PoL Physics of Life TU Dresden

CENTER FOR SYSTEMS BIOLOGY DRESDEN

Introduction to Bio-Image Analysis

Robert Haase

Reusing some materials made by Beth Cimini,
Anne Carpenter & colleagues (Broad Institute)

@haesleinhuepf

October 2022

Quantitative bio-image analysis

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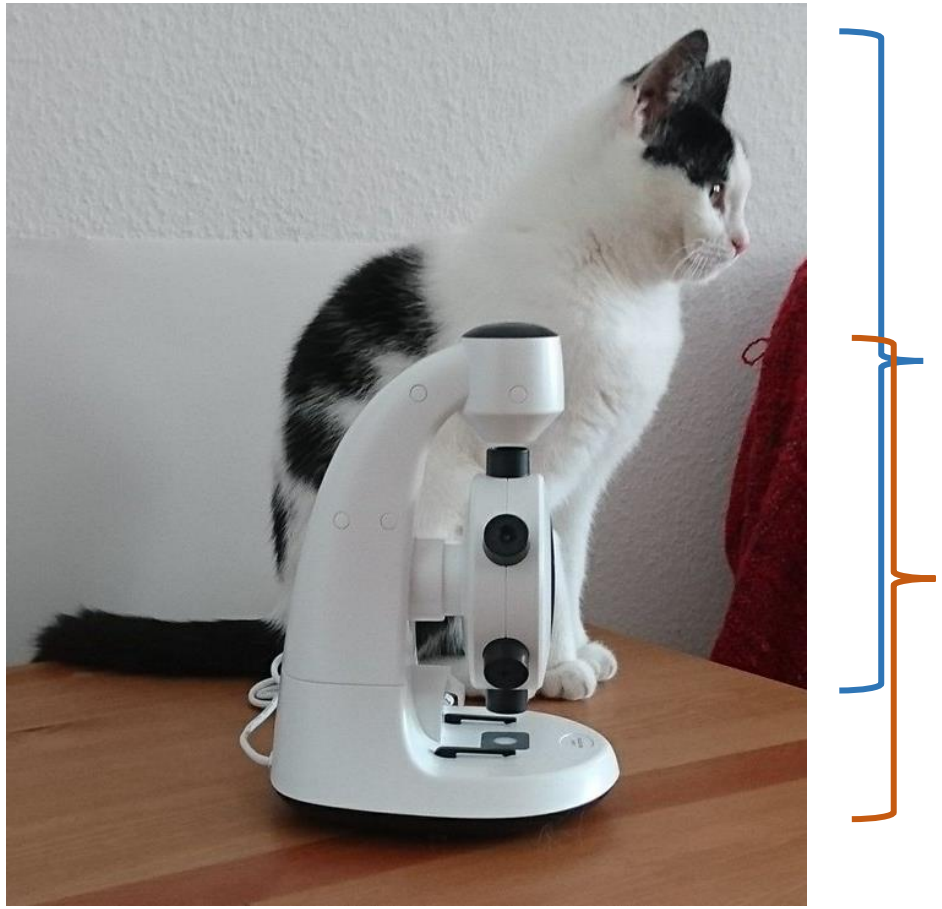
- Deriving quantitative information from images of biological samples taken with microscopes



cat height = 1.5 x microscope height

Quantitative bio-image analysis

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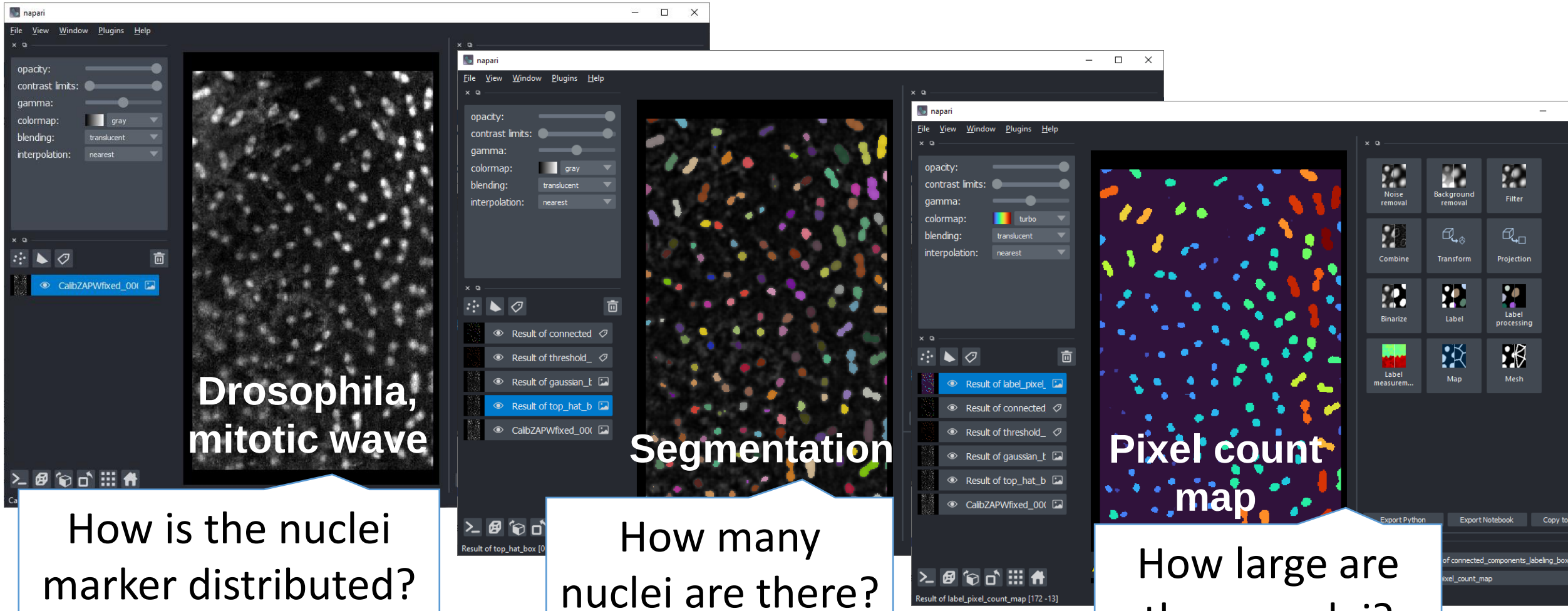
- Deriving quantitative information from images of biological samples taken with microscopes + visualization



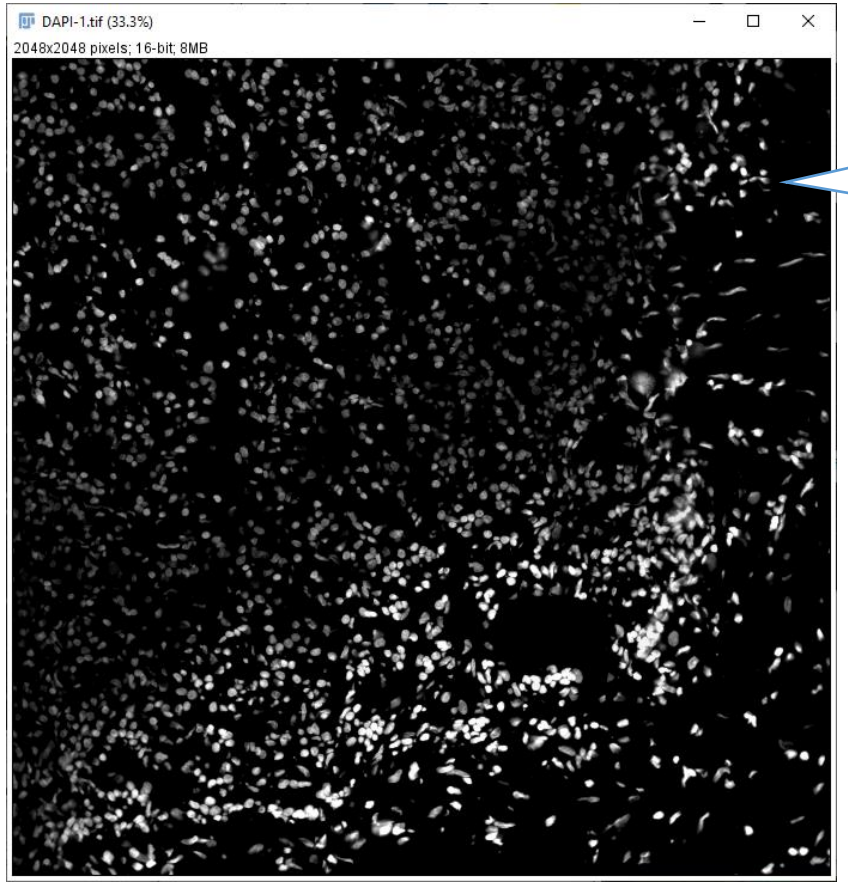
How is the nuclei marker distributed?

How many nuclei are there?

How large are these nuclei?

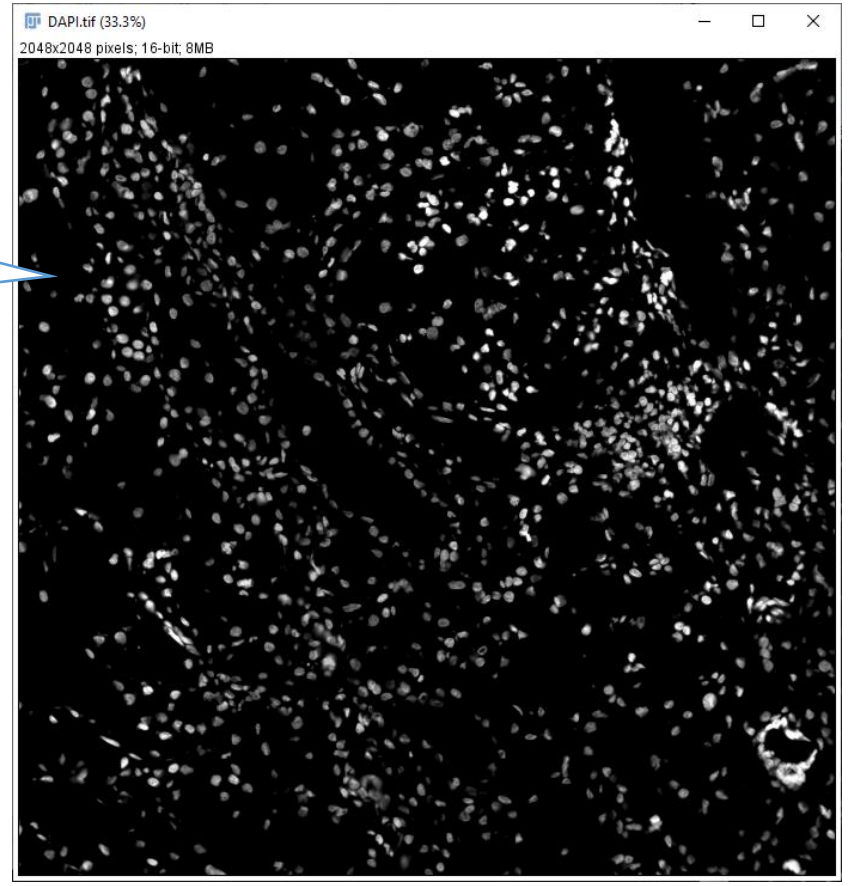
Objective bio-image analysis

- Measurements should be objective, not influenced by human interpretation



Nuclei in this image are …

… more dense than in this image.



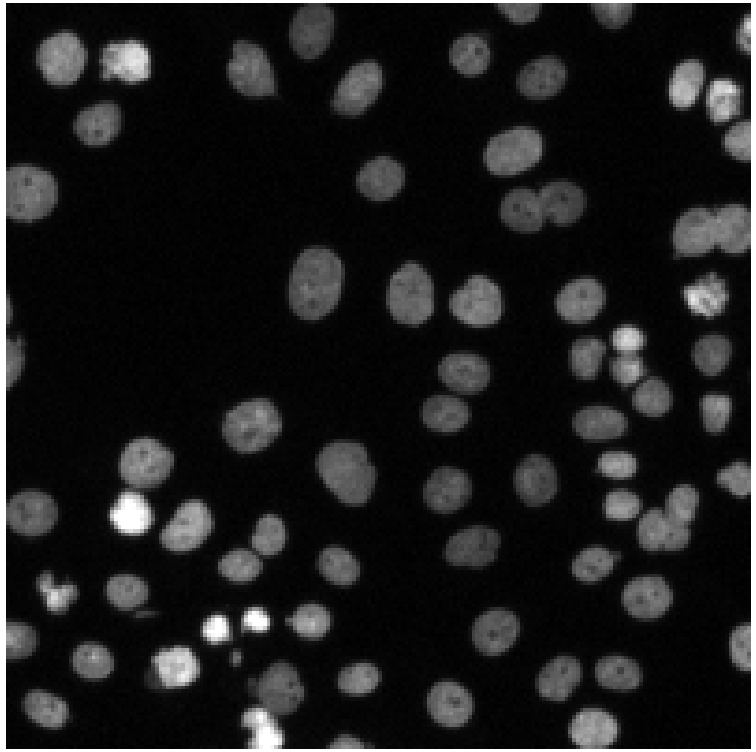
Use automation for less subjective analysis.

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

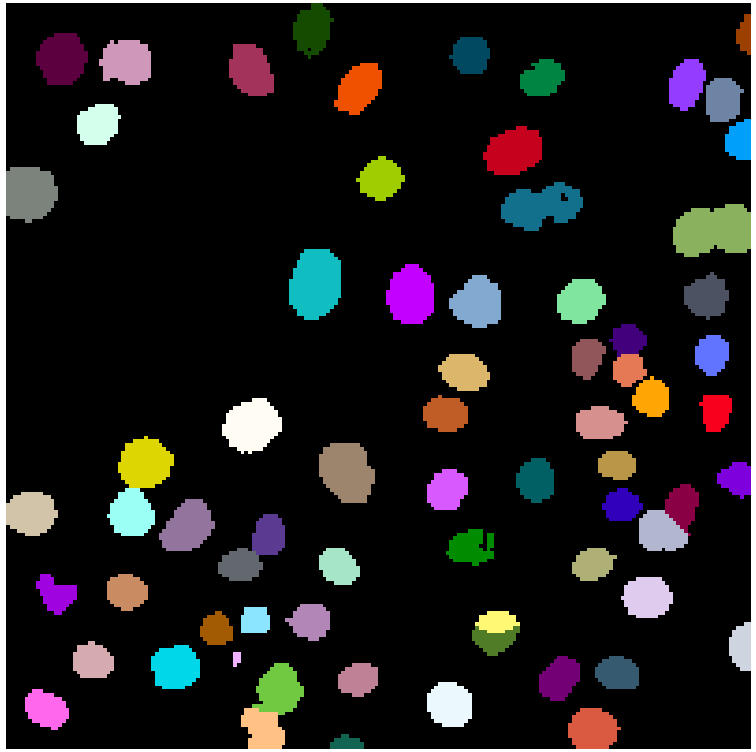
Reliable bio-image analysis

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

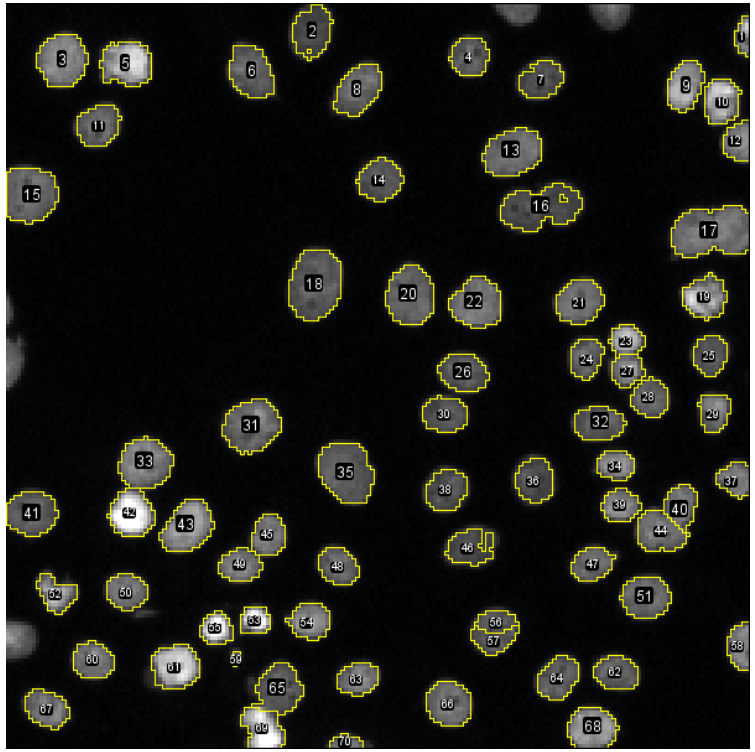
Original image



Label image



Overlay

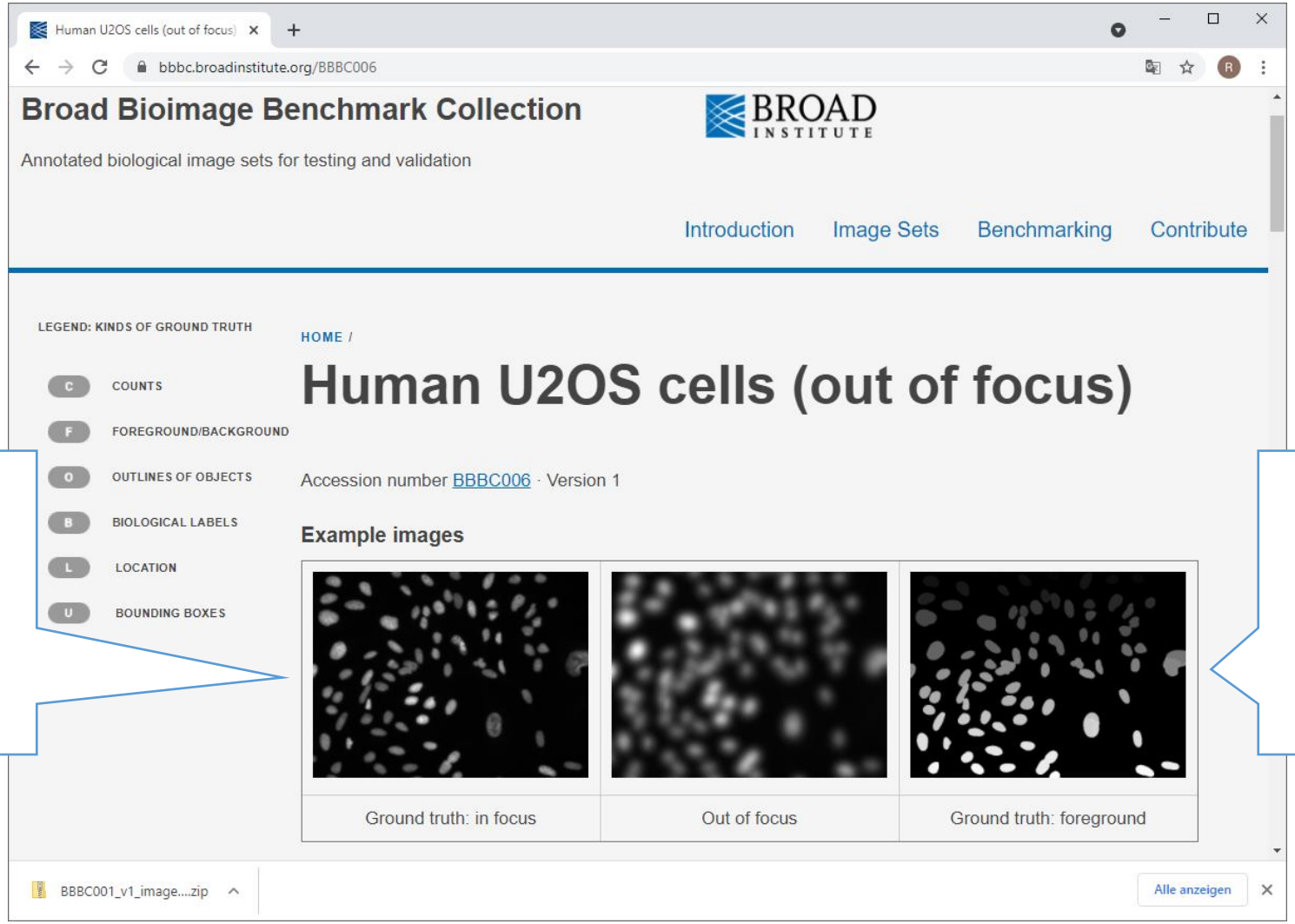


There are 70 nuclei in this image.

Image Data source: BBBC001v1 [Carpenter et al., *Genome Biology*, 2006] from the Broad Bioimage Benchmark Collection [Ljosa et al., *Nature Methods*, 2012]

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 bio-image analysis

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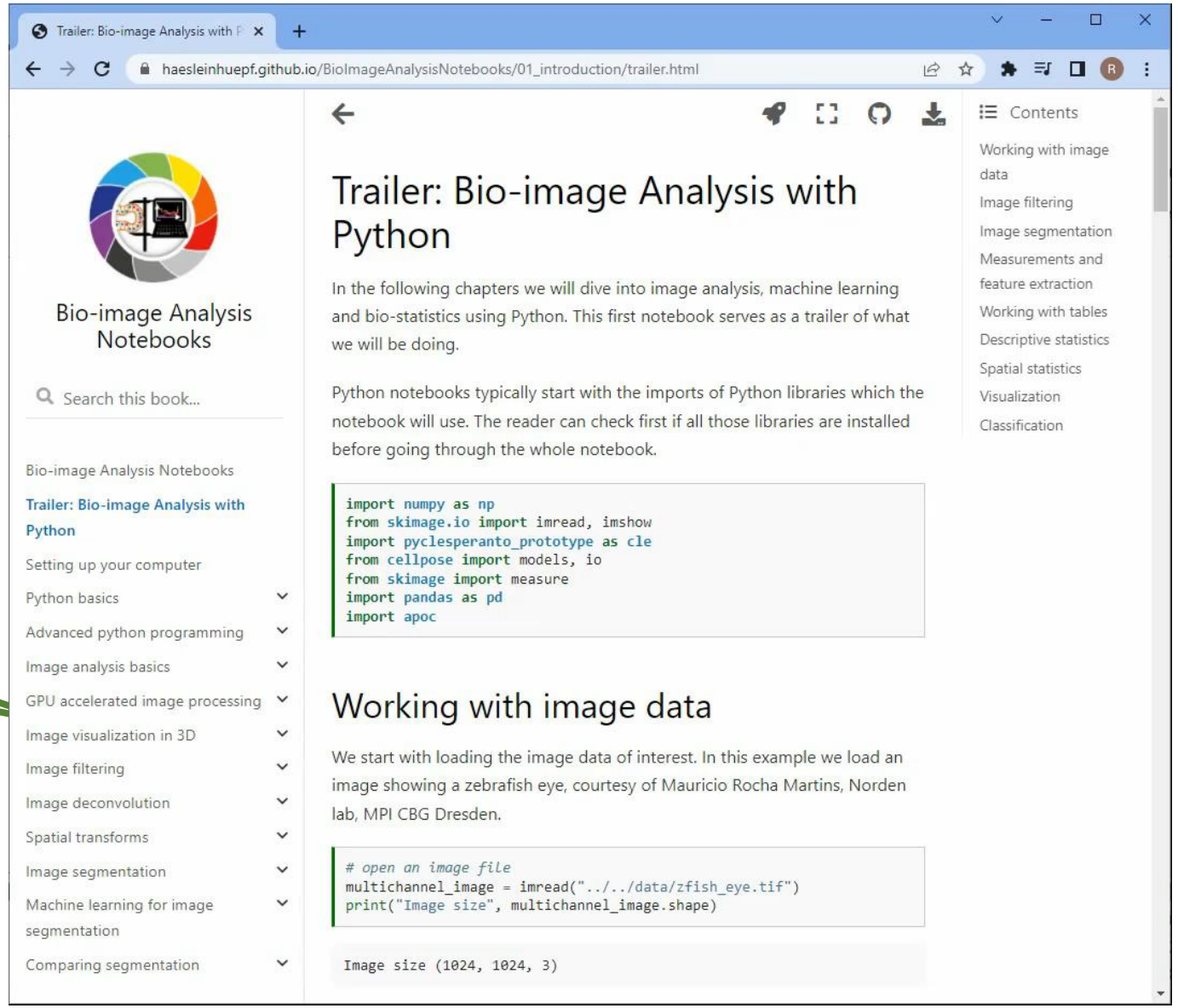
- “The image data was analyzed with ImageJ.”

Can you reproduce what they did?

Reproducible bio-image analysis

- “The image data was analyzed with ImageJ.”

Can you reproduce what they did?



Can you reproduce what they did?

Interactivity versus reproducibility

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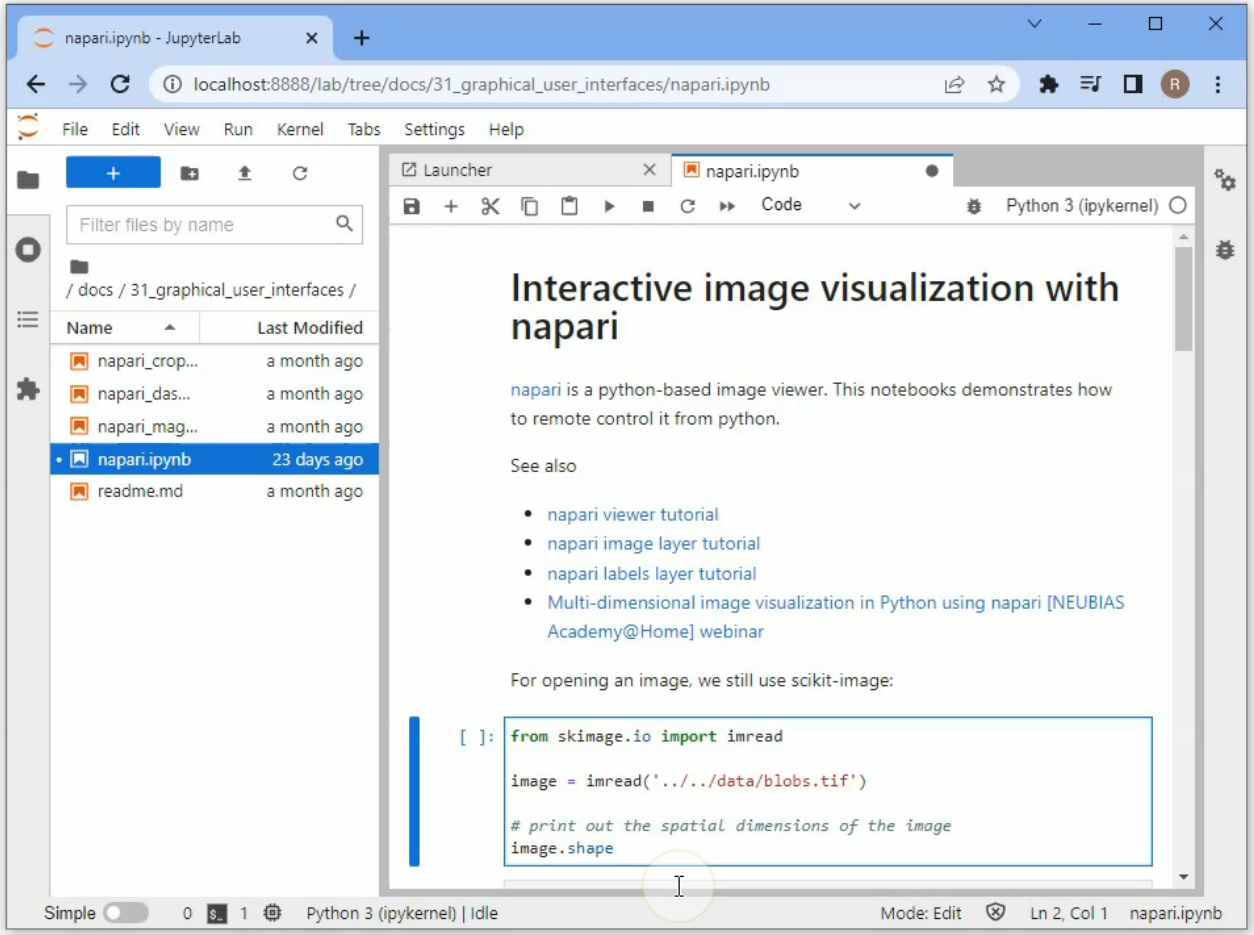
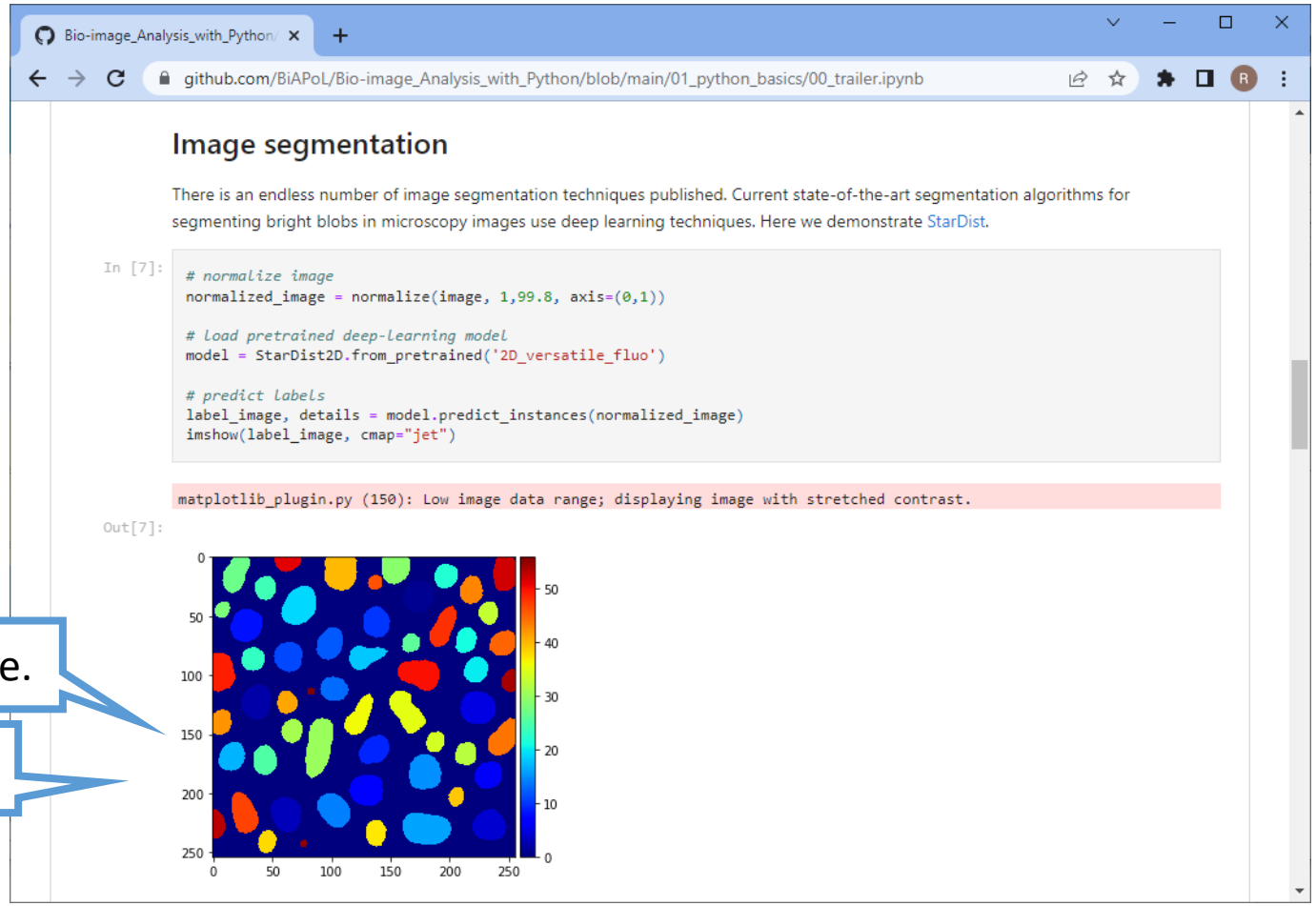
- Remote controlling graphical user interfaces



Repeatable Bio-image analysis

- Compared to wet-lab experiments, image analysis (in-silico) experiments are typically repeatable.
- In wet-lab experiments, samples may get destroyed while executing the experiment.
- Repeatability is a property of the experiment. You cannot improve repeatability by better documentation.

- However, you need to pay some extra attention to have repeatable image processing
  - Scripts need to be written in a way that their execution is repeatable.
  - Test it!



Run it once.

Run it twice.

Are results identical?

Introduction to bio-image analysis

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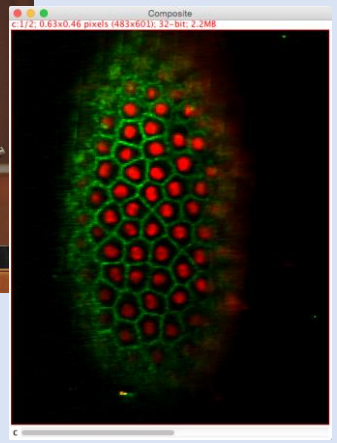
- Bio-image analysis is supposed to be
  - **Quantitative**
    - We derive numbers from images which describe physical properties of the observed sample.
  - **Objective**
    - The derived measurement does not depend on who did the measurement. The measurement is free of interpretation.
  - **Reliable (trustworthy / validated)**
    - We are confident that the measurement is describing what it is supposed to describe.
  - **Reproducible**
    - Somebody else can do the experiment under *different conditions* and gets similar measurements. For this, documentation is decisive!
  - **Repeatable**
    - We can do the same experiment twice under the *same conditions* and get similar measurements.

Image analysis is part of the experiment

Observation

$$p_{ij}(t) = \frac{[\tau_{ij}(t)]^{\alpha} \cdot [\eta_{ij}]^{\beta}}{\sum_{j=1}^{n} [\tau_{ij}(t)]^{\alpha} \cdot [\eta_{ij}]^{\beta}}$$

Modeling



Imaging

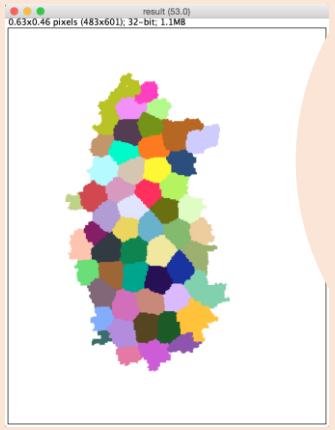
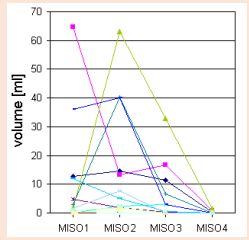


Image processing



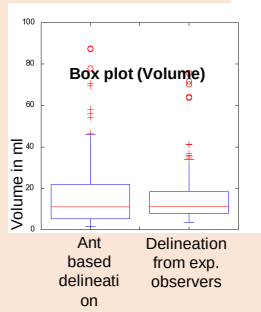


Image analysis
Bio-statistics

Image analysis is part of the experiment

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- Think about how to analyze your images before starting the experiment.
  - Consider adapting your experiment so that quantitative image analysis can be performed easily.
  - Do small test-experiments.

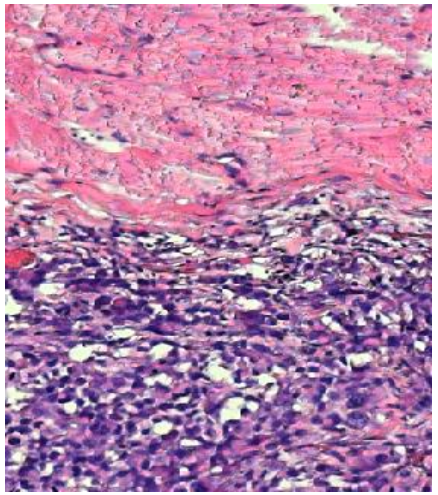
- Think about controls, counter-proves, an easy to falsify null-hypothesis.
  - Be a lazy scientist. Do simple experiments.

- How can you exclude yourself from the experiment?
  - Think of blinding yourself or fully automate analysis.

- One experiment usually answers one question. Or less.

Common questions

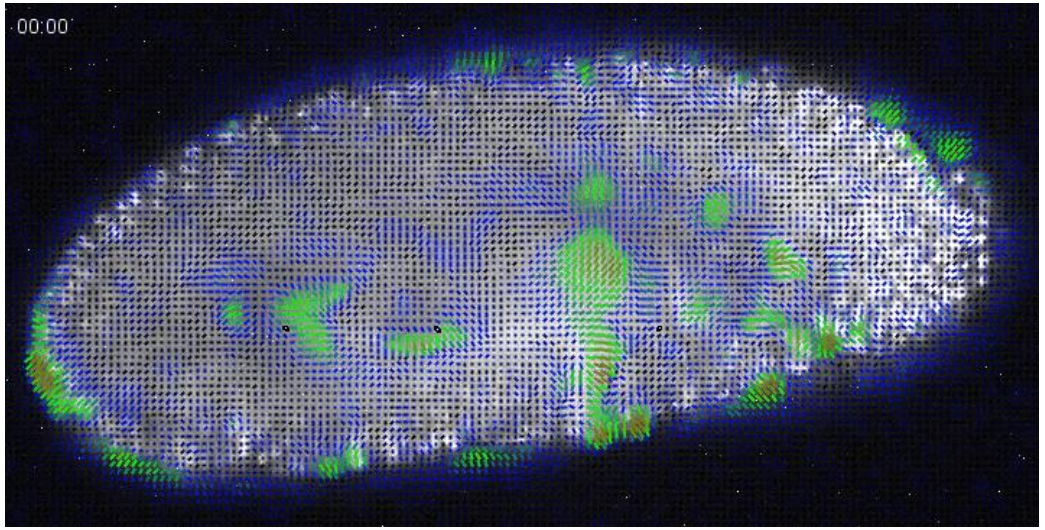
- Typical questions bio-image analysts deal with
  - Is signal intensity different under varying conditions?
  - How many cells are in my image?
  - How high is cell density?
    - ➢ Bio-statistics / medicine
  - How are different tissues characterized?
    - ➢ Machine learning



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

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- Hypothesis: Cell shape can be influenced by modifying X.

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

Shall we use a different microscope?

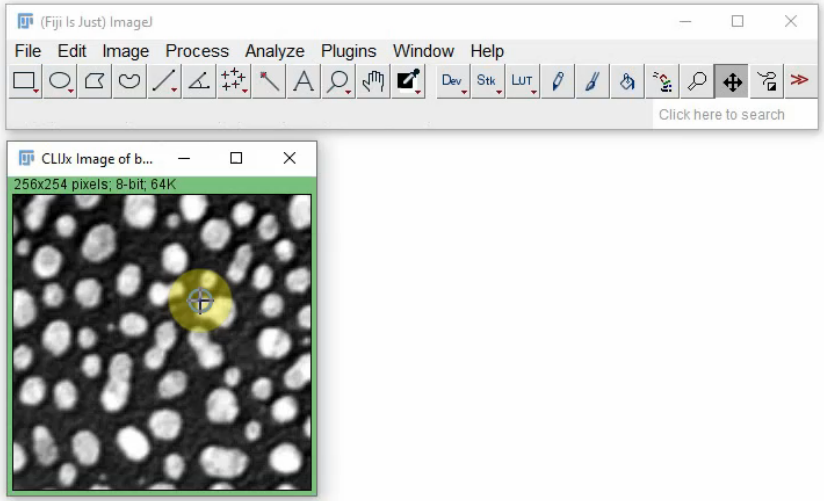
Should we use a different segmentation algorithm?

Is circularity the right parameter to measure?

Hypothesis-driven quantitative biology

PoL Physics of Life TU Dresden

- Should we use a different segmentation algorithm?



Hypothesis-driven quantitative biology

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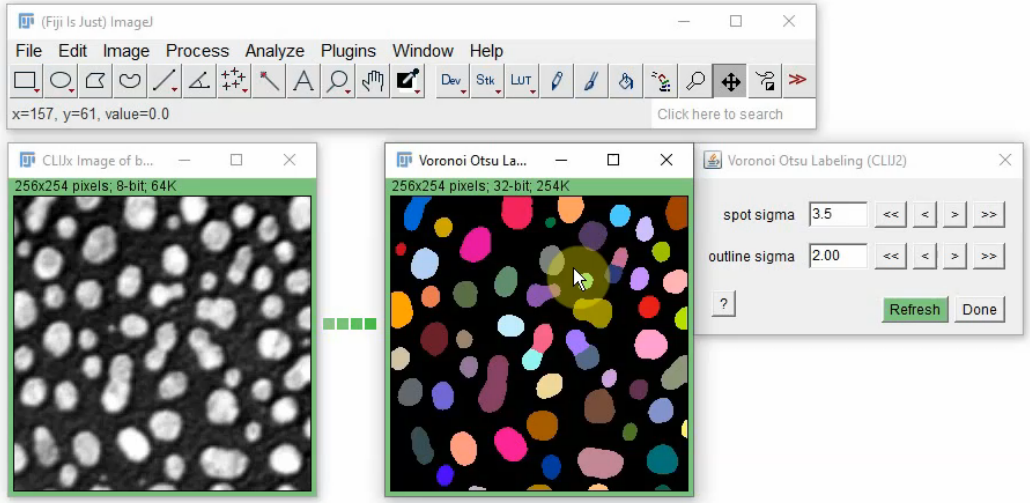
Is circularity the right parameter to measure?



Hypothesis generating quantitative biology

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

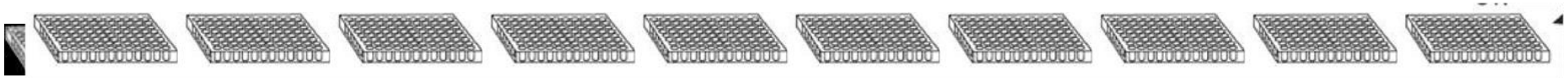
Which segmentation algorithms allow measurements that show a relationship with X?

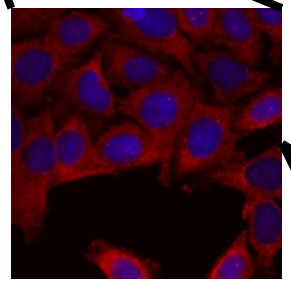
Why?

Which parameter shows any relationship with X?

Cell Profiler

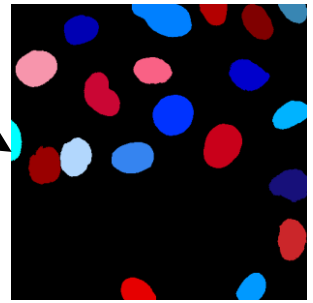
- Cells or organisms in multiwell plates, each well treated with a gene or chemical perturbant

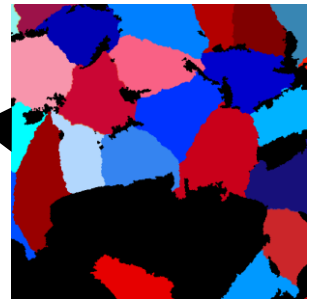




Automated
microscopy
(any manufacturer)

CellProfiler™
cell image analysis software





Cell measurements

(size, shape, intensity,
texture, etc.)

CellProfiler
Analyst

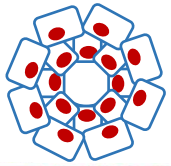
Data exploration
& machine learning

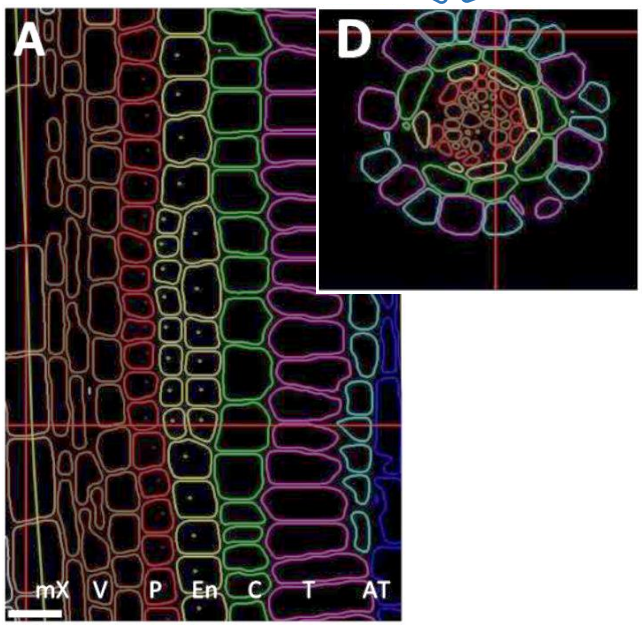
Image analysis beyond segmentation

PoL Physics of Life TU Dresden

- Spatial pattern / image analysis for cell biology

Membranes
Nuclei





Structural biology of plants[1]

15 years ago!

Joint Pattern Recognition Symposium
DAGM 2006: Pattern Recognition pp 182-191 | Cite as

Fast Scalar and Vectorial Grayscale Based Invariant Features for 3D Cell Nuclei Localization and Classification

Authors | Authors and affiliations

Janina Schulz, Thorsten Schmidt, Olaf Ronneberger, Hans Burkhardt, Taras Pasternak, Alexander Dovzhenko, Klaus Palme

Conference paper

10 Citations | 1.8k Downloads

Part of the Lecture Notes in Computer Science book series (LNCS, volume 4174)

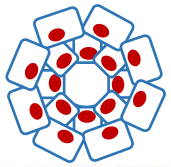
1) Image data source: Pasternak et al. (CC-BY 4.0) https://www.biorxiv.org/content/10.1101/2021.01.01.425043v2

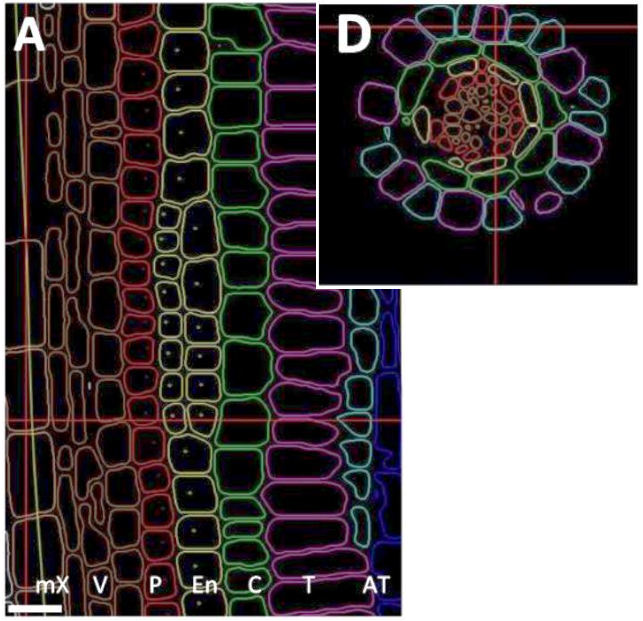
Image analysis beyond segmentation

PoL Physics of Life TU Dresden

- Spatial pattern / image analysis for cell biology

Membranes
Nuclei





Structural biology of plants[1]

Kay Schneitz @PlantDevTUM · Mar 29

Just in case you hadn't yet noticed our supercool new toolbox for MorphoGraphX. Provides organ-centric spatial context to cellular features in 3D digital plant organs.
tinyurl.com/2n6rb6ds
@athul_r_v @StraussSoe @tejasvinee_m @kareninglelee @RichardSmithLab @PlantPhys

21 76

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1) Image data source: Pasternak et al. (CC-BY 4.0) https://www.biorxiv.org/content/10.1101/2021.01.01.425043v2
2) Palla et al https://academic.oup.com/plphys/advance-article/doi/10.1093/plphys/kiac145/6555043

Image analysis beyond segmentation

PoL Physics of Life TU Dresden

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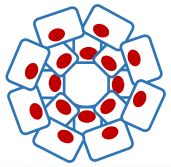
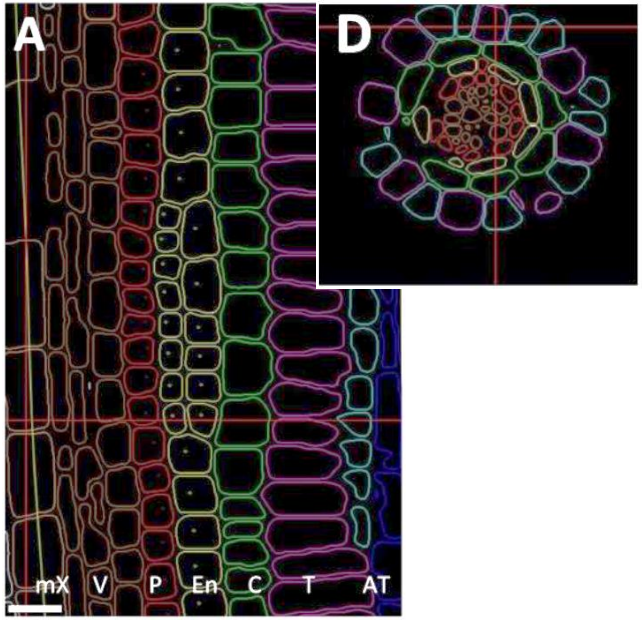
Nuclei





Structural biology of plants[1]

2

Cell Reports
Resource

CytoMAP: A Spatial Analysis Toolbox Reveals Features of Myeloid Cell Organization i

Caleb R. Stoltzfus,[1] Jakub Fili
Miranda R. Lyons-Cohen,[1] Jes
Kevin B. Urdahl,[1,2,3] Mario Per
[1]Department of Immunology, Unive
[2]Seattle Children's Research Institu
[3]Department of Pediatrics, Univers
[4]School of Medicine, University of
[5]Roche Innovation Center Munich,
Penzberg, Germany
[6]Roche Innovation Center Zurich,
[7]Lead Contact
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https://doi.org/10.1016/j.celrep.20

3

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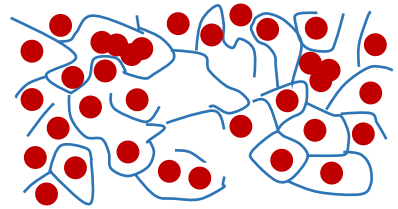
Article | Open Access | Published: 31 January 2022

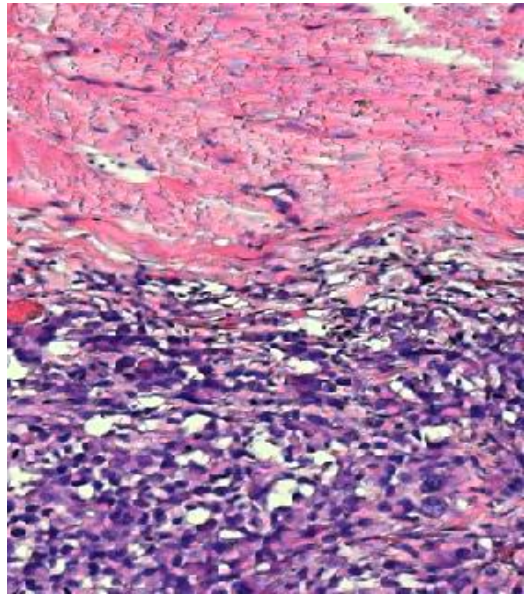
Squidpy: a scalable framework for spatial omics analysis

Giovanni Palla, Hannah Spitzer, Michal Klein, David Fischer, Anna Christina Schaar, Louis Benedikt Kuemmerle, Sergei Rybakov, Ignacio L. Ibarra, Olle Holmberg, Isaac Virshup, Mohammad Lotfollahi, Sabrina Richter & Fabian J. Theis

Nature Methods 19, 171–178 (2022) | Cite this article

19k Accesses | 3 Citations | 375 Altmetric | Metrics





Cancer research / histology

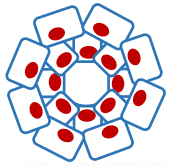
1) Image data source: Pasternak et al. (CC-BY 4.0) https://www.biorxiv.org/content/10.1101/2021.01.01.425043v2
2) https://www.cell.com/cell-reports/fulltext/S2211-1247(20)30423-X
3) Solorzano et al. https://www.nature.com/articles/s41592-021-01358-2

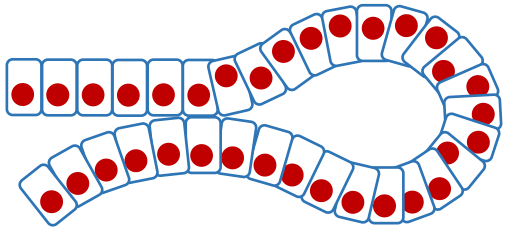
Image analysis beyond segmentation

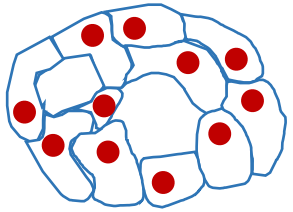
PoL Physics of Life TU Dresden

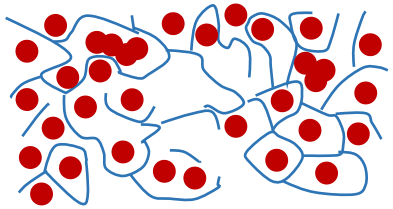
- Spatial pattern / image analysis for cell biology

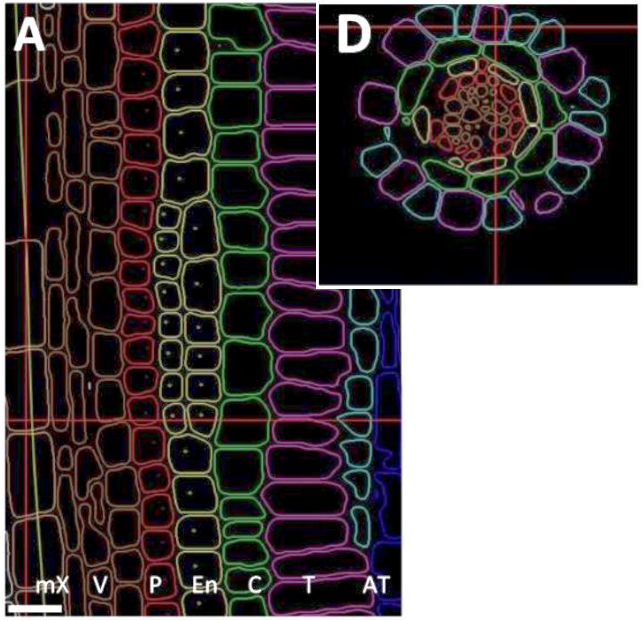
Membranes
Nuclei

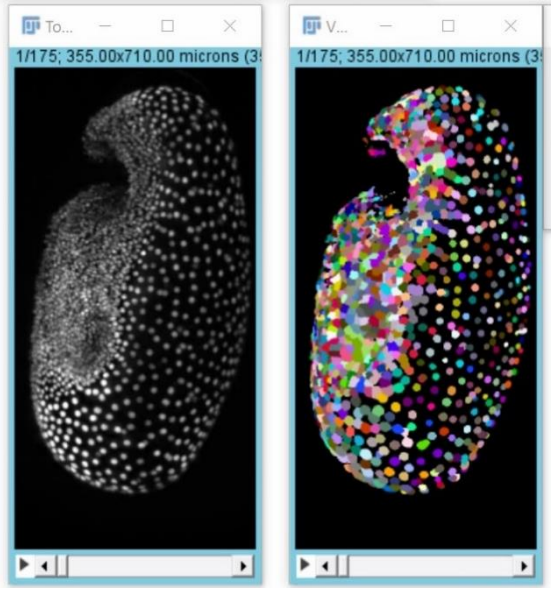


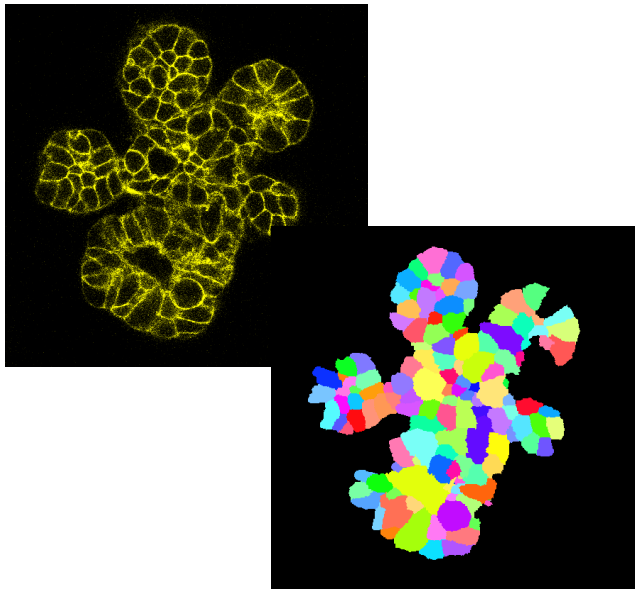


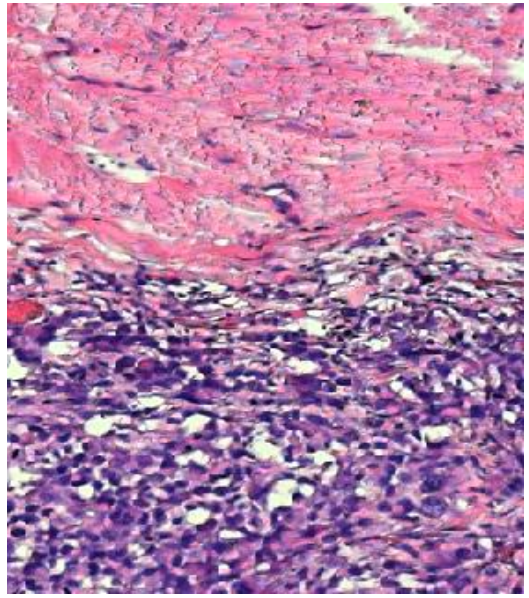












Structural biology of plants[1]

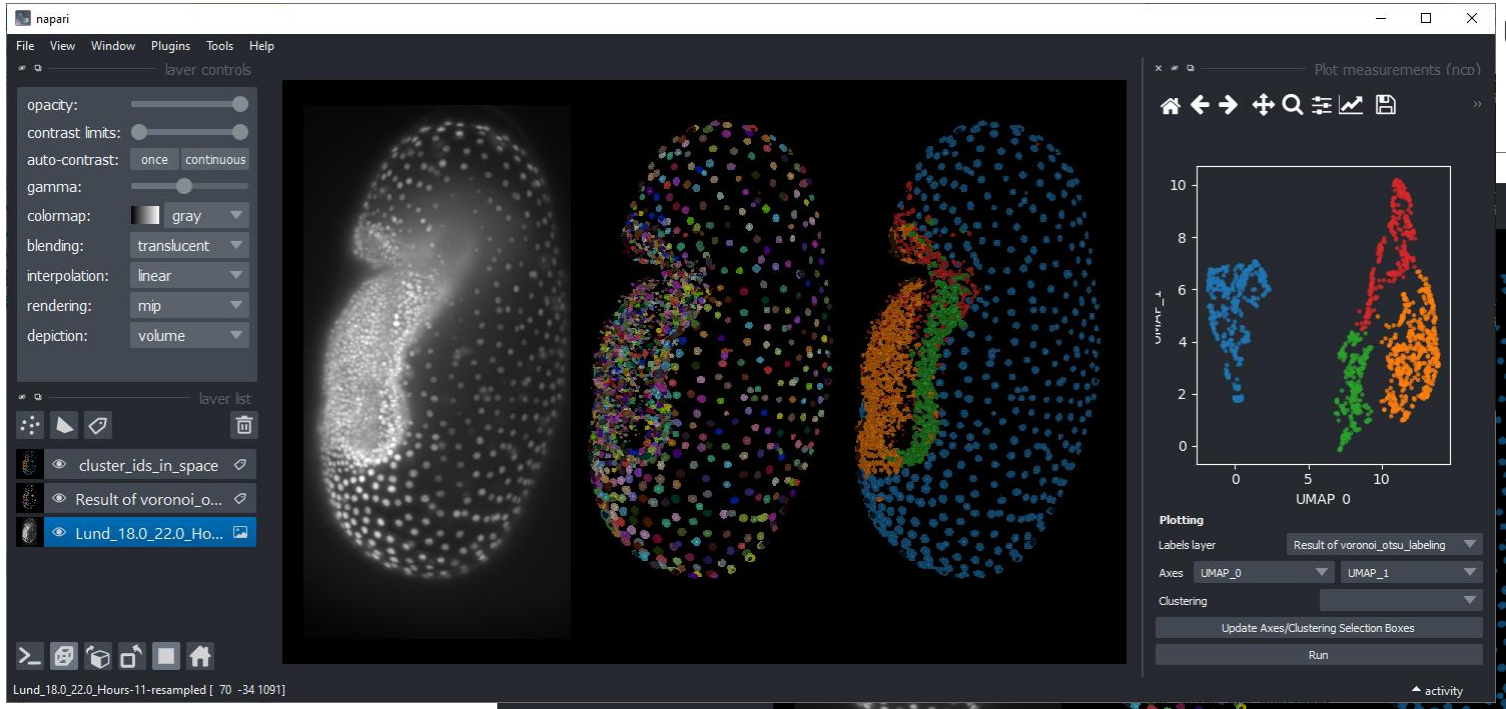
Early embryo development

Organoid formation[3]

Cancer research / histology

1) Image data source: Pasternak et al. (CC-BY 4.0) https://www.biorxiv.org/content/10.1101/2021.01.01.425043v2
3) Image data source: ByungHo Lee, Grapin-Botton Lab

Dimensionality reduction



Uniform manifold approximation and projection (UMAP)

t-distributed stochastic neighbor embedding (t-SNE)

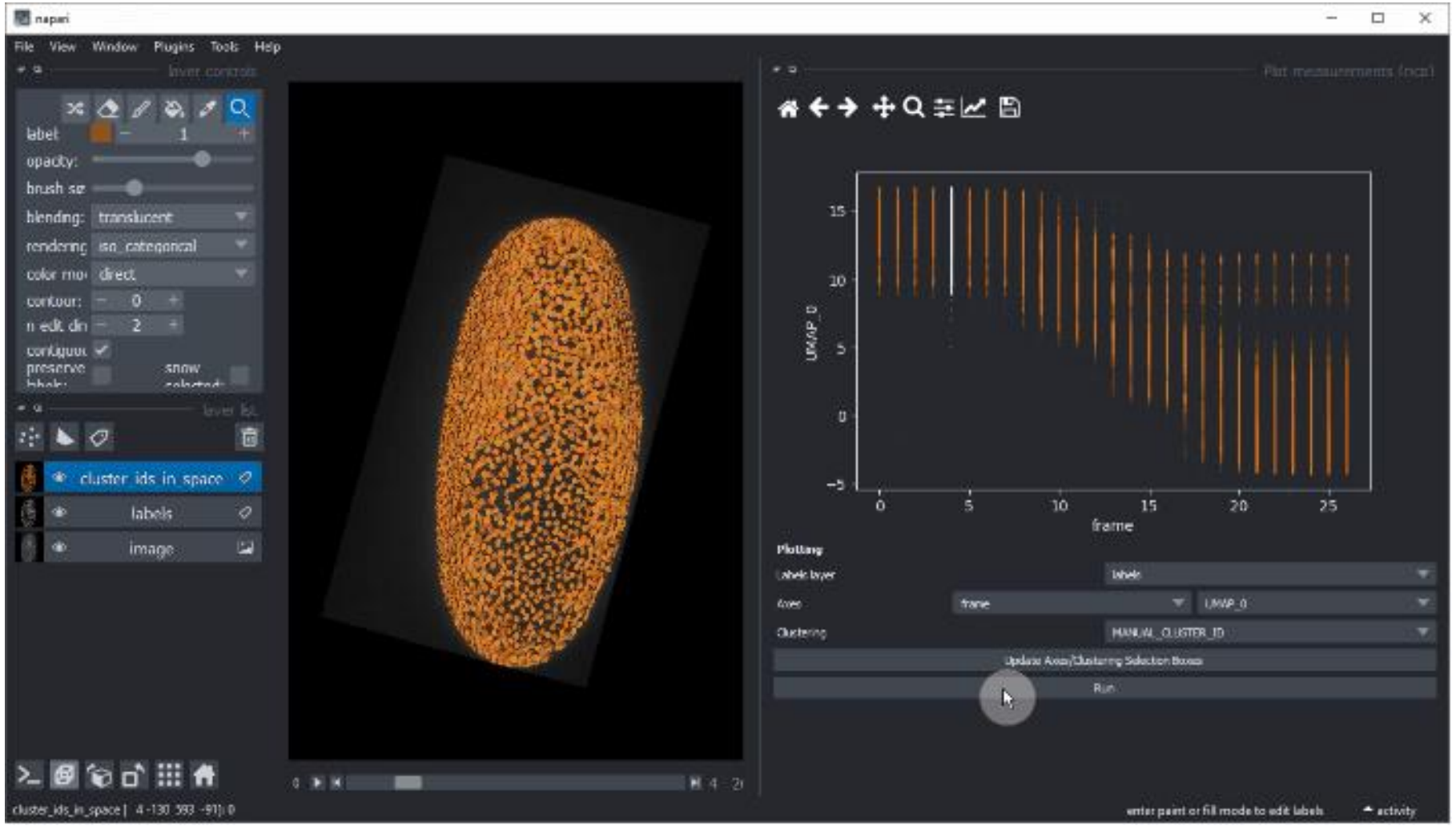
Principal component analysis (PCA)

Laura Žigutytė
@zigutyte

Ryan Savill
@RyanSavill4

Data exploration

- Manual clustering to gain deeper insights in relationships between measured parameters



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

Image data source: Daniela Vorkel, Myers lab, MPI-CBG/CSBD

Image analysis is part of the experiment

Start talking to image-analysis / data-science experts early during your project.

Not in the final year of your PhD.

Summary

So far, you learned

- *Bio-image analysis*
  - Quantitative
  - Objective
  - Reproducible
  - Repeatable
  - Reliable

- When to talk to an expert

Coming up next

- Working with images from Python
- Image filtering