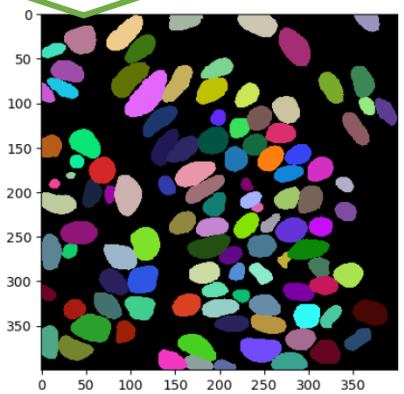
Machine learning: Pixel classification, Instance segmentation

Machine learning: Dimensionality reduction

Machine learning: Object classification

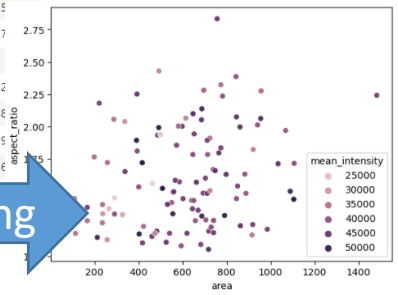






	area	mean_intensity	major_axis_length	minor_axis_length
0	594.0	40572.809764	28.611591	26.537947
1	645.0	43764.872868	33.511511	24.566916
2	1105.0	51970.561991	45.232031	31.456308
3	718.0	47015.487465	31.023274	29.5
4	791.0	49132.515803	36.382253	27.7
...	...	...	...	
105	238.0	30477.126050	20.252197	15.2
106	615.0	32886.154472	41.030760	19.8
107	110.0	33042.445455	14.366347	9.9
108	222.0	43304.180180	25.370549	11.6
109	167.0	43378.808383		

Plotting



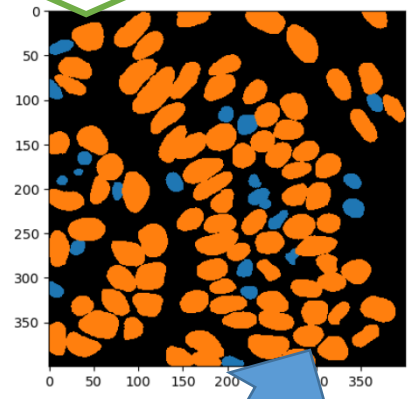
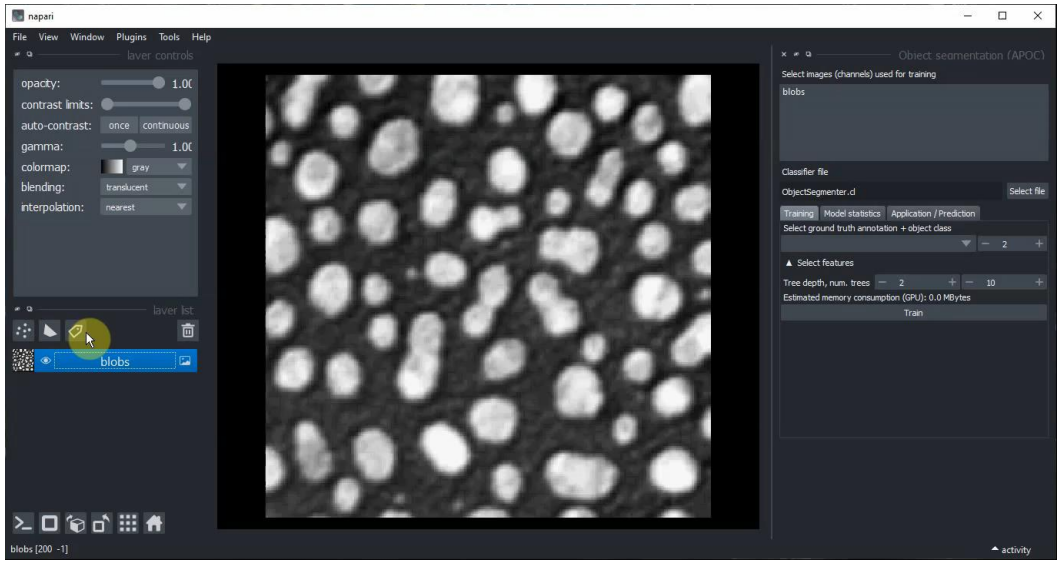
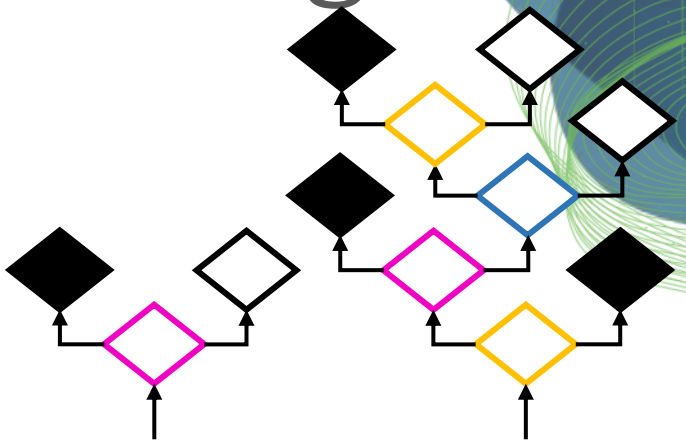


Image filtering → Image segmentation → Feature extraction → Object classification → Visualization

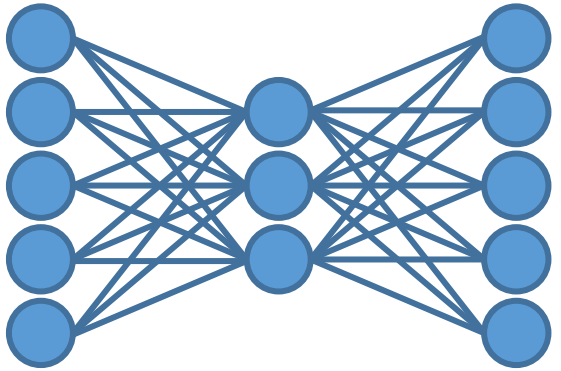
Lecture overview: Machine learning

- Machine learning
  - in the context of bioimage analysis
- Computers can *learn* from data, potentially revealing relationships that are not obvious to a human
- Goal: **Give you an insight into state-of-the-art methods**





Random forest classifiers

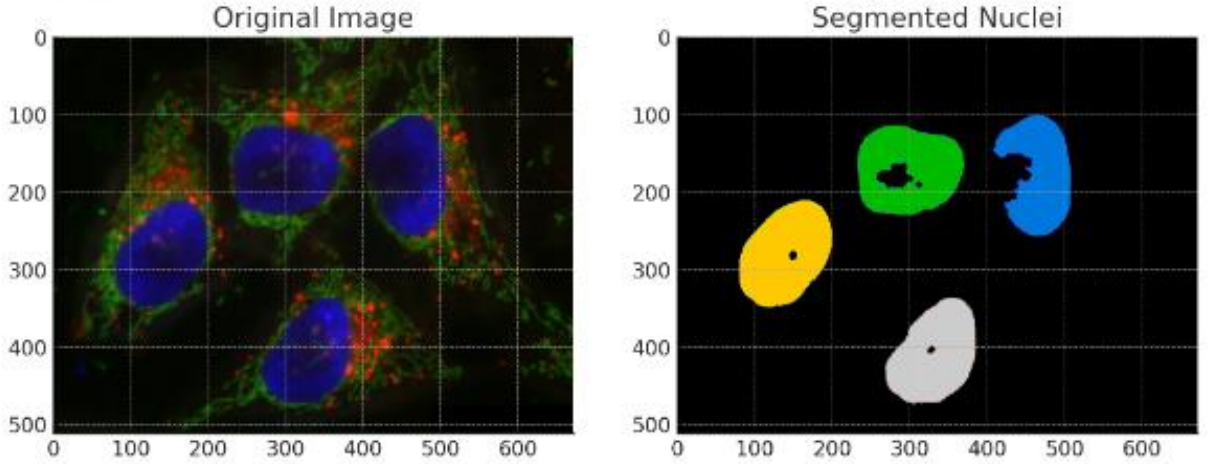


Neural networks

Lecture Overview: Generative AI Multi-modal / Large Language Models

Prompt:
“Please segment the nuclei in the blue channel of the image”

ChatGPT



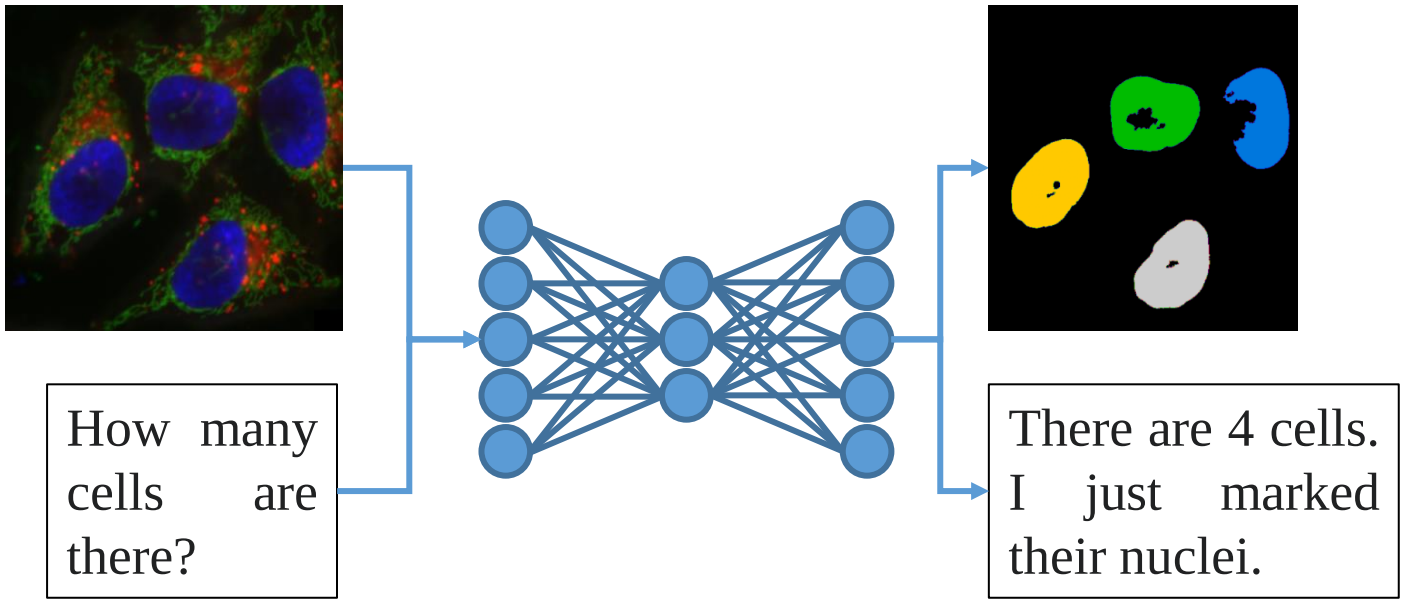
The segmentation of the nuclei has been performed on the blue channel of the image. The right side of the figure shows the segmented nuclei in different colors, each corresponding to a different nucleus in the field of view. Each color represents a unique label assigned to the individual nuclei detected in the image.

Analysis

```
from skimage import io, filters, measure, morphology
import matplotlib.pyplot as plt
import numpy as np

# Load the image
image = io.imread('/mnt/data/hela-cells-8bit.png')

# Since it's a color image, we'll just take the blue chann
blue_channel = image[:, :, 2]

# Threshold the image to get the binary image for nuclei
thresh = filters.threshold_otsu(blue_channel)
binary = blue_channel > thresh

# Remove small objects and perform morphological closing t
cleaned = morphology.remove_small_objects(binary, 50)
closed = morphology.closing(cleaned, morphology.disk(3))

# Label the image
label_image = measure.label(closed)

# Plot the original image and the binary image
fig, ax = plt.subplots(1, 2, figsize=(12, 6))
ax[0].imshow(image)
ax[0].set_title('Original Image')
ax[1].imshow(label_image, cmap='nipy_spectral')
ax[1].set_title('Segmented Nuclei')
plt.show()
```

Lecture Overview: Generative AI Multi-modal / Large Language Models

After we learned how analyze images, we will teach an LLM to do it.



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Basics of Microscopy

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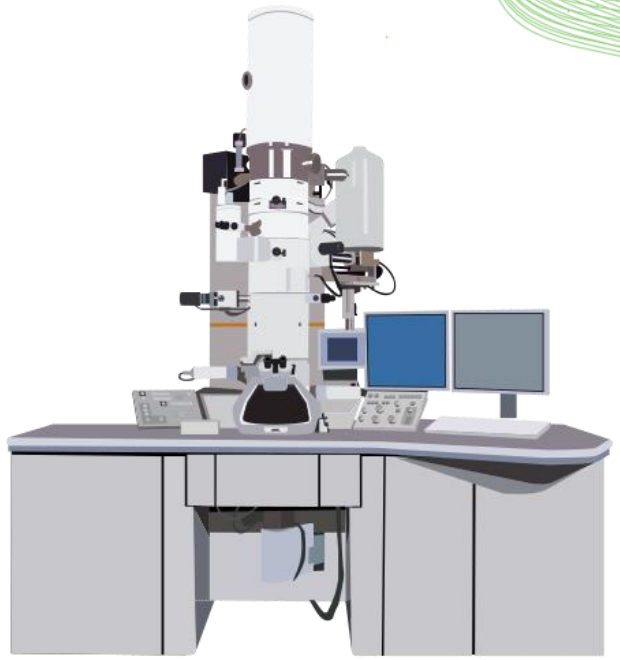
Microscopy

- Common tool to answer biological questions

Transmitted light microscope

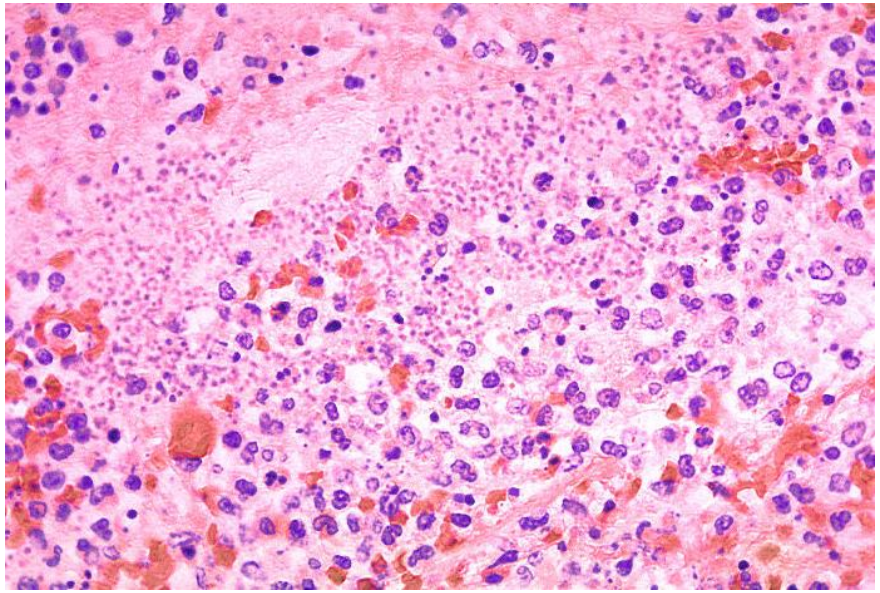
Fluorescence microscope



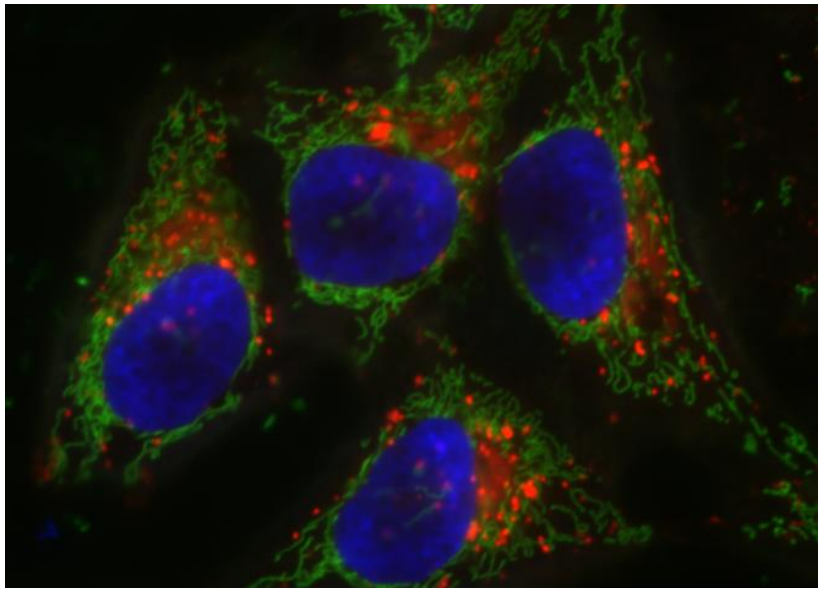
Electron microscope

Microscopy

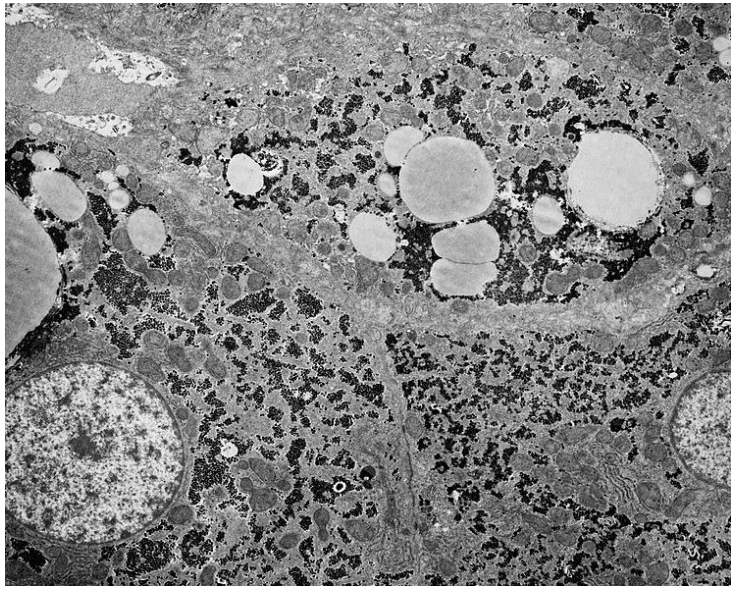
- Common tool to answer biological questions



Transmitted light microscope



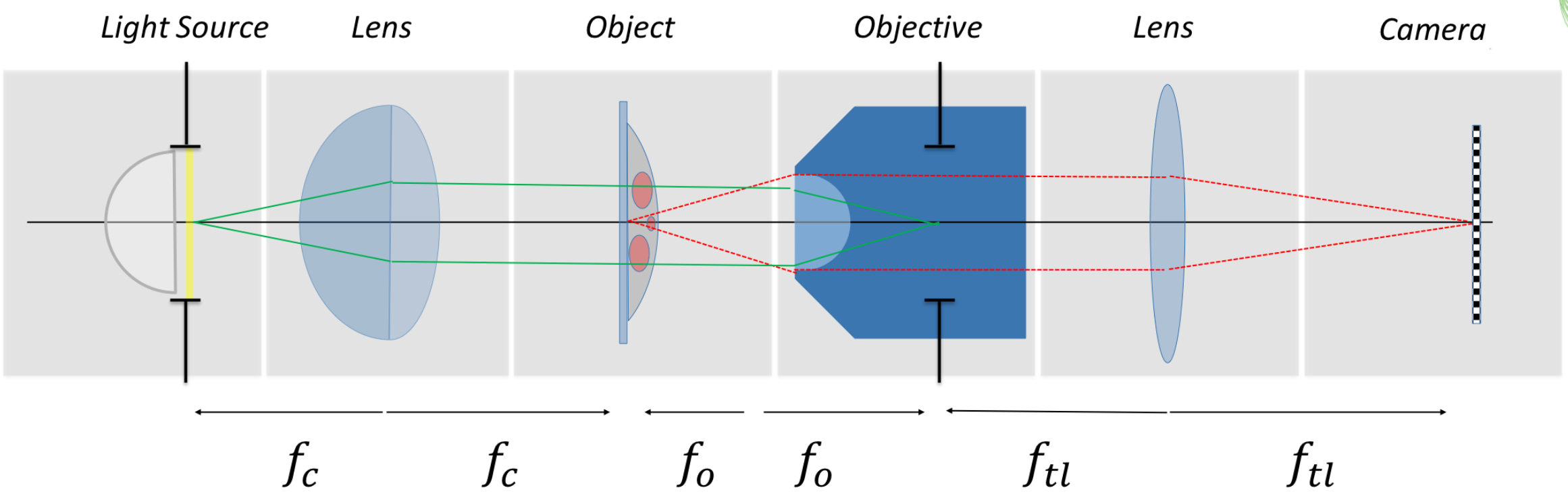
Fluorescence microscope



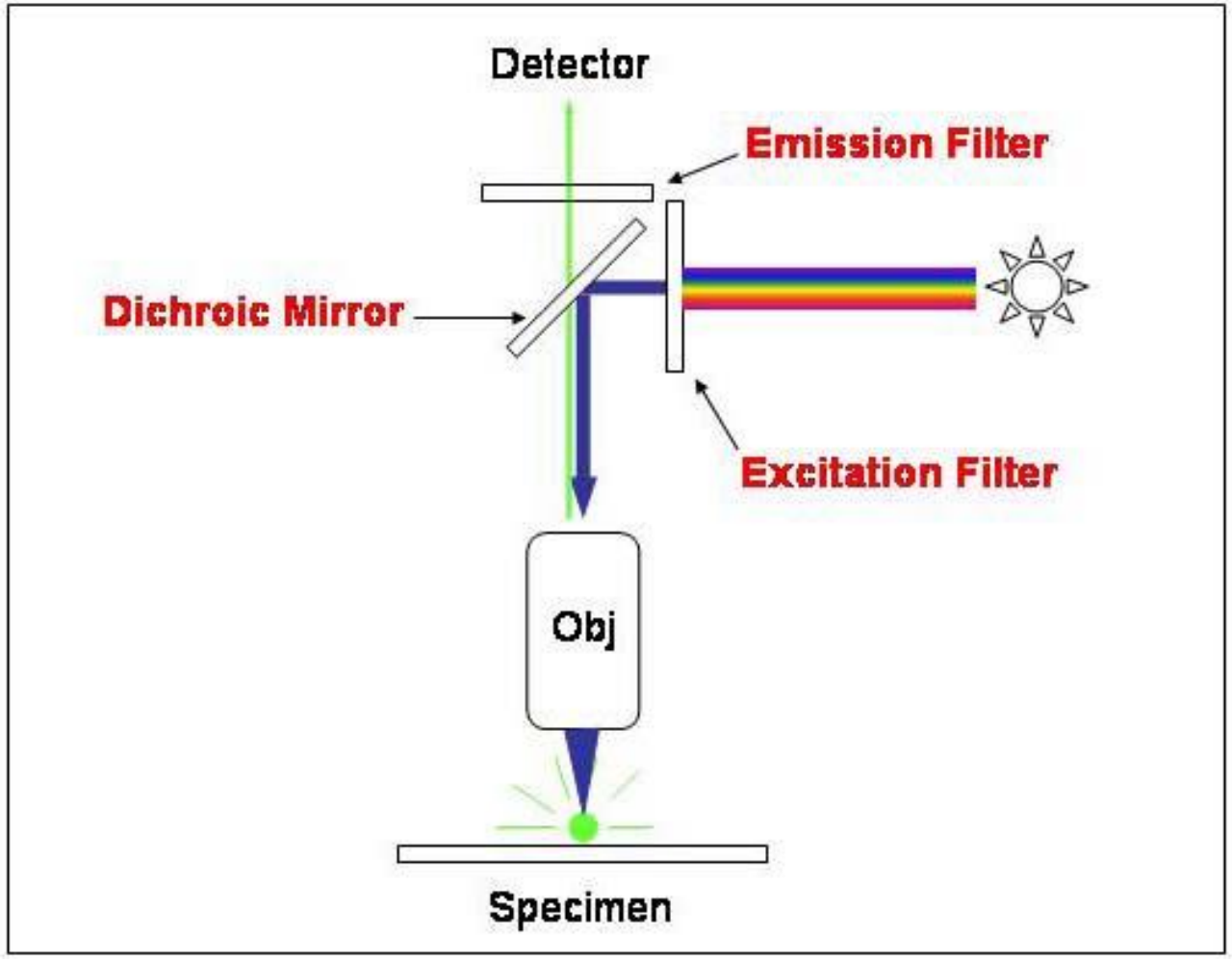
Electron microscope

Sources:
https://commons.wikimedia.org/wiki/File:Histo_Lungenpest.jpg, by PHIL, license public domain
Example image of ImageJ, by Tony Collins, license: public domain
https://commons.wikimedia.org/wiki/File:Electron_microscopy_of_cells_with_cytoplasmic_glycogen_granules.jpg , by Mike Kayser, licensed CC-BY 4.0

Transmitted light microscopy



Fluorescence Microscopy

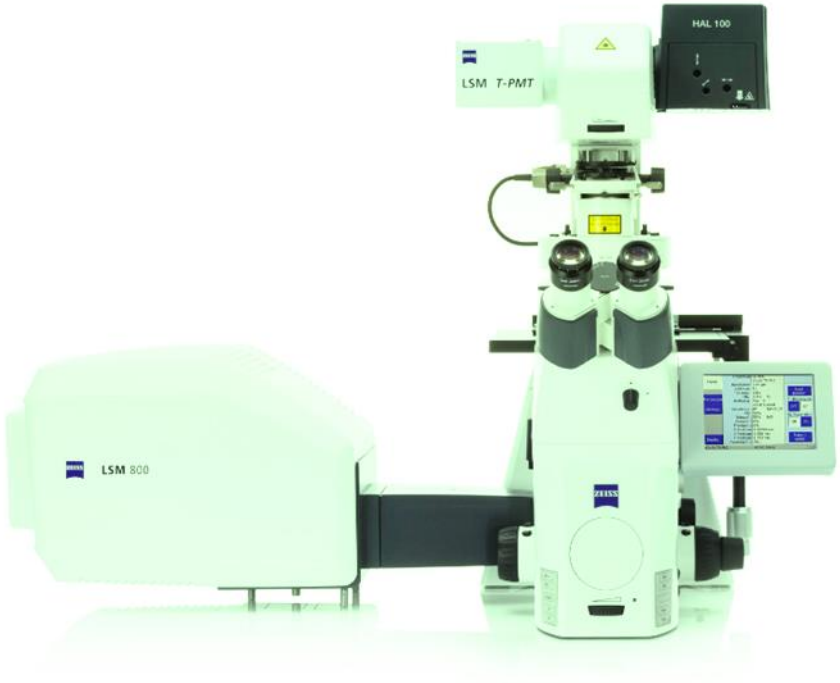


Source: https://commons.wikimedia.org/wiki/File:FluorescenceFilters.jpg by Jennifer Waters, license: public domain

Fluorescence Microscope

- NOT Fluorescent microscope

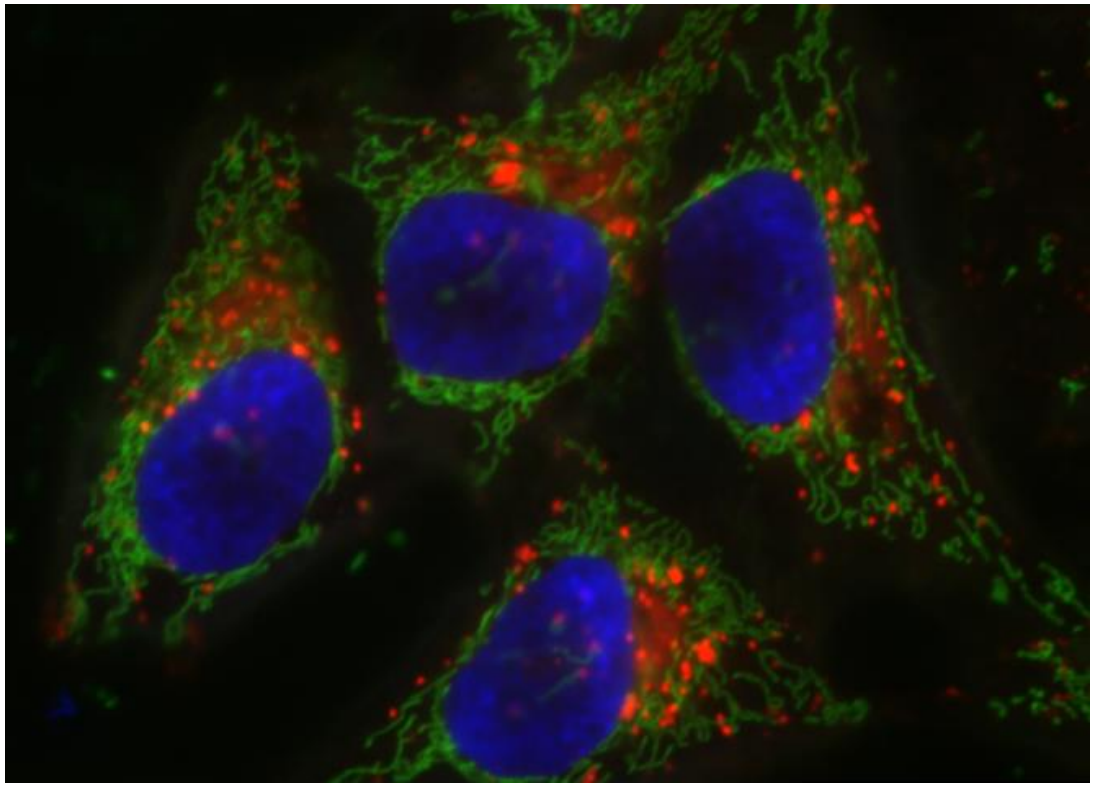
Fluorescence microscope

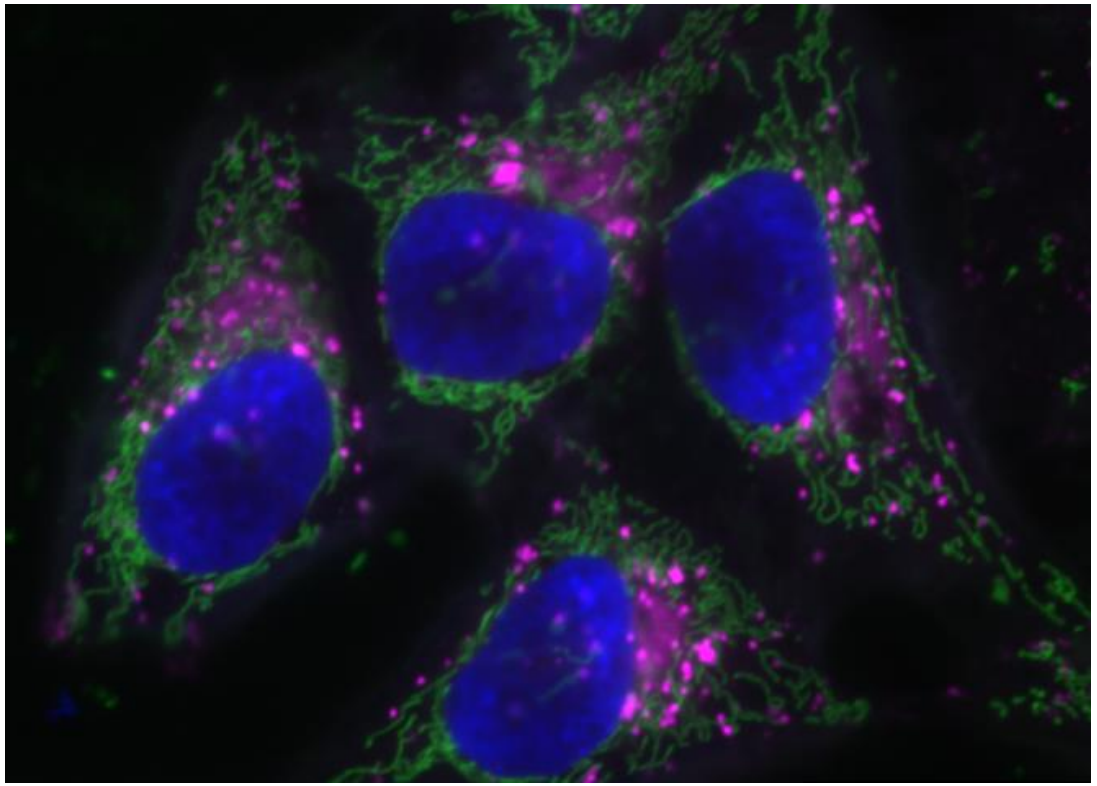


Fluorescent microscope

Color maps / lookup tables

- Just because you see something in red, doesn't mean it was imaged emitting red light.



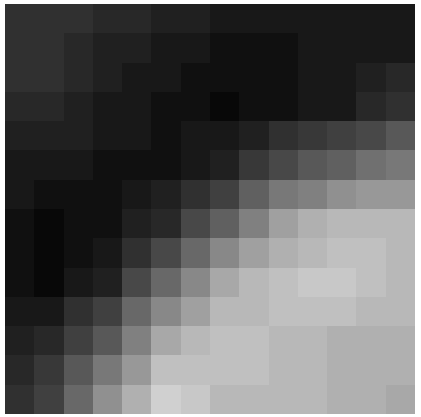


Source: Example image of ImageJ, by Tony Collins, license: public domain

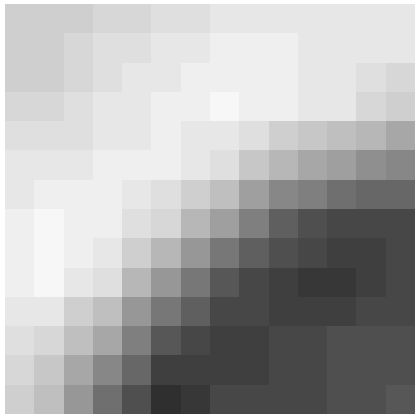
Color maps / lookup tables

- The lookup table decides how the image is displayed on screen.
- Applying a different lookup table doesn't change the image. All pixel values stay the same, they just appear differently

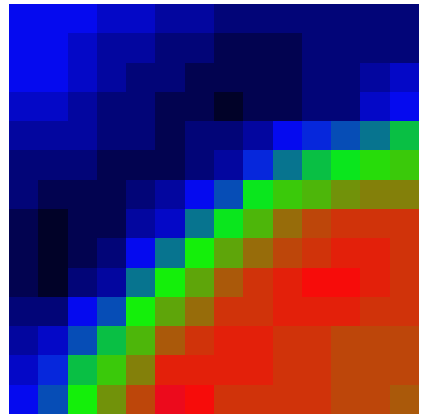
Pixel value	Display color
0 1 2 … 255	



Pixel value	Display color
0 1 2 … 255	



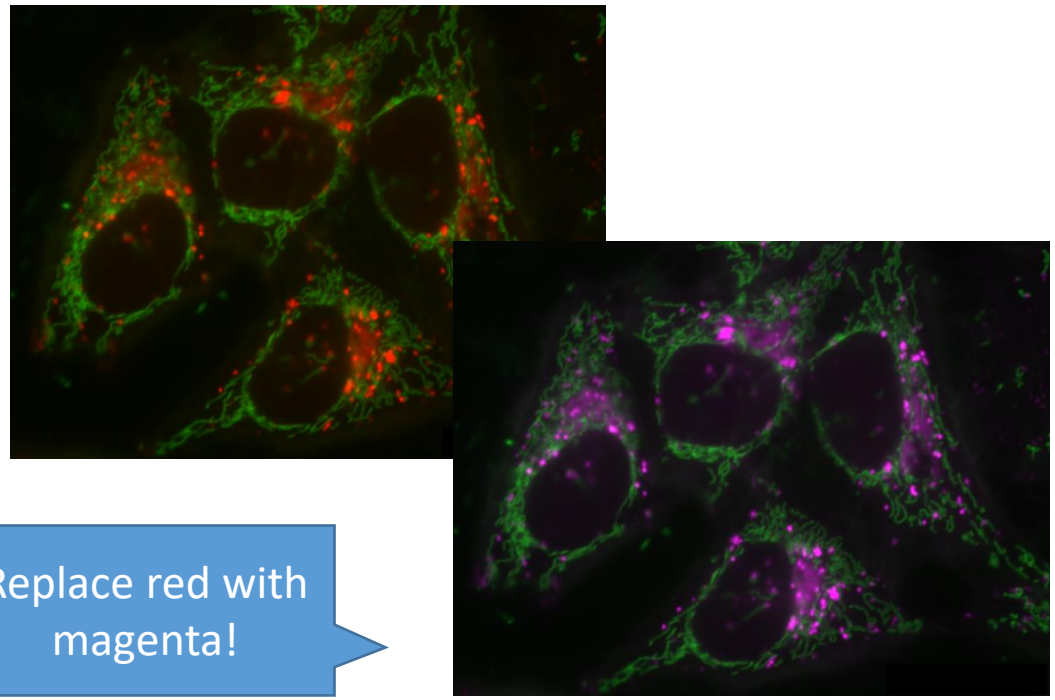
Pixel value	Display color
0 1 2 … 255	



Color maps / lookup tables

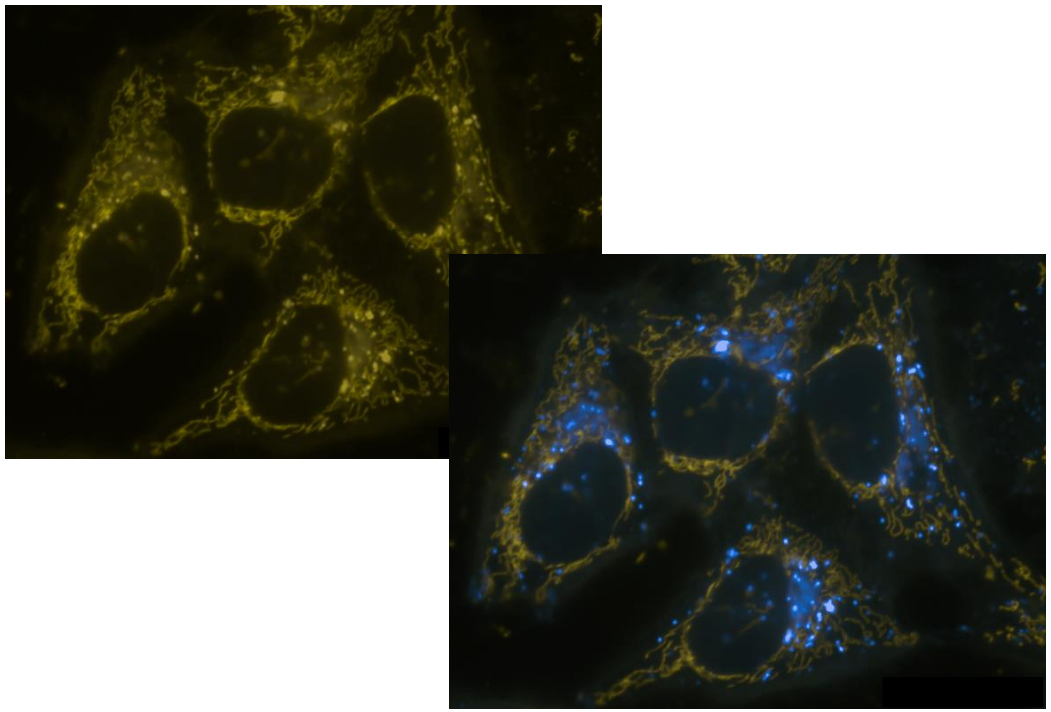
- Choose visualization of your color tables wisely!
- Think of people with red/green blindness!

Default view



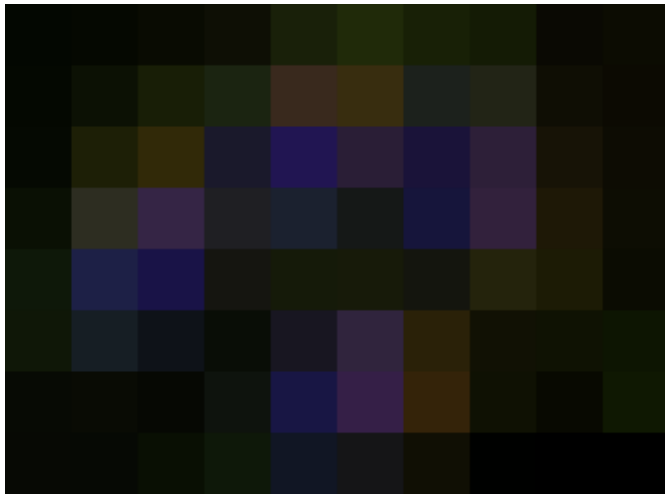
Replace red with magenta!

Red/green blind people see it like this

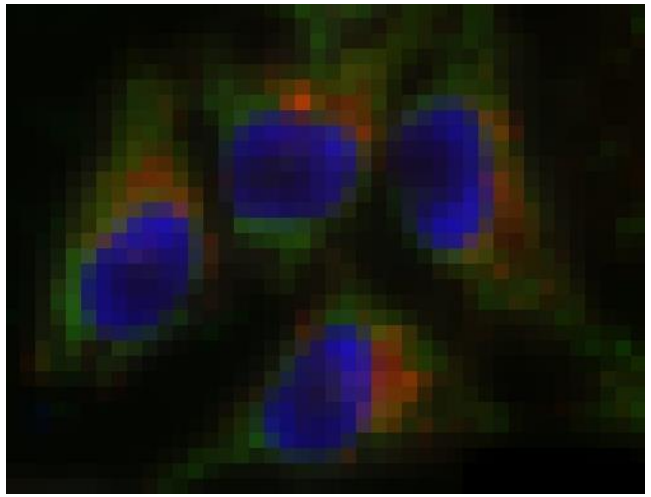


Pixel size versus resolution

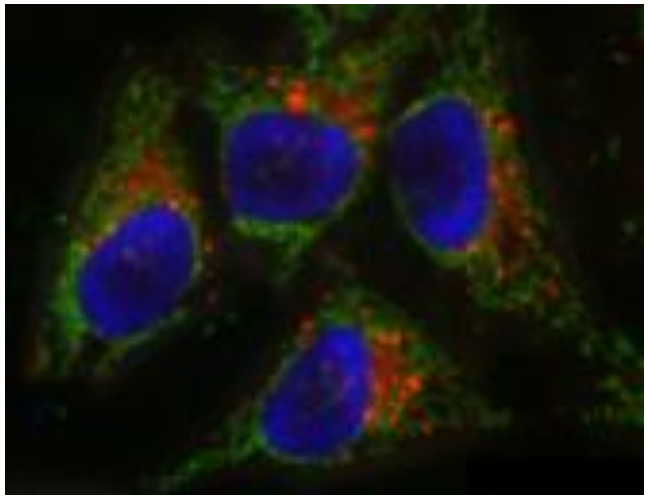
- Pixel size is a property of a digital image.
- You configure it during the imaging session at the microscope.



Pixel size: 3.3 µm



Pixel size: 0.8 µm

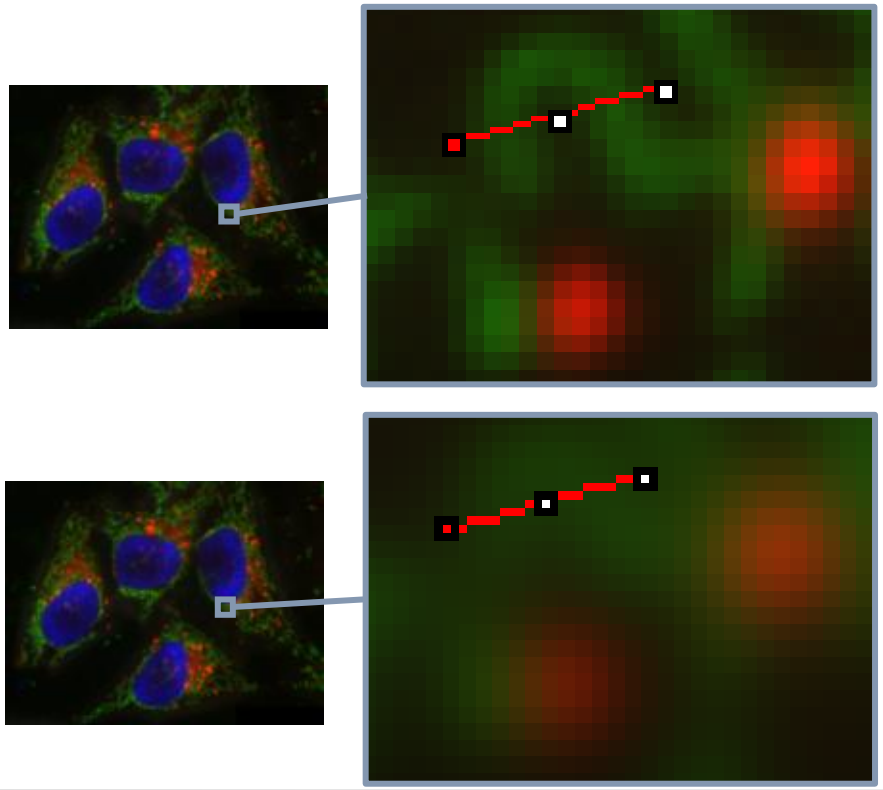


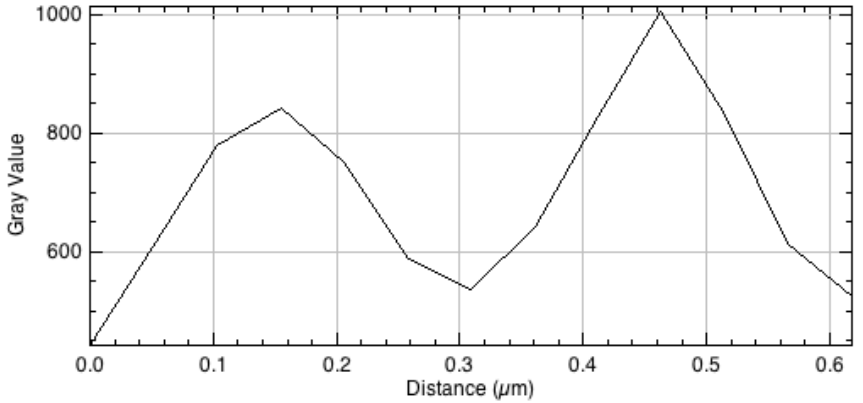
Pixel size: 0.05 µm

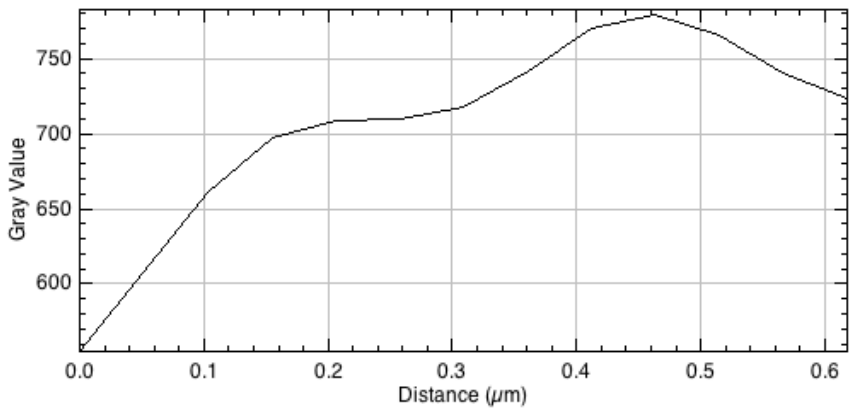
- We are not talking about resolution!

Pixel size versus resolution

- Resolution is a property of your imaging system.
- How small can objects be, to be still differentiable?







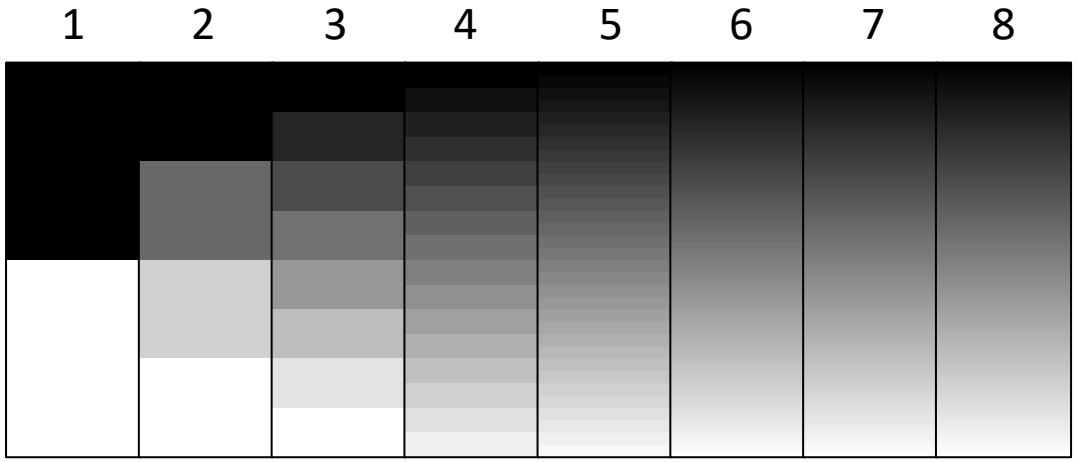
Quiz

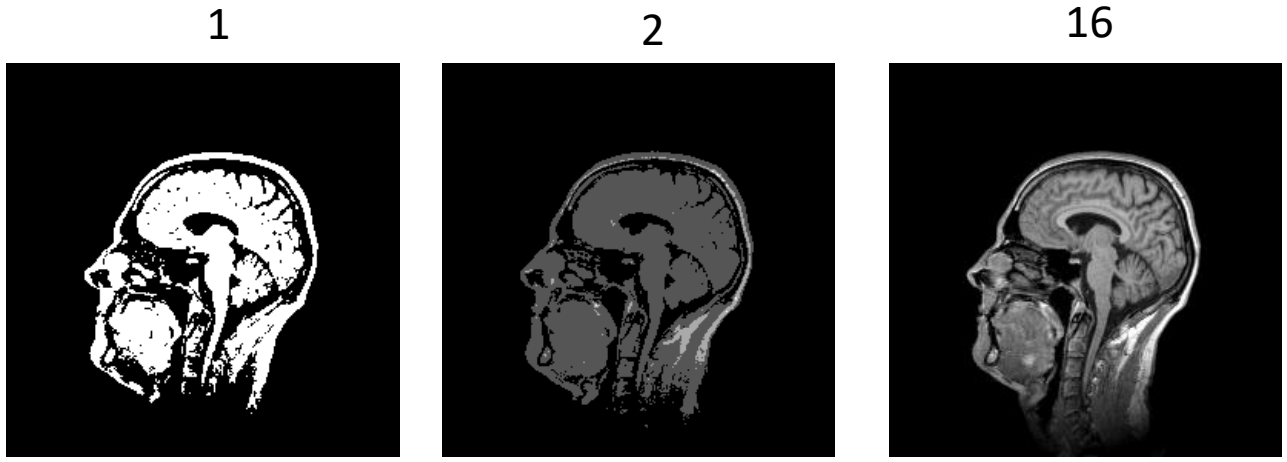
- How can I change the pixel size at a microscope practically?

Move sample	Enter in software	Change camera	Change objective

Bit-depth

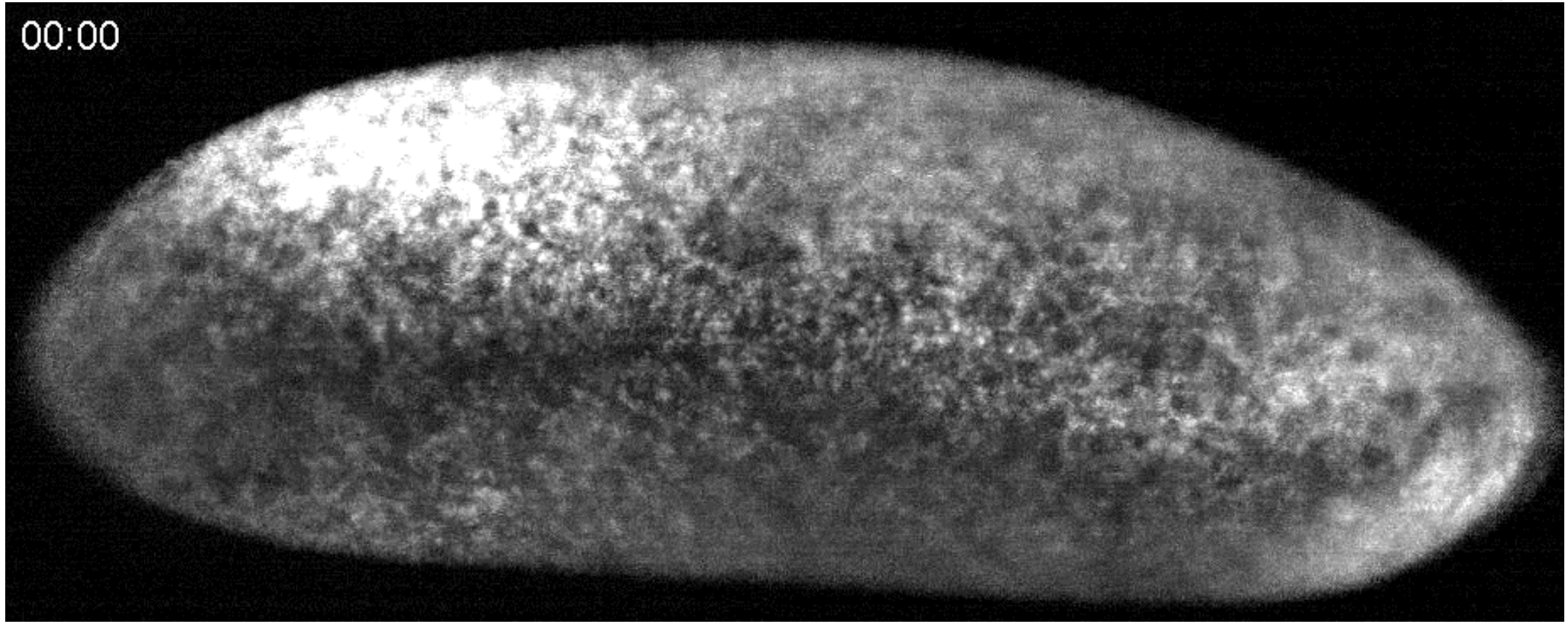
- A bits is the smallest memory unit in computers, *atomic data*.
- The bit-depth *n* enumerates how many different intensity values are present in an image:
  - 2^n grey values
- In microscopy, images are usually stored as 8, 12 or 16-bit images.
- In computer vision, 8-bit integer and 32-bit float (range 0…1) is more common





Light-sheet fluorescence microscopy

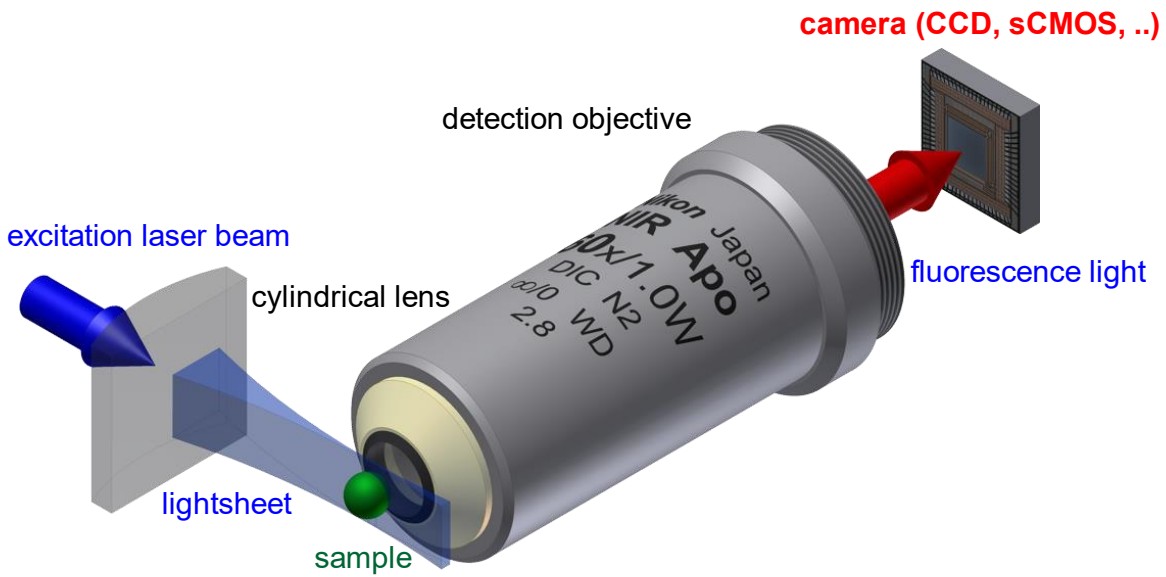
- Popular tool for large-volume imaging

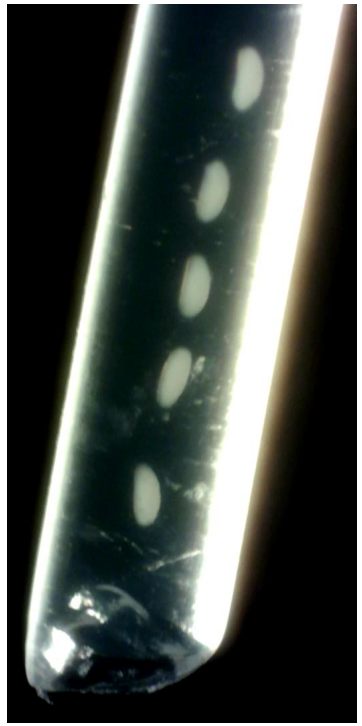


Drosophila melanogaster, histone-GFP marked

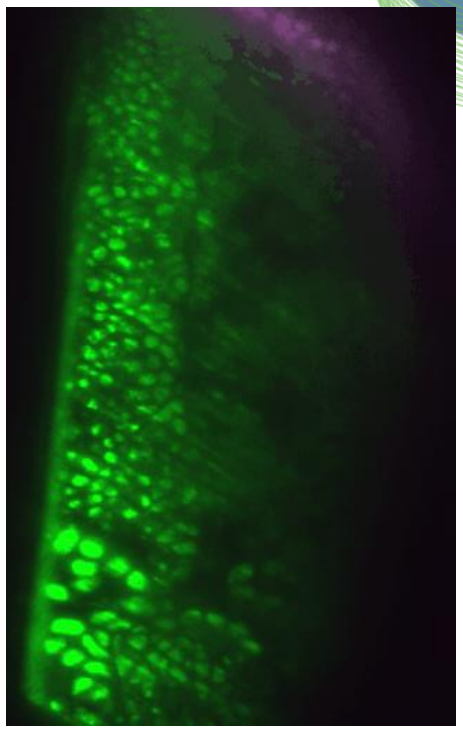
Light-sheet fluorescence microscopy

- Single-plane illumination microscopy (SPIM)





Sample mounting

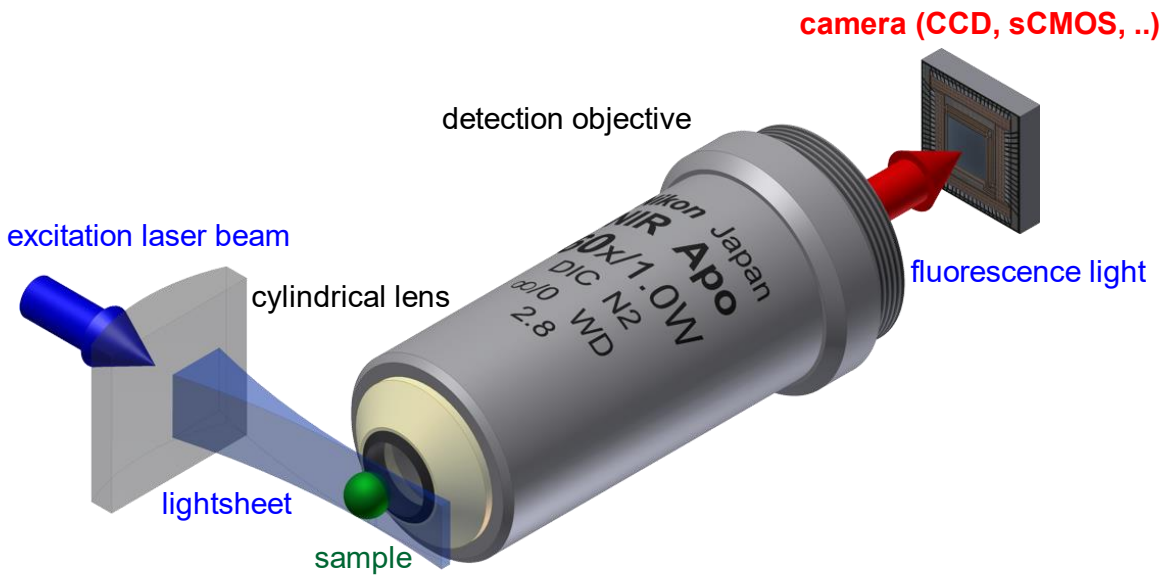


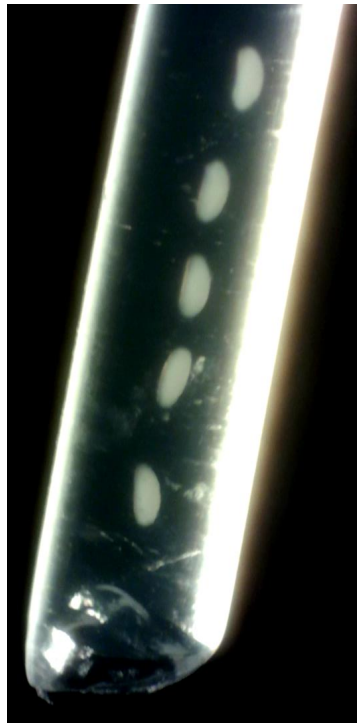
Laser beam scanning

Figure left, source: Wikimedia Commons by Jan Krieger, licensed CC BY-SA 3.0

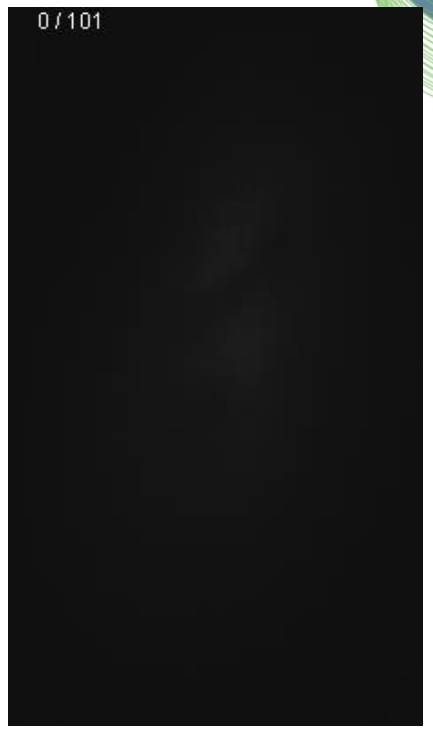
Light-sheet fluorescence microscopy

- Single-plane illumination microscopy (SPIM)





Sample mounting

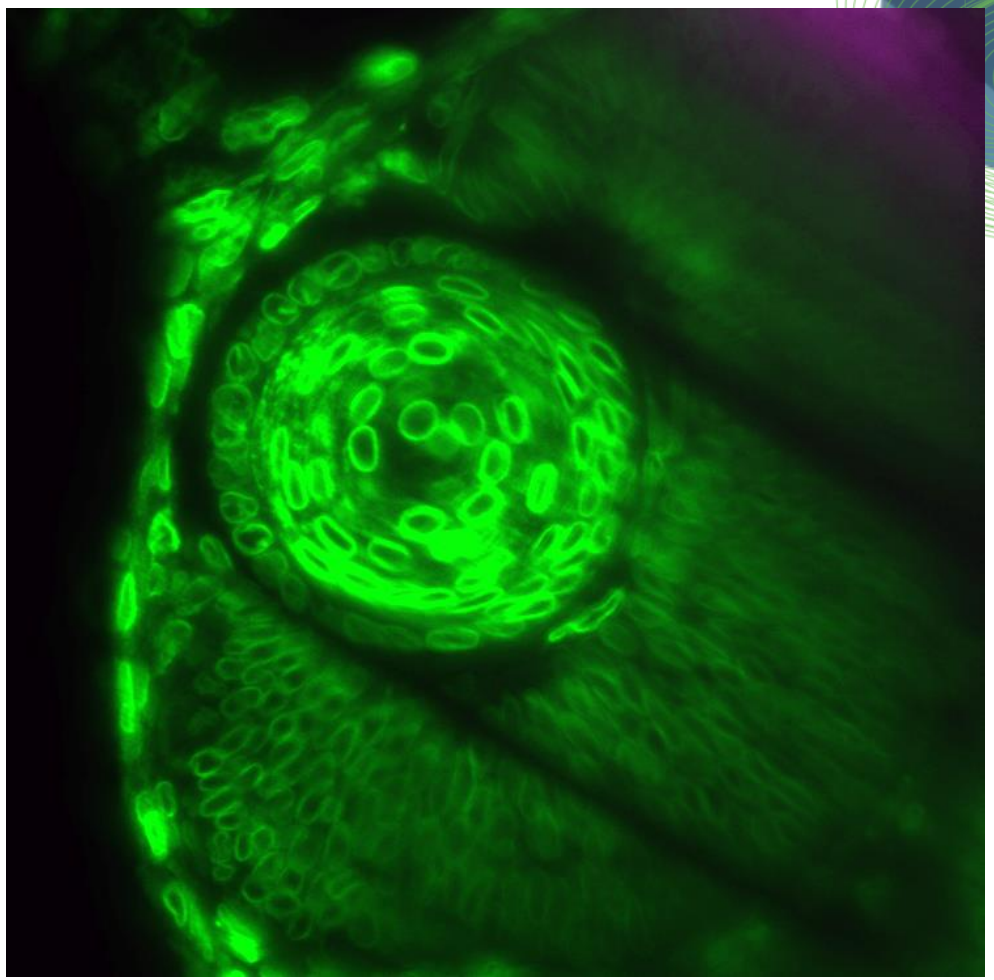


Plane scanning

Figure left, source: Wikimedia Commons by Jan Krieger, licensed CC BY-SA 3.0

Light-sheet fluorescence microscopy

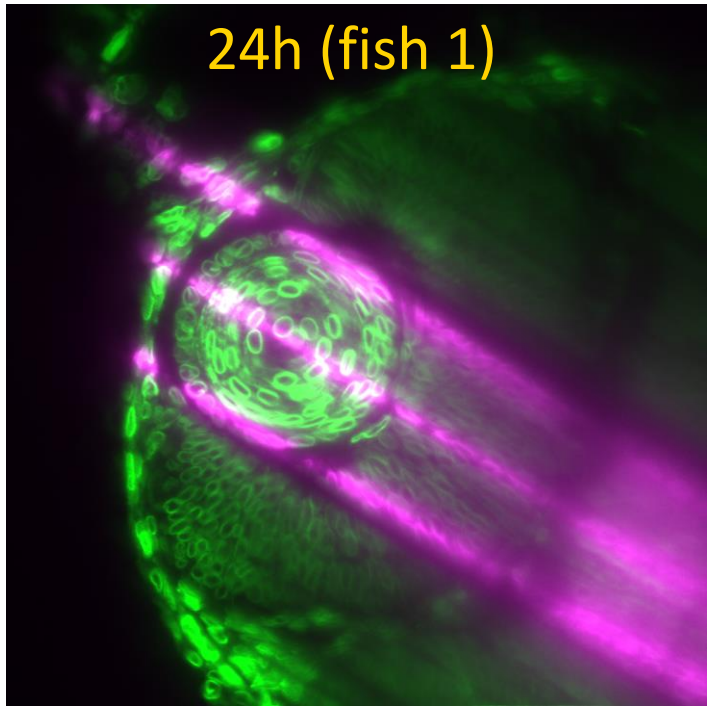
- The beam interacts with the tissue while passing matter
  - Refraction
  - [Reflection]
  - Scattering
- Challenge for quantitative analysis: image quality varies within the image

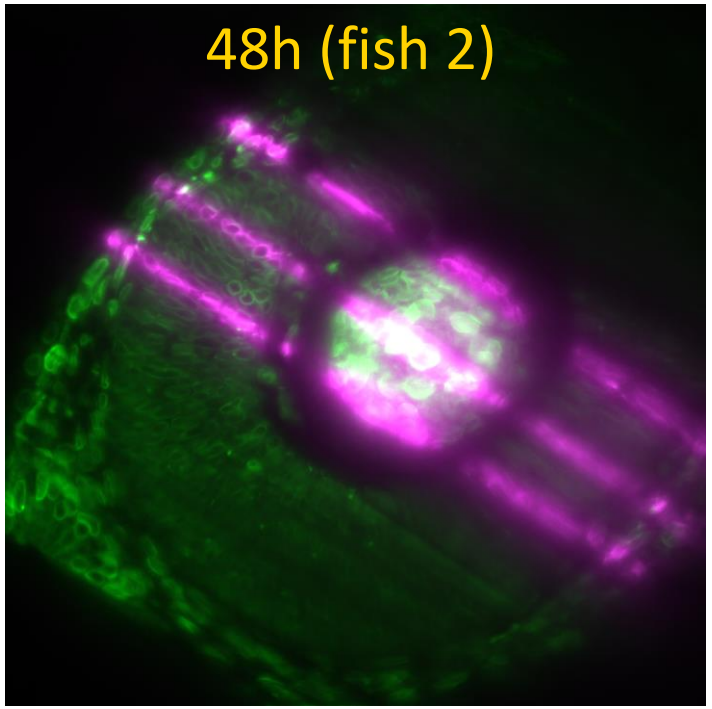


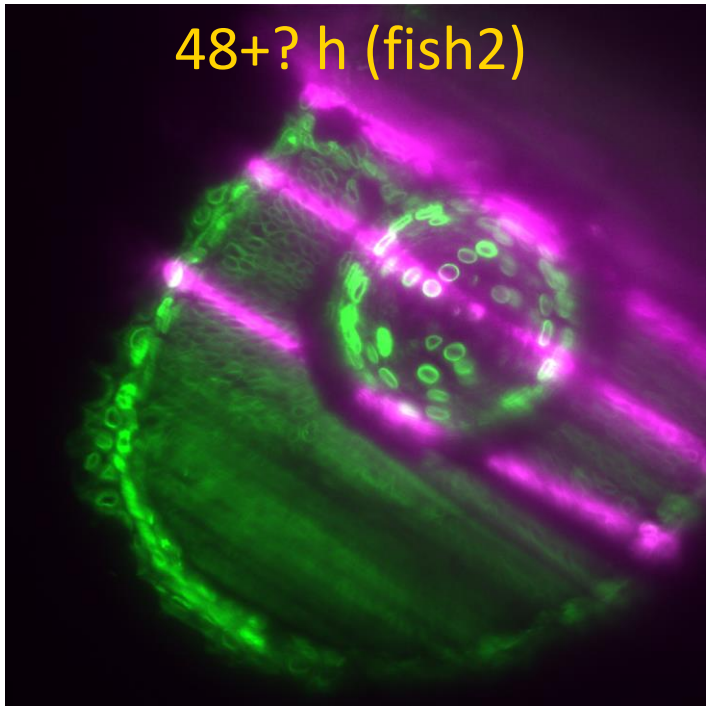
Zebrafish [eye] sample kindly provided by Mauricio Rocha Martins (Myers/Norden lab, MPI-CBG Dresden)

Light-sheet fluorescence microscopy

- These effects depend on the sample
- Big challenge in practice: *Image deep inside*







Zebrafish [eye] sample kindly provided by Mauricio Rocha Martins (Myers/Norden lab, MPI-CBG Dresden)

Light-sheet fluorescence microscopy

- These effects depend on the sample
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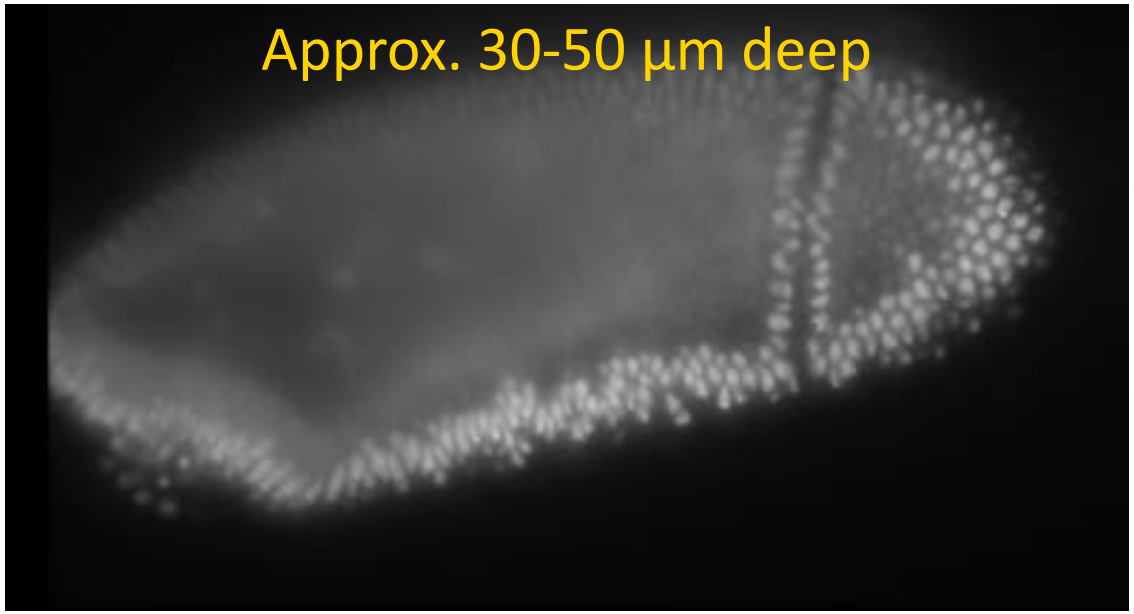
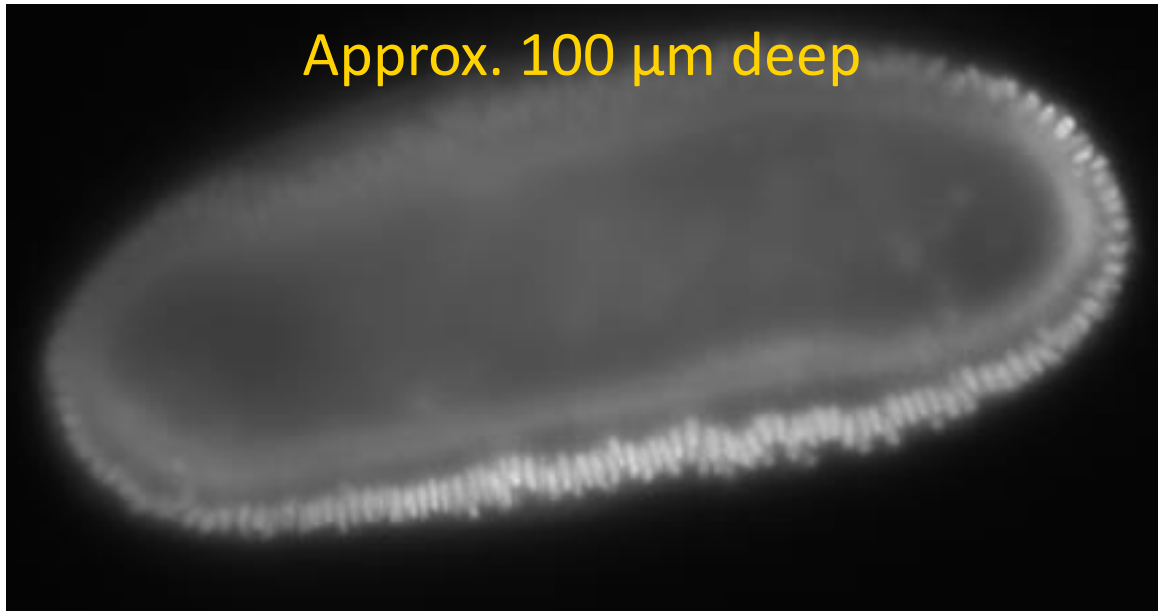
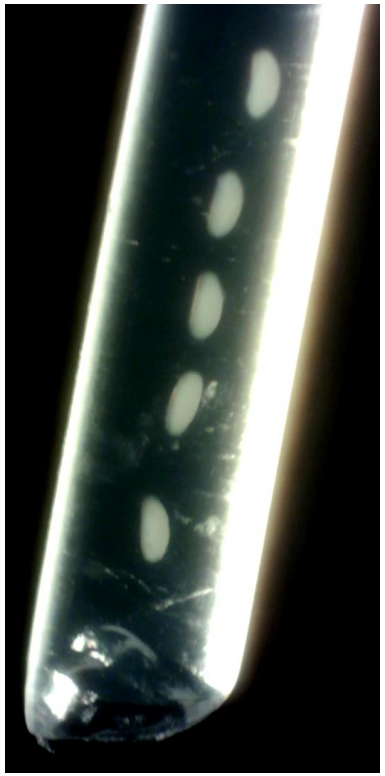
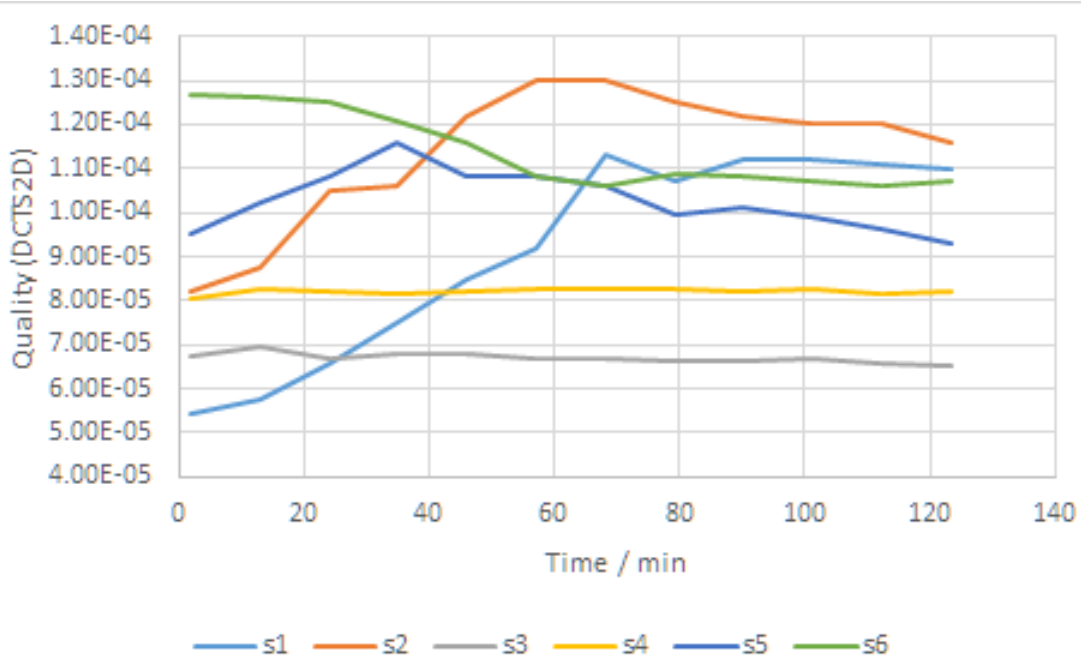




High-throughput imaging

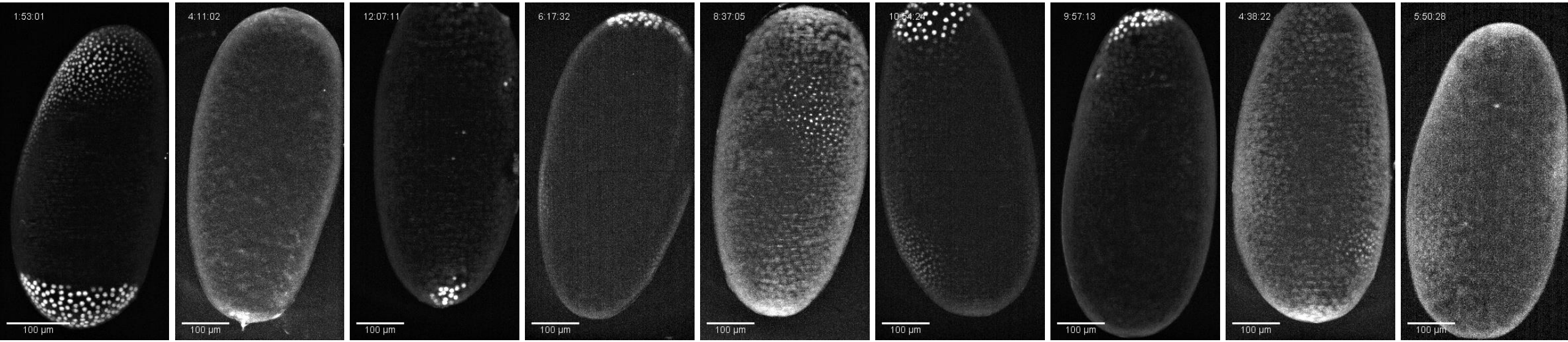
- Challenge: Online decision making while acquisition
  -> AI





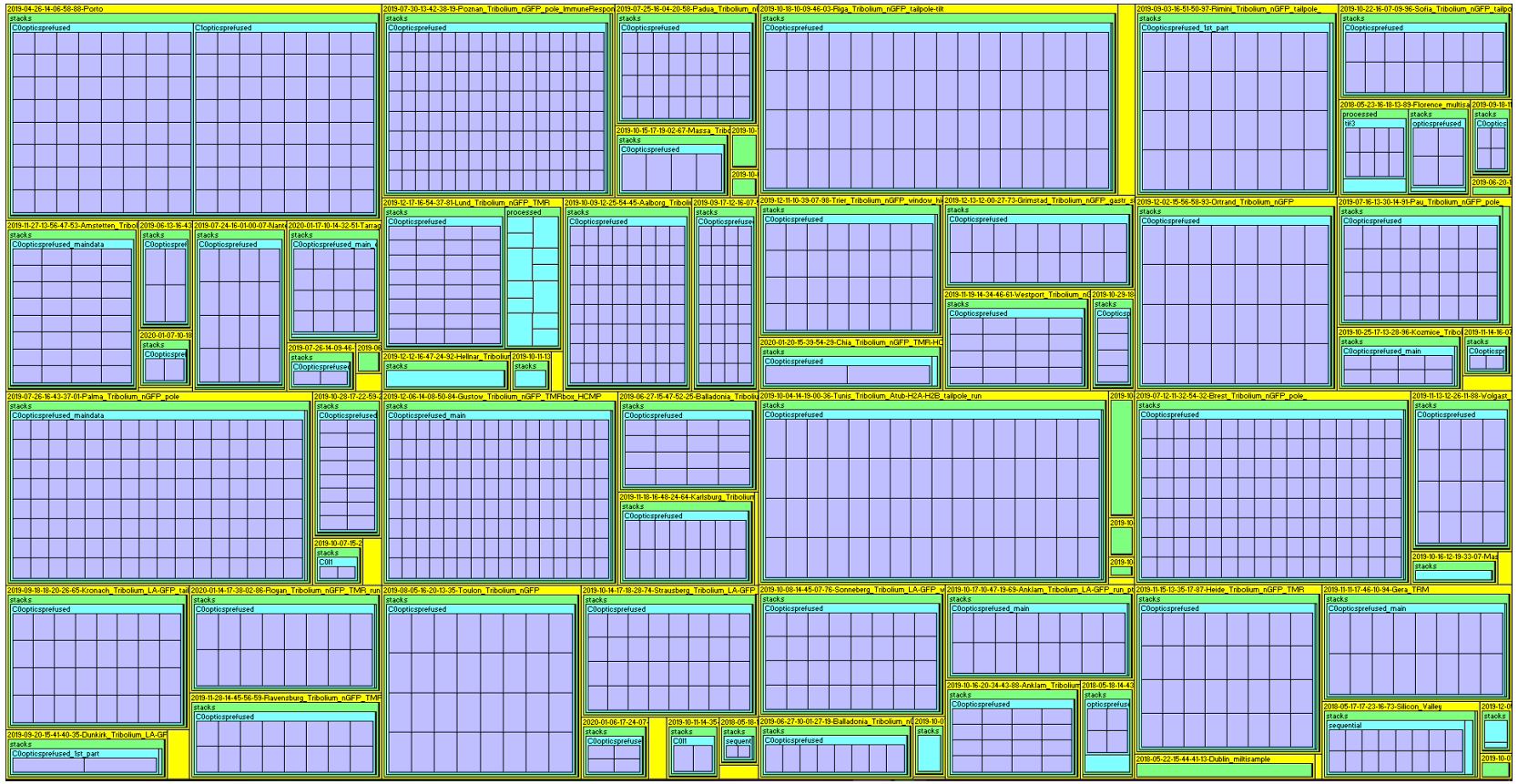
High-throughput imaging

- Quality variation between samples, in space and time [and algorithms which process the data]



High-throughput imaging

- “Big data”



Postdoc 2 years imaging

- 35 TB imaging data

Assuming

- one frame is about 200 MB
- counting nuclei takes 30 sec per frame

Just counting nuclei in all the data would take 2 months.

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AND ARTIFICIAL INTELLIGENCE

Introduction to Bioimage Analysis

Robert Haase

GEFÖRDERT VOM
Bundesministerium
für Bildung
und Forschung

SACHSEN
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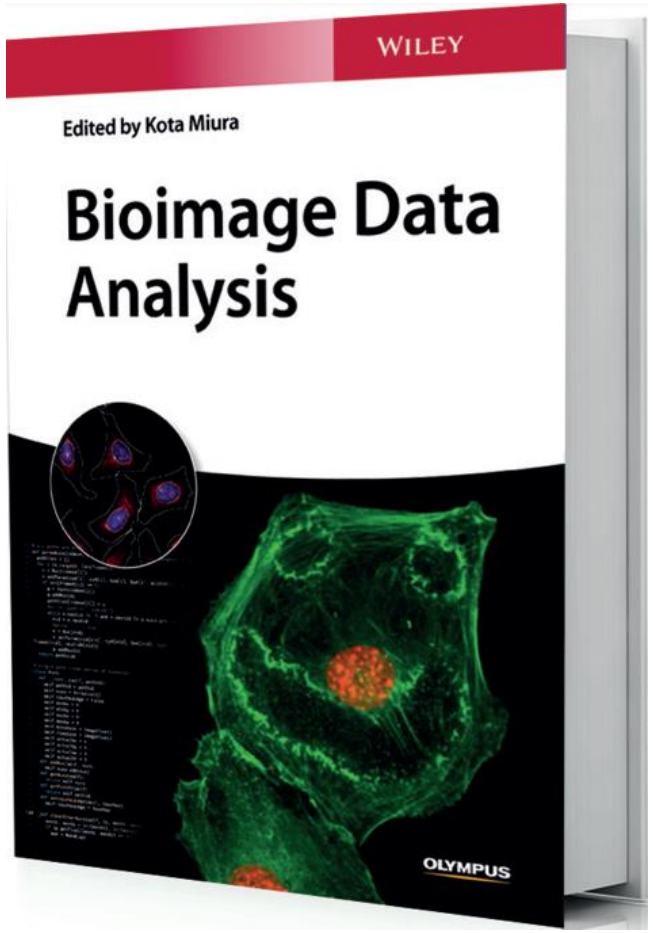
Diese Maßnahme wird mitfinanziert durch Steuermittel auf der Grundlage des von den Abgeordneten des Sächsischen Landtags beschlossenen Haushaltes.

Bioimage Analysis

- Kota Miura & Sebastien Tosi 2015:

In the light of this definition, image analysis, which is also called "computer vision," aims at mimicking the way we see the world and how we identify its visible structures. Image analysis in biology does undeniably also hold this element, but more importantly, its main goal is to *measure* biological structures and phenomena in order to study and understand biological systems in a quantitative way.

To achieve this task, we in fact do not have to be bothered with similarity to the human recognition – we have more emphasis on the objectivity of quantitative measurement, rather than how that computer-based recognition becomes in agreement with human recognition. Therefore, in biology, image analysis is a process of identifying spatial distribution of biological components in images and measuring their characteristics to study their underlying mechanisms in an unbiased way. To underline this difference in the goals of image analysis in the two fields and to distinguish them from each other, we will now on refer to image analysis in biology as *bioimage analysis.*



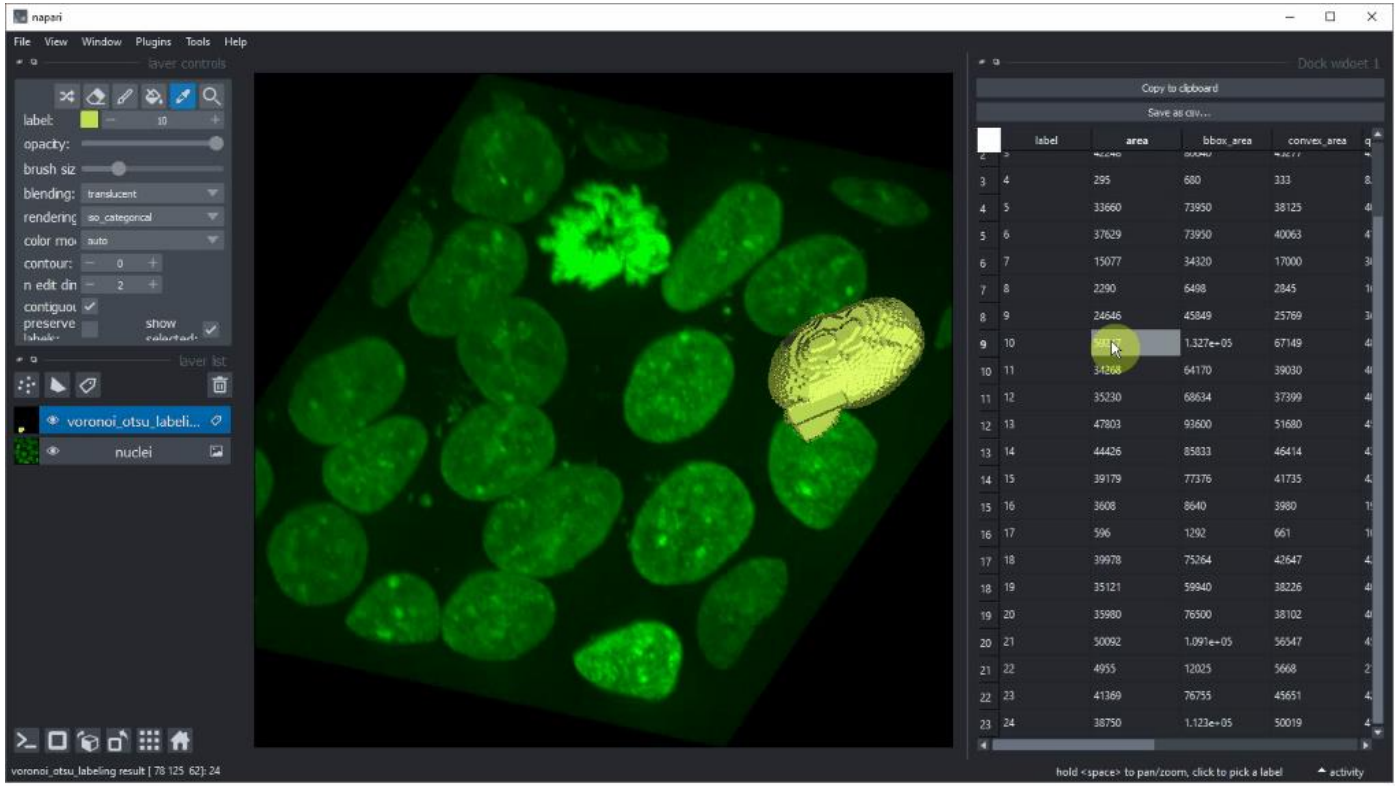
Quantitative bio-image analysis

- Deriving quantitative information from images of biological samples taken with microscopes

cat height = 1.5 x microscope height

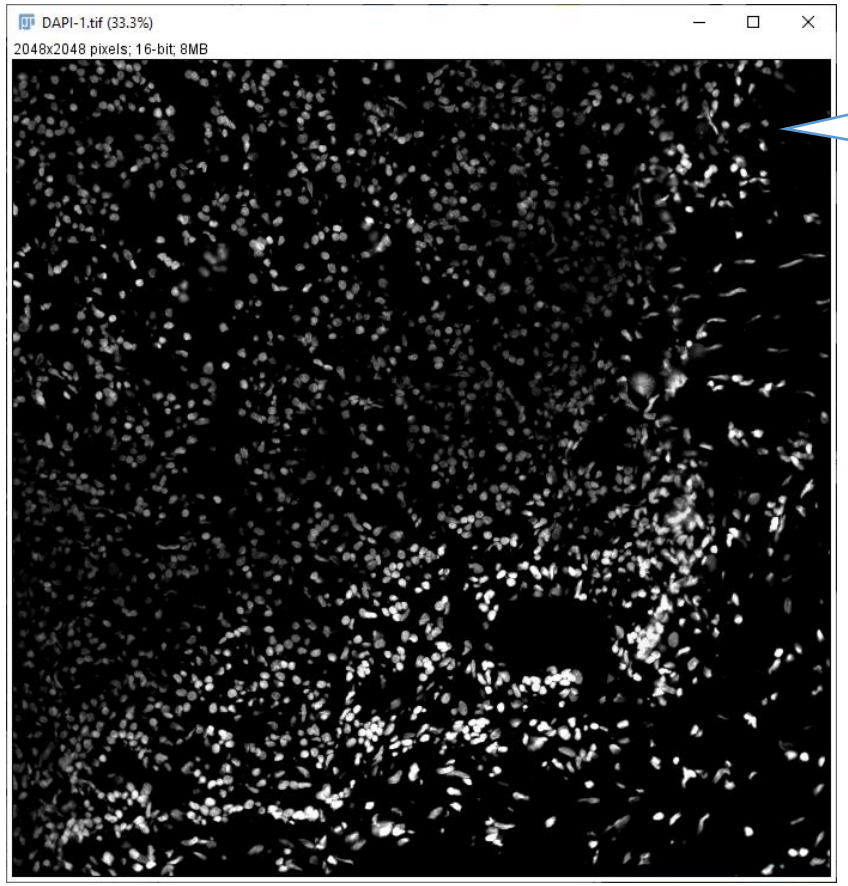
Quantitative bio-image analysis

- Deriving quantitative information from images of biological samples taken with microscopes + visualization



Objective bio-image analysis

- Measurements should be objective, not influenced by human interpretation



Nuclei in this image are …

… more dense than in this image.

Use automation for less subjective analysis.

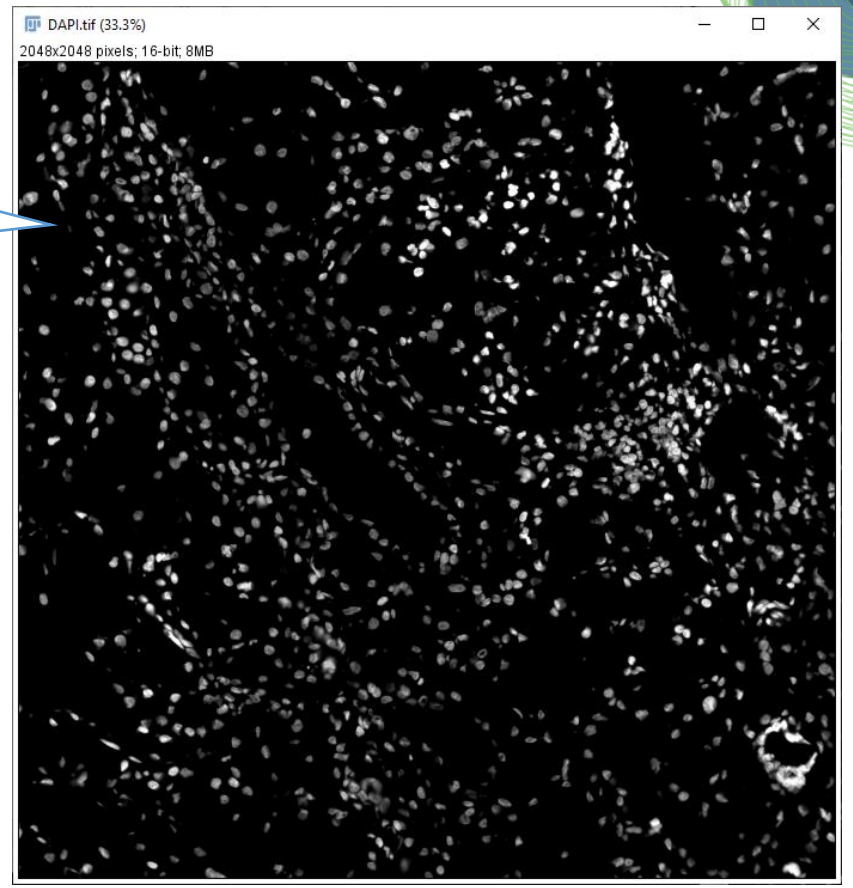
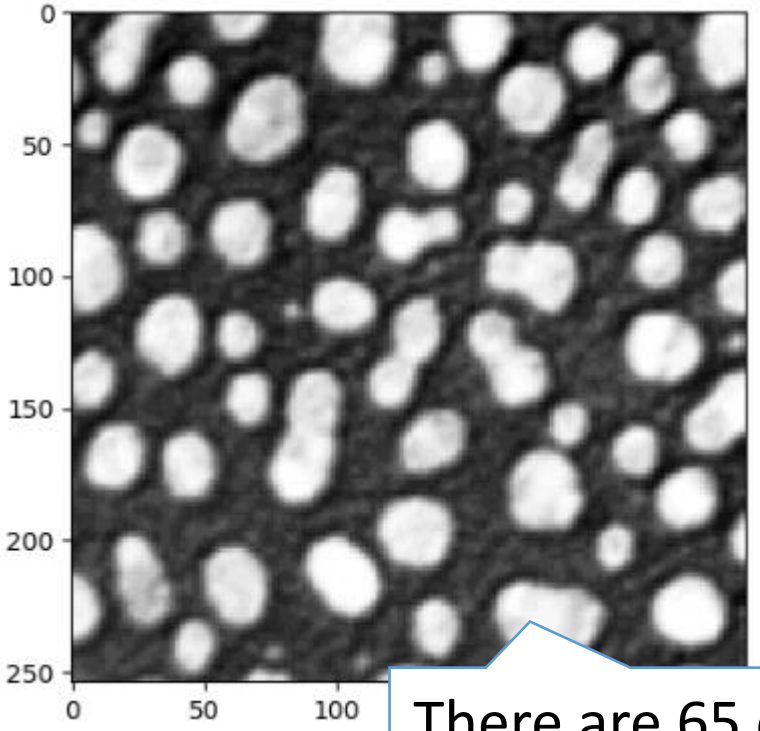


Image data source: Pascual-Reguant, Anna. (2021). Immunofluorescence staining of a human kidney (#2, peri-tumor area) obtained by MELC [Data set]. Zenodo. http://doi.org/10.5281/zenodo.4434462 licensed CC-BY 4.0

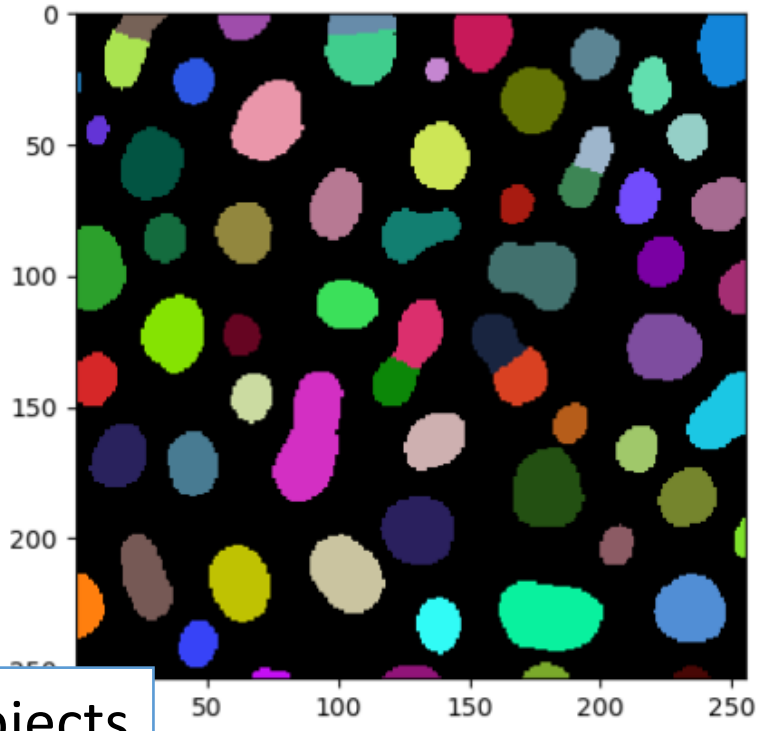
Reliable bio-image analysis

- Algorithms must be reliable (trustworthy).
- Visualization helps gaining trust in automated methods.

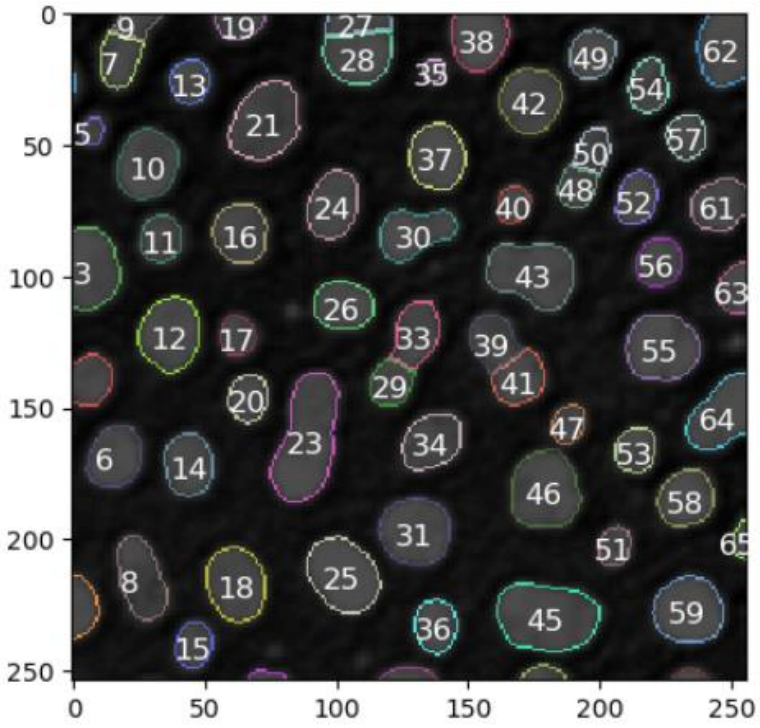
Original image



Label image



Overlay

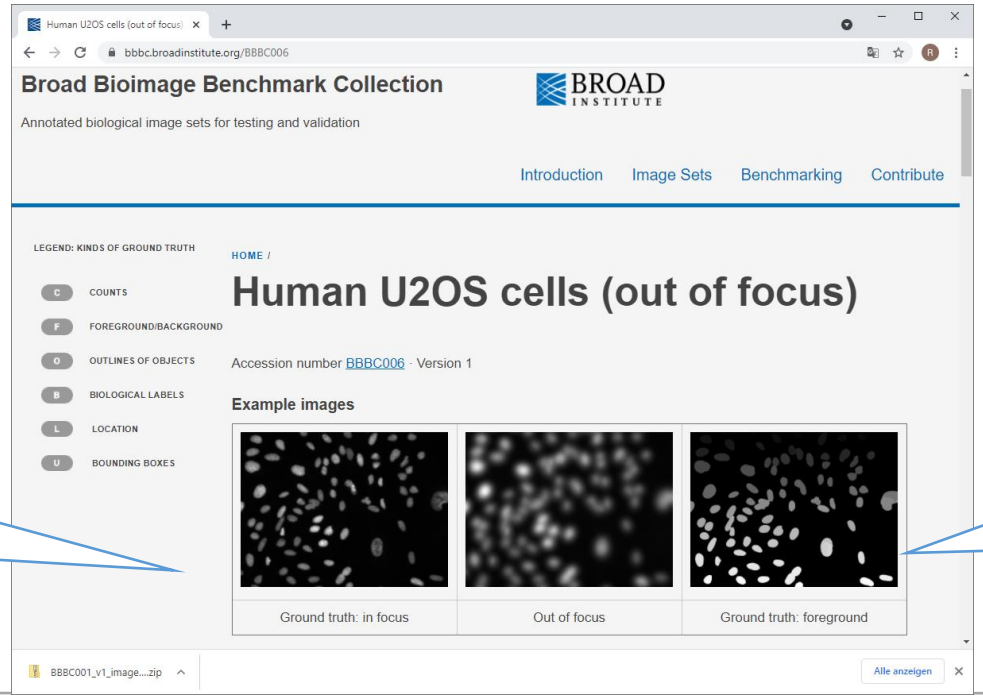


There are 65 objects in this image.

Reliable bio-image analysis

- Algorithms must be reliable (validated methods).
- Publicly available benchmark data sets allow to compare algorithms on common data.

Original image data



"Ground truth" label images

Reproducible data analysis

- Allowing others to do your experiment again.

- “The image data was analyzed with Python.”

Can you reproduce what they did?

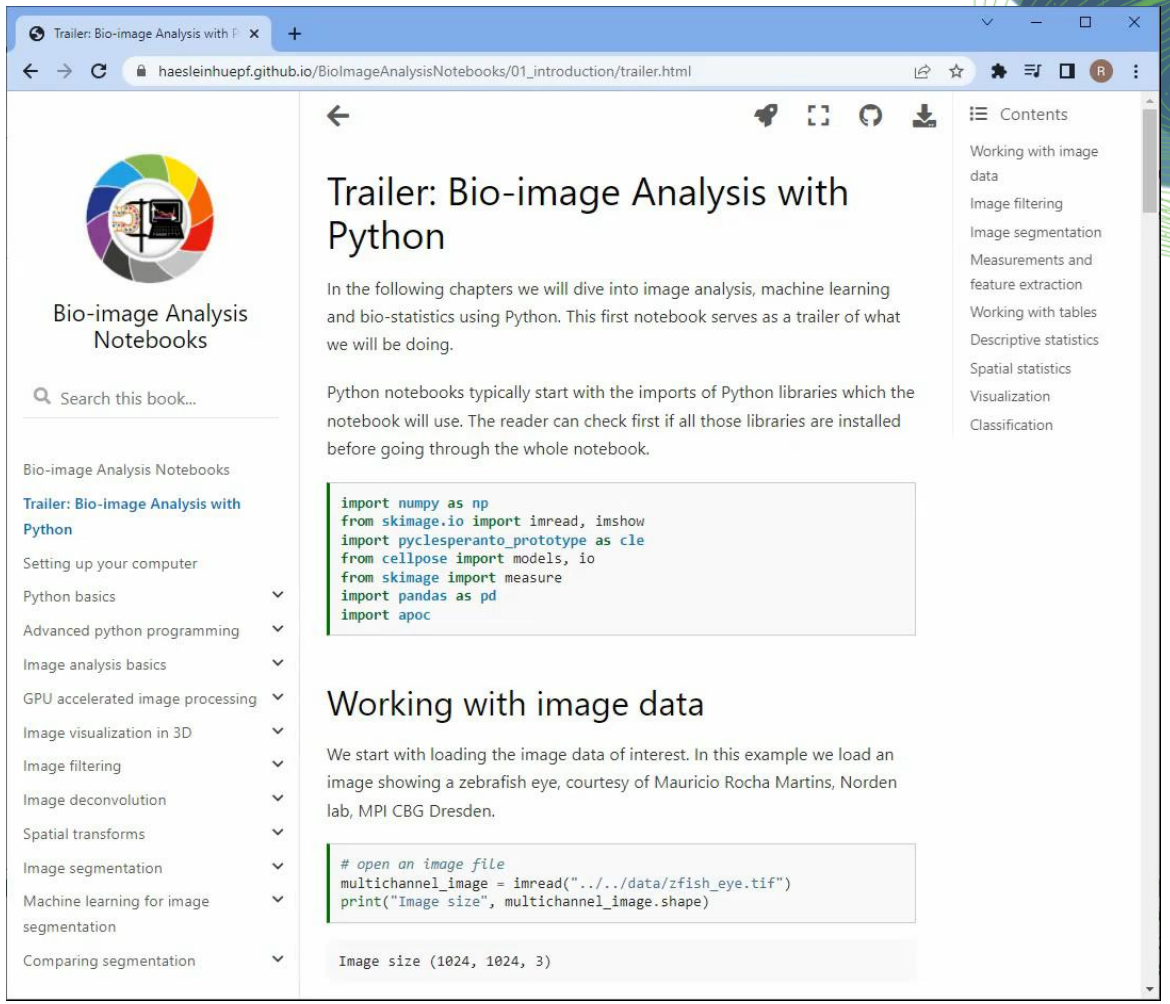
Reproducible bio-image analysis

- Allowing others to do your experiment again.

- “The image data was analyzed with Python.”

Can you reproduce what they did?

Can you reproduce what they did?



Replicable bio-image analysis

- Others run the same analysis on their data and have consistent results / same conclusions.
- Can only be achieved if data analysis protocol was documented reproducibly.

- See also: *Replication crisis*
  - In Psychology (surveys)
  - In Medicine (clinical trials)
  - In Computer Science (executable code)
  - …

Open access, freely available online

Essay

Why Most Published Research Findings Are False

John P. A. Ioannidis

Summary

There is increasing concern that most current published research findings are false. The probability that a research claim is true may depend on study power and bias, the number of other studies on the same question, and, importantly, the ratio of true to no relationships among the relationships probed in each scientific field. In this framework, a research finding is less likely to be true when the studies conducted in a field are smaller; when effect sizes are smaller; when there is a greater number and lesser preselection of tested relationships; where there is greater flexibility in designs, definitions, outcomes, and analytical modes; when there is greater financial and other interest and prejudice; and when more teams are involved in a scientific field in chase of statistical significance. Simulations show that for most study designs and settings, it is more likely for

factors that influence this problem and some corollaries thereof.

Modeling the Framework for False Positive Findings

Several methodologists have pointed out [9–11] that the high rate of nonreplication (lack of confirmation) of research discoveries is a consequence of the convenient, yet ill-founded strategy of claiming conclusive research findings solely on the basis of a single study assessed by formal statistical significance, typically for a *p*-value less than 0.05. Research is not most appropriately represented and summarized by *p*-values, but, unfortunately, there is a widespread notion that medical research articles

It can be proven that most claimed research findings are false.

is characteristic of the field and can vary a lot depending on whether the field targets highly likely relationships or searches for only one or a few true relationships among thousands and millions of hypotheses that may be postulated. Let us also consider, for computational simplicity, circumscribed fields where either there is only one true relationship (among many that can be hypothesized) or the power is similar to find any of the several existing true relationships. The pre-study probability of a relationship being true is $R/(R+1)$. The probability of a study finding a true relationship reflects the power $1 - \beta$ (one minus the Type II error rate). The probability of claiming a relationship when none truly exists reflects the Type I error rate, α. Assuming that c relationships are being probed in the field, the expected values of the 2×2 table are given in Table 1. After a research

https://journals.plos.org/plosmedicine/article?id=10.1371/journal.pmed.0020124
https://en.wikipedia.org/wiki/Replication_crisis

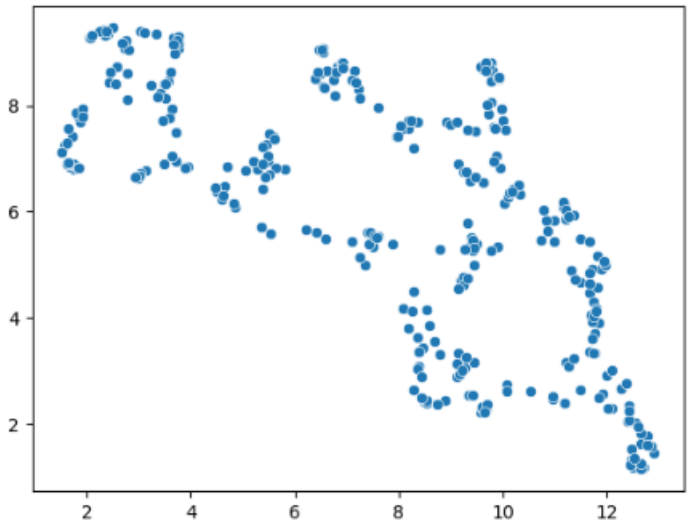
Repeatable data analysis

- In wet-lab experiments, samples may get destroyed while executing the experiment.
- Repeatab … annot improve …

```
[11]: reducer = umap.UMAP()
      embedding2 = reducer.fit_transform(scaled_statistics)

      seaborn.scatterplot(x=embedding2[:, 0],
                          y=embedding2[:, 1])
```

```
[11]: <AxesSubplot: >
```




```
[12]: reducer = umap.UMAP()
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                          y=embedding2[:, 1])
```
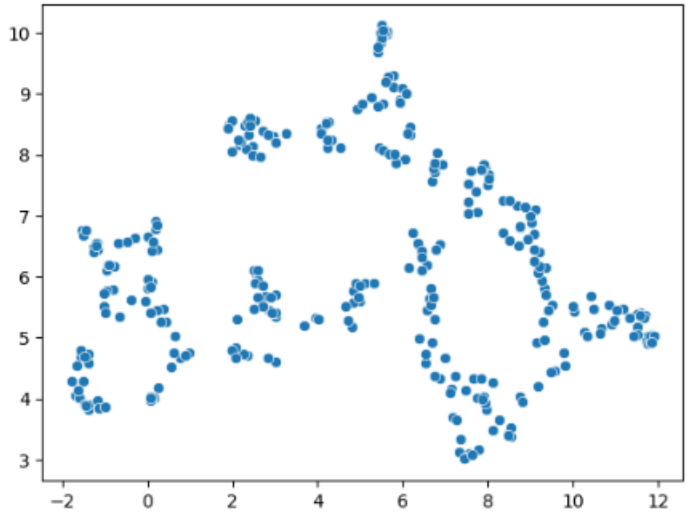
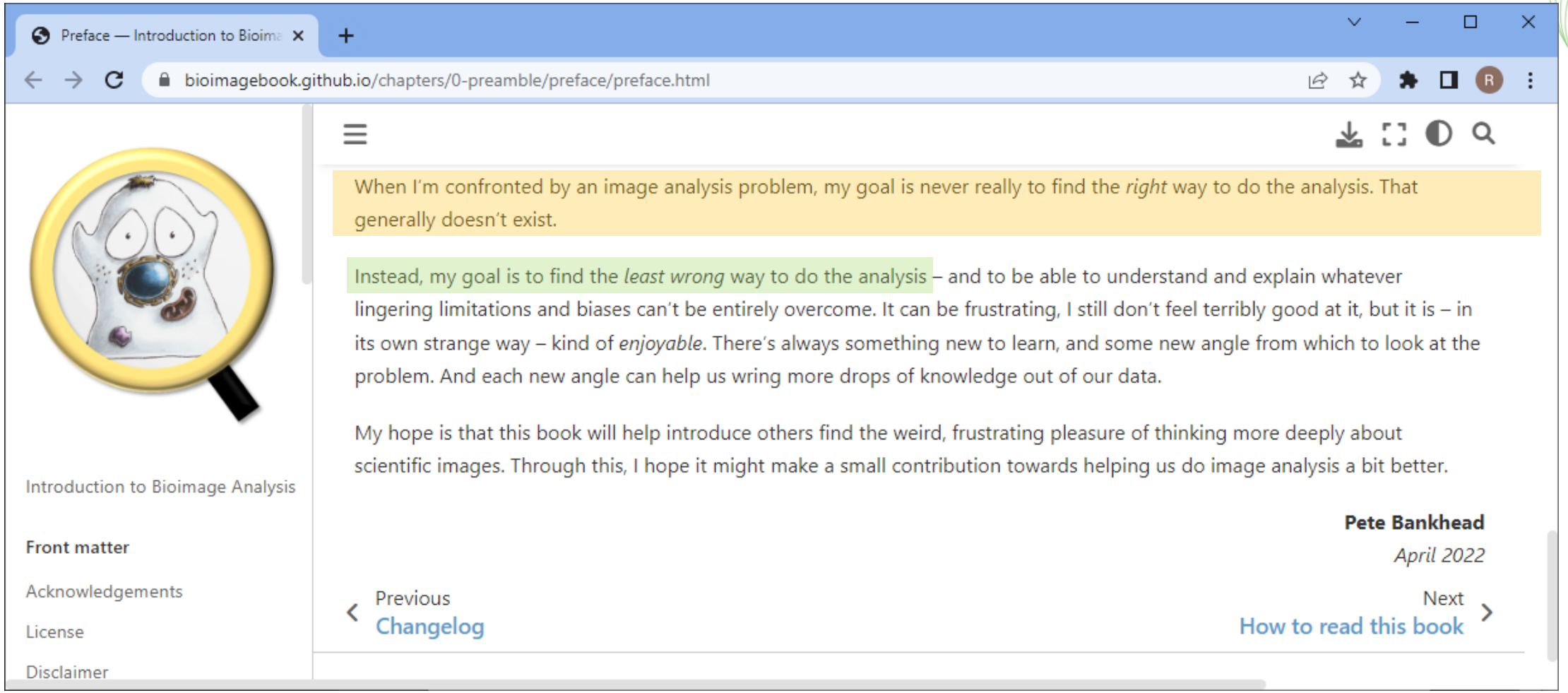

```
[12]: <AxesSubplot: >
```



Bio-image Analysis: good scientific practice

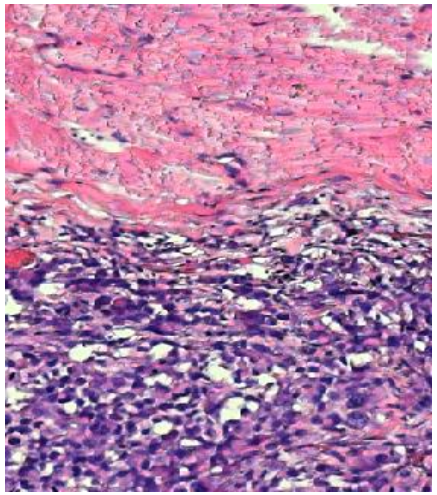


Bio-image analysis is supposed to be

- **Quantitative**
  - We derive numbers from images which describe physical properties of the observed sample.
- **Objective**
  - The derived measurement does not depend on who did the measurement. The measurement is free of interpretation.
- **Reliable (trustworthy / validated)**
  - We are confident that the measurement is describing what it is supposed to describe.
- **Reproducible**
  - Enabling others to re-do the experiment. For this, documentation is crucial!
- **Replicability**
  - Others *do* execute the same analysis, potentially on other data, and see consistent results.
- **Repeatable**
  - We can do the same experiment twice under the *same conditions* and get the same measurements.

Common topics

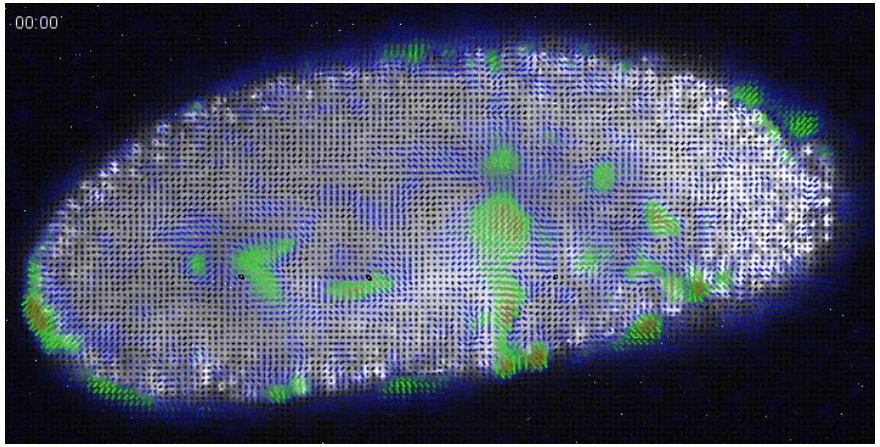
- Typical questions bio-image analysts deal with
  - Is signal intensity different under varying conditions?
  - How many cells are in my image?
  - How high is cell density?
    - ➢ Bio-statistics / medicine / disease staging
  - How are different tissues characterized?



muscle, normal tissue

squamous-cell carcinoma

- Typical questions bio-image analysts struggle with
  - What force drives the observed processes?
  - What is the lineage tree of one particular cell?
  - Are observation A and observation B related?
  - Are structures observed in different color channels colocalized?



Hypothesis-driven quantitative biology

- Hypothesis: Cell shape can be influenced by modifying X.
- Null-Hypothesis: Circularity of modified cells is similar to cells in the control group.

- Sample preparation
- Imaging
- Cell segmentation
- Circularity measurement
- Statistics

Shall we use a different microscope?

Should we use a different segmentation algorithm?

Is circularity the right parameter to measure?

Hypothesis *generating* quantitative biology

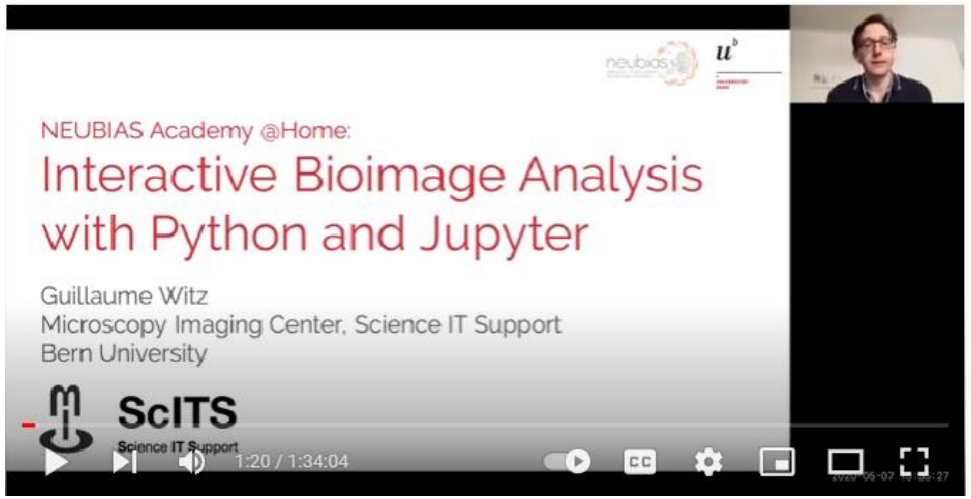
- ~~Hypothesis: Cell shape can be influenced by modifying X.~~
- Question: Which image-derived parameter is influenced when modifying X?
  - Sample preparation
  - Imaging
  - Cell segmentation algorithm A, algorithm B, algorithm C
  - Measurement of circularity, solidity, elongation, extend, texture, intensity, topology …
  - Statistics

Which segmentation algorithms allow measurements that show a relationship with X?

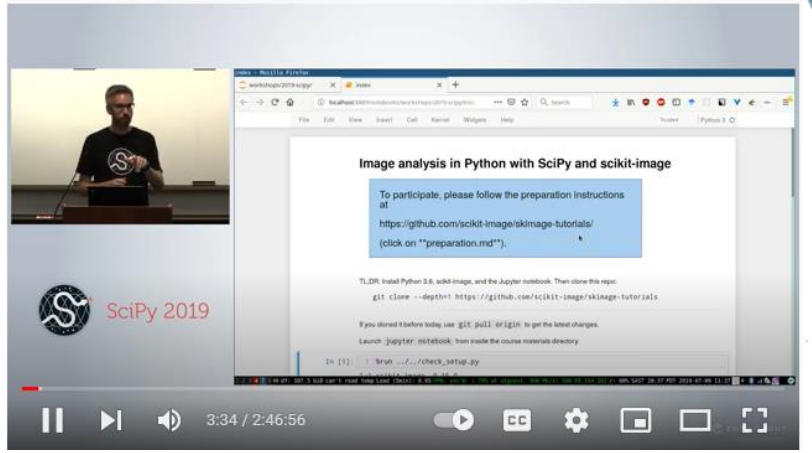
Why?

Which parameter shows any relationship with X?

More resources



Guillaume Witz, NEUBIAS Academy 2020



Stéfan van der Walt, Juan Nunez-Iglesias, SciPy 2019

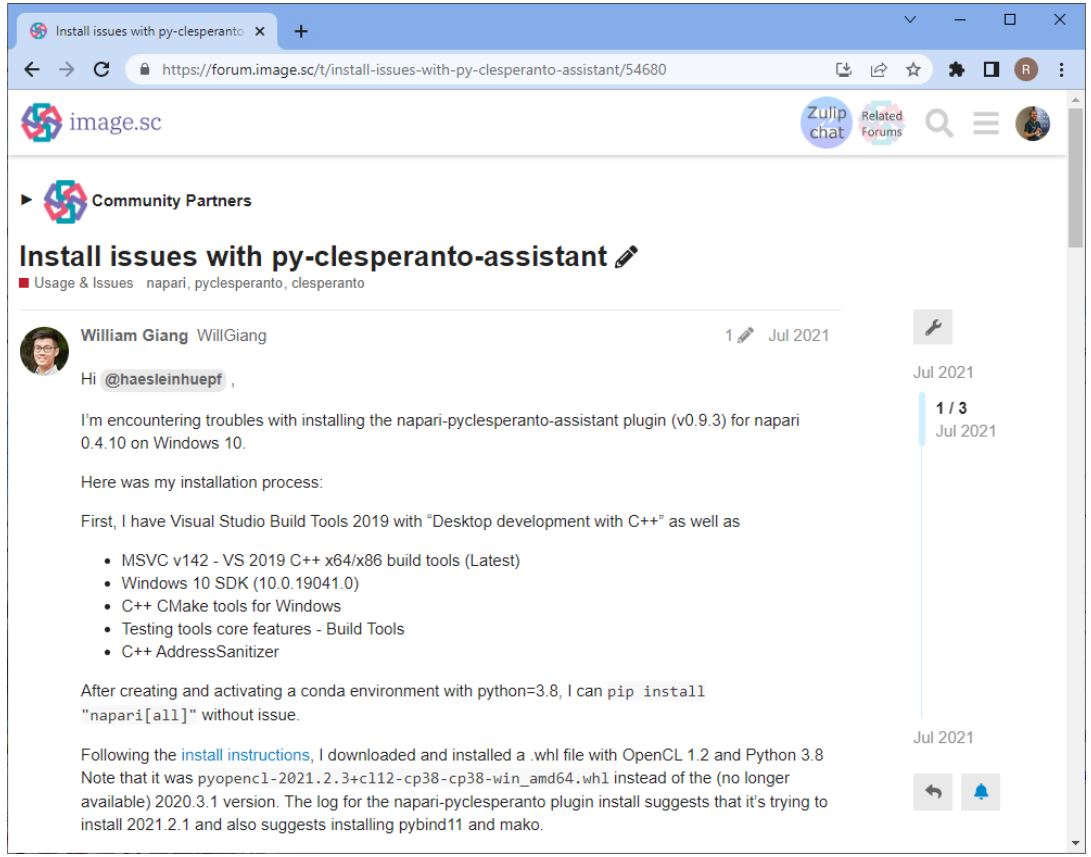
Sreenivas Bhattiprolu, Python for Microscopists @Youtube 2019-…

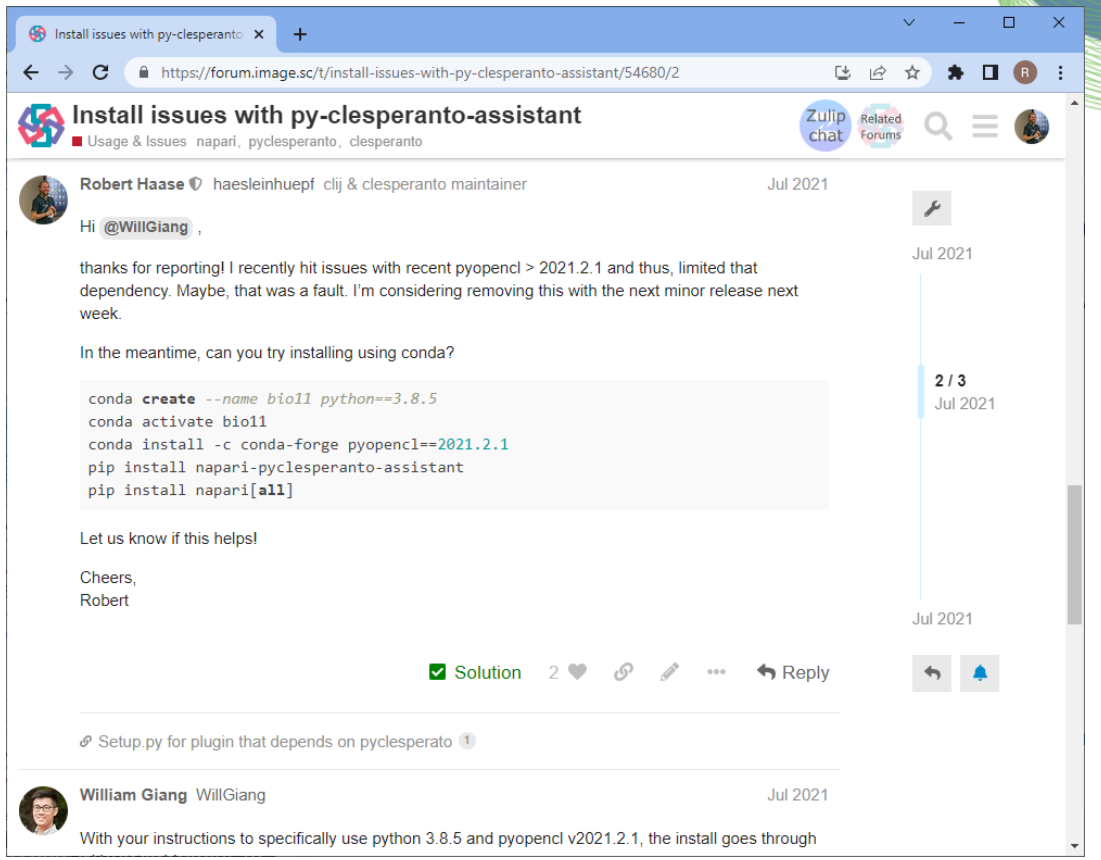
Watch more:

- https://www.youtube.com/watch?v=2KF8vBrp3Zw
- https://www.youtube.com/watch?v=d1CIV9irQAY
- https://www.youtube.com/watch?v=X_pCiVQ4c4E

The Image Science Community

- Ask your question online and an expert will likely reply the same day ☺





Quiz

- Enabling others to do your experiment is about …

Repeatability

Reproducibility

Replicability

Reliability

Quiz

- Reproducibility can be achieved by

Writing documentation

Writing code

Providing example data

Recording Video tutorials

Quiz

- Resolution in imaging describes…

Size of pixels on a screen	Size of pixels on a camera chip	Maximum size of objects in relation to the image	Minimum size of objects that can be differentiated in an image

Summary

Today, you learned

- Microscopy
  - Fluorescence microscopy
  - Light-sheet microscopy
- Bio-image analysis
  - Quantitative
  - Objective
  - Reproducible
  - Repeatable
  - Reliable

Coming up next

- Exercises: Setting up your environment

Next week(s):

- Research Data Management
- Image processing for microscopy

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AND ARTIFICIAL INTELLIGENCE

Exercises

Robert Haase

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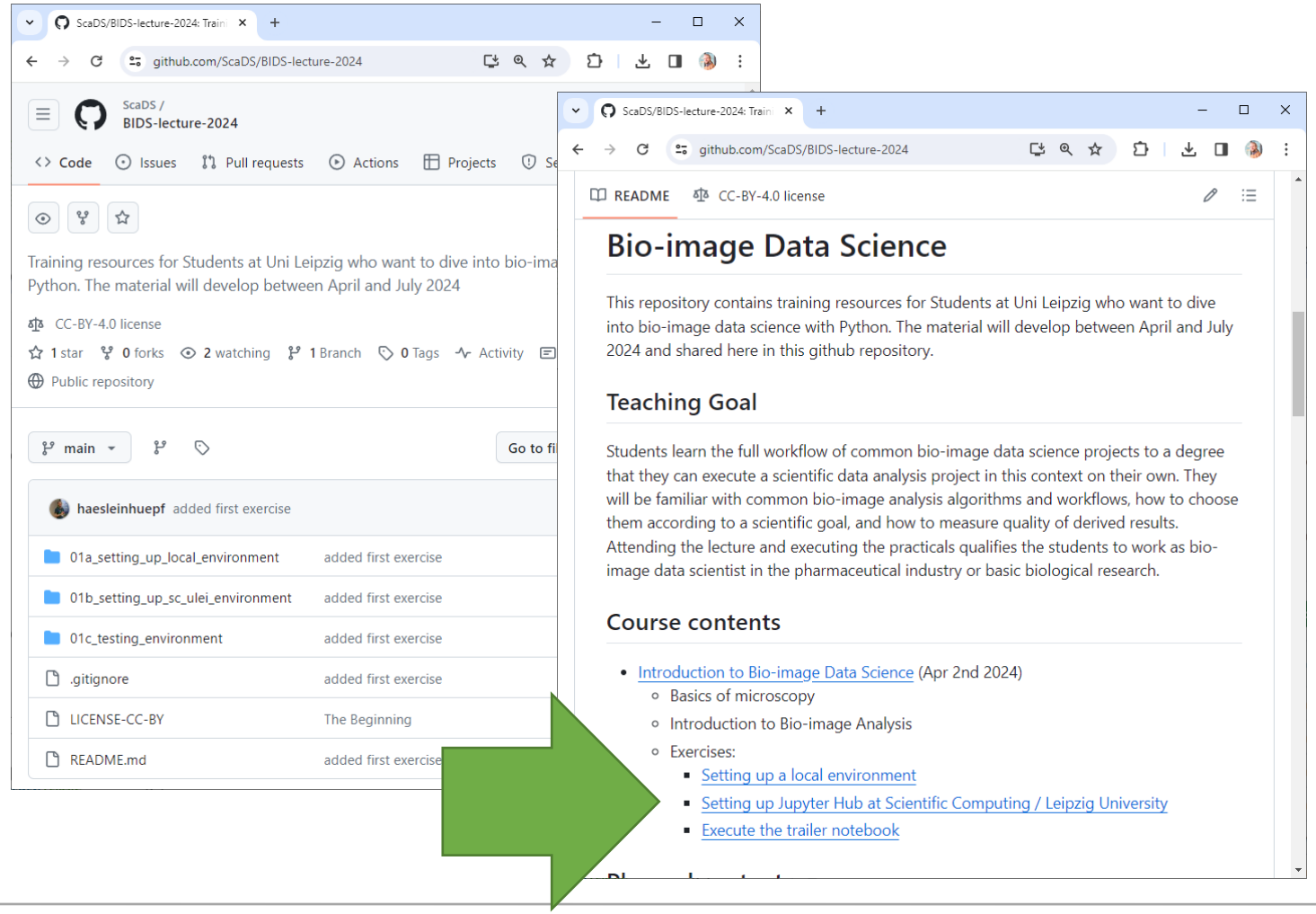
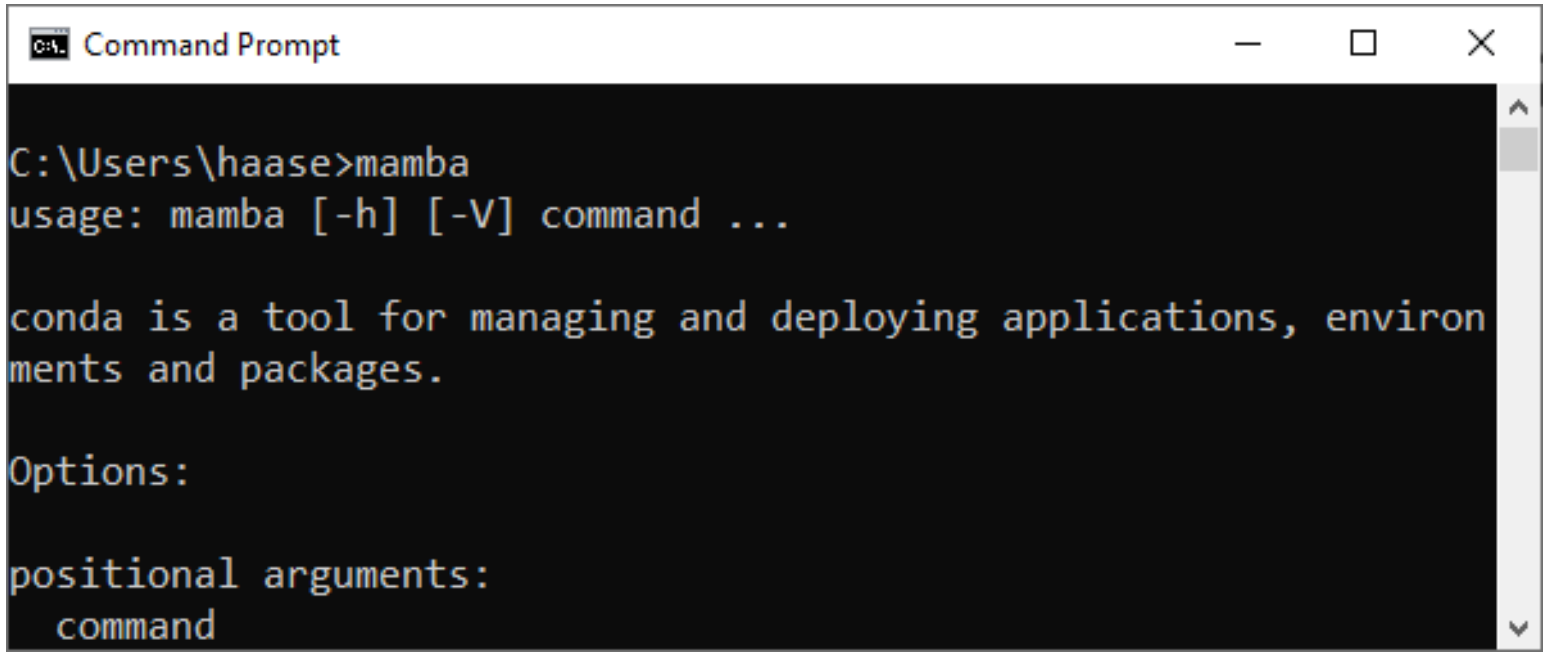
Exercises


ScaDS/BIDS-lecture-2024: Train
github.com/ScaDS/BIDS-lecture-2024
ScaDS /
BIDS-lecture-2024
Code
Issues
Pull requests
Actions
Projects
Training resources for Students at Uni Leipzig who want to dive into bio-ima
Python. The material will develop between April and July 2024
CC-BY-4.0 license
1 star
0 forks
2 watching
1 Branch
0 Tags
Activity
Public repository
main
Go to fi
haesleinhuepf added first exercise
01a_setting_up_local_environment added first exercise
01b_setting_up_sc_ulei_environment added first exercise
01c_testing_environment added first exercise
.gitignore added first exercise
LICENSE-CC-BY The Beginning
README.md added first exercise
README
CC-BY-4.0 license
Bio-image Data Science
This repository contains training resources for Students at Uni Leipzig who want to dive into bio-image data science with Python. The material will develop between April and July 2024 and shared here in this github repository.
Teaching Goal
Students learn the full workflow of common bio-image data science projects to a degree that they can execute a scientific data analysis project in this context on their own. They will be familiar with common bio-image analysis algorithms and workflows, how to choose them according to a scientific goal, and how to measure quality of derived results. Attending the lecture and executing the practicals qualifies the students to work as bio-image data scientist in the pharmaceutical industry or basic biological research.
Course contents
Introduction to Bio-image Data Science (Apr 2nd 2024)
Basics of microscopy
Introduction to Bio-image Analysis
Exercises:
Setting up a local environment
Setting up Jupyter Hub at Scientific Computing / Leipzig University
Execute the trailer notebook

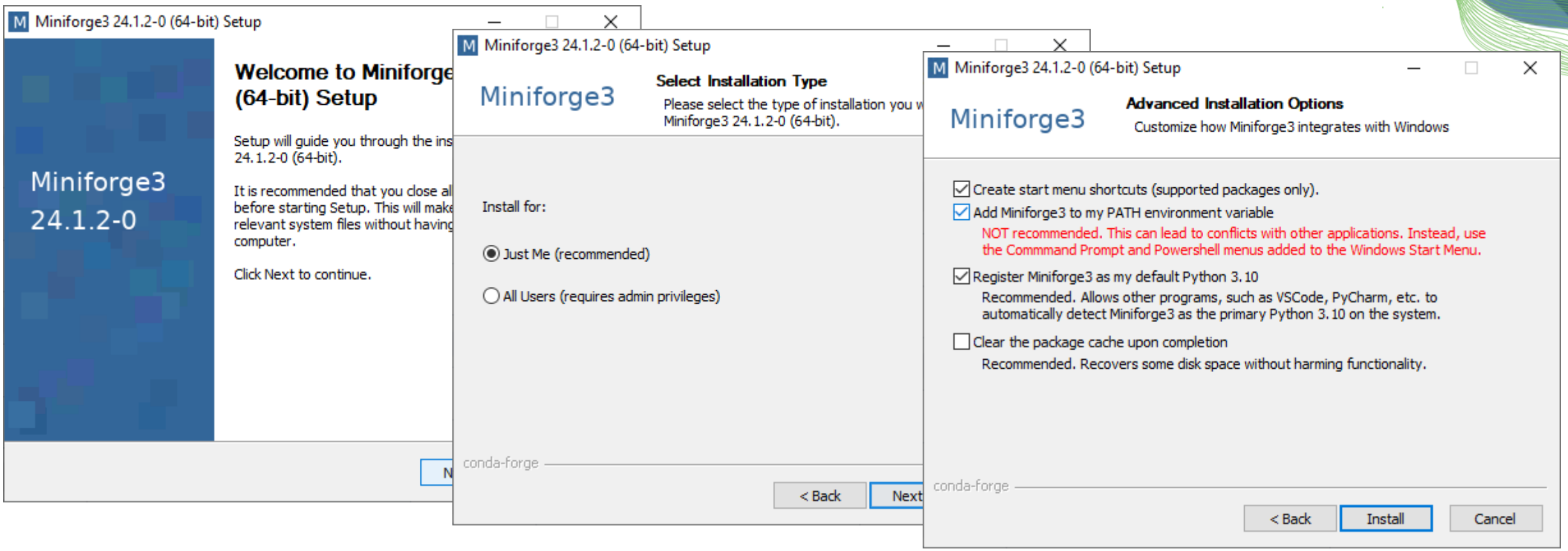
Installation

- You can skip the first local installation steps if you already use `mamba`



Installation

- Install mini-forge



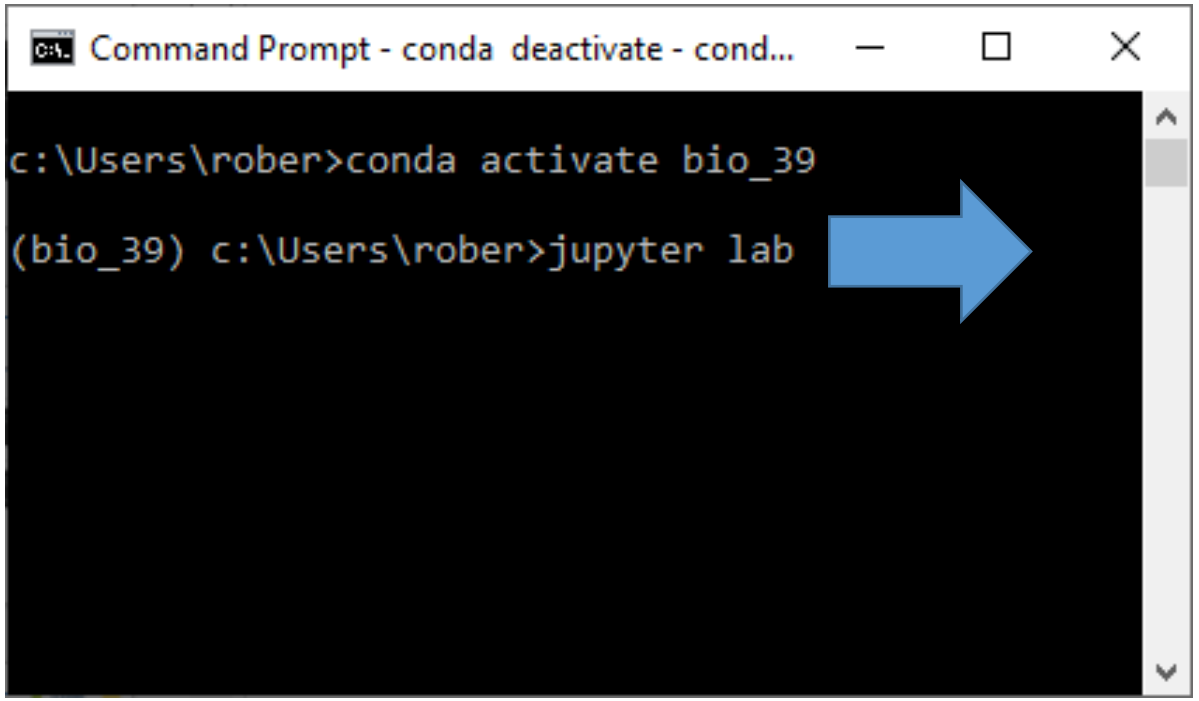
Setting up an environment

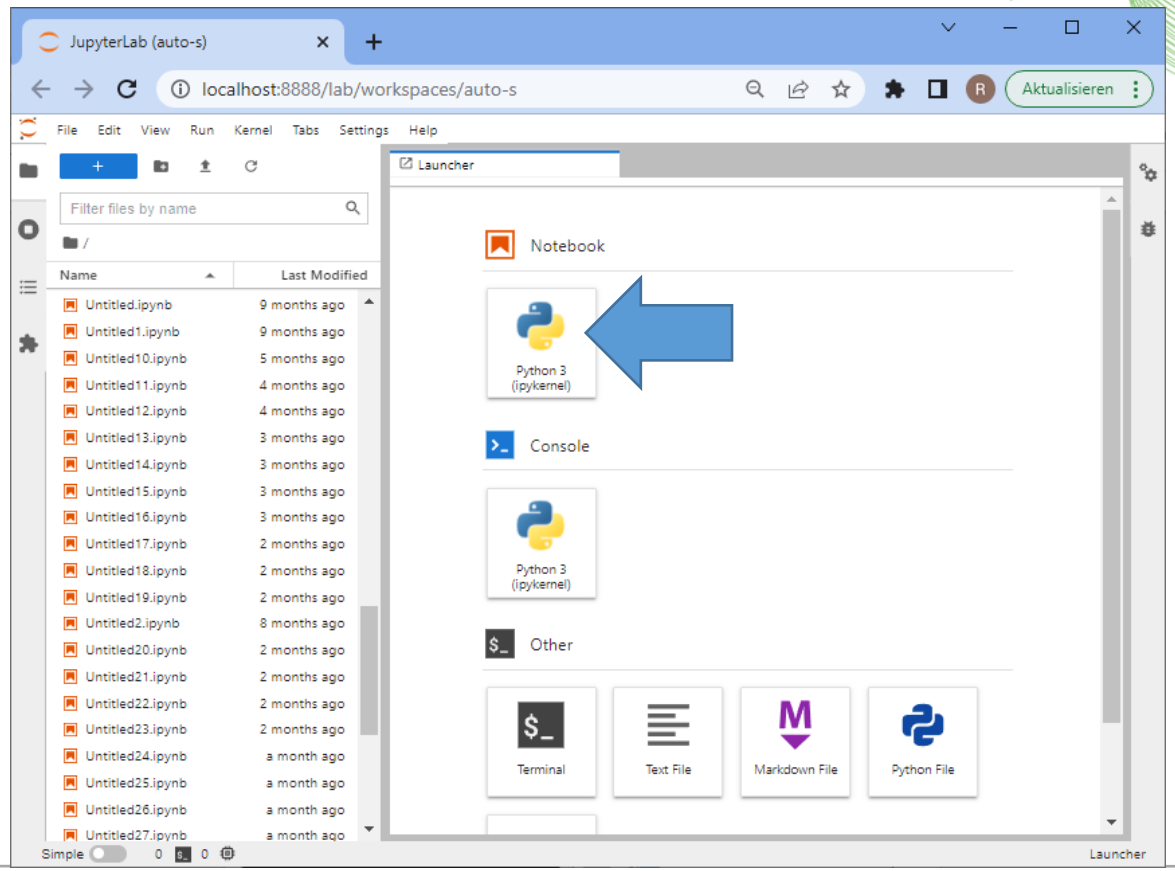
- Create a conda/mamba environment

```
mamba create --name my_fist_env
python=3.9 devbio-napari pyqt -c
conda-forge
```

Jupyter lab

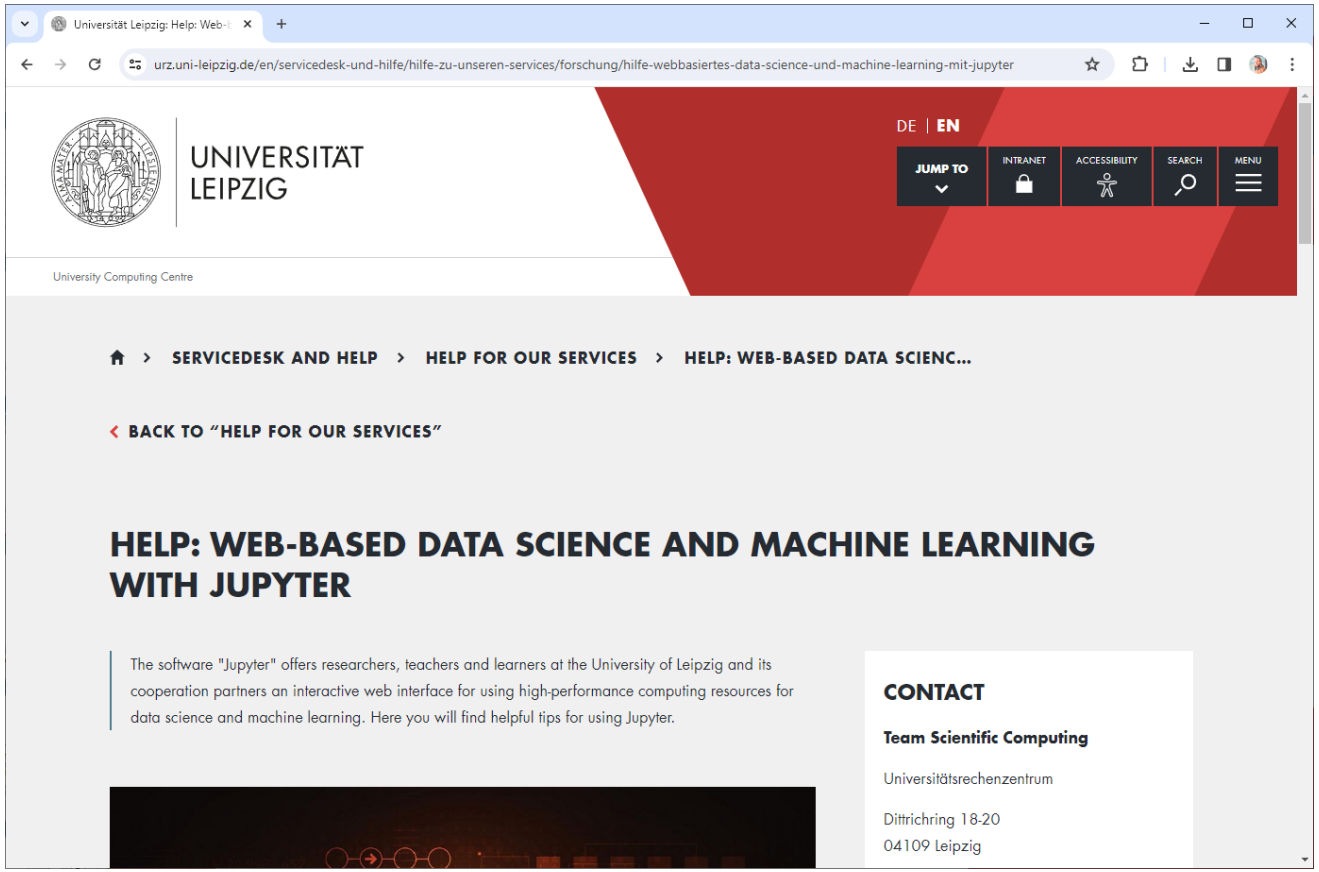
- Start Jupyter lab from the folder you want to work in
- Create a new notebook



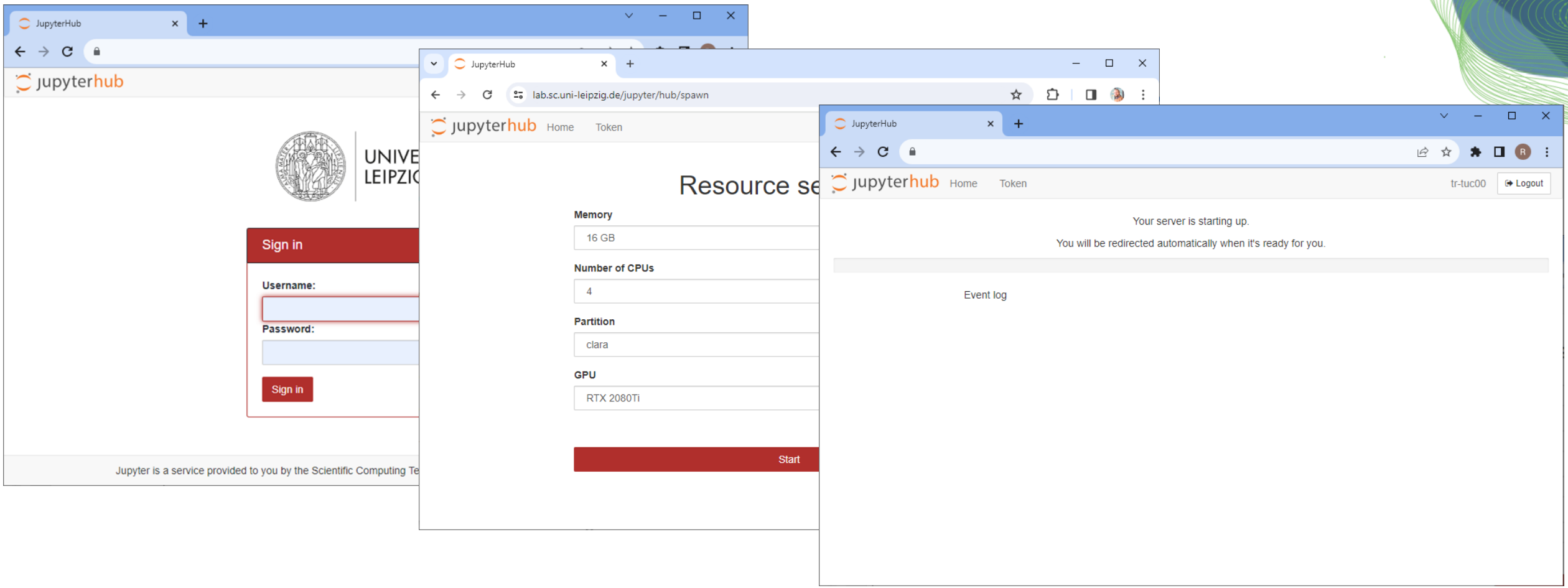


Setting up the JupyterHub @ Scientific Computing ULEI

- Alternatively: Register @ Scientific Computing / Uni Leipzig

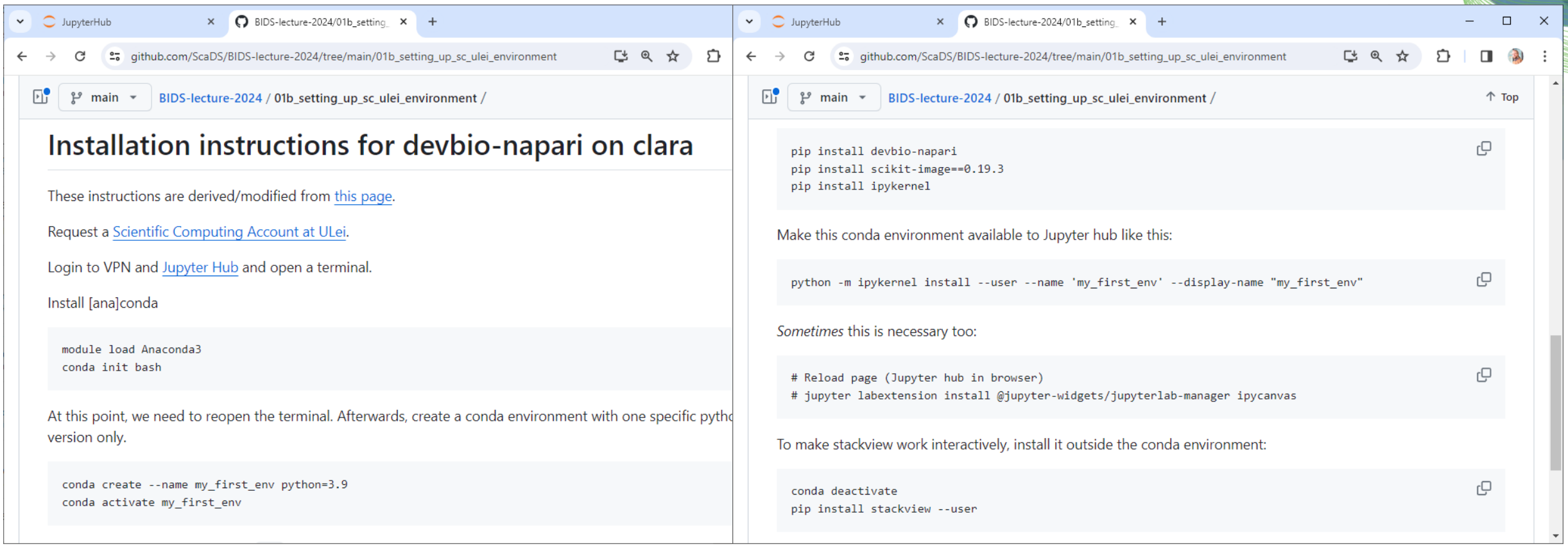


Setting up the JupyterHub @ Scientific Computing ULEI



Setting up the JupyterHub @ Scientific Computing ULEI

- Detailed instructions



Exercise: Test the environment

- Download and test the trailer notebook