

QM Course 2025
Charles University, Prague

Bio-Image Analysis with napari Plugins

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Re-using material from:

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- Feature Extraction with **napari-skimage-regionprops**
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Break

- Object Classification with Machine Learning using **napari-apoc** and **napari-clusters-plotter**





Installation

- napari as a Python Library
- napari as a Bundle App
- Installing Plugins

Installing napari and napari plugins

For this course, an environment with containing napari and a few plugins should already be installed in the local computers (napari-intro-env or devbio-napari). In case you have problems, the installation instructions can be found here:

- https://biapol.github.io/QM_Course_Bio_Image_Analysis_with_napari_2025/intro.html



The following slides are meant to clarify what these instructions mean. You do not need to actually do them.

Installing napari as a Python package

napari is a Python library

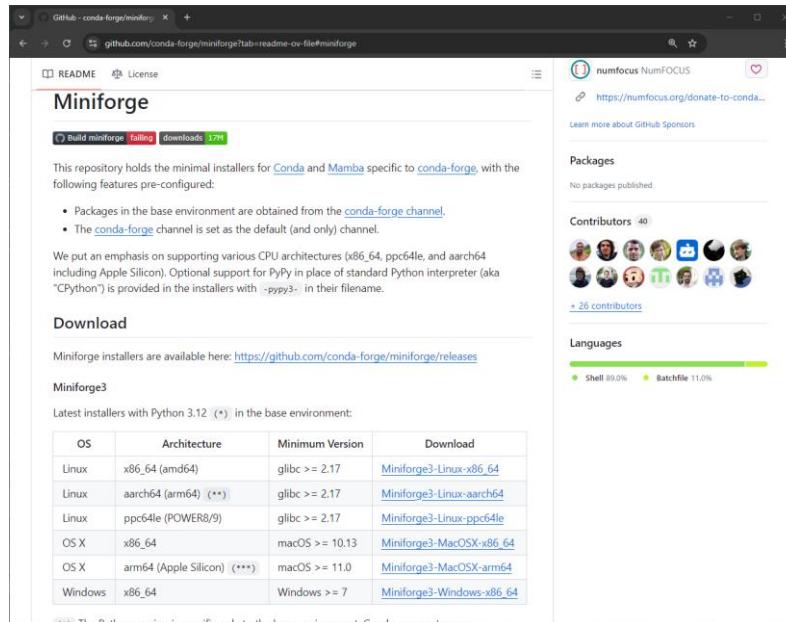
The recommended way to install napari is as a Python package

<https://napari.org/stable/tutorials/fundamentals/installation.html#install-as-python-package-recommended>

Your computer needs Python to run a Python package

- How do I install Python?

Install Miniforge!



<https://github.com/conda-forge/miniforge?tab=readme-ov-file#miniforge>

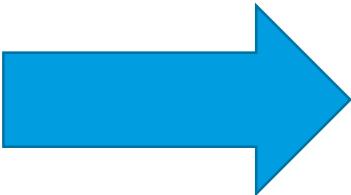
- Miniforge is a minimal installer for `conda` and `mamba` (efficient `conda` in C++)
- `conda` is an environment and a package manager
- `conda` can create Virtual Environments and install Python (and other) packages, including napari

https://hackmd.io/@talley/SJB_lObBi#Terms

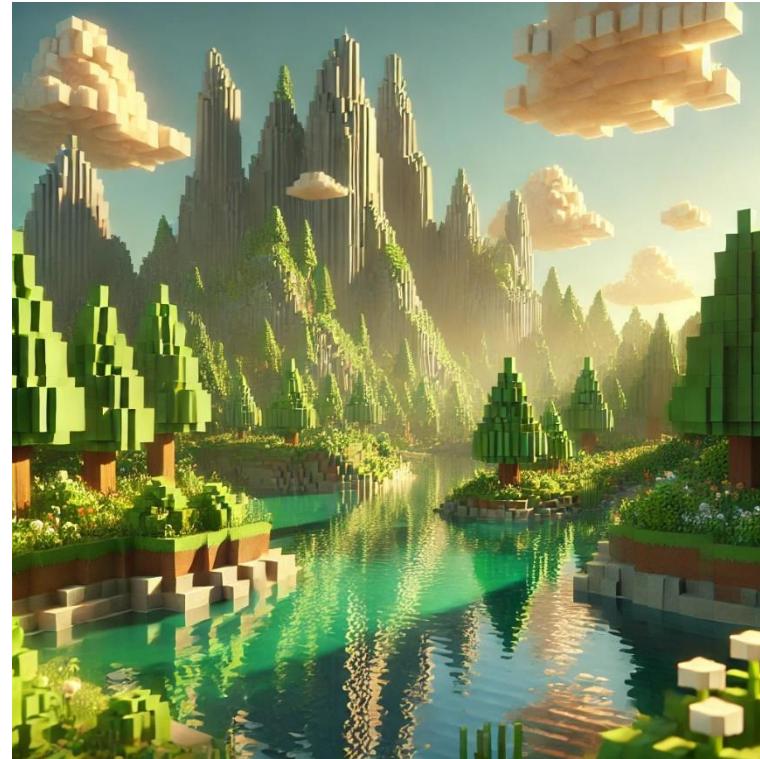
Installing napari as a Python package



Wait, environments ?



Yes, virtual environments!



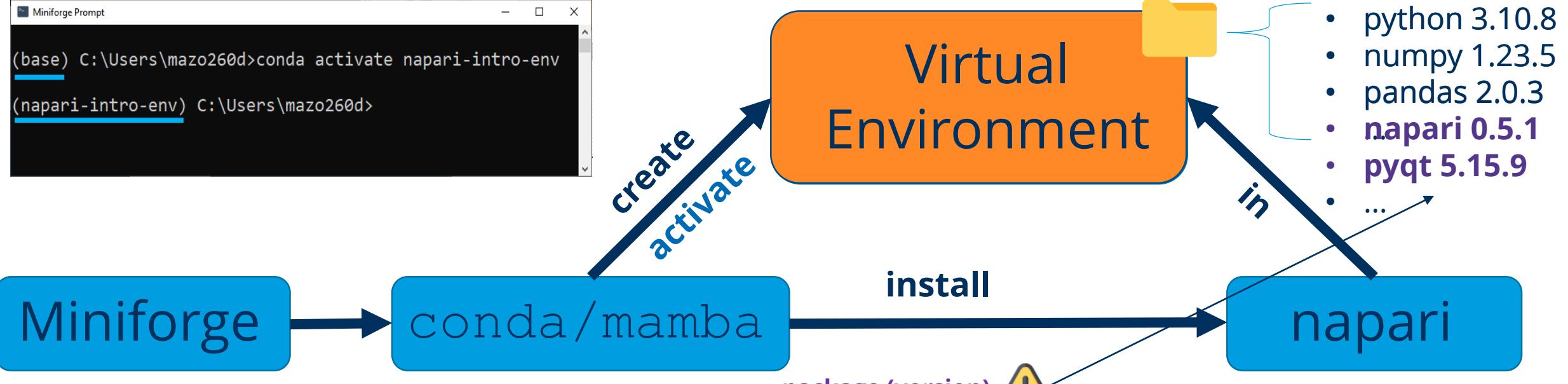
Images generated by DALL-E, developed by OpenAI.

Smiley icons from Flaticon.com

Installing napari as a Python package

Install it as a Python package in a Virtual Environment

- A virtual environment is an isolated collection of packages, settings, and an associated python interpreter



Commands:

mamba **create -y -n** napari-intro-env **-c** conda-forge **python=3.10**

mamba **activate** napari-intro-env

mamba **install -c** conda-forge napari pyqt

Some packages can only be installed with `pip`

`conda` checks for version compatibility before installing

- `pip` tries to install, then lets you know if something went wrong
- `pip` cannot create environments

Installing napari

Napari can also be downloaded and installed like other software

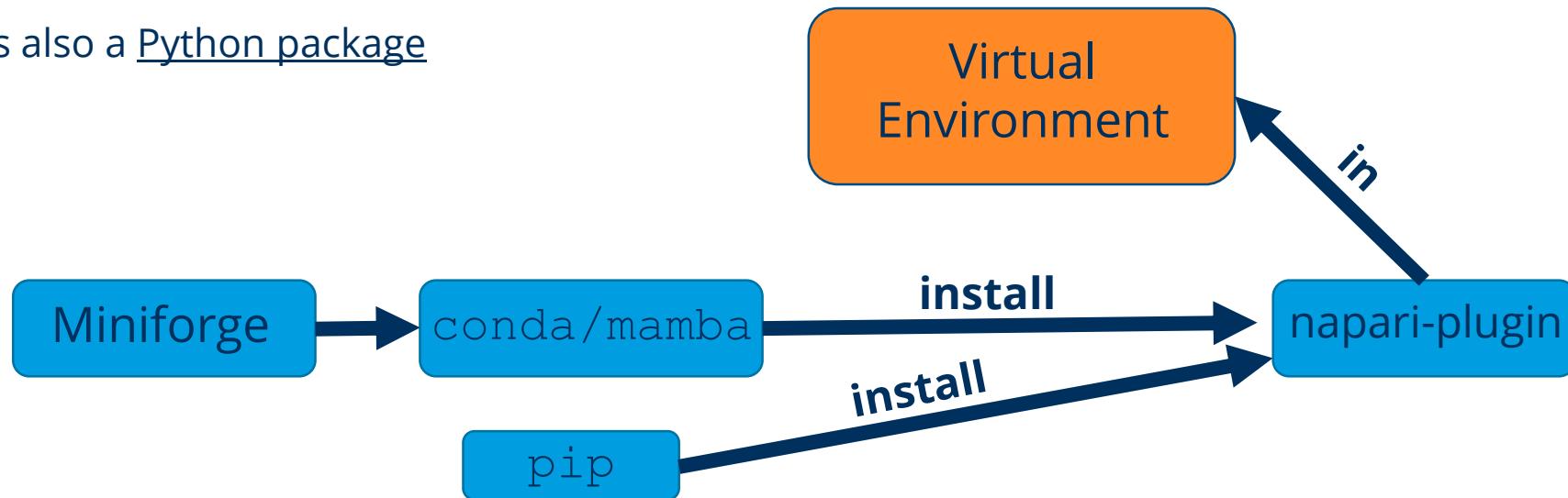
- https://napari.org/stable/tutorials/fundamentals/installation_bundle_conda.html#how-to-install-napari-as-a-bundled-app

This is more convenient, however it may be difficult to manage different plugin versions mid-long term

- In case of a dependency version mismatch, it may be necessary to uninstall and re-install the software and all the plugins again

Installing a plugin

A napari plugin is also a Python package



Commands:

`mamba activate napari-intro-env`

`mamba install napari-plugin`

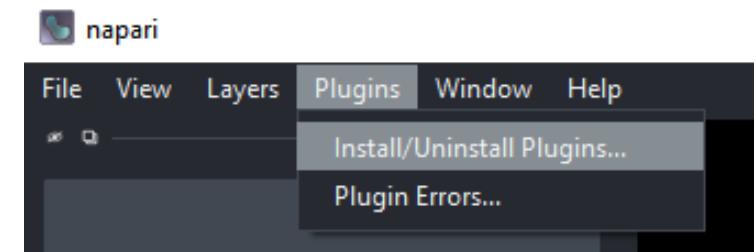
`pip install napari-plugin`

OR

`conda install napari-plugin`

Always check plugin
installation instructions!

Via Plugins Menu:



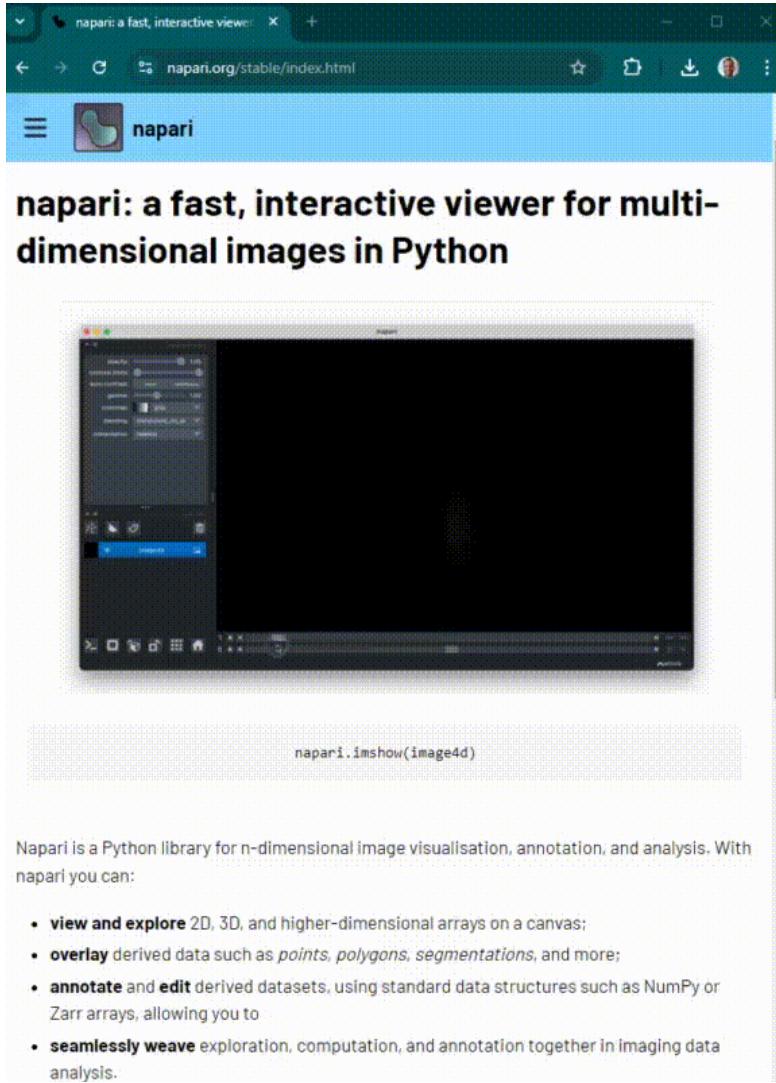
Introduction to napari

- napari Viewer
- Layer Types
- napari Plugins

Napari: 3D viewer for Python

Multi-dimensional image viewer in python

<https://napari.org/>



Napari is a Python library for n-dimensional image visualisation, annotation, and analysis. With napari you can:

- **view and explore** 2D, 3D, and higher-dimensional arrays on a canvas;
- **overlay** derived data such as *points*, *polygon*s, *segmentations*, and more;
- **annotate and edit** derived datasets, using standard data structures such as NumPy or Zarr arrays, allowing you to
- **seamlessly weave** exploration, computation, and annotation together in imaging data analysis.

Napari: 3D viewer for Python



<https://napari.org/>

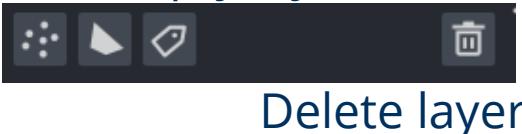
Napari user interface

Menus

View configuration / tools

```
layer.opacity = 0.5
```

Add empty layers



Delete layer

Layers

```
layer.visible = False
```

Viewer controls



Open Python Console



2D/3D view



Change axes order



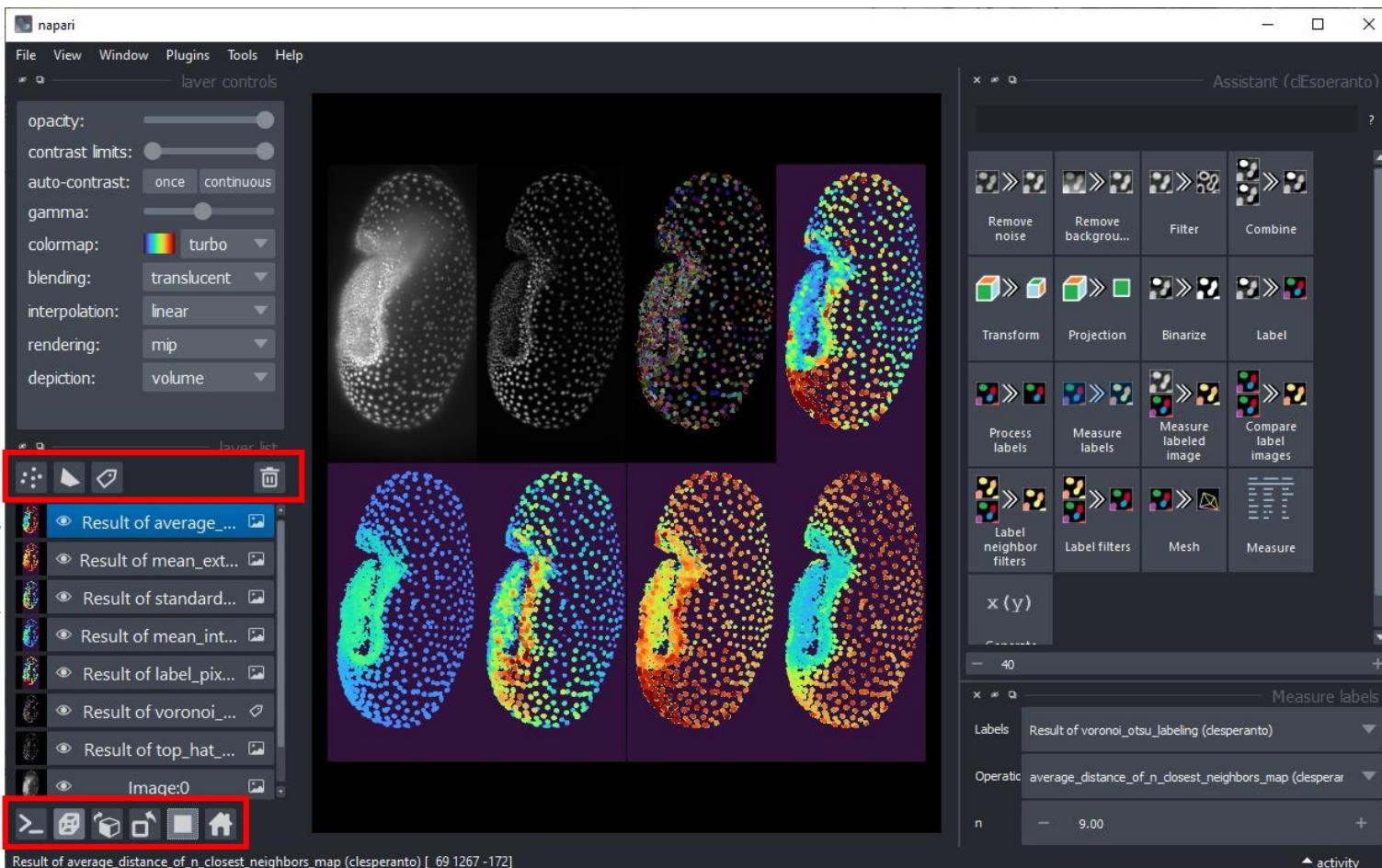
Transpose visible axes



Grid mode



Reset view

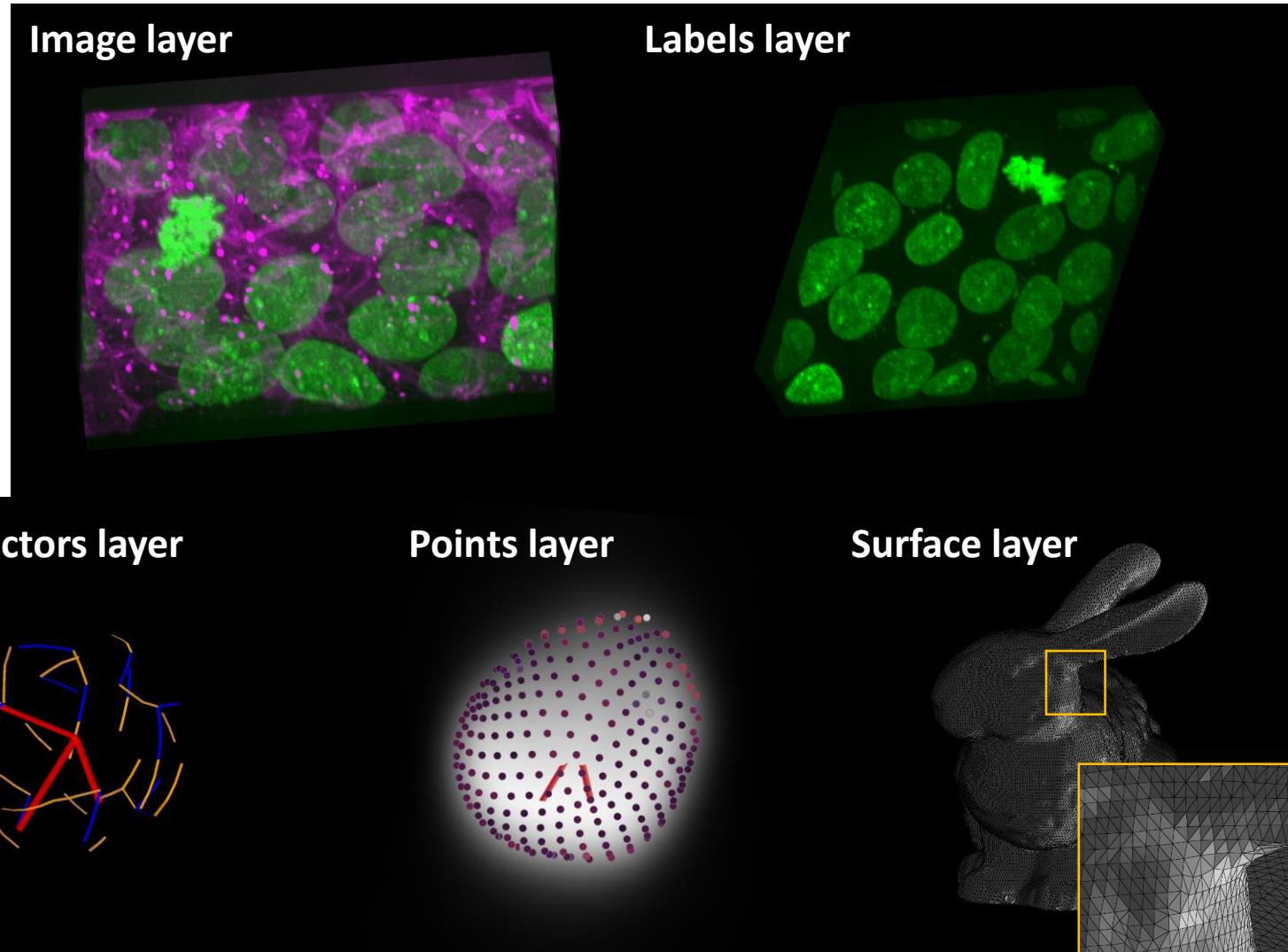


Dock widgets
(custom plugins)

Function widgets
(custom plugins)

Layer types

- **Image layer:** Can be n-dimensional grayscale data (e.g., [CTZYX])
- **Labels layer:** Similar to image layer, but contains only integer numbers (e.g., 0,1,2,3,...)
- **Points layer:** List of coordinates in space
- **Vectors layer:** Direction from point A to point B
- **Surface layer:** Mesh (Vertices, Faces, Values)
- **Tracks layer:** Follow objects through time



<https://github.com/quantumjot/arboretum> licensed under [MIT license](#)

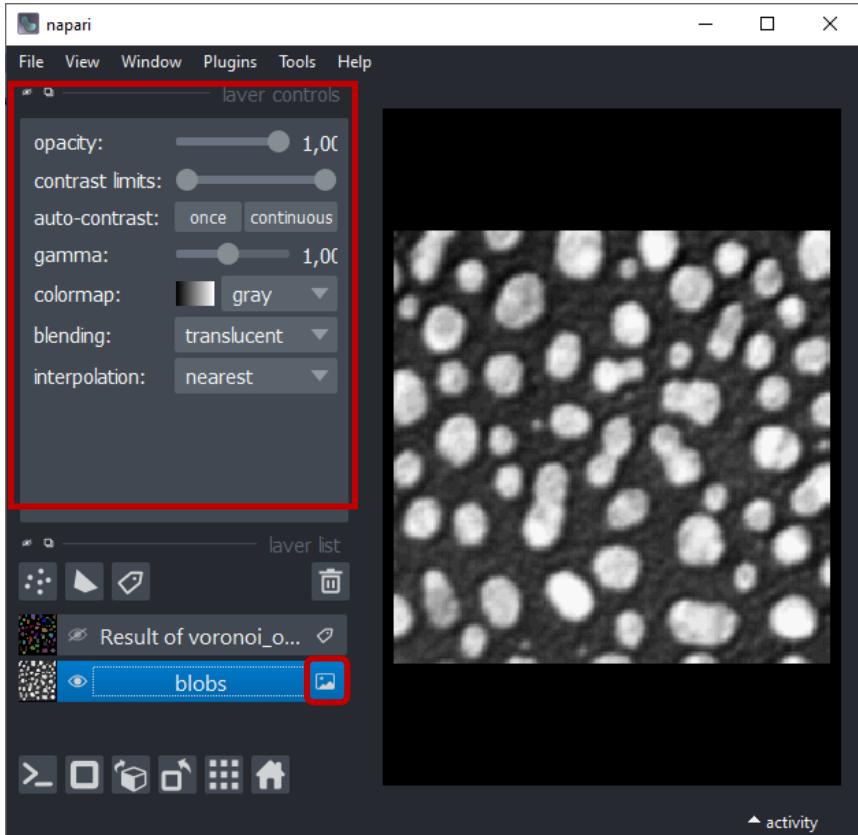
<https://github.com/campaslab/napari-stress>

<https://gitlab.kitware.com/vtk/vtk>

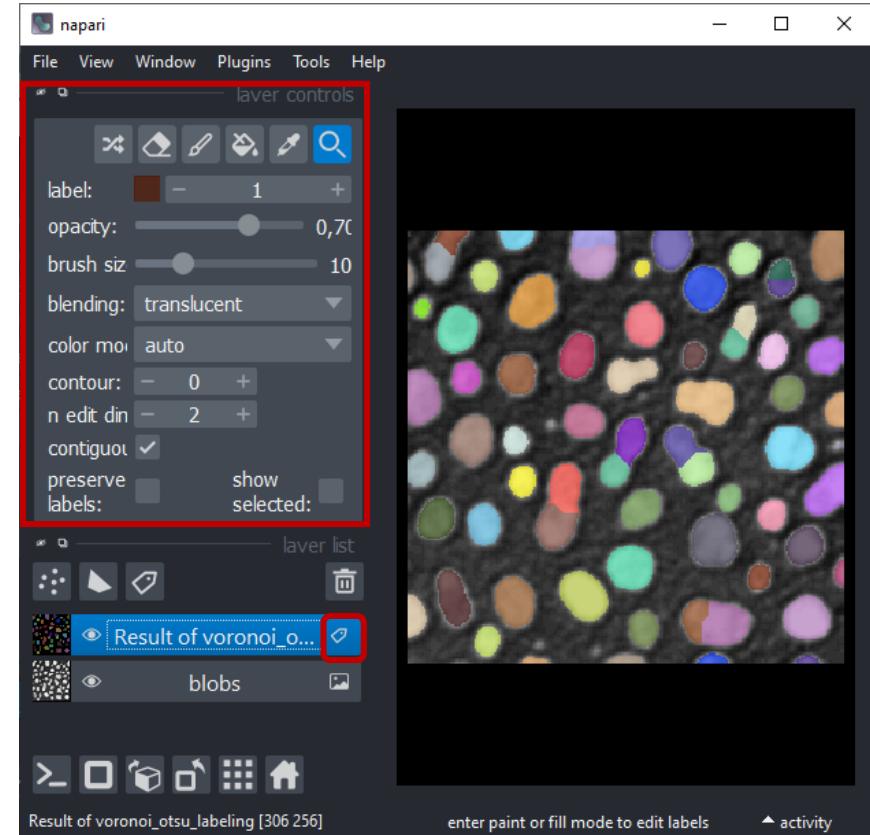
Layer types

Different layers have different tools and options

Image Layer



Labels Layer



Python image & data analysis tools are powerful!



Python scientific image computing
toolbox
<https://github.com/scikit-image>



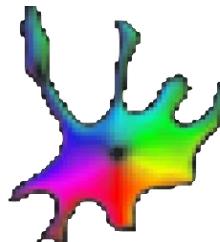
Object detection with Stardist
<https://github.com/stardist>



Python machine-learning toolbox
<https://github.com/scikit-learn>



Data visualization & exploration
<https://github.com/matplotlib>
<https://github.com/seaborn>



Cell segmentation
<https://github.com/MouseLand/cellpose>



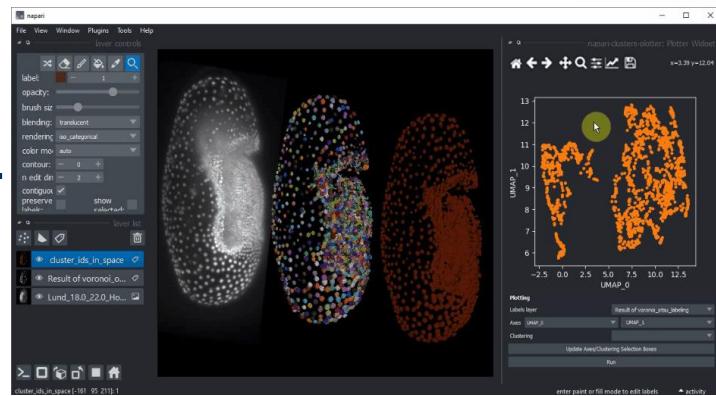
GPU-accelerated image processing
<https://github.com/cLEsperanto>

Napari



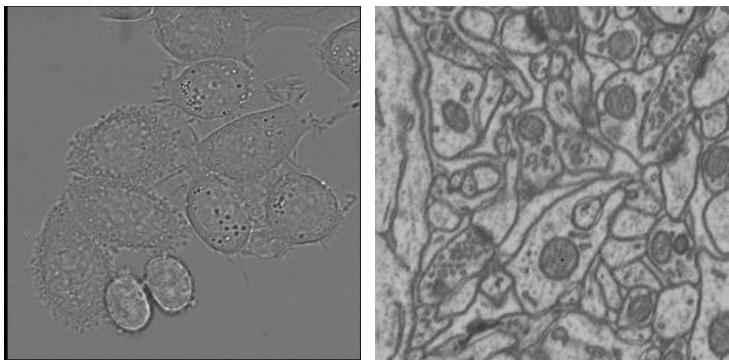
Existing functionality can
be turned into plugins!
→ Interactivity
→ Automatic GUI
generation

clusters-plotter



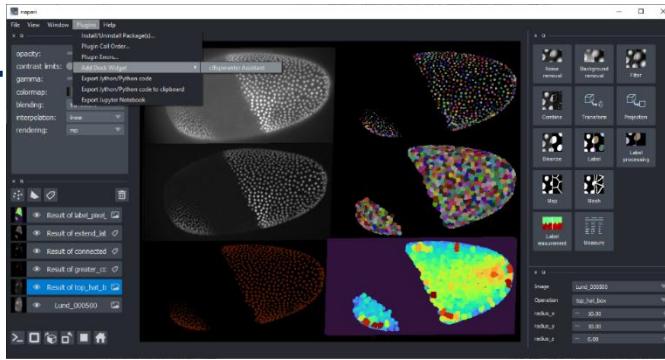
<https://github.com/BiAPoL/napari-clusters-plotter>

micro-sam



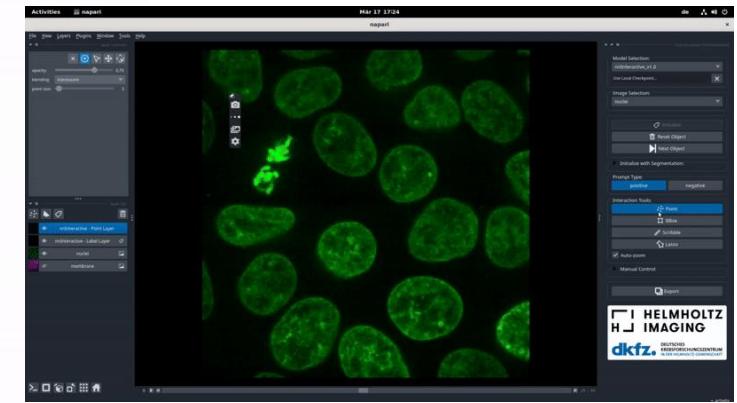
<https://github.com/computational-cell-analytics/micro-sam>

devbio-napari



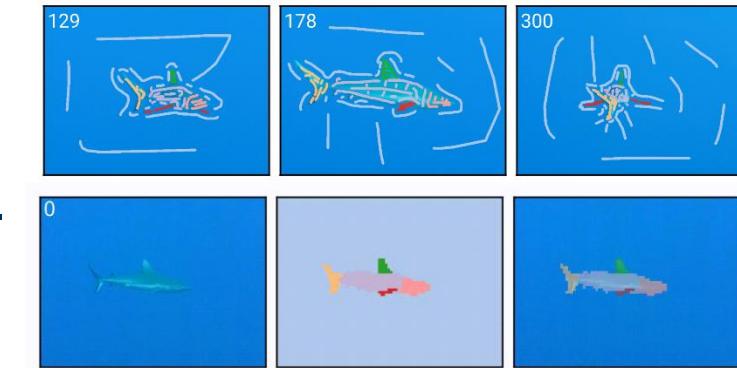
<https://github.com/haesleinhuepf/devbio-napari>

segmentation



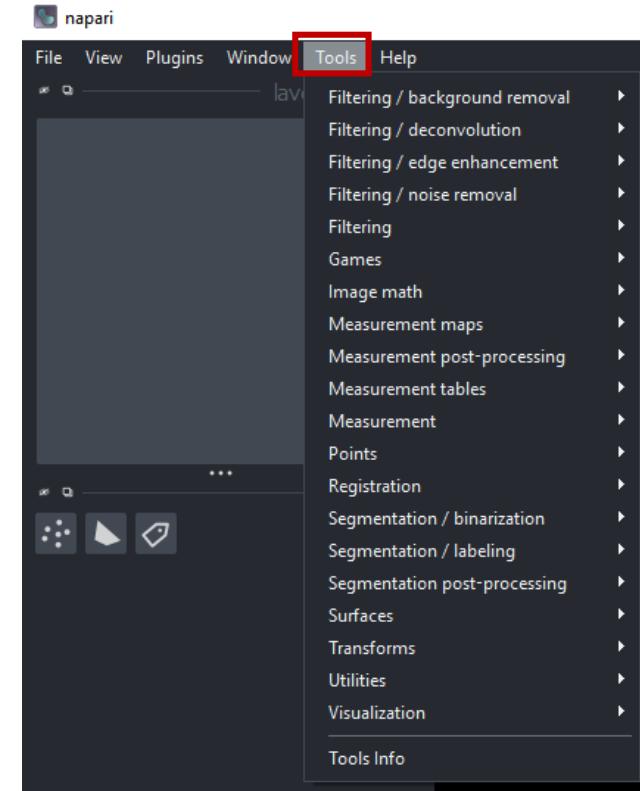
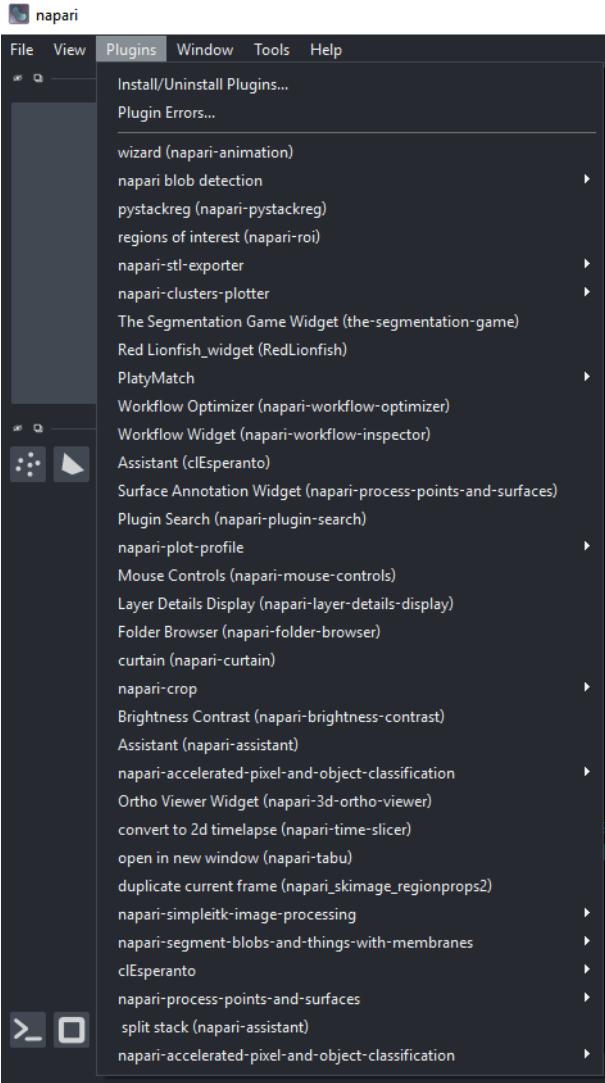
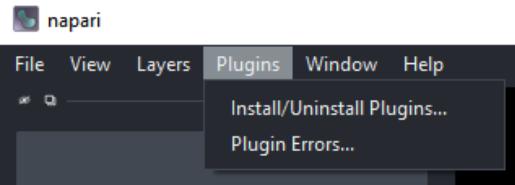
<https://github.com/MIC-DKFZ/napari-nninteractive>

conv-paint

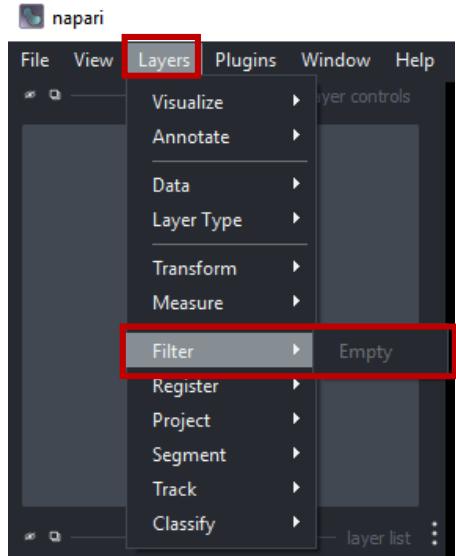


<https://github.com/guiwitz/napari-convpaint>

Plugins and Layers Menus



devbio-napari plugin bundle



napari >= 0.5.0

Layers sub-menu items depend on plugin developers populating them

The Napari Hub

The plugin you are looking for may be near you!
Search engine for napari plugins

The Napari Hub homepage features a large blue header with the text "Discover, install, and share napari plugins". Below this, a message states "Since October 1, 2024, this version is no longer actively maintained and will not be updated. New plugins and plugin updates will continue to be listed." A search bar at the top allows users to search for plugins by keyword or author. The main content area displays "Browse plugins: 227" and includes filters for Workflow step, Image modality, and Supported data. A sidebar shows the number of plugins by category: napari-cell-segmentation (14), napari-feature-extraction (1), napari-blob-detection (1), and napari-skimage-regionprops (1). The footer includes social media links for Mastodon (@mazoc.bsky.social) and Technische Universität Dresden.

Two screenshots of the Napari Hub search interface are shown. The left screenshot shows the search results for "cell segmentation", displaying a list of plugins including "voluseg-napari" and "Varun Kapoor". The right screenshot shows the search results for "feature extraction", displaying a list of plugins including "napari-features" and "napari-blob-detection". Both pages include filters for Workflow step, Image modality, and Supported data, along with sorting options (Relevance, Recently updated, Newest). The footer of the right screenshot includes links to BioDIP, PoL, and Dresden Concept.

Inspecting plugin usage and maintenance - GitHub

The screenshot shows a GitHub repository page for 'empanada-napari'. The repository has 210 commits, with the most recent one being 'Merge pull request #34 from volume-em/empanada_v1.1.1' by 'barrybarry9' 8 months ago. The commit history is highlighted with a red box and labeled 'Latests updates'. A red arrow points from this box to the 'Documentation' section on the right, which includes links to 'empanada.readthedocs.io/en/latest/emp...' and 'Zenodo First Release'. Another red arrow points from the bottom left to the repository name 'empanada-napari'.

Latests updates

Documentation

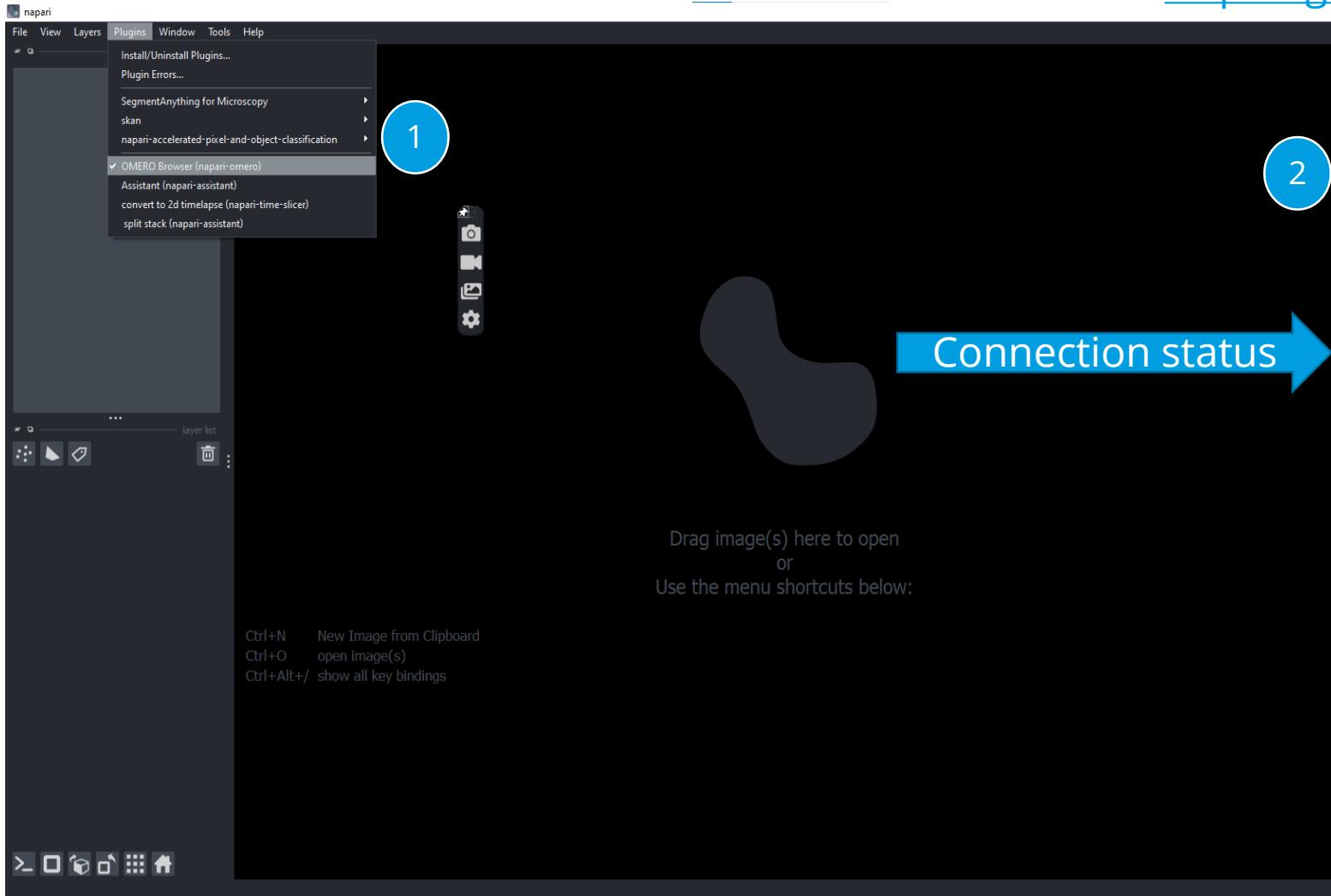
empanada-napari

File / Commit	Date
.github/workflows deployment ready	3 years ago
.napari model and data docs	3 years ago
custom_configs allow model architecture customization	3 years ago
empanada_napari Updated requirements.txt and Count labels module.	8 months ago
images docs	3 years ago
.gitignore v1.1 release	last year
LICENSE Initial commit	4 years ago
MANIFEST.in deployment ready	3 years ago
README.md Updated setup.cfg and requirements.txt file to include comp...	8 months ago
pyproject.toml code addition/deletion for project package install	last year
requirements.txt Updated setup.cfg and requirements.txt file to include comp...	8 months ago
setup.cfg Updated setup.cfg and requirements.txt file to include comp...	8 months ago
tox.ini preprint citation	3 years ago

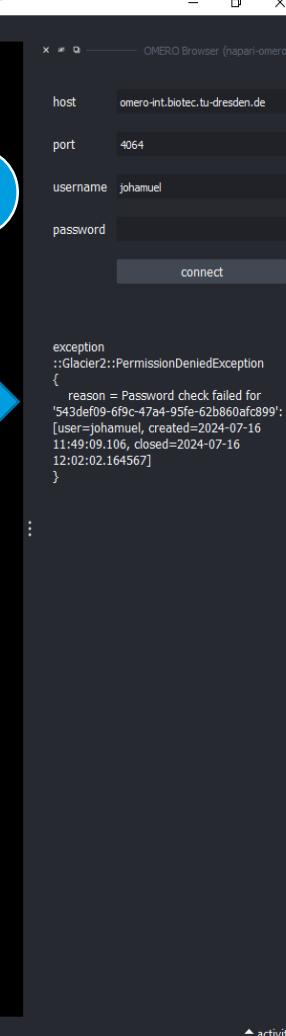
Napari - OMERO integration

- Demonstration: napari-OMERO plugin

Napari-OMERO usage



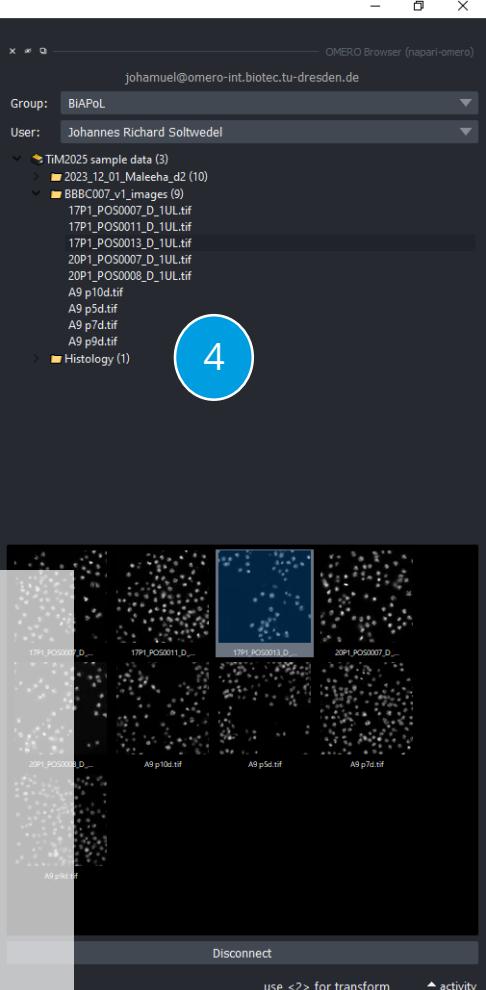
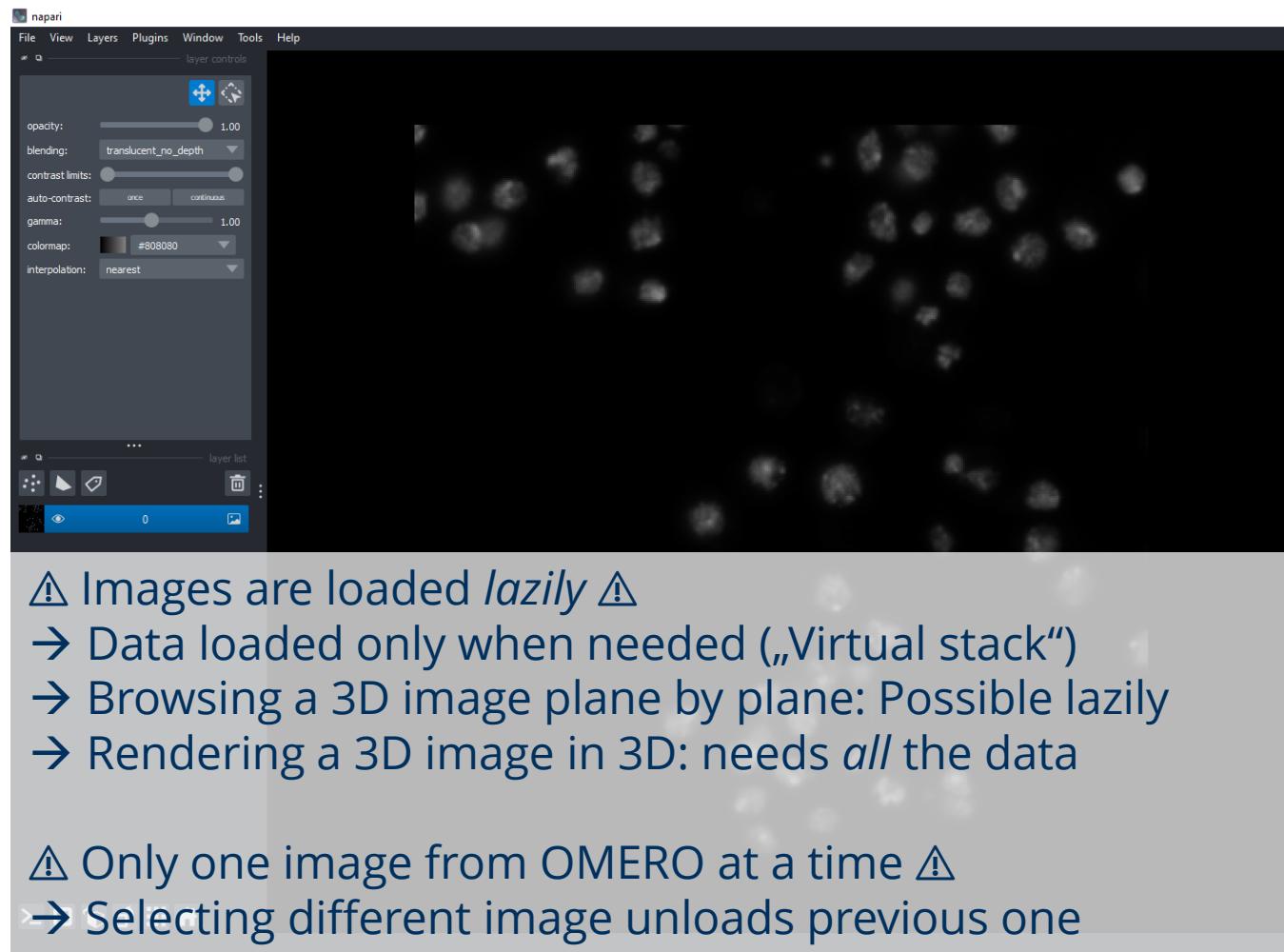
<https://github.com/tlambert03/napari-omero>



1. Select Plugin from plugin menu
2. Log into TiM OMERO server with your TiM credentials

Napari-OMERO usage

3. Select group and user
4. Selecting item from file tree loads image into viewer



Some tips and tricks for OMERO from Python: <https://biapol.github.io/omero-tools>

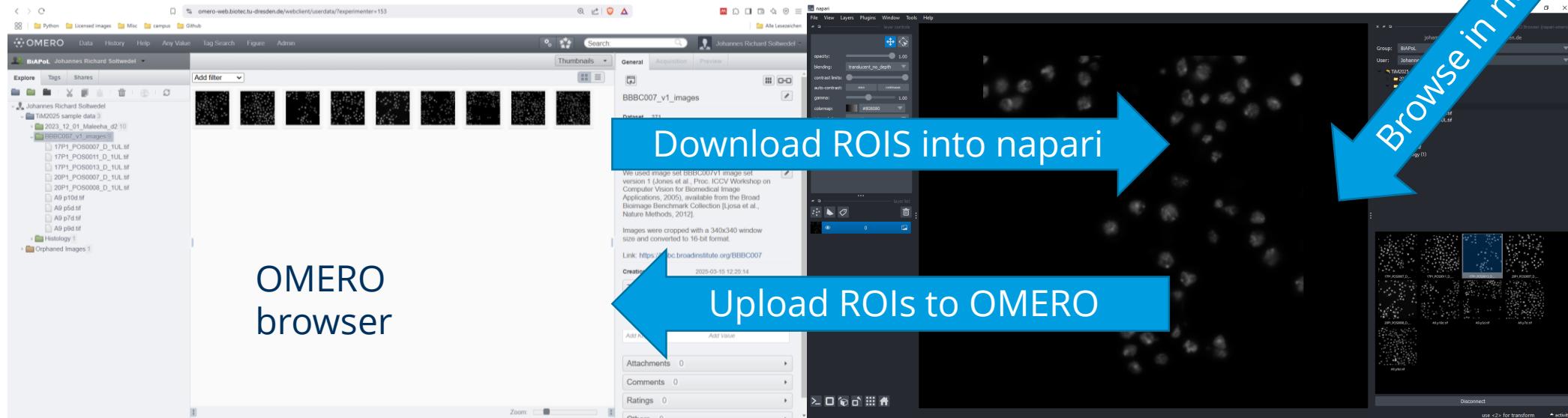
Napari-OMERO usage

Coming soon:

- ROI support
- Multiscale support: Browse larger-than-RAM samples (3D limitation remains)



Browsing multiscale data in OMERO



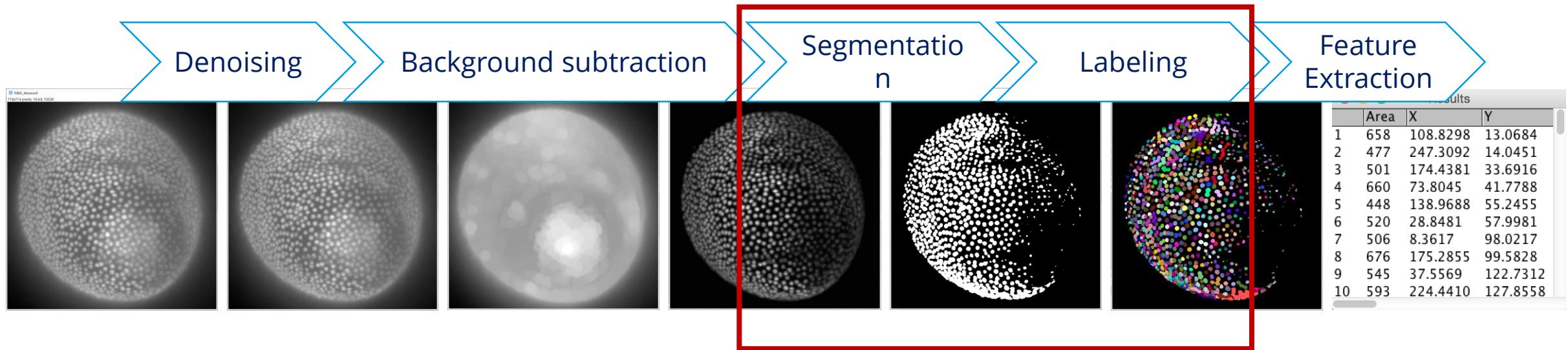
Download ROIS into napari

Upload ROIs to OMERO

Image Analysis Classic Workflows

Image analysis workflow is a series of processes/functions (not always linear) applied to images to achieve a certain goal (usually some measurements)

Here is a classic example:



Segmentation and Supervised Machine Learning

Random Forest Classifiers

- Pixel Classifier

Application: Segmentation

Aim:

Separate background from foreground

Vocabulary:

- **Segmentation:**

- **Segmentation:**
 - Assigning a meaningful *label* to each pixel
 - Segmentation is a *classification* problem

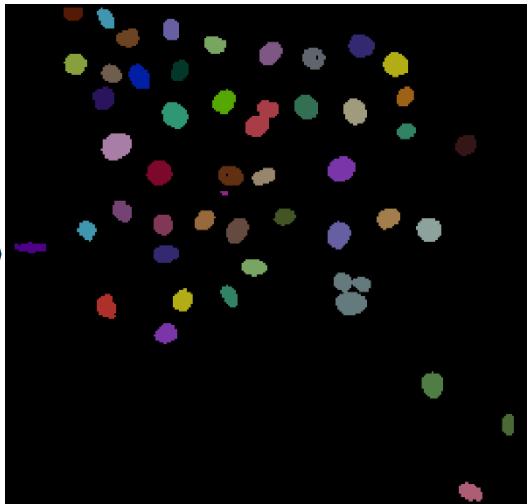
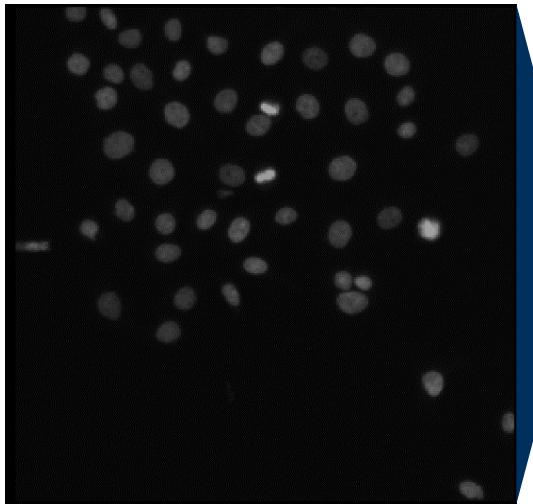
- **Semantic segmentation:**

Differentiate pixels into multiple *classes* (e.g., membrane, nucleus, cytosol, etc.)

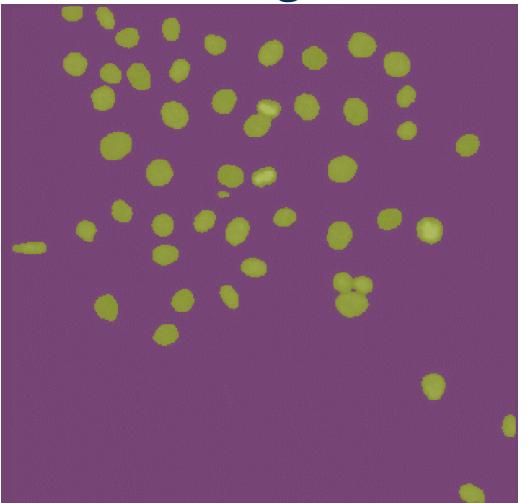
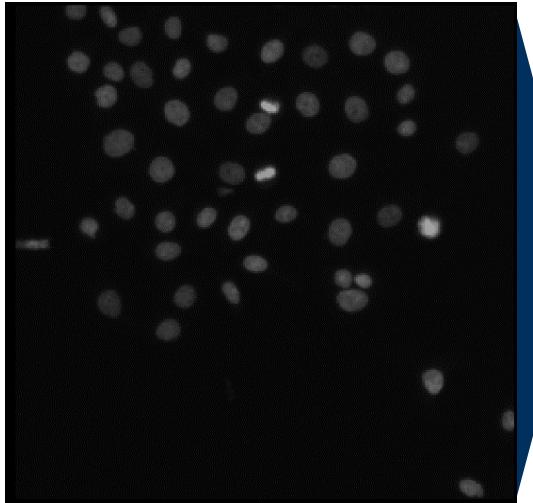
- **Instance segmentation:**

Differentiate multiple occurrences of the same class into separate instances of this class (e.g., separate *label* for each cell in image)

<https://scikit-image.org/docs/stable/api/skimage.data.html>



Instance segmentation



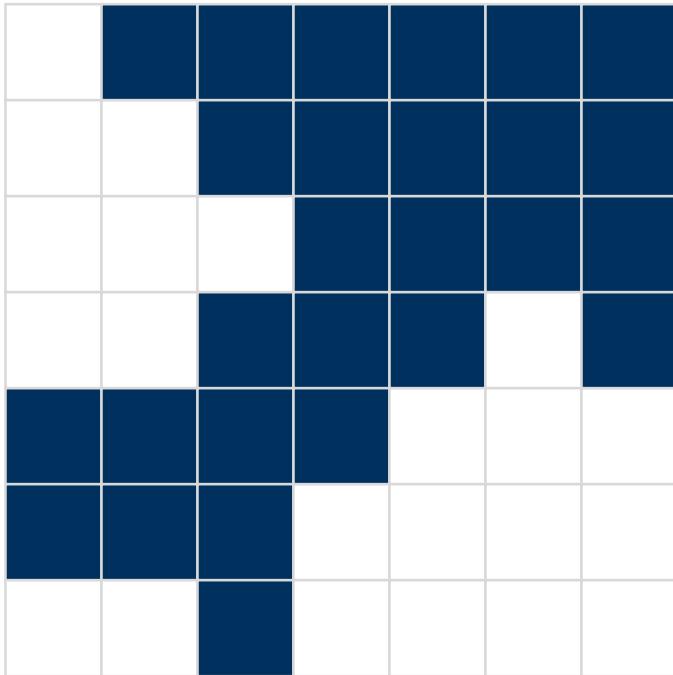
Semantic segmentation

Instance segmentation

In order to allow the computer differentiating objects, connected component analysis (CCA) is used to mark pixels belonging to different objects with different numbers

Background pixels are marked with 0.

The maximum intensity of a labelled map corresponds to the number of objects.



CCA

1	0	0	0	0	0	0
1	1	0	0	0	0	0
1	1	1	0	0	0	0
1	1	0	0	0	3	0
0	0	0	0	3	3	3
0	0	0	3	3	3	3
2	2	0	3	3	3	3

Image segmentation using thresholding

Finding the right workflow towards a good segmentation takes time

A priori, we usually don't know which information in the image is useful for a good segmentation

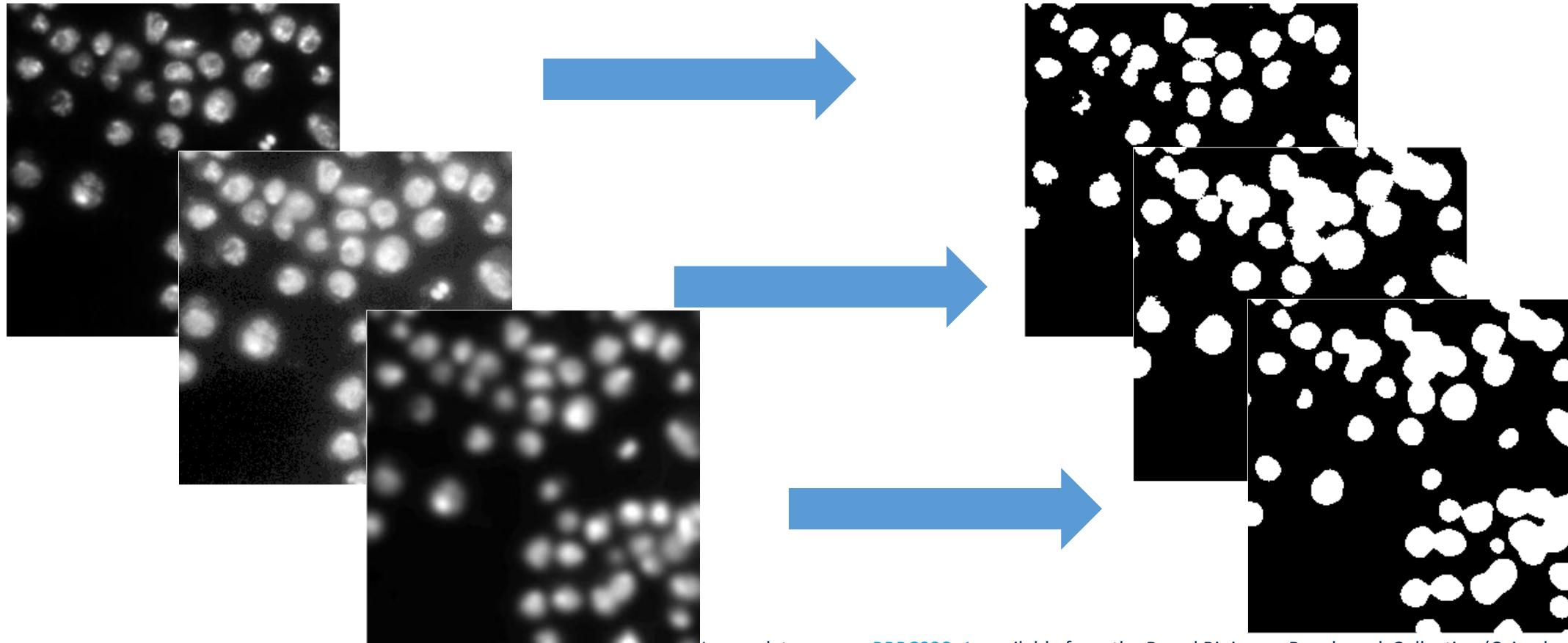


Image data source: [BBBC038v1](#), available from the Broad Bioimage Benchmark Collection (Caicedo et al., Nature Methods, 2019)

Machine learning

- A research field in computer science
- Finds more and more applications, also in life sciences.

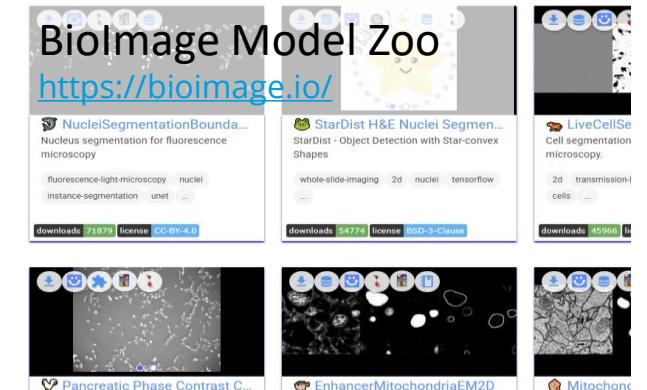
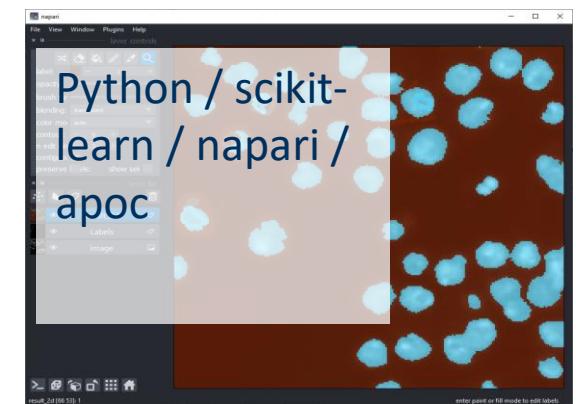
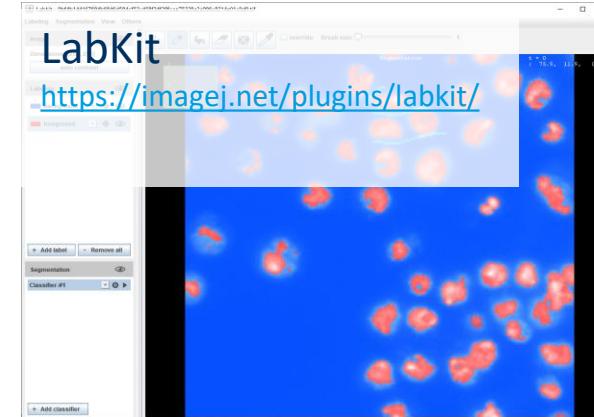
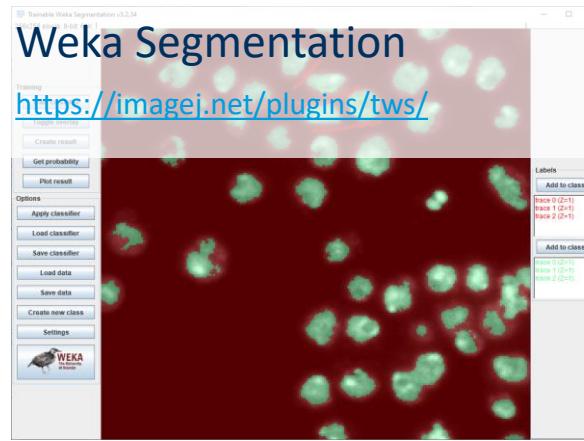
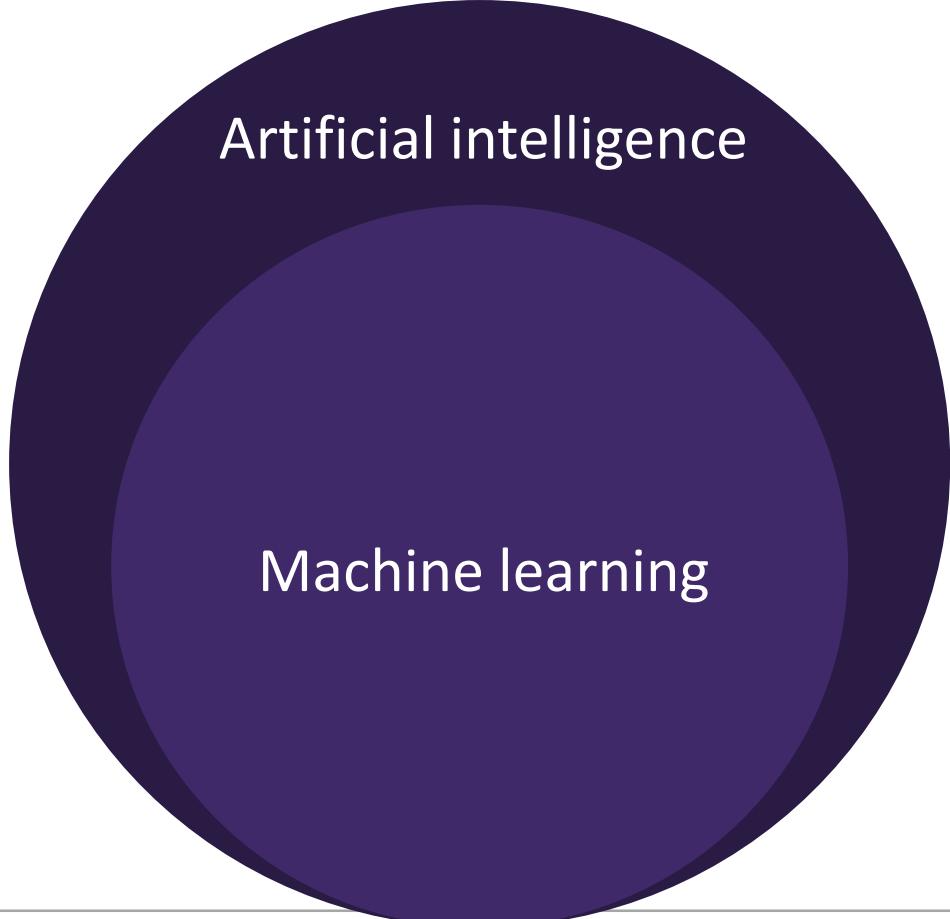
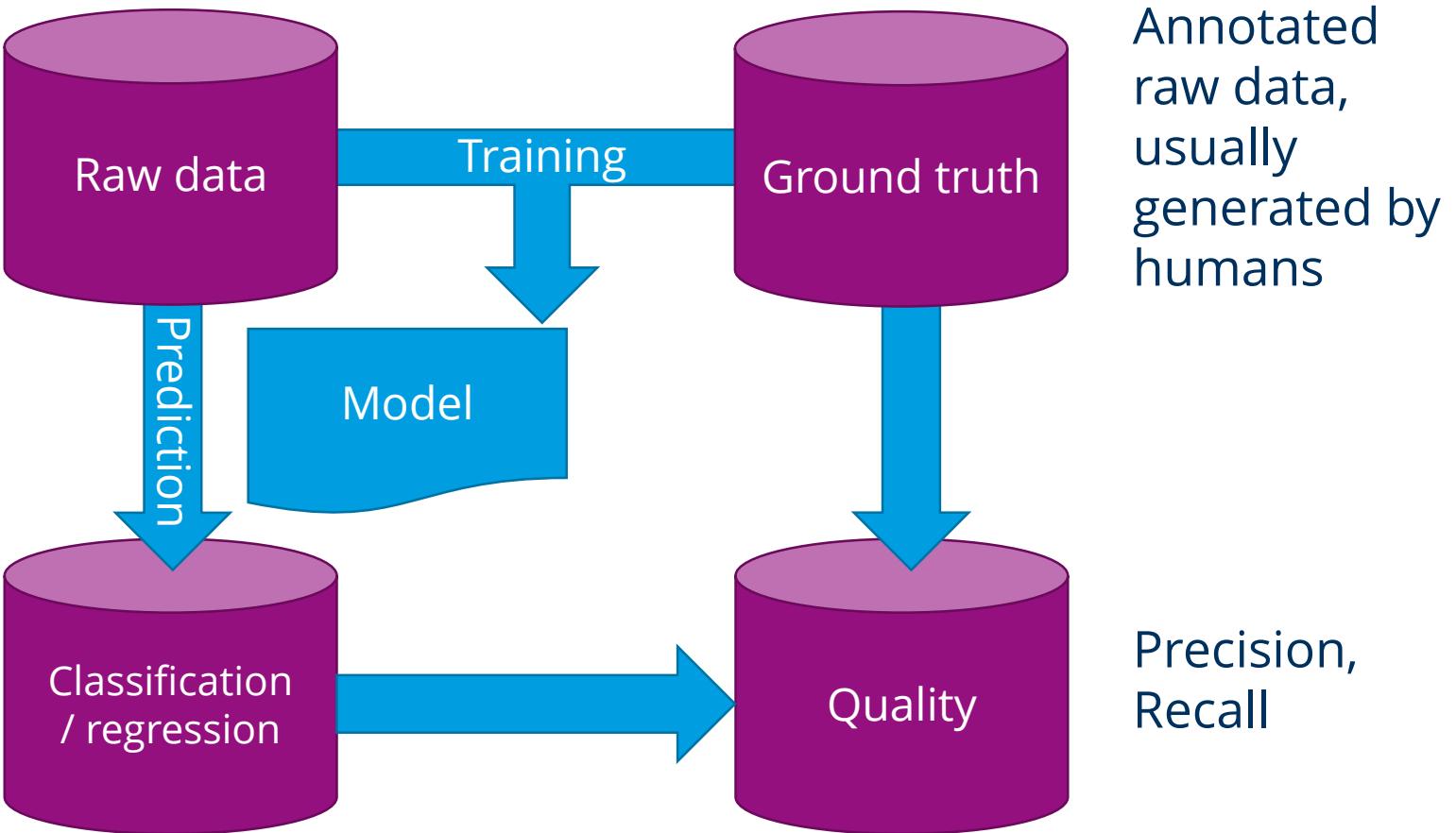
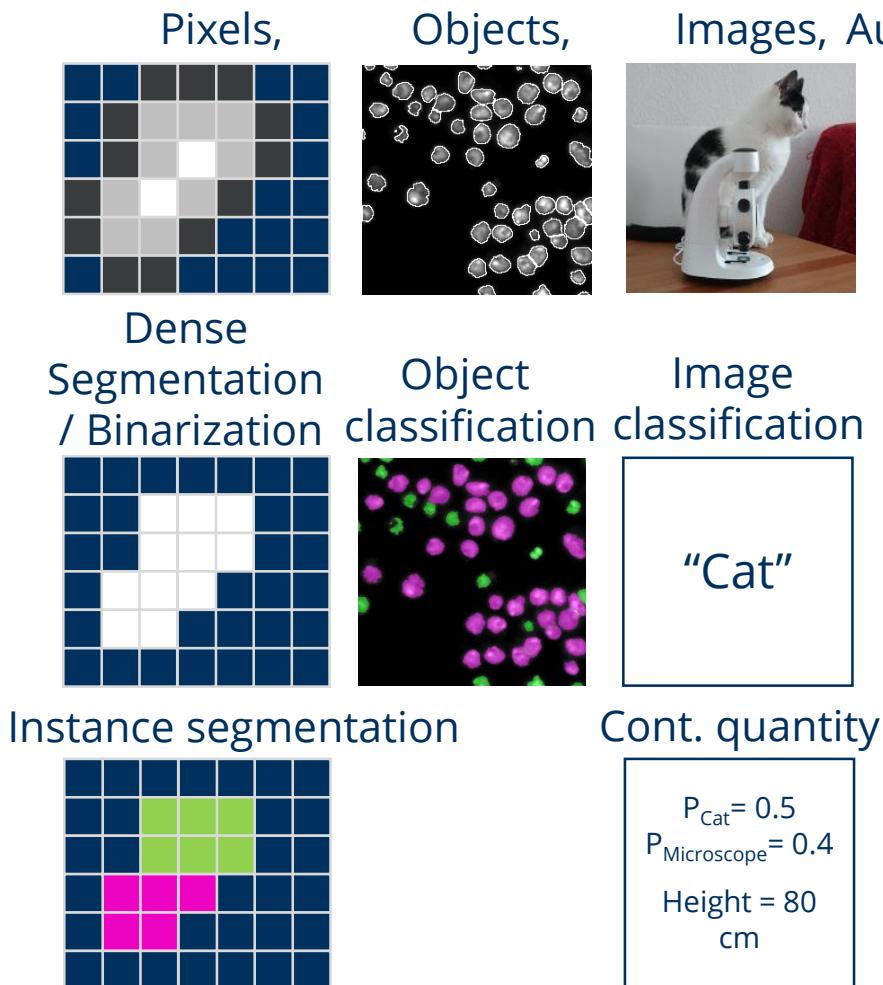


Image data source: [BBBC038v1](https://www.broadinstitute.org/bbbc/bbbc038v1), available from the Broad Bioimage Benchmark Collection (Caicedo et al., Nature Methods, 2019)

Machine learning

Automatic construction of predictive models from given data



Segmentation: Latest developments

1970s-2010: Filtering,
thresholding

A Threshold Selection Method
from Gray-Level Histograms
NOBUYUKI OTSU

Abstract—A nonparametric and unsupervised method of automatic threshold selection for picture segmentation is presented. An optimal threshold is selected by the discriminant criterion, namely, so as to maximize the separability of the resultant classes in gray levels. The procedure is very simple, utilizing only the zeroth- and the first-order cumulative moments of the gray-level histogram. It is straightforward to extend the method to multithreshold problems. Several experimental results are also presented to support the validity of the method.

2010s: Random
forests et al.



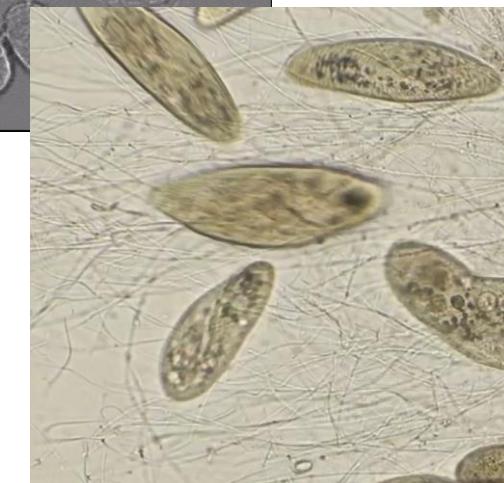
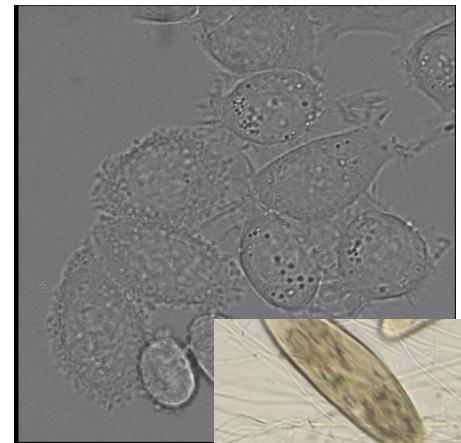
Deep
learning

2015: UNet
2018: Stardist



Foundational
models

2023: Segment
anything (SAM)
2024: SAM 2



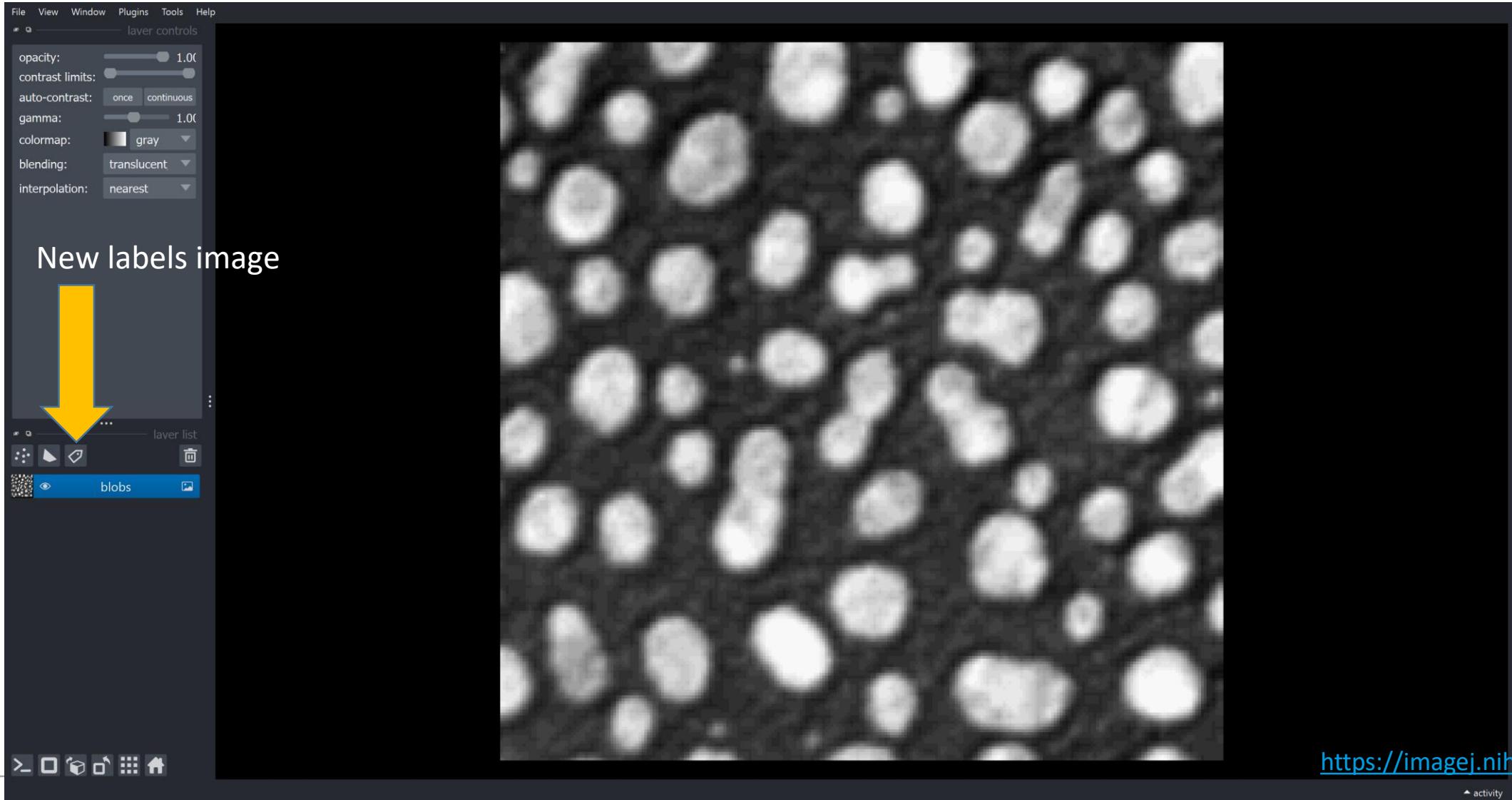
Computational demand

Segmentation and Supervised Machine Learning in napari

Random Forest Classifiers

- Pixel Classifier
- **napari-apoc plugin**

In napari: annotation



In napari: annotation



In napari: annotation

The screenshot shows the napari software interface. On the left is a toolbar with various tools like selection, drawing, and measurement. A sidebar on the left contains settings for 'label' (set to 2), 'opacity' (0.70), 'brush size' (1), 'blending' (translucent), 'color mode' (auto), 'contour' (0), 'n edit dim' (2), 'contiguous' (checked), 'preserve labels' (unchecked), and 'show selected' (unchecked). Below these are 'layer controls' and a 'layer list' containing 'Labels' and 'blobs'. At the bottom are standard window control icons.

Tips for annotations:

- Use **small brush size**: Pixels next to each other do not give much additional information to the classifier
- Annotate only pixels the class of which (e.g., background, foreground, etc) is **unambiguous** to you
- **Annotate few pixels**: If you already annotated 100k pixels, annotating 100 more will not change the result – annotating few pixels allows you to **tune the result**

A large yellow arrow points from the text box to a cell boundary in the main image. To the right, three smaller images show examples of annotation quality: 'good' (blue outline), 'Not so good' (red outline), and 'bad' (multiple overlapping outlines).

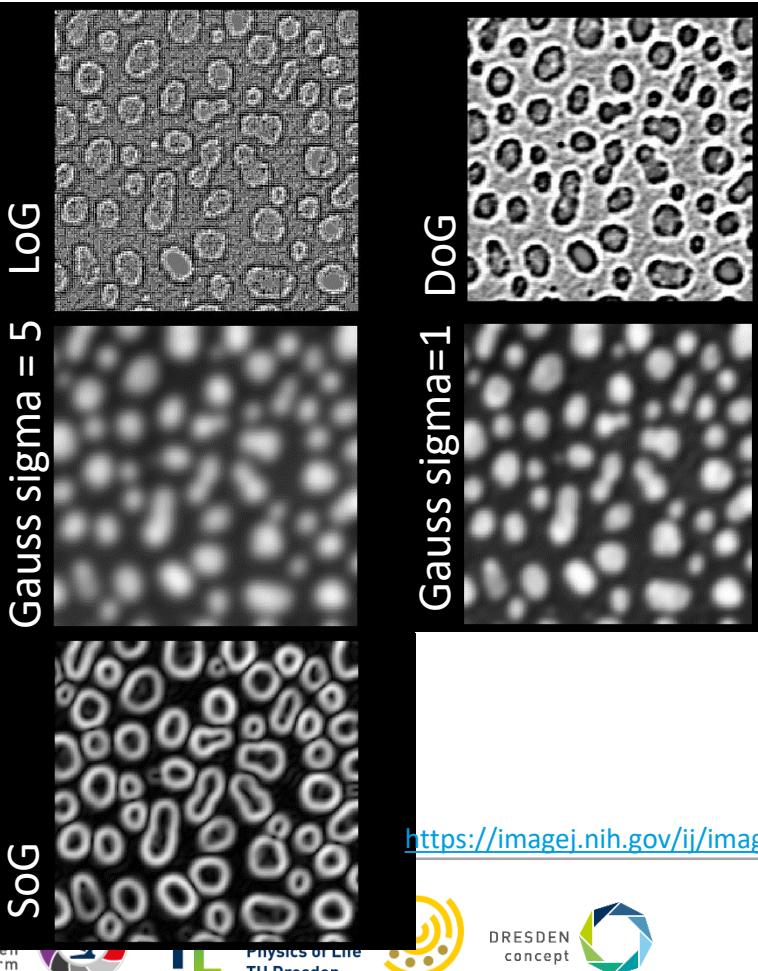
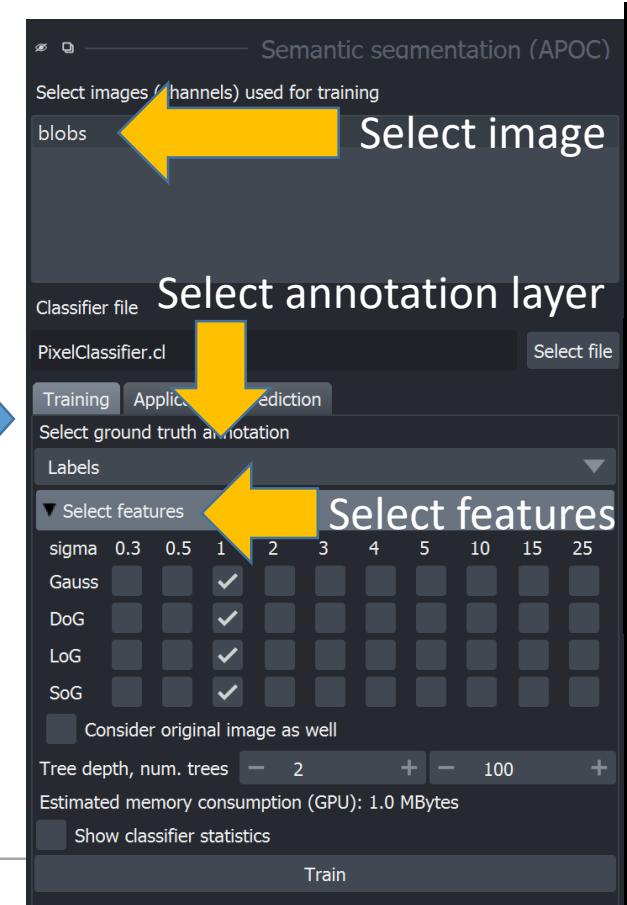
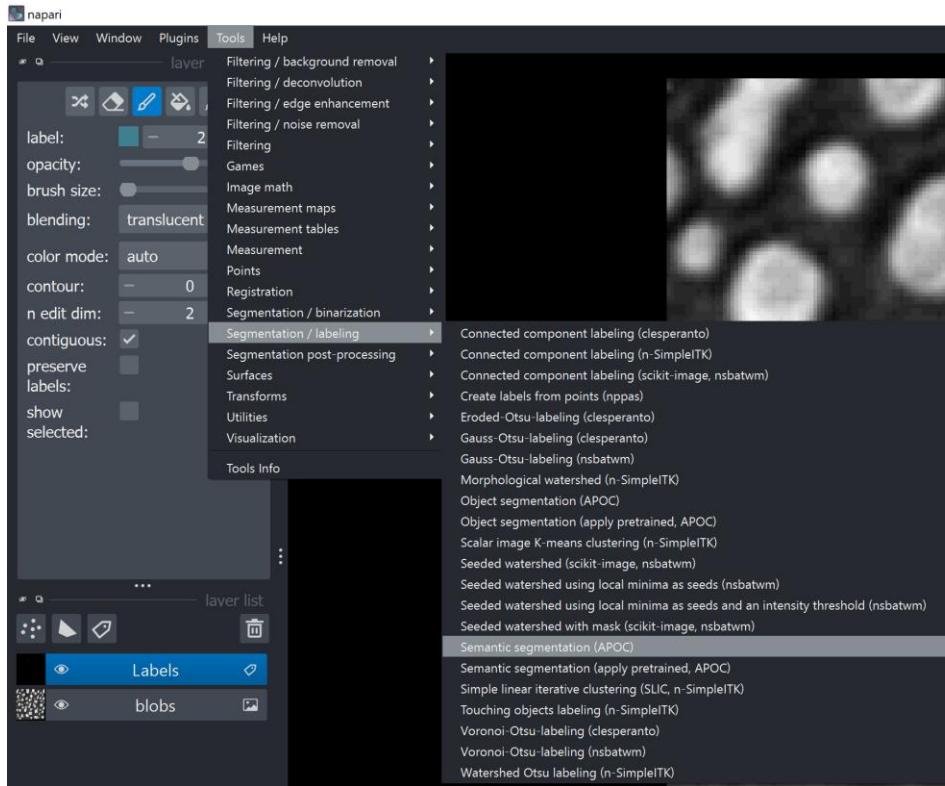
<https://imagej.nih.gov/ij/images/>

In napari: Semantic segmentation (training)

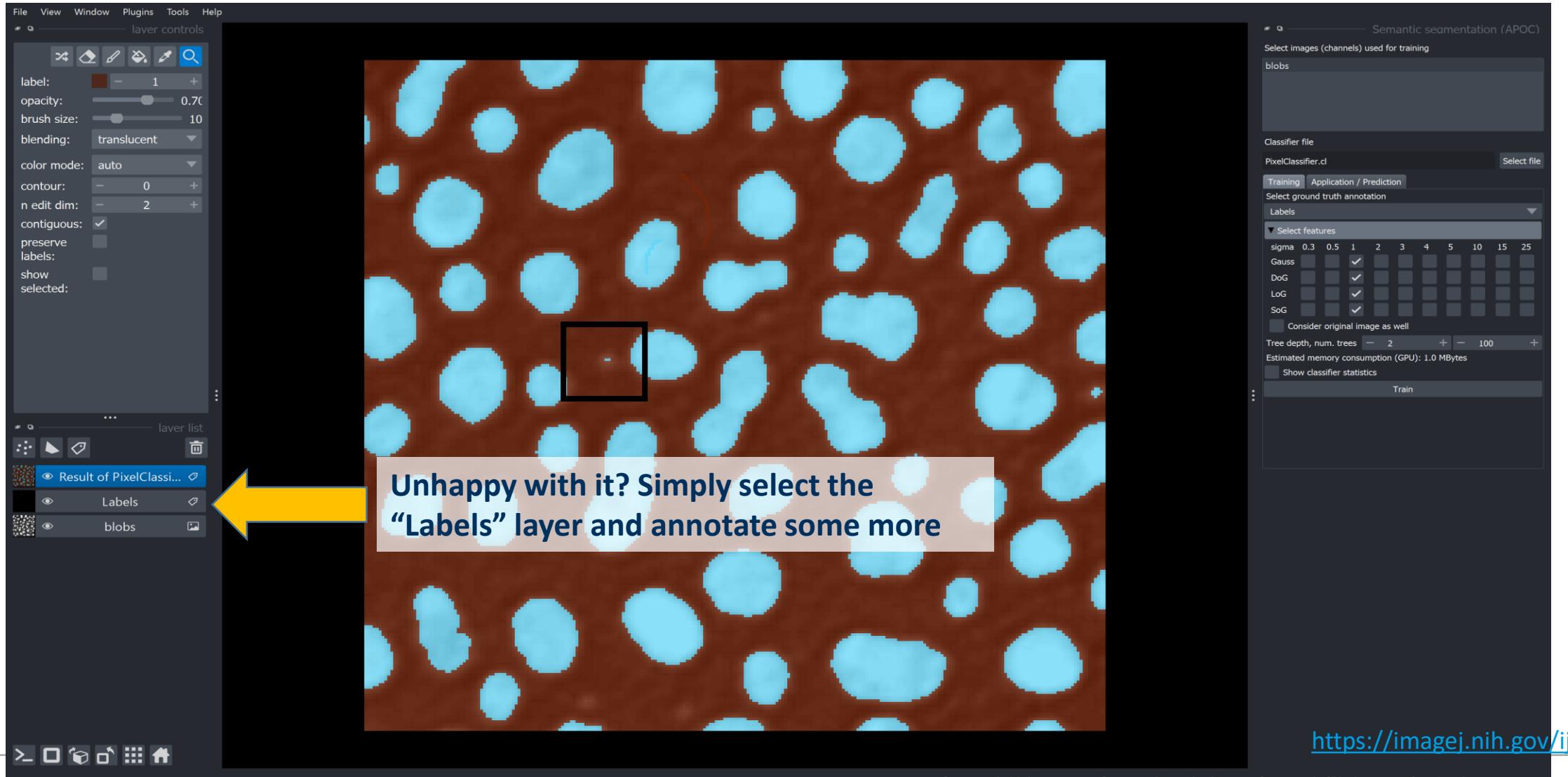
Two options:

Semantic segmentation: Predict class of every pixel according to annotation

Object segmentation: Assumes that class “1” refers to background – applies connected component analysis to other class



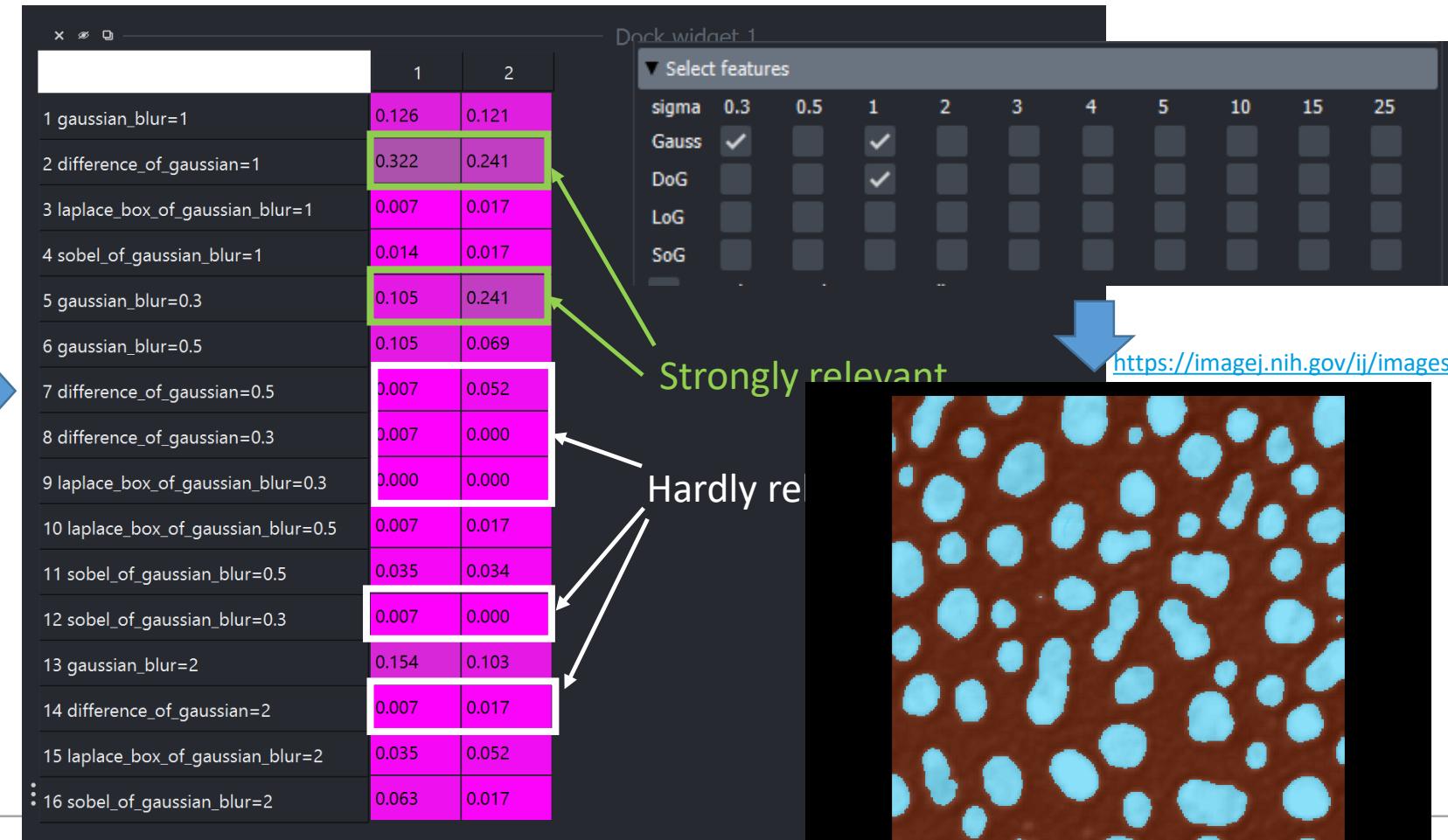
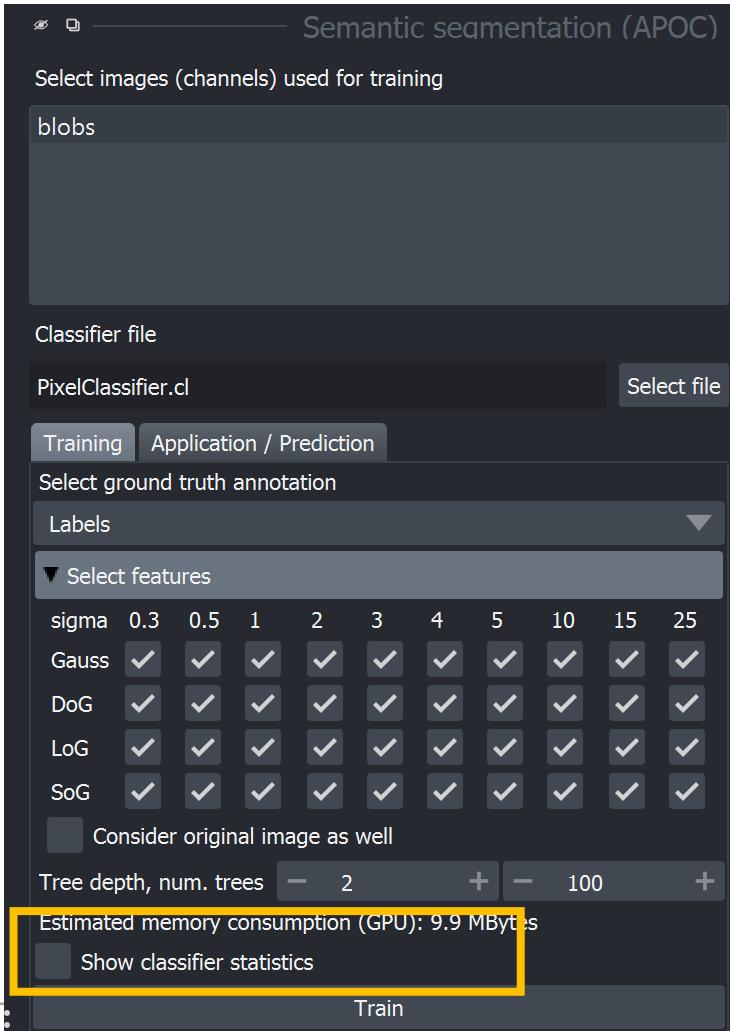
In napari: Semantic segmentation (training)



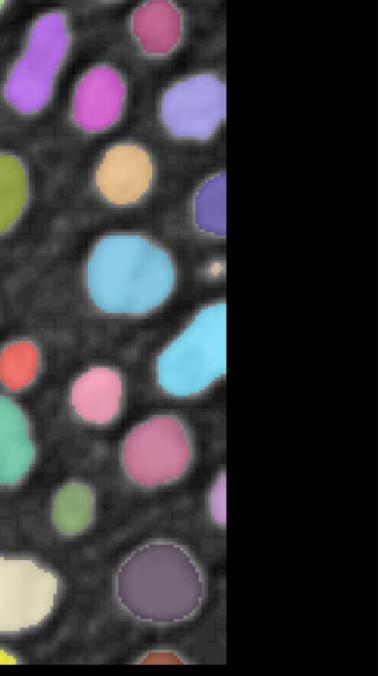
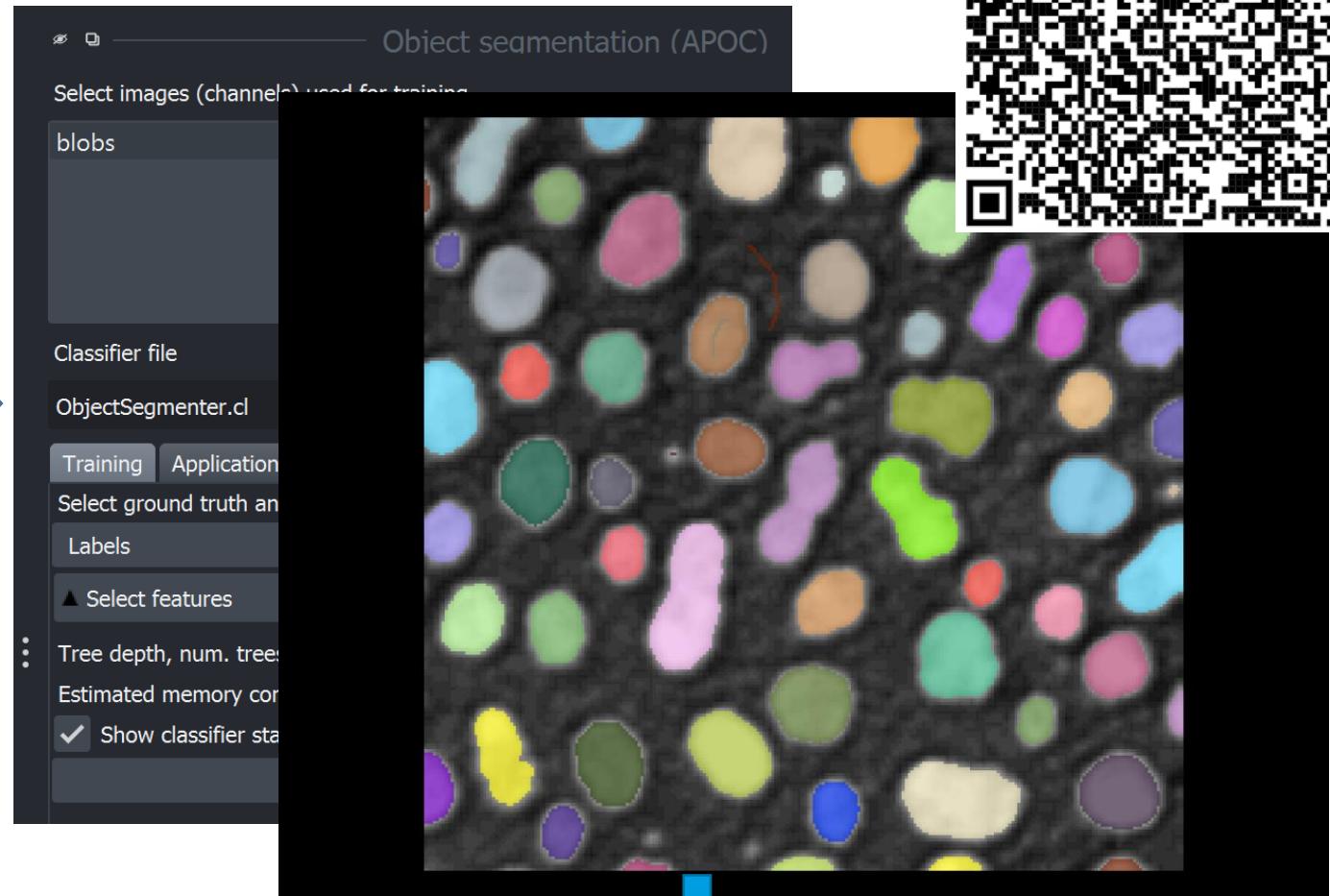
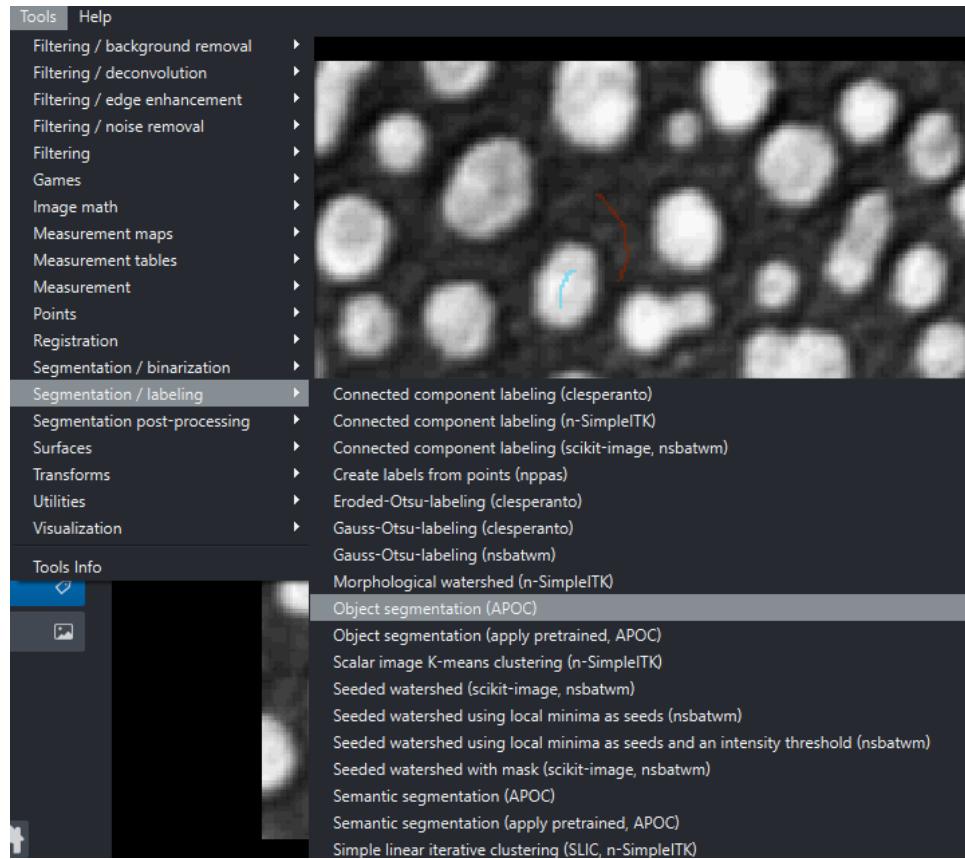
Semantic segmentation: Choosing the right features

Why not just do this?

- Not all features are equally relevant!
- Calculating the features takes time and computation resources!



Exercise: Object segmentation



<https://imagej.nih.gov/ij/images/>

https://biapol.github.io/QM_Course_Bio_Image_Analysis_with_napari_2025/interactive_pixel_classification/readme.html#readme.html#

1. Activate the environment and open napari

```
mamba activate napari-intro-env  
napari
```

2. Open the blobs image (File -> Open Sample -> c1Esperanto -> Blobs)

3. Perform object segmentation on blobs sample dataset

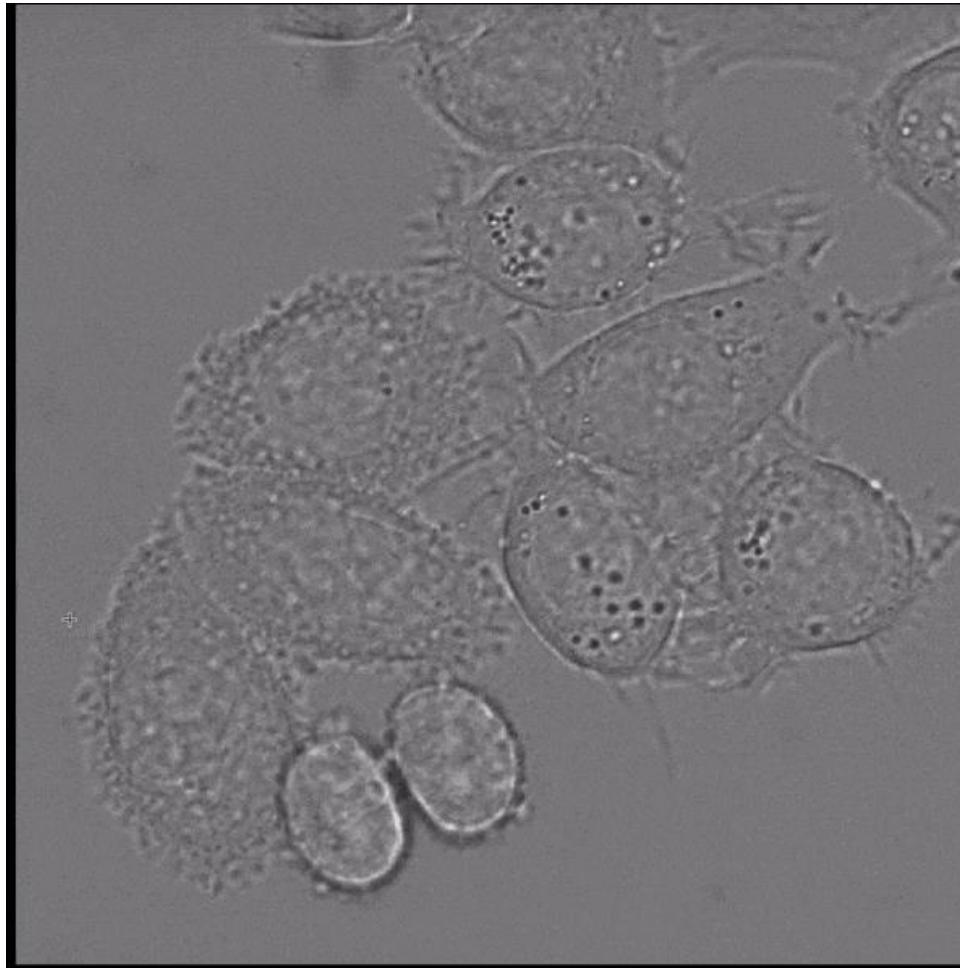
A photograph of a broken window pane against a blue sky with white clouds. The pane is shattered into many shards, with the largest shard at the top left containing the word "Break". The sun is visible in the center, creating a bright starburst effect.

Break

Segmentation and Supervised Machine Learning in napari

- Demonstration: Micro-sam plugin

Micro-sam



<https://github.com/computational-cell-analytics/micro-sam>



Segmentation and Supervised Machine Learning in napari

- Demonstration: napari-nninteractive plugin

<https://github.com/MIC-DKFZ/napari-nninteractive>



Feature Extraction

Feature extraction

- A feature is a countable or measurable property of an image or object.
- Goal of feature extraction is finding a minimal set of features to describe an object well enough to differentiate it from other objects.

- **Intensity based**

- Mean intensity
- Standard deviation
- Total intensity
- Textures

- **Shape based /spatial**

- Area / Volume
- Roundness
- Solidity
- Circularity / Sphericity
- Elongation
- Centroid
- Bounding box

- **Spatio-temporal**

- Displacement,
- Speed,
- Acceleration

- **Others**

- Overlap
- Colocalization
- Neighborhood

- **Mixed features**

- Center of mass
- Local minima / maxima

Further reading:

<https://focalplane.biologists.com/2023/05/03/feature-extraction-in-napari/>

Multichannel Analysis

- napari-skimage-regionprops plugin

Exercise: Multichannel Analysis with napari-skimage-regionprops

1. Start up a terminal
2. Activate the environment using:
mamba activate napari-intro-env

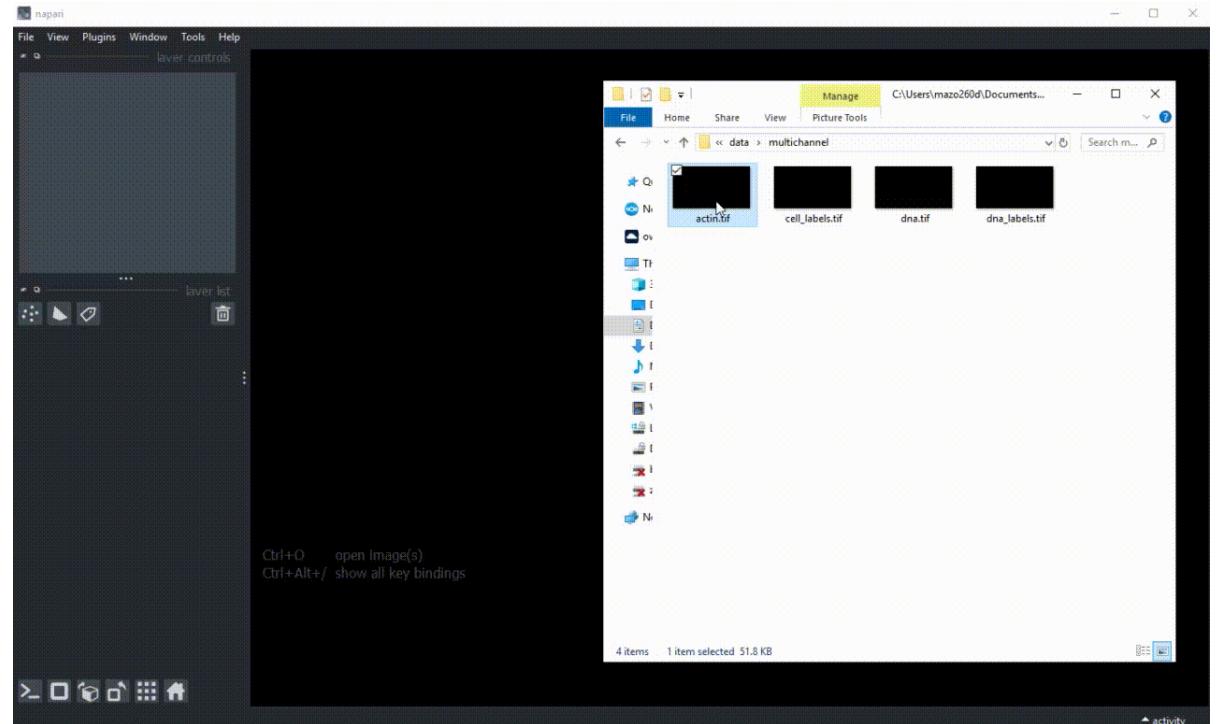
3. Run:
napari

4. Open the following images in napari

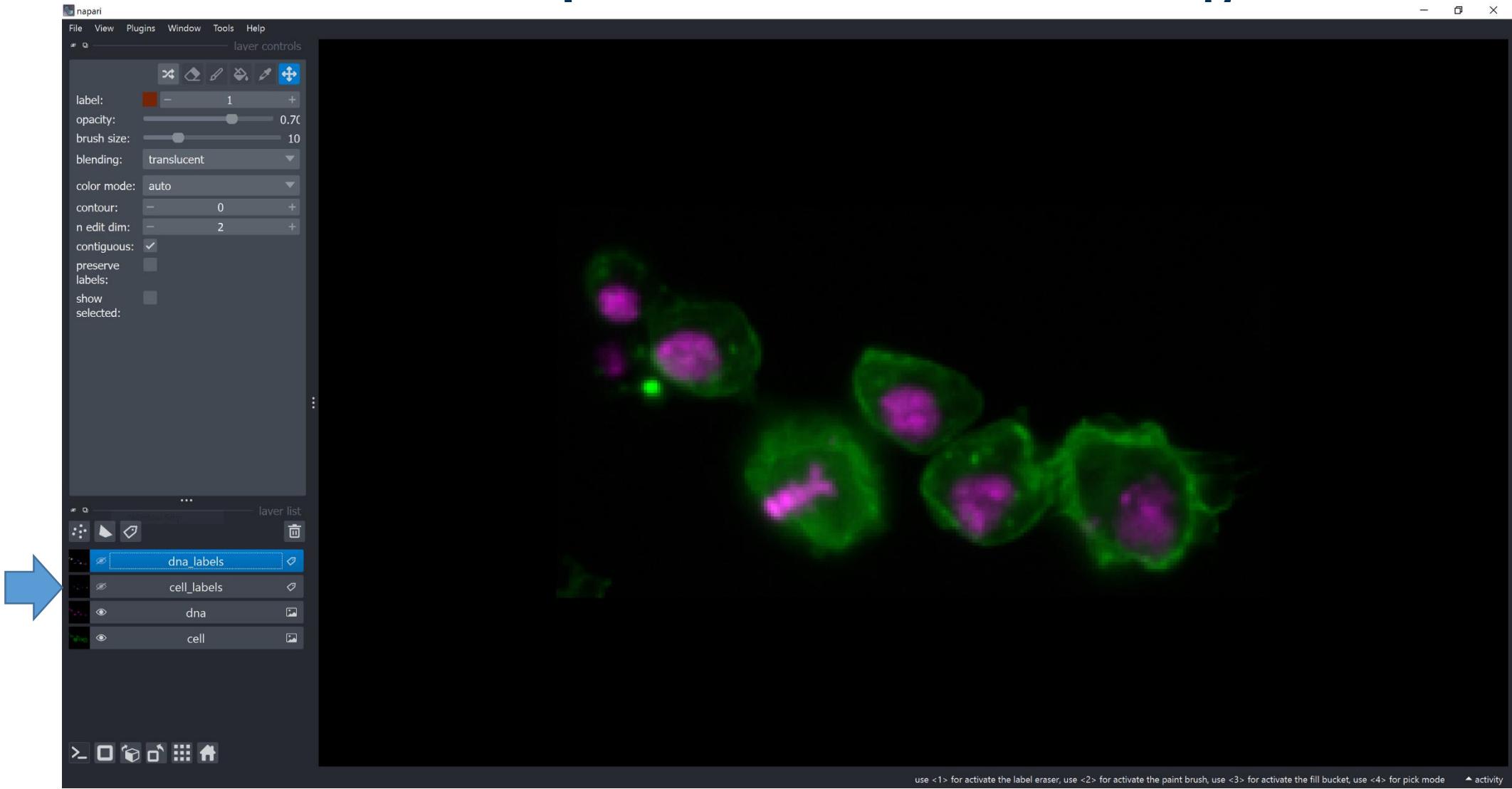
(located in the [data/multichannel](#) folder from the GitHub repository):

- actin.tif
- dna.tif
- cell_labels.tif
- dna_labels.tif

5. Change blending mode of 'actin' and 'dna' Image layers to 'additive' (it is a dropdown in the Image layer controls)
6. Select the 'cell_labels' Labels layer, and change the contour parameter from 0 to 1 (it is a spinbox in the Labels layer controls)
7. Change the colormaps of these layers to green and magenta, respectively
https://biapol.github.io/QM_Course_Bio_Image_Analysis_with_napari_2025/multichannel_analysis/readme.html#

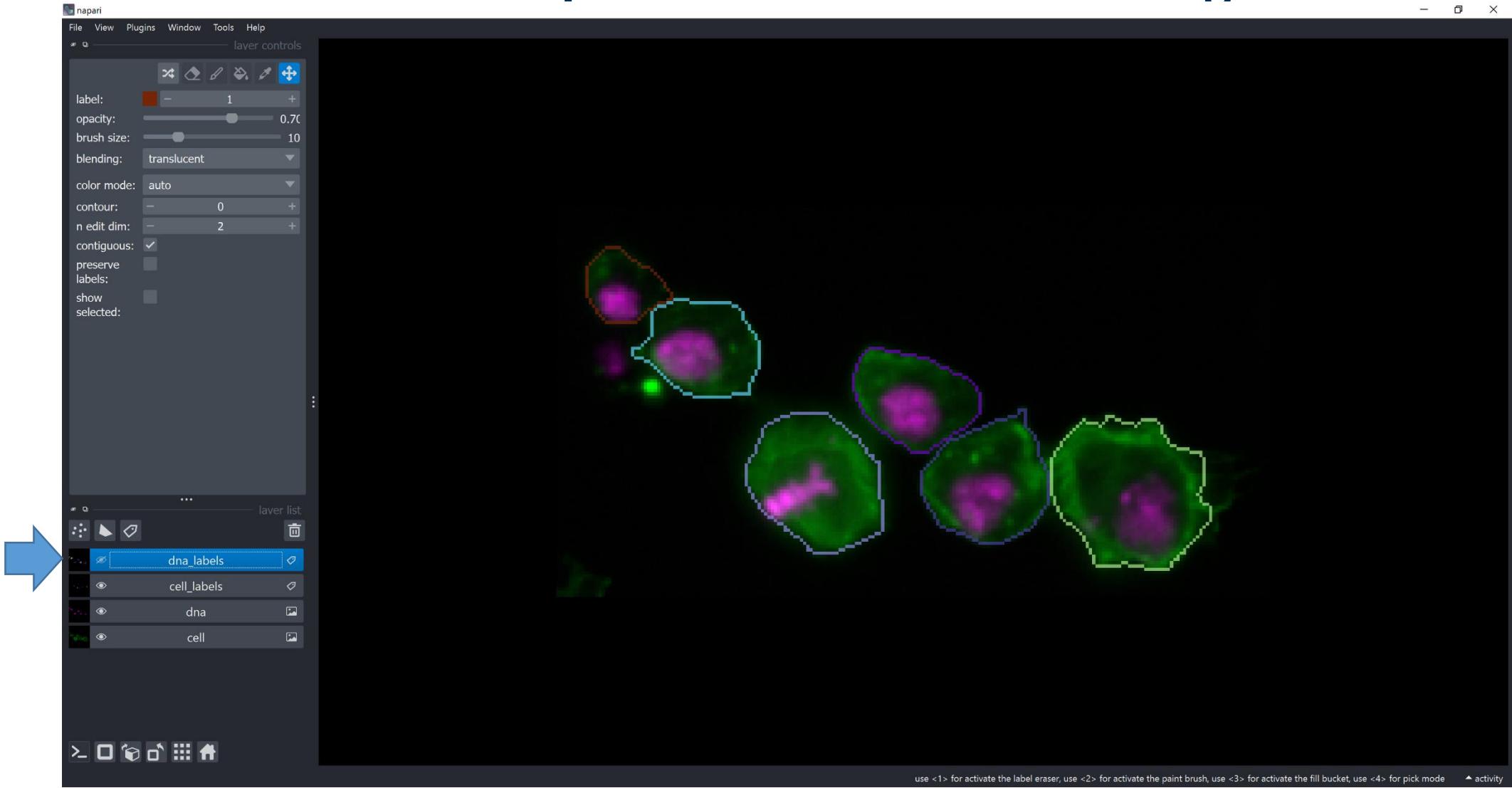


Feature extraction in napari with multichannel images



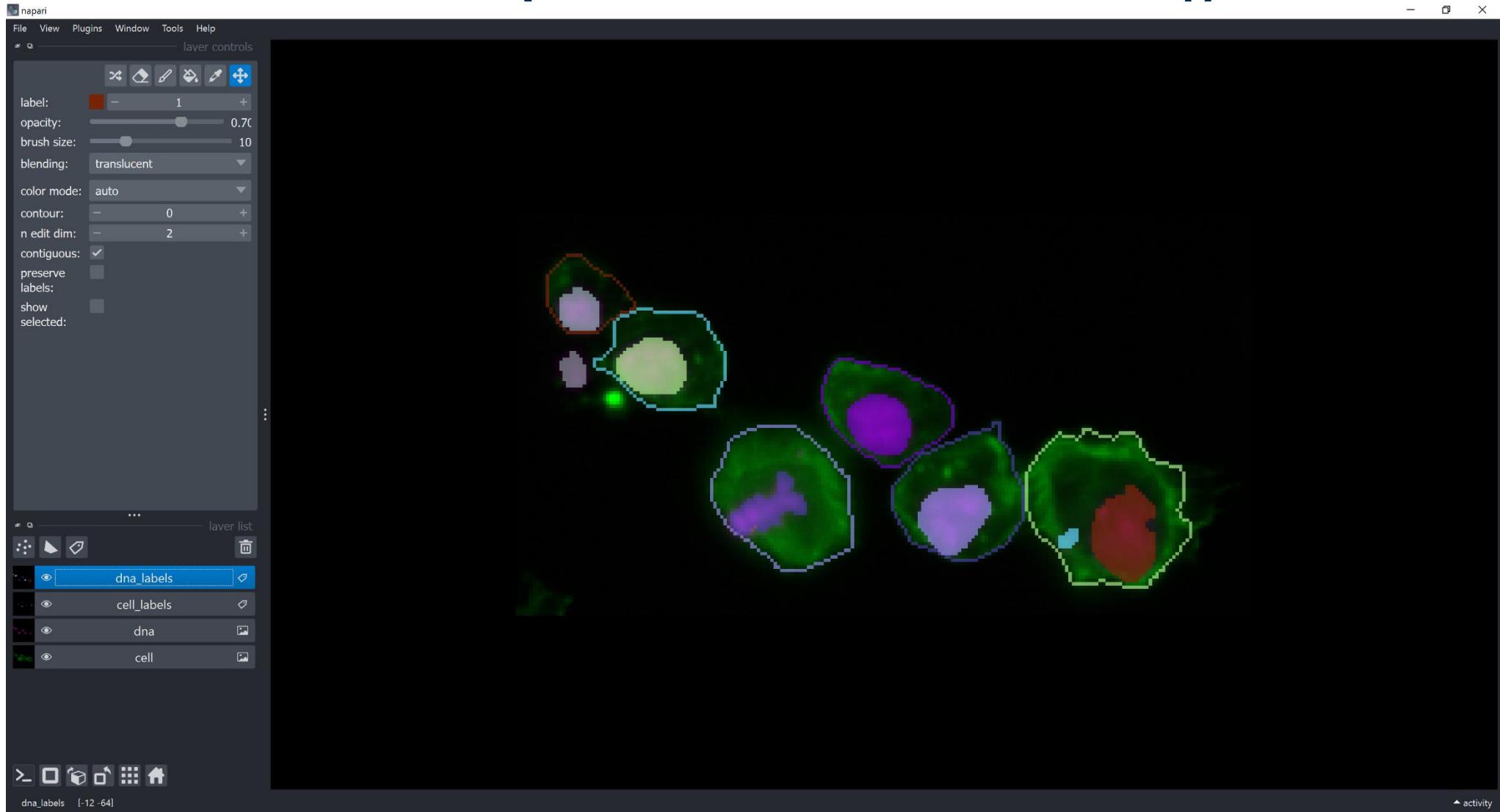
<https://github.com/haesleinhuepf/napari-skimage-regionprops>

Feature extraction in napari with multichannel images



<https://github.com/haesleinhuepf/napari-skimage-regionprops>

Feature extraction in napari with multichannel images



<https://github.com/haesleinhuepf/napari-skimage-regionprops>

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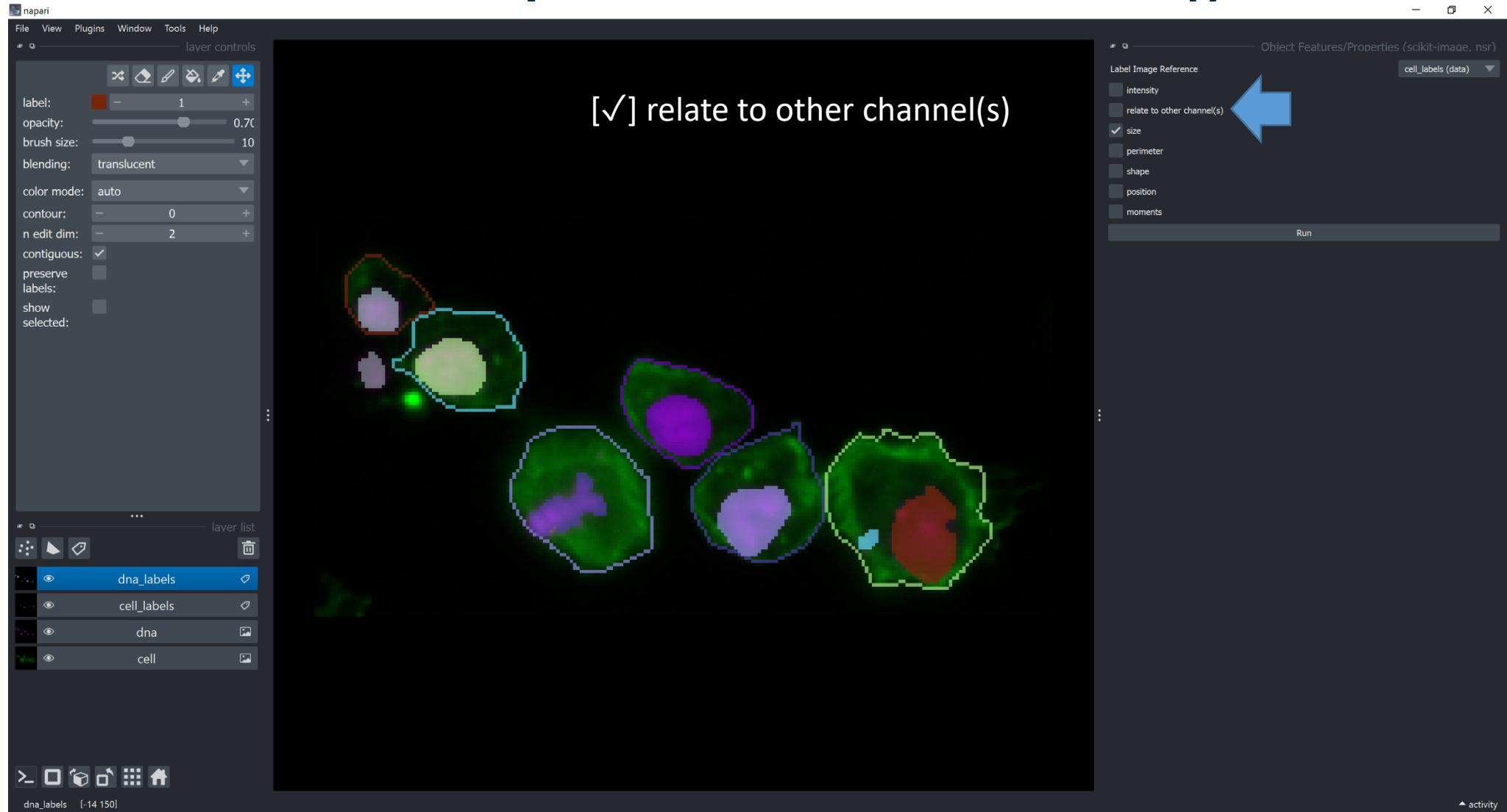
Feature extraction in napari with multichannel images



Tools ->
Measurement tables ->
Object Features/Properties

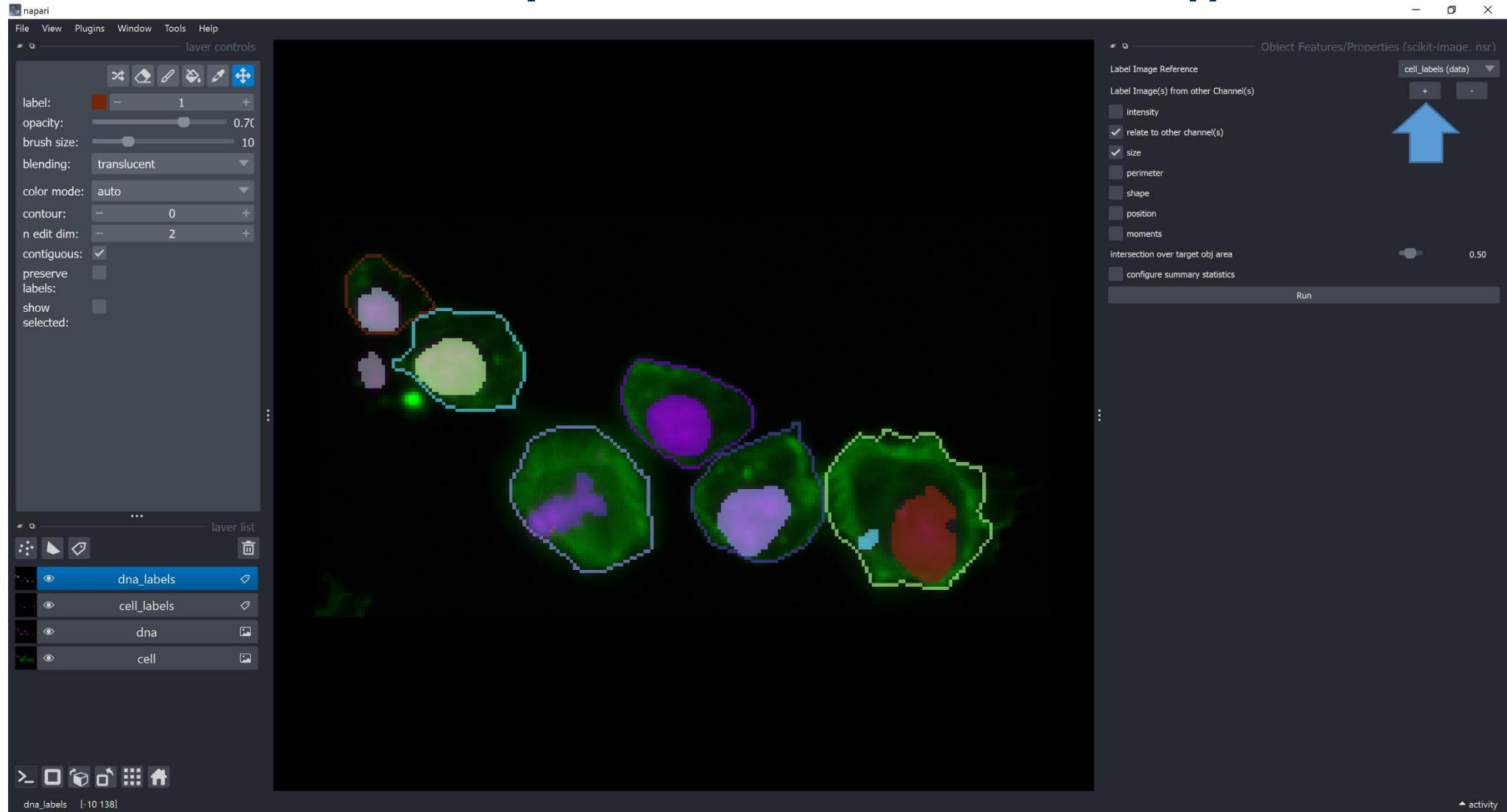
<https://github.com/haesleinhuepf/napari-skimage-regionprops>

Feature extraction in napari with multichannel images



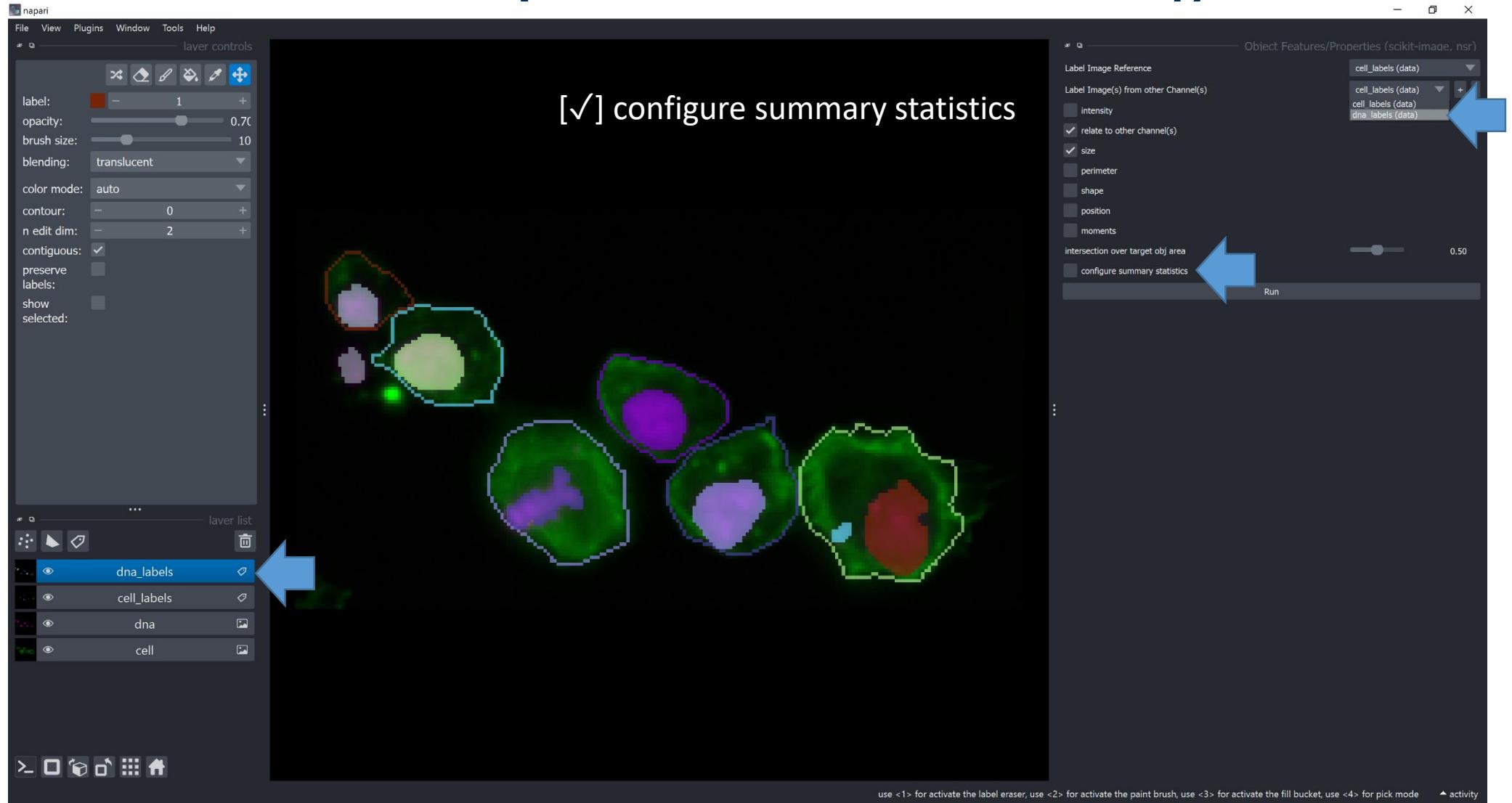
<https://github.com/haesleinhuepf/napari-skimage-regionprops>

Feature extraction in napari with multichannel images



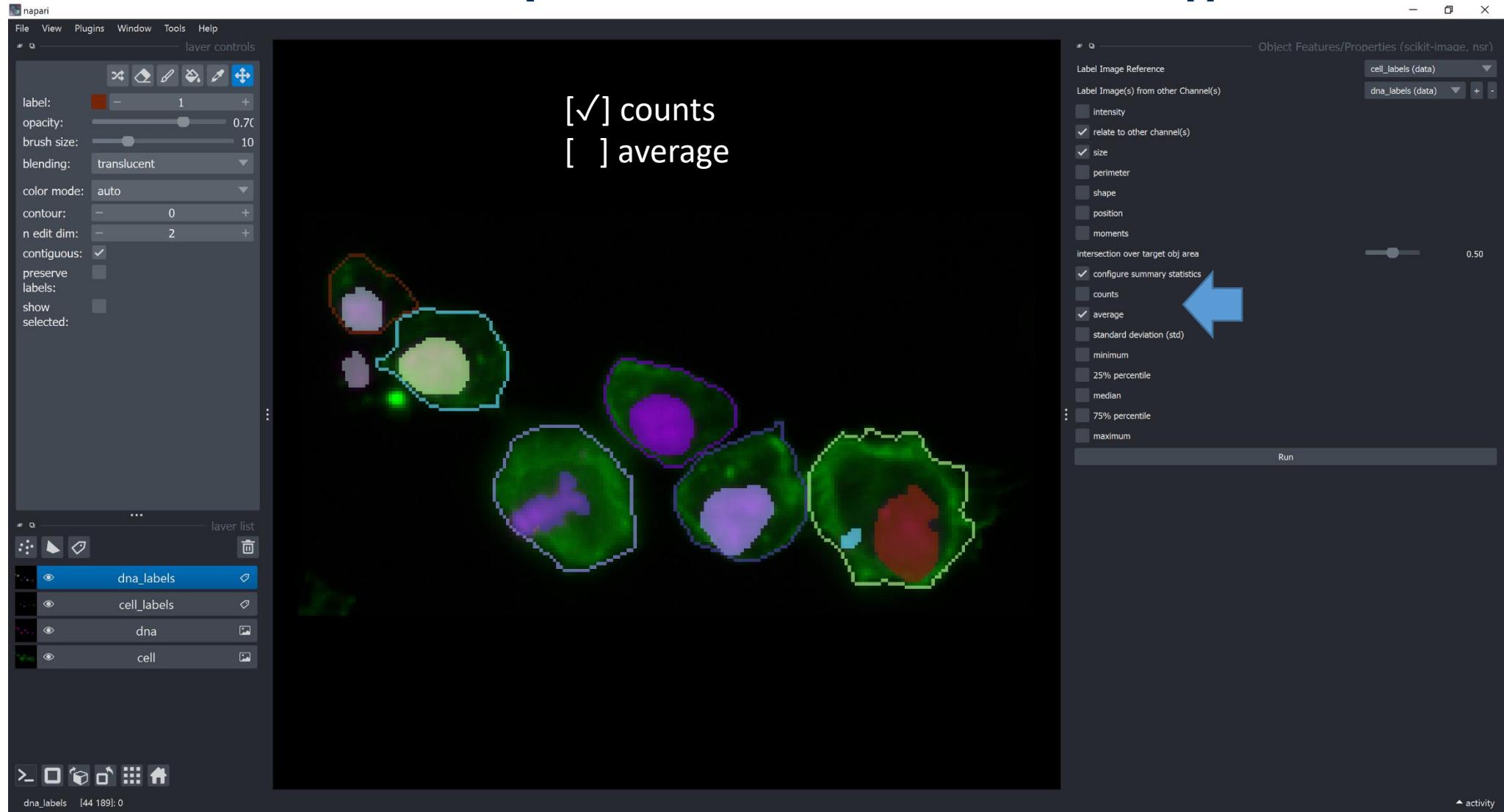
<https://github.com/haesleinhuepf/napari-skimage-regionprops>

Feature extraction in napari with multichannel images



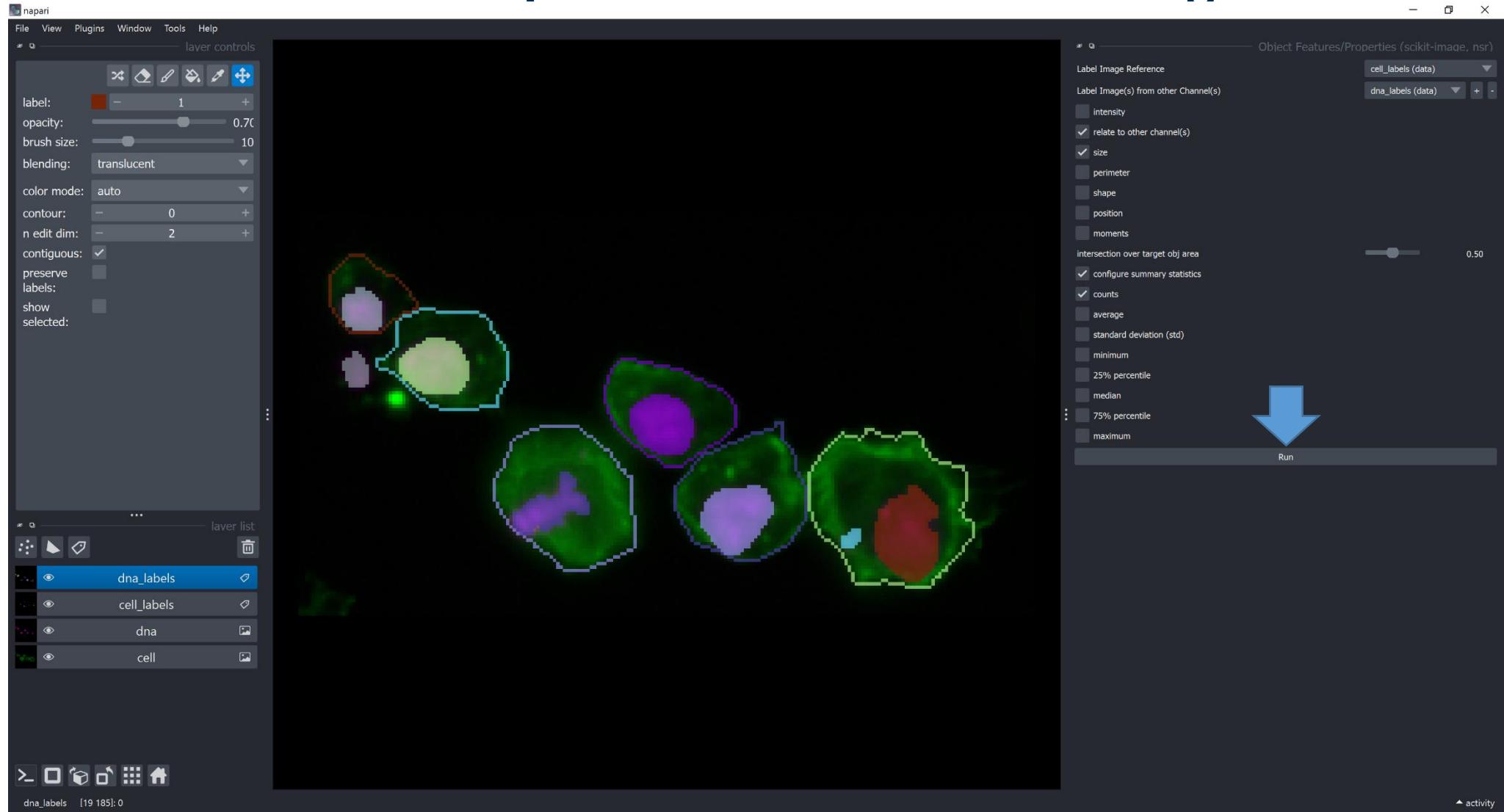
<https://github.com/haesleinhuepf/napari-skimage-regionprops>

Feature extraction in napari with multichannel images



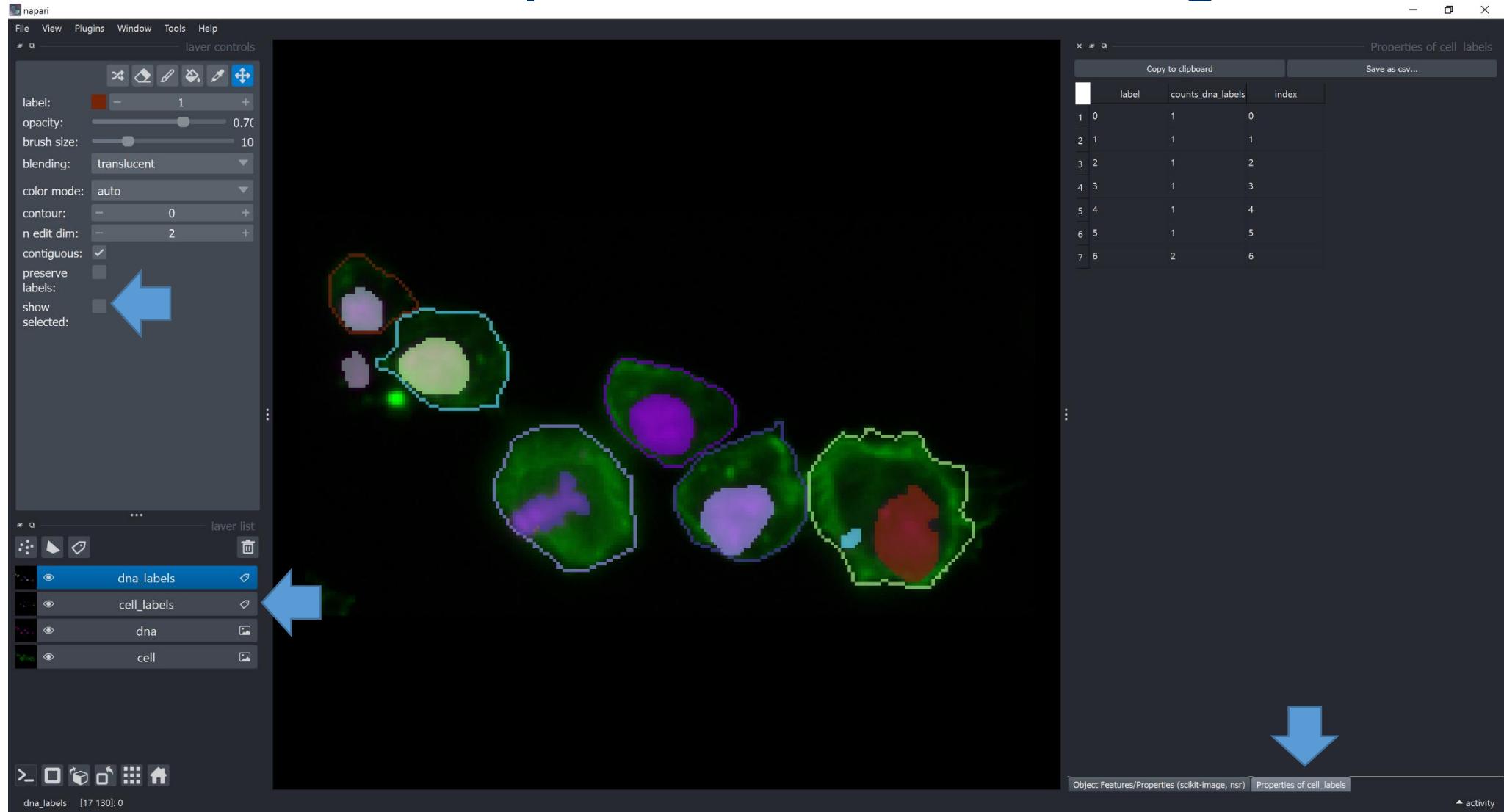
<https://github.com/haesleinhuepf/napari-skimage-regionprops>

Feature extraction in napari with multichannel images



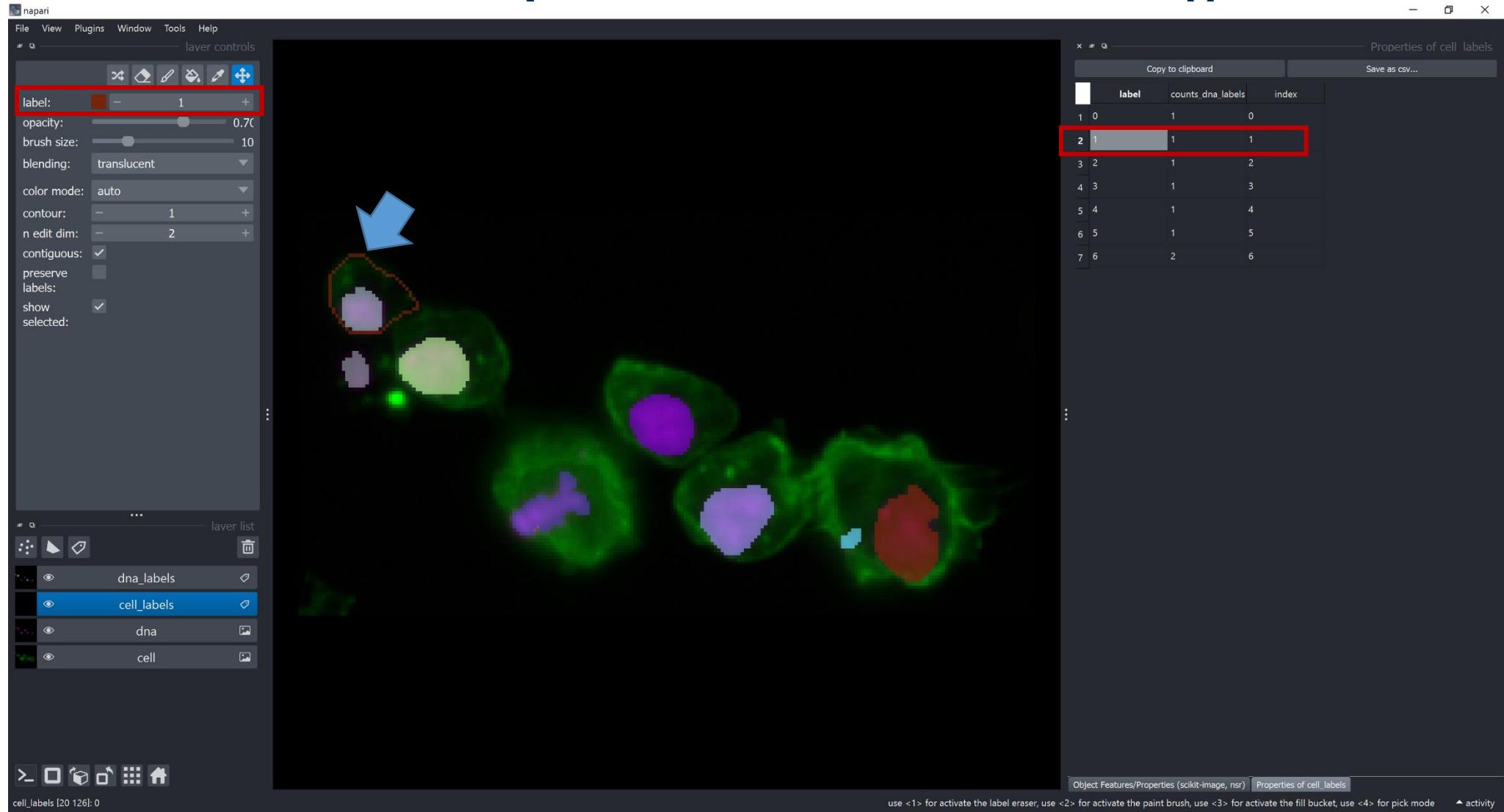
<https://github.com/haesleinhuepf/napari-skimage-regionprops>

Feature extraction in napari with multichannel images



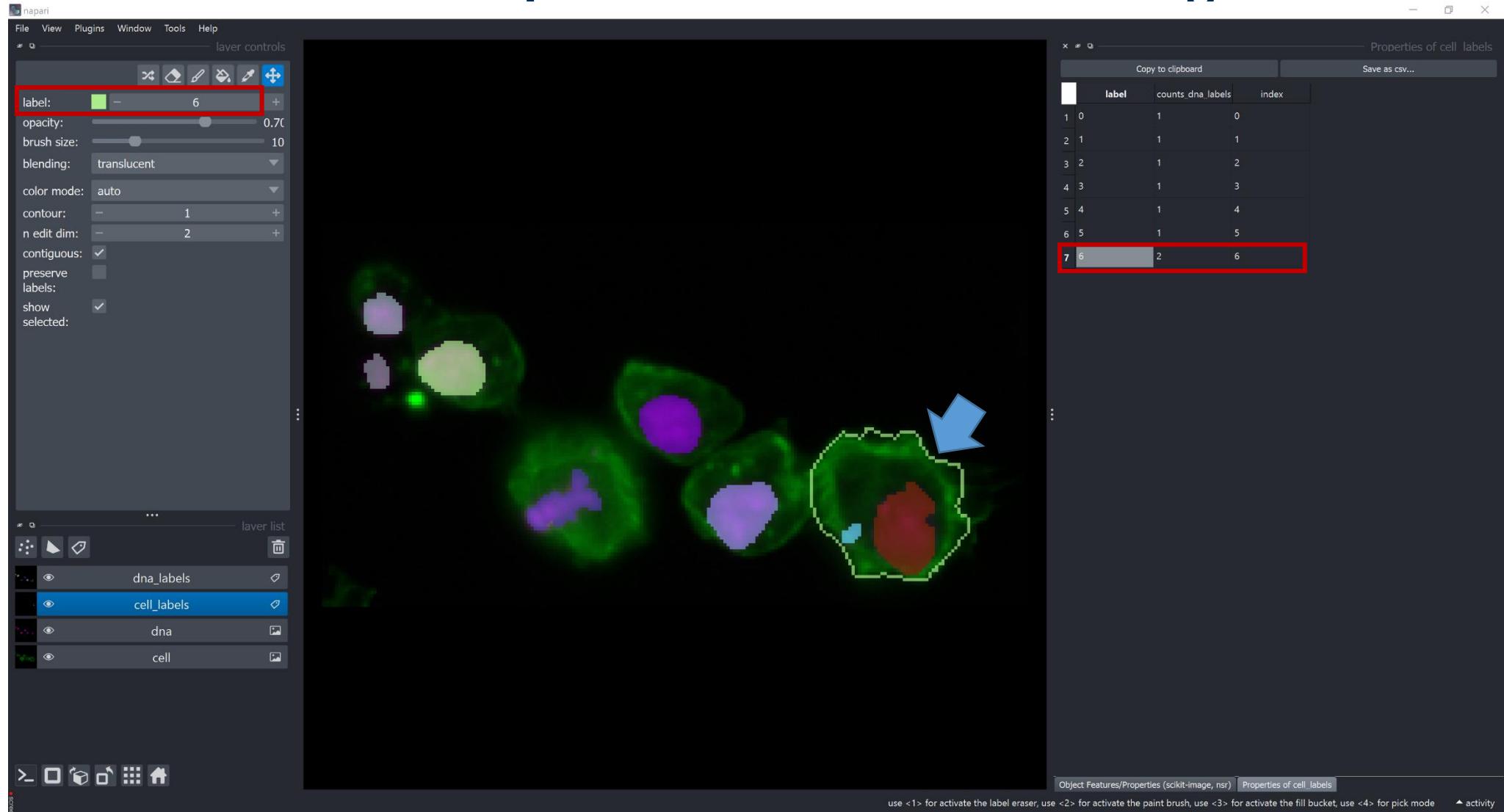
<https://github.com/haesleinhuepf/napari-skimage-regionprops>

Feature extraction in napari with multichannel images



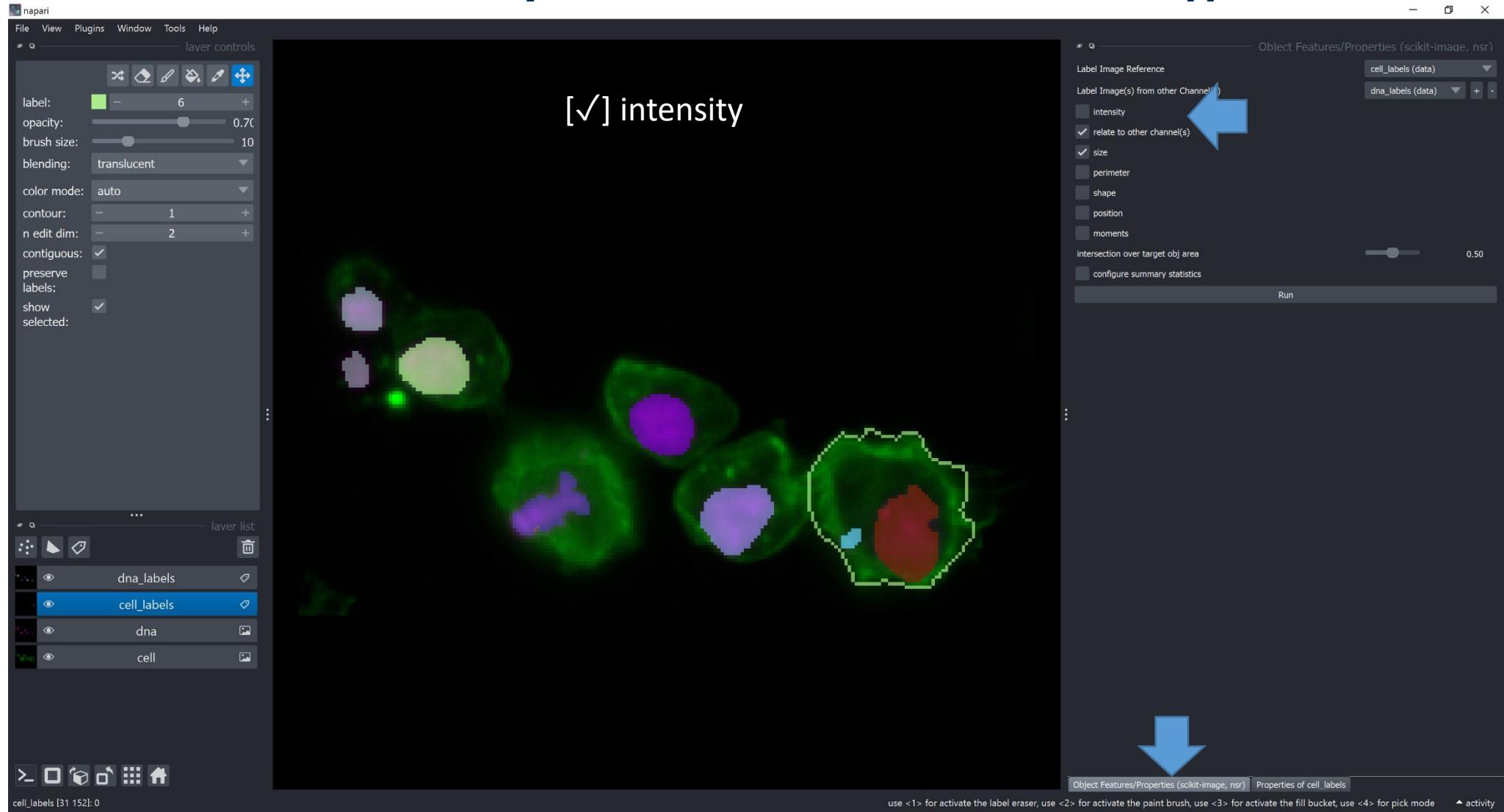
<https://github.com/haesleinhuepf/napari-skimage-regionprops>

Feature extraction in napari with multichannel images



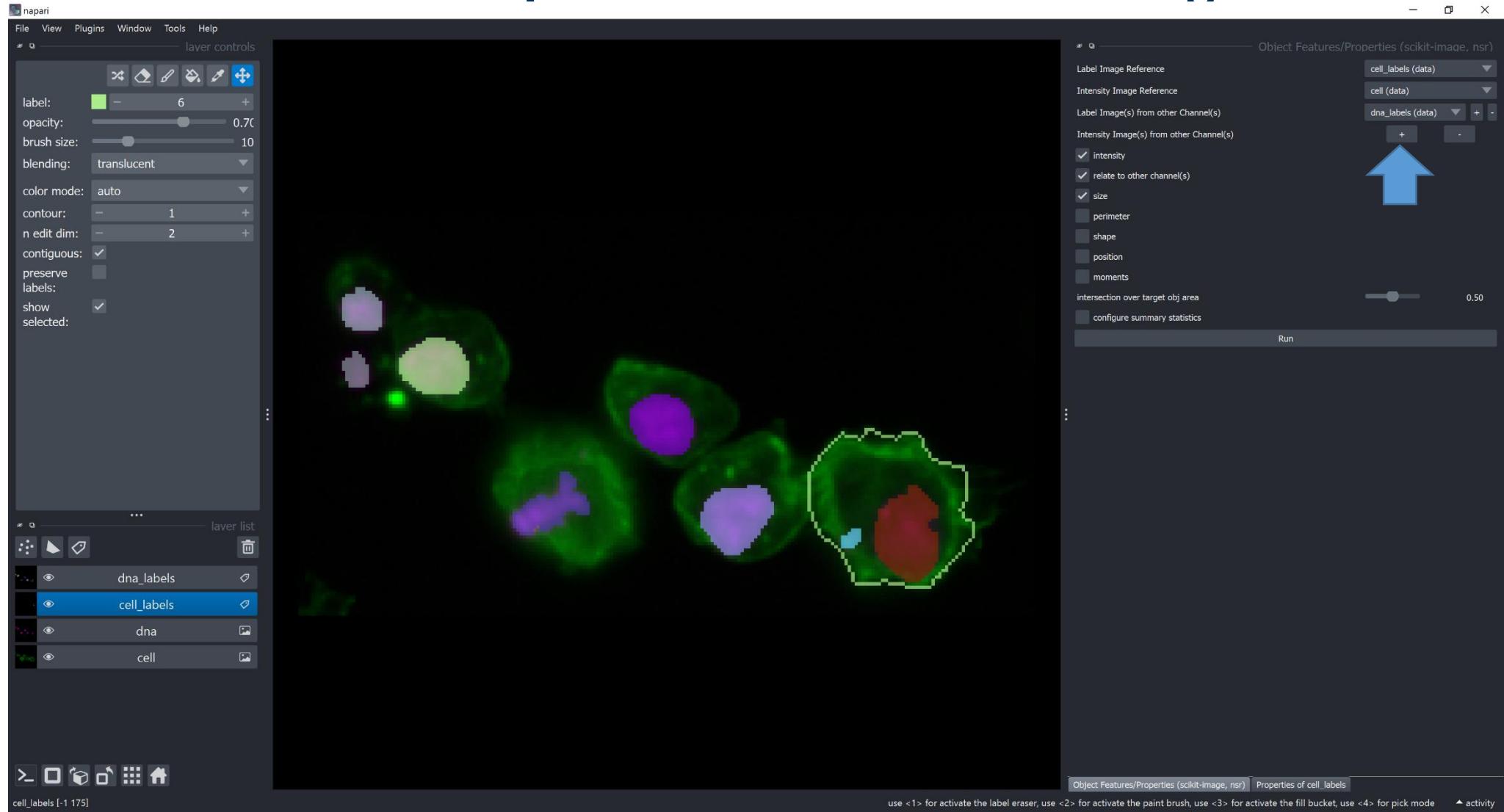
<https://github.com/haesleinhuepf/napari-skimage-regionprops>

Feature extraction in napari with multichannel images



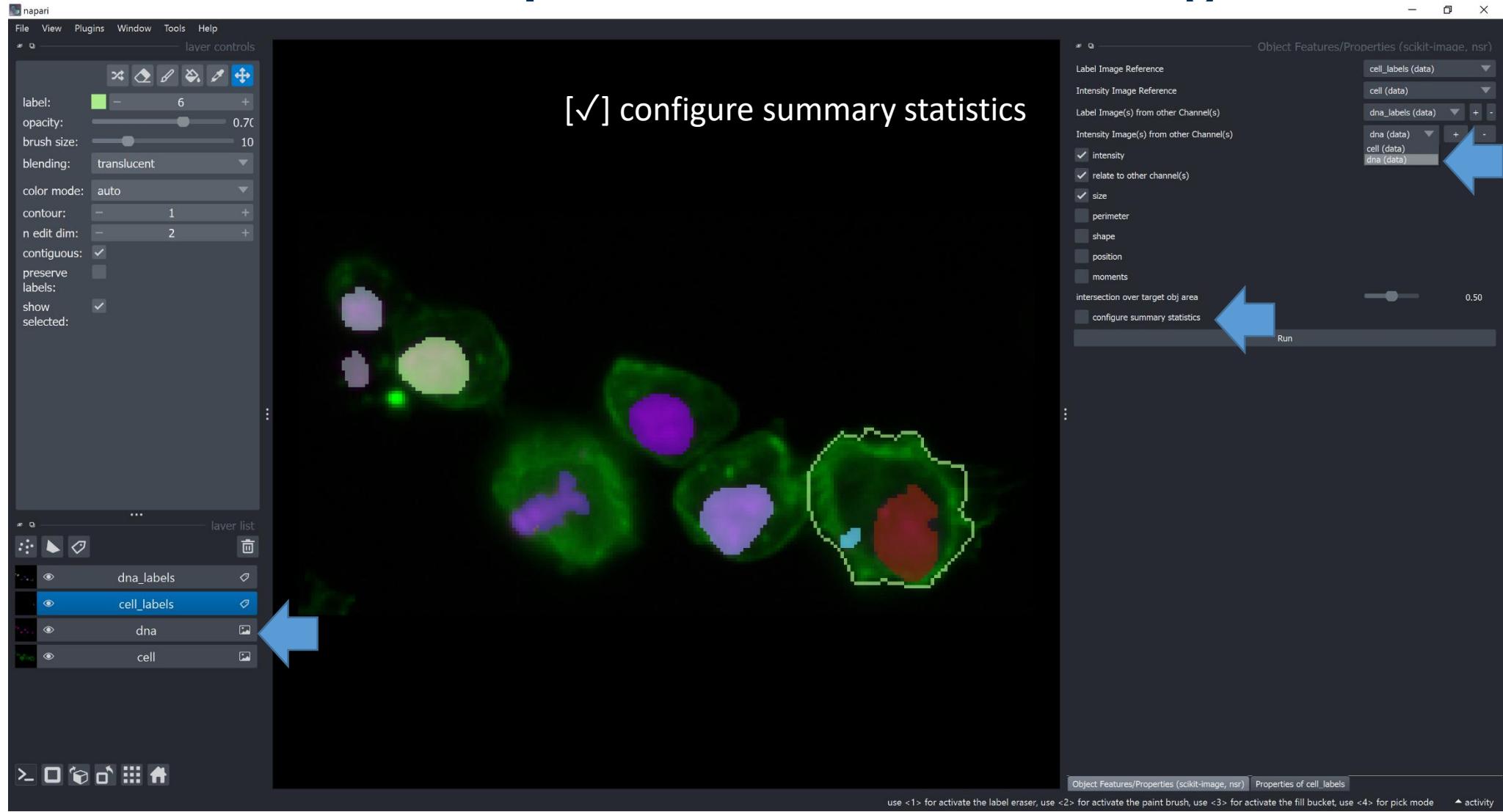
<https://github.com/haesleinhuepf/napari-skimage-regionprops>

Feature extraction in napari with multichannel images



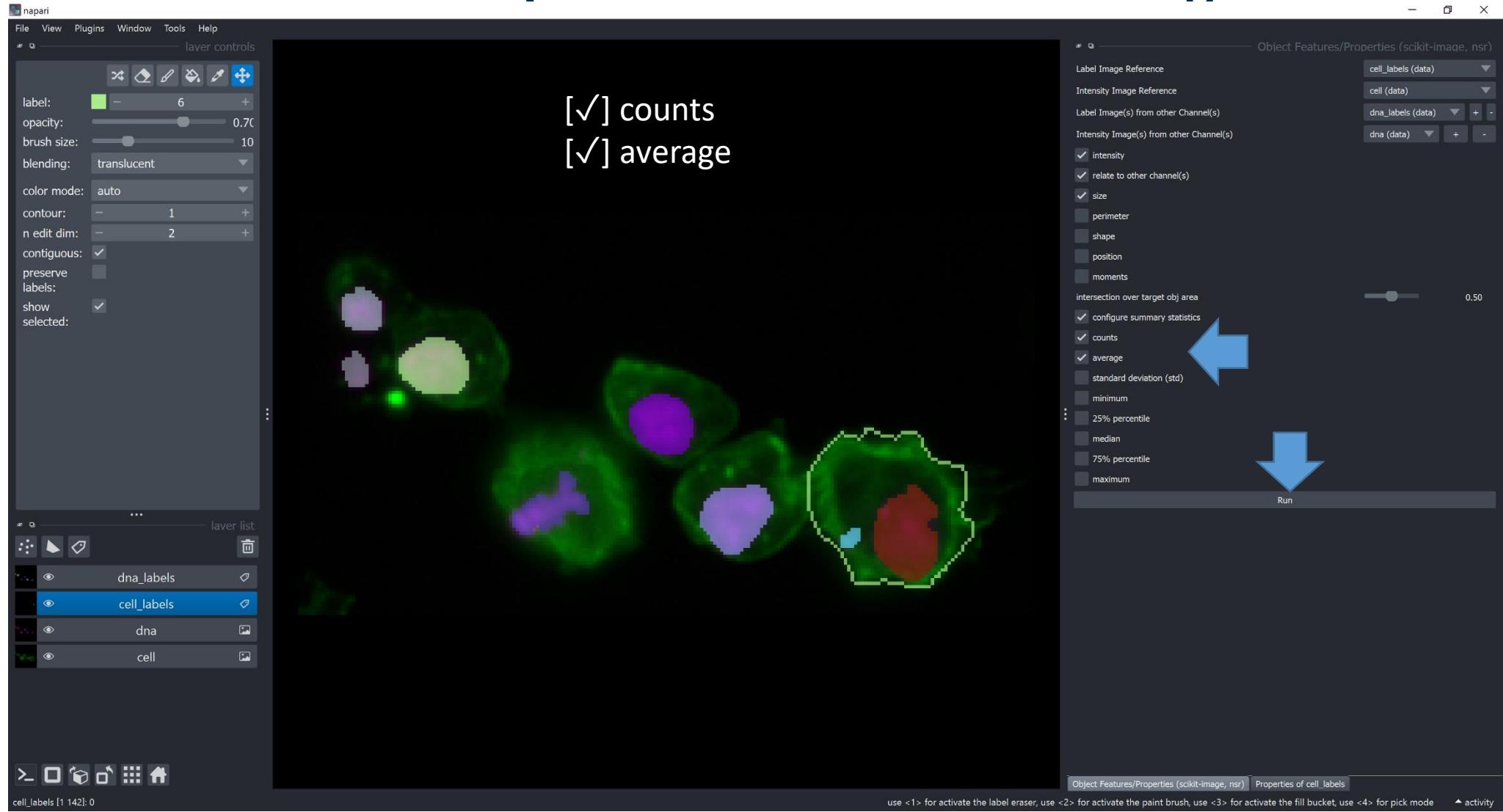
<https://github.com/haesleinhuepf/napari-skimage-regionprops>

Feature extraction in napari with multichannel images



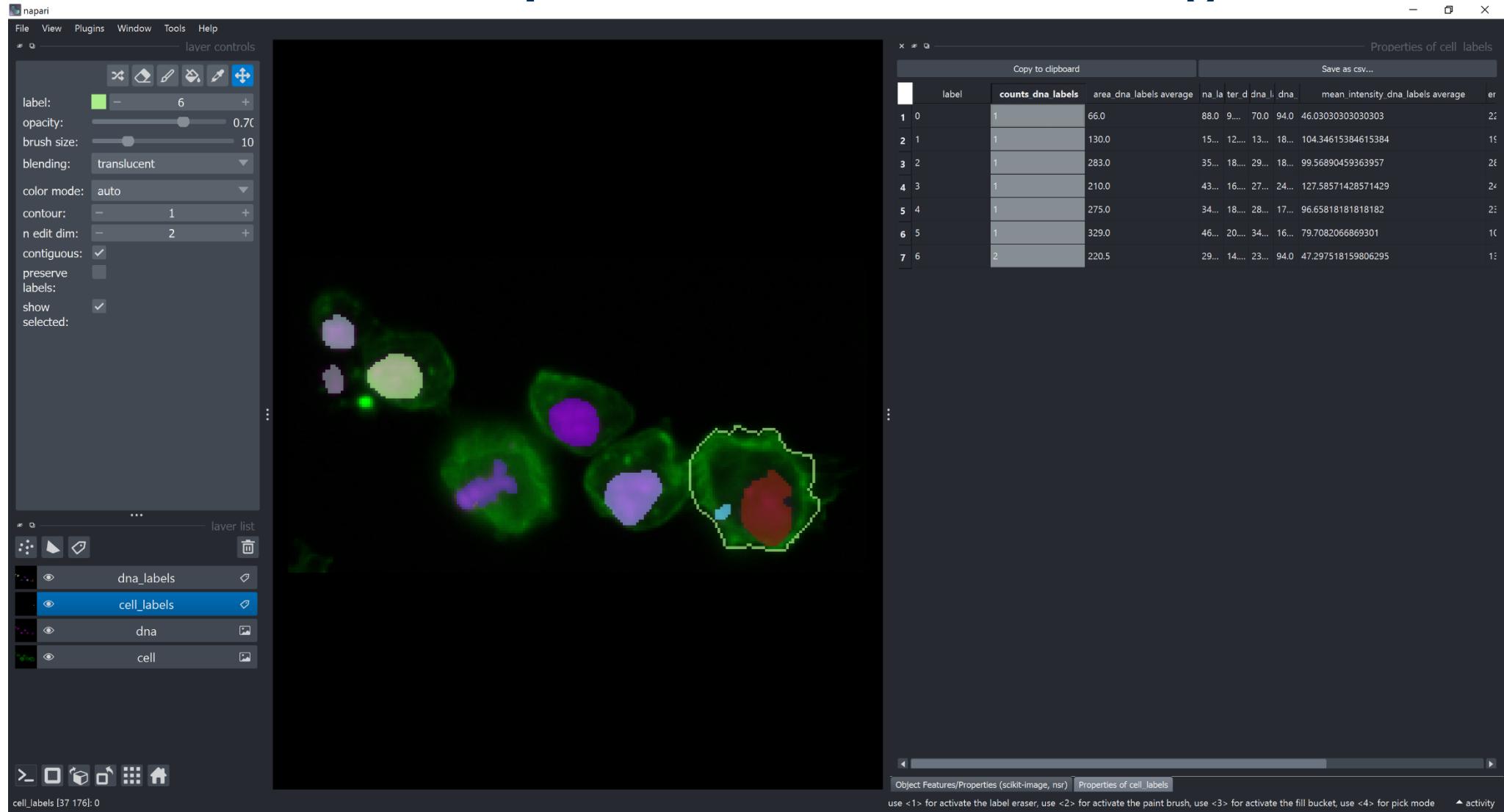
<https://github.com/haesleinhuepf/napari-skimage-regionprops>

Feature extraction in napari with multichannel images



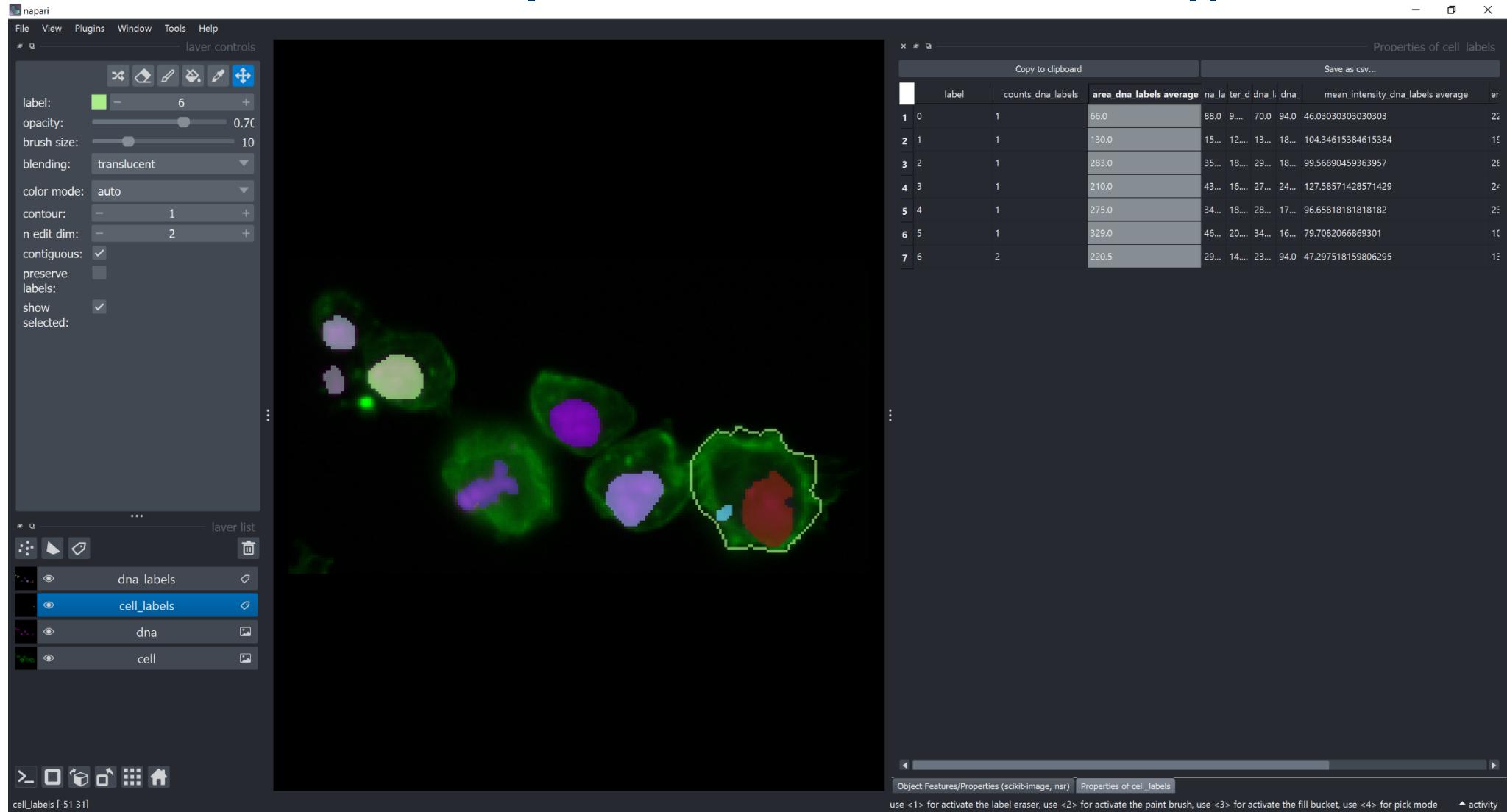
<https://github.com/haesleinhuepf/napari-skimage-regionprops>

Feature extraction in napari with multichannel images



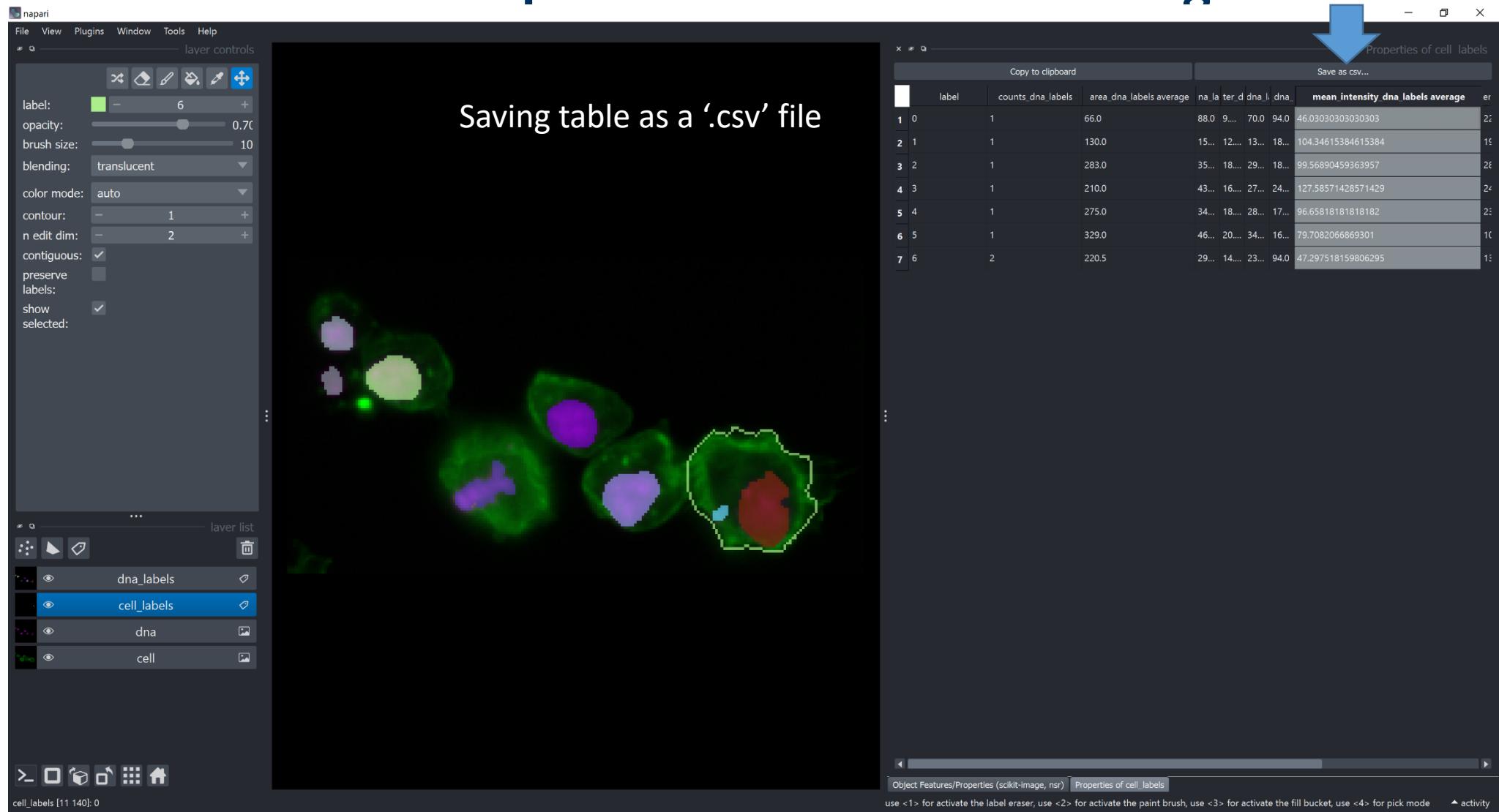
<https://github.com/haesleinhuepf/napari-skimage-regionprops>

Feature extraction in napari with multichannel images



<https://github.com/haesleinhuepf/napari-skimage-regionprops>

Feature extraction in napari with multichannel images



Saving table as a '.csv' file

<https://github.com/haesleinhuepf/napari-skimage-regionprops>

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Feature extraction in napari with multichannel images

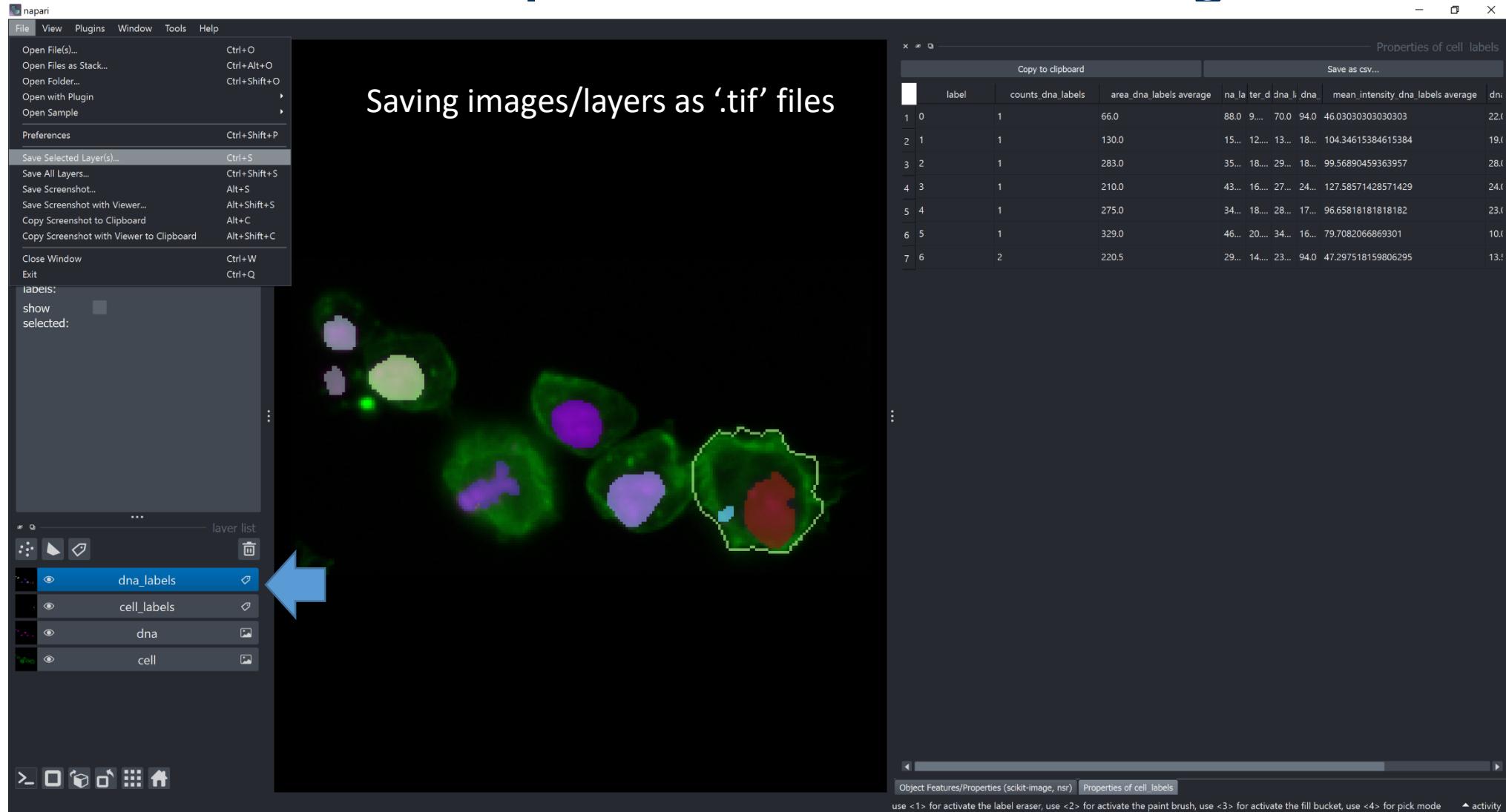
	A	B	C	D	E	F	G	H	I	J	K						
1	label	counts_dna_labels	area_dna_labels	average	bbox_area_dna_labels	average	equivalent_diameter_dna_labels	average	convex_area_dna_labels	average	max_intensity_dna_labels	average	mean_intensity_dna_labels	average	min_intensity_dna_labels	average	standard_deviation_intensity_dna_labels
2	0	0	1	66		88	9.166995688		70		94		46.03030303		22		
3	1	1	1	130		156	12.86550197		135		183		104.3461538		19		
4	2	2	1	283		357	18.98227571		293		182		99.56890459		28		
5	3	3	1	210		437	16.35176762		271		247		127.5857143		24		
6	4	4	1	275		342	18.71205159		284		173		96.65818182		23		
7	5	5	1	329		462	20.46694433		345		161		79.70820669		10		
8	6	6	2	220.5		293.5	14.45109786		233		94		47.29751816		13.5		
9																	
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<https://github.com/haesleinhuepf/napari-skimage-regionprops>

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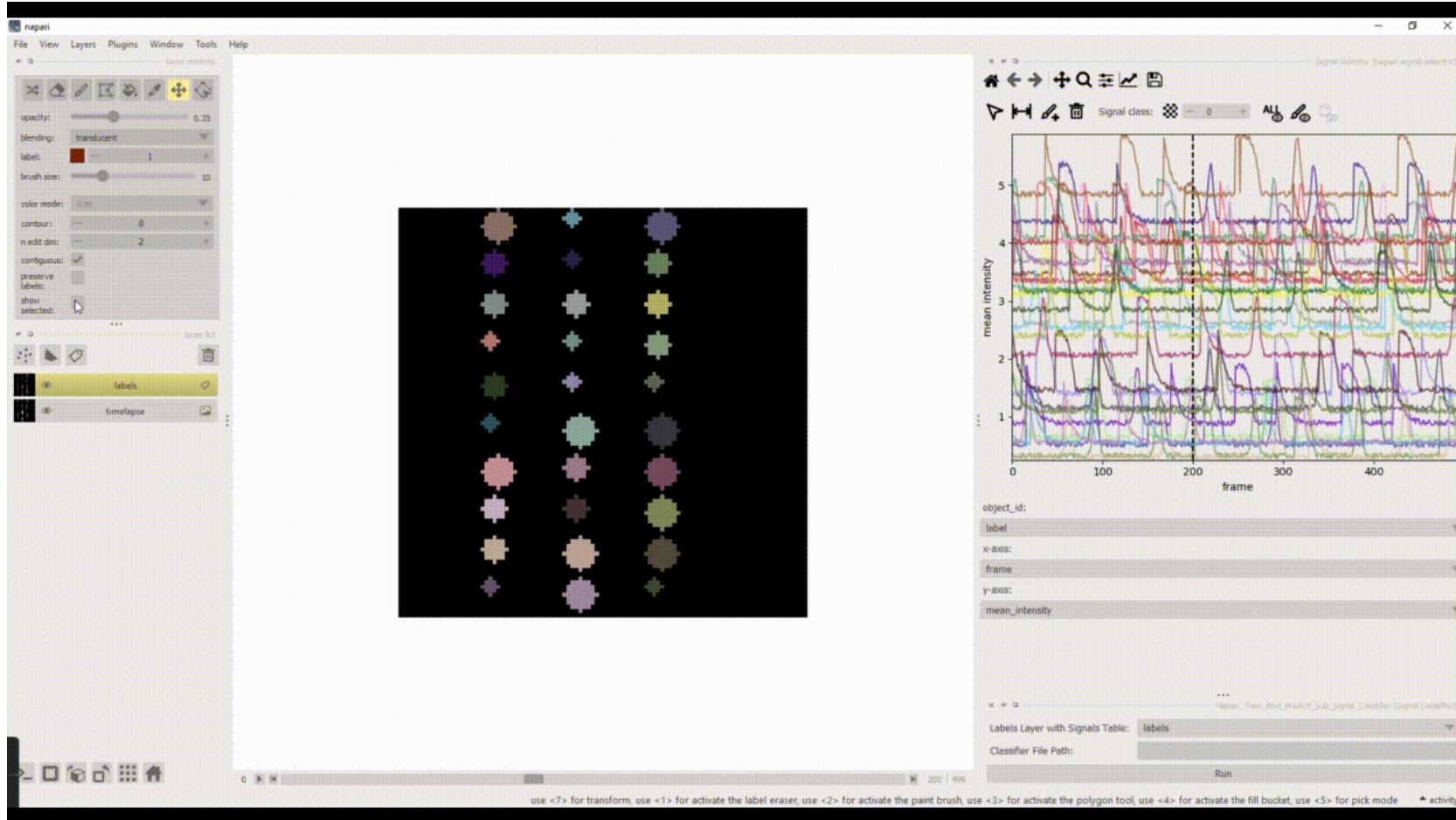


Feature extraction in napari with multichannel images



<https://github.com/haesleinhuepf/napari-skimage-regionprops>

Outlook: Temporal Features Annotation and Classification



- <https://github.com/zoccoler/napari-signal-selector>
 - <https://github.com/zoccoler/napari-signal-classifier>

A photograph of a smartphone screen that has been shattered into many pieces. The cracks radiate from the center, where a bright sunburst is visible against a clear blue sky with a few wispy clouds.

**Another
Break**

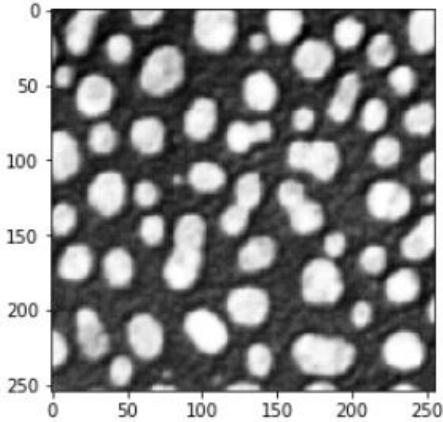
Object Classification and Supervised Machine Learning in napari

Random Forest Classifiers

- Object Classifier
- `napari-apoc` plugin

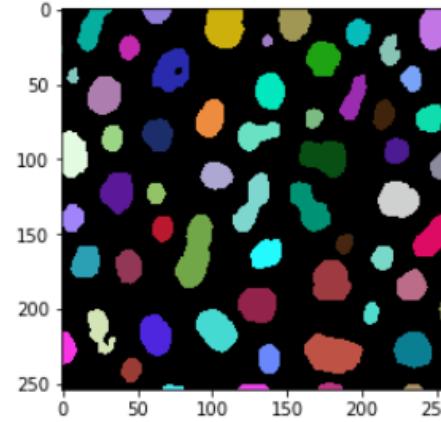
Object classification

- Object classification is a task that can be applied to an existing instance segmentation in order to identify a particular group of objects



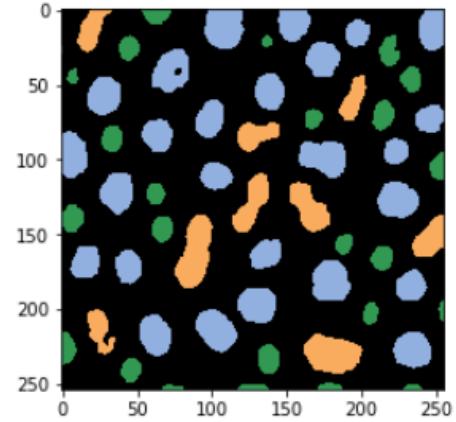
Raw image

Object
segmentation

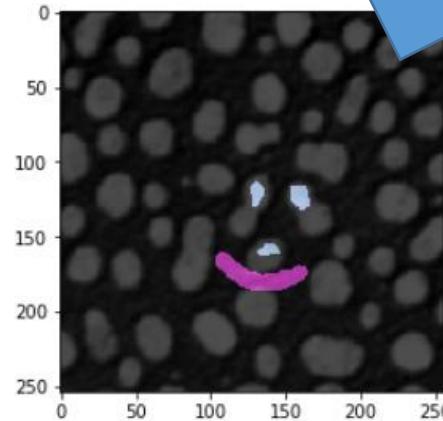


Object label image

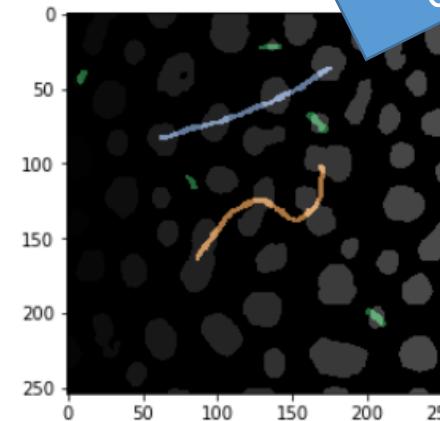
Object
classification



Class label image



Pixel annotation



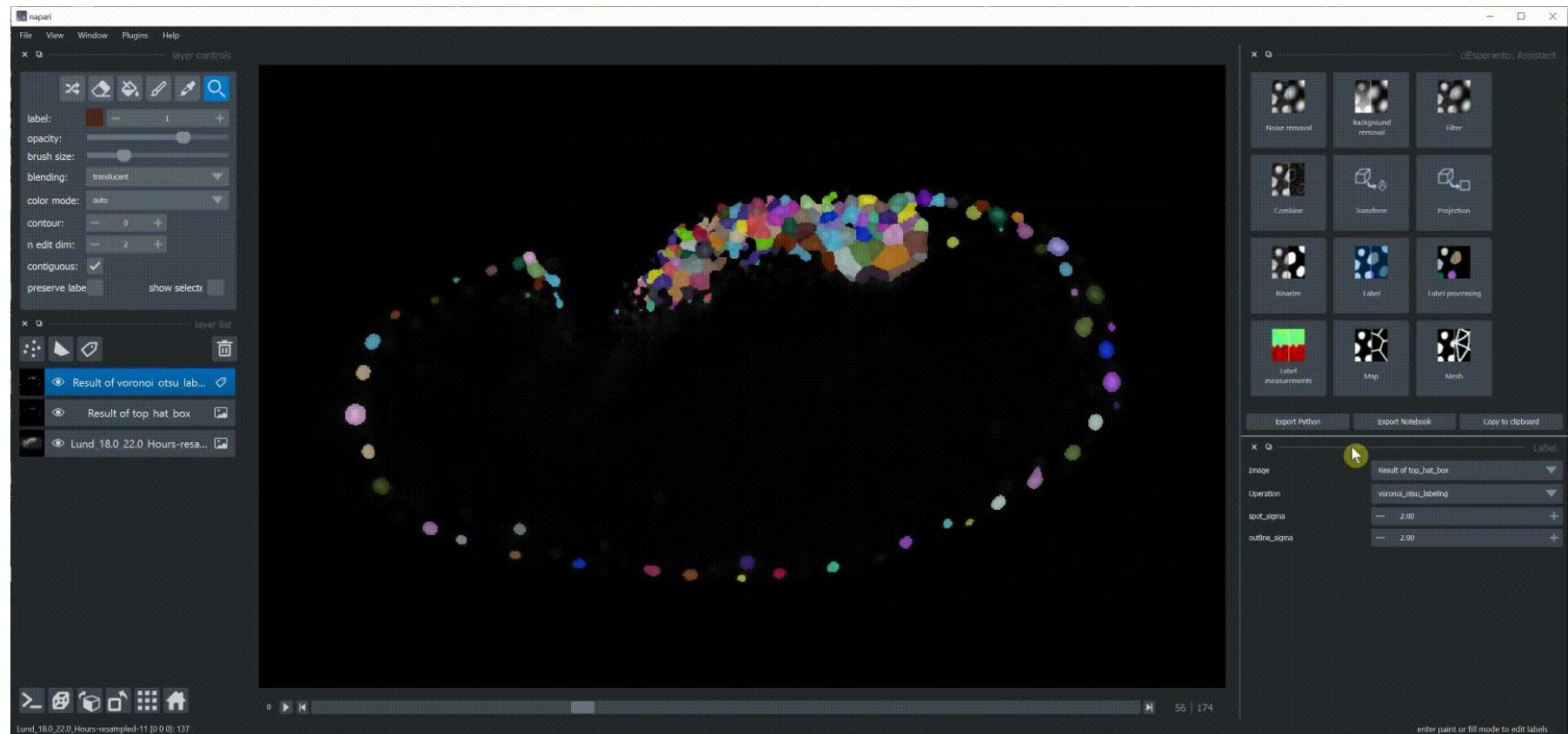
Object annotation

<https://imagej.nih.gov/ij/images/>

Object classification

Random Forest Classifiers based on

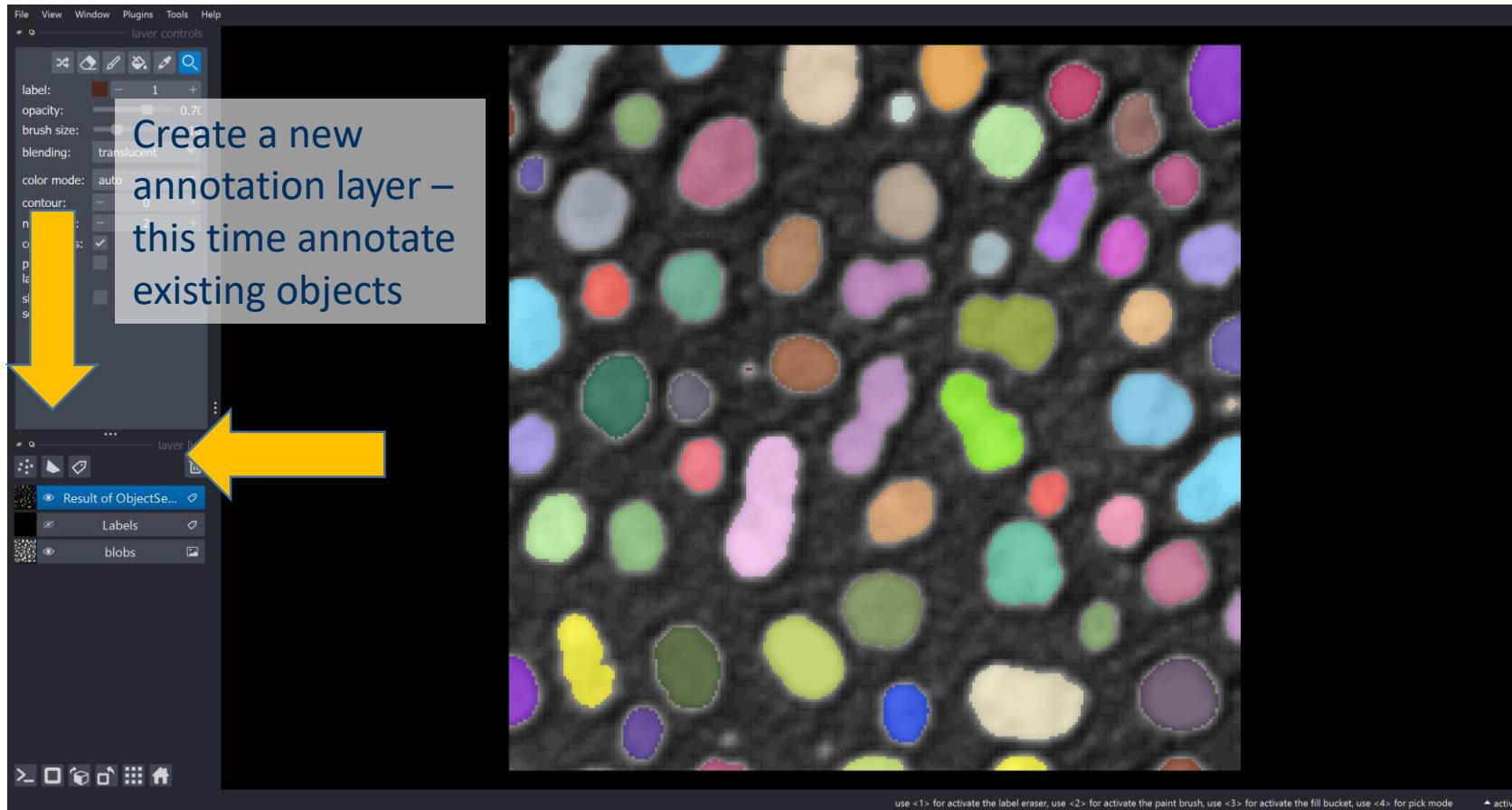
- scikit-learn and
- clesperanto



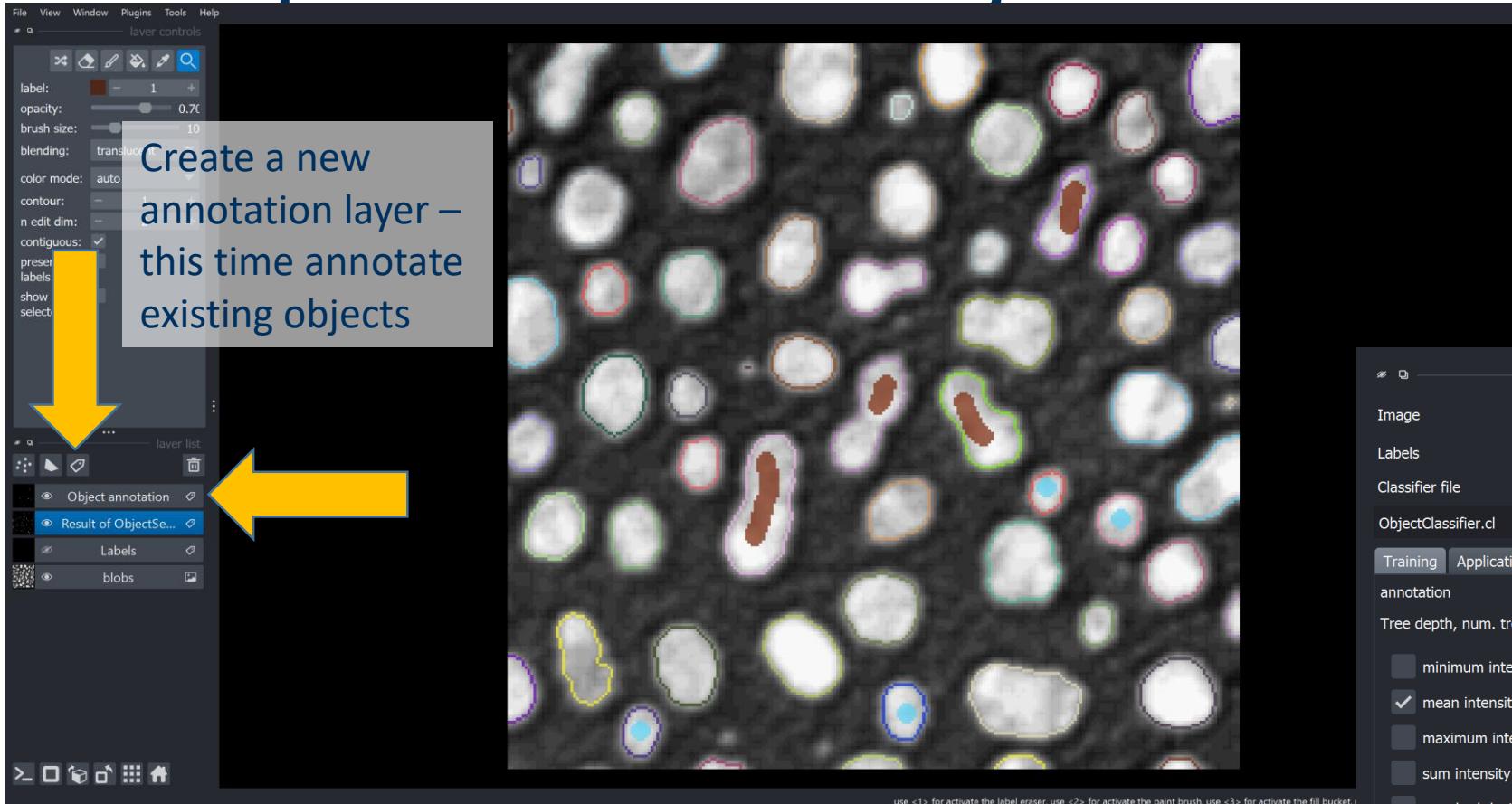
<https://github.com/haesleinhuepf/napari-accelerated-pixel-and-object-classification>

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

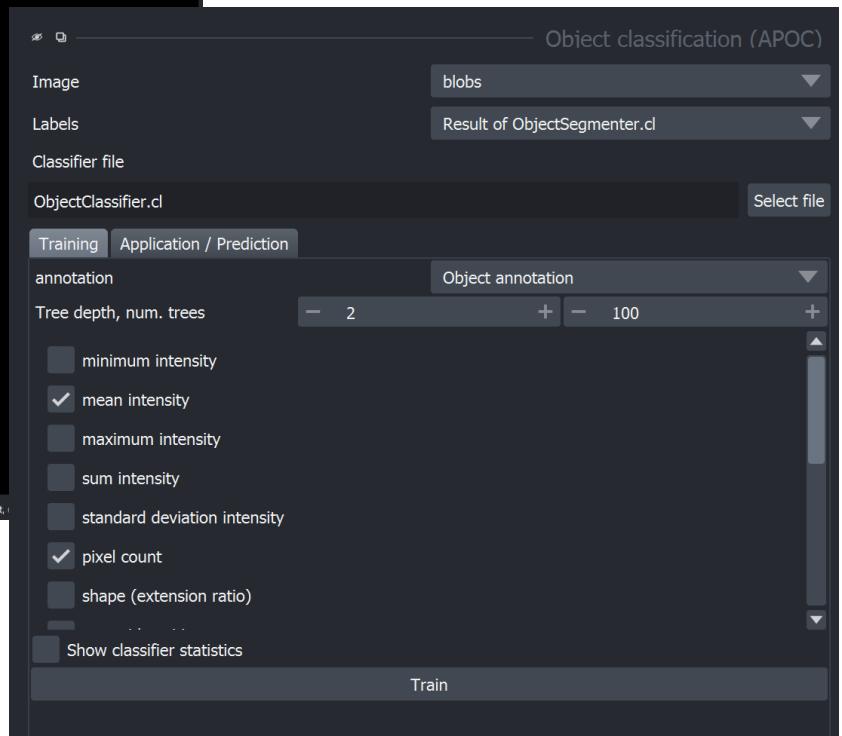
In napari: annotation



In napari: annotation and object classification



Tools ->
Segmentation post-processing ->
Object Classification (APOC)



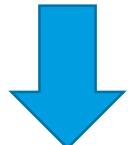
Telling different classes of objects apart requires:

- An **annotation** for some example objects
- **Features** for each objects upon which to make a prediction

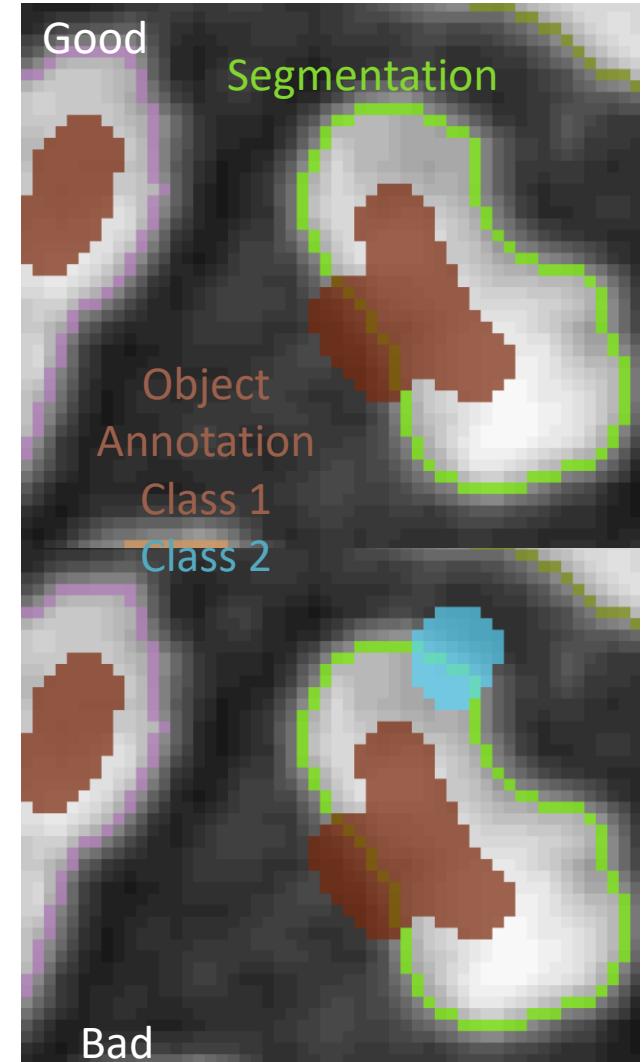
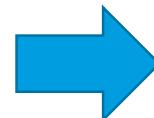
<https://imagej.nih.gov/ij/images/>

Exercise: Object Classification

1. Activate the environment and open napari
mamba activate napari-intro-env
napari
 2. Train an object classifier on sample data: Differentiate **good nuclei** (nicely separated from other objects) from **bad nuclei** (two nuclei sticking together)
- Hints for annotation:
- The annotation **does not have to overlap exactly** with the painted object
 - Every annotation should **only touch** the correct objects
 - **Save your annotations**
 - In this case, it makes no difference whether we annotate many or few pixels. The number of annotated objects is more important in this context.



https://biapol.github.io/QM_Course_Bio_Image_Analysis_with_napari_2025/interactive_object_classification/readme.html



<https://imagej.nih.gov/ij/images/>

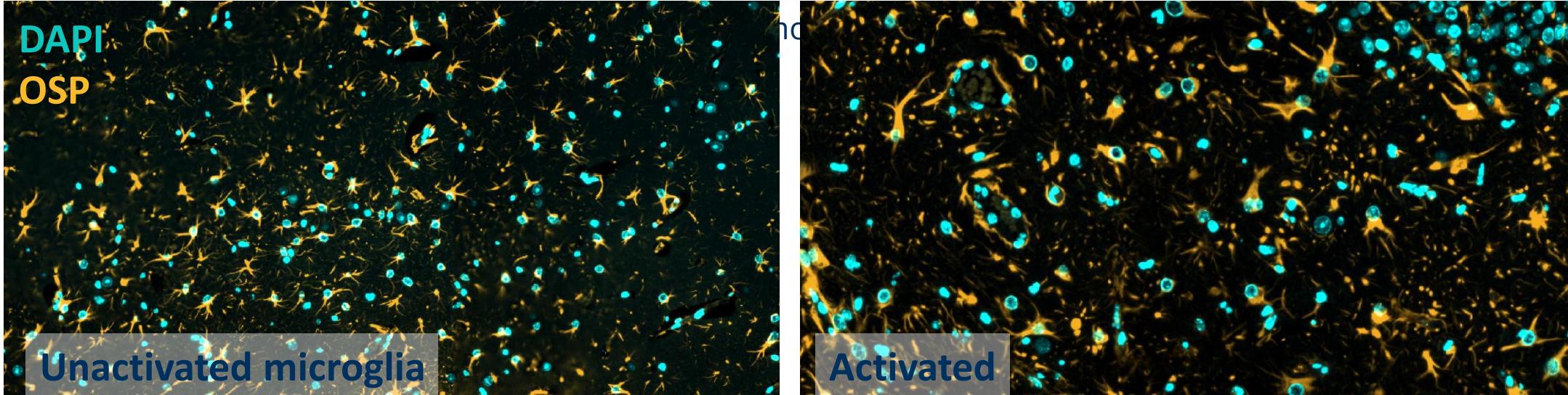


Object Classification and Unsupervised Machine Learning

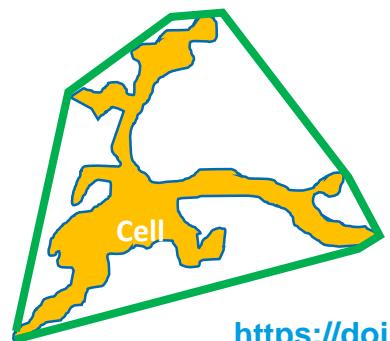
- Dimensionality Reduction
- Clustering

Introduction

Ideal situation: Biological property is related to a known, measurable feature in image data

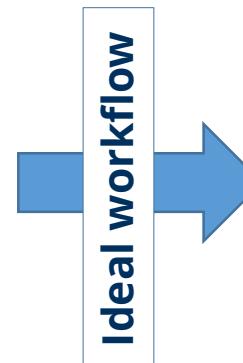


Existing scores: "Ramification index" (Wittekindt et al., 2022)



$$\text{Rammificationindex} = \frac{A_C}{A_P}$$

<https://doi.org/10.3390/cells11111723>

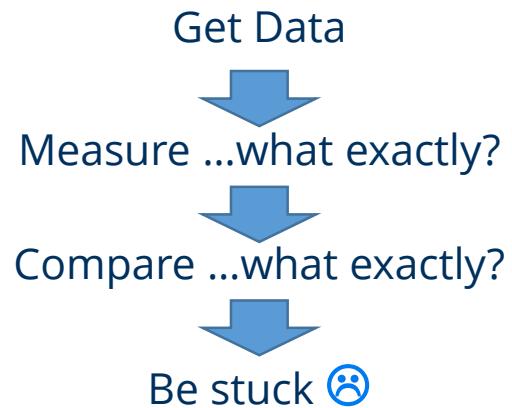


- Get Data
- Measure defined score/features
- Compare conditions
- Be done 😊

Introduction

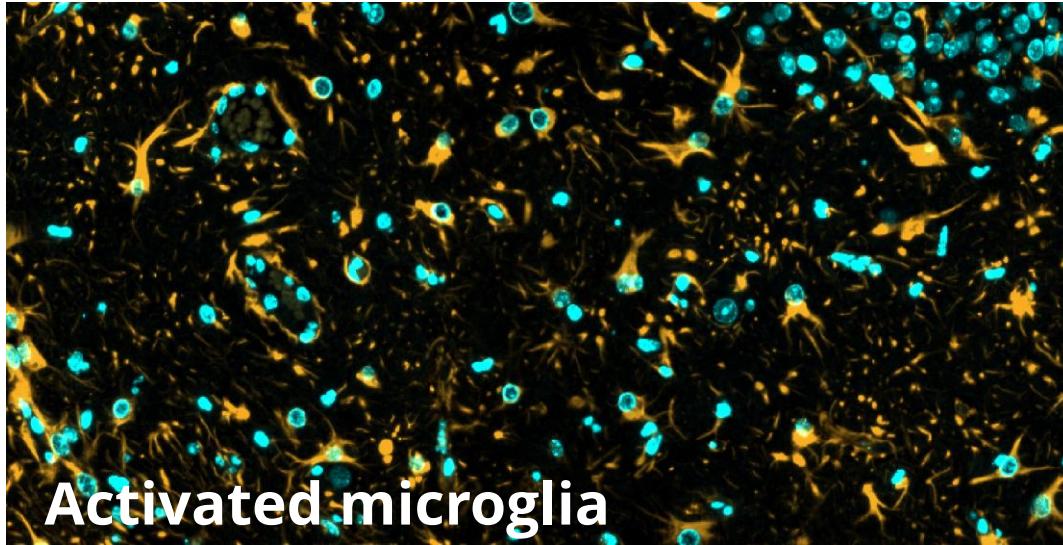
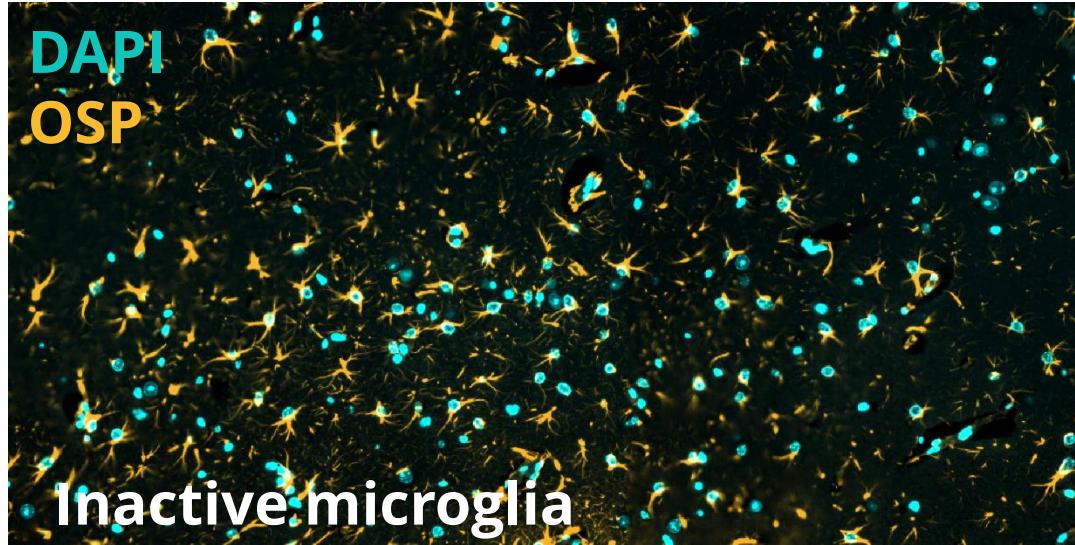
More typical situation:

- We expect or know of a biological effect (e.g., through external cues, cell growth stages, etc.)
- We do not know how this effect can be measured or how it manifests itself



Identifying features to measure

Example: Inactive vs. activated microglia in mouse brain

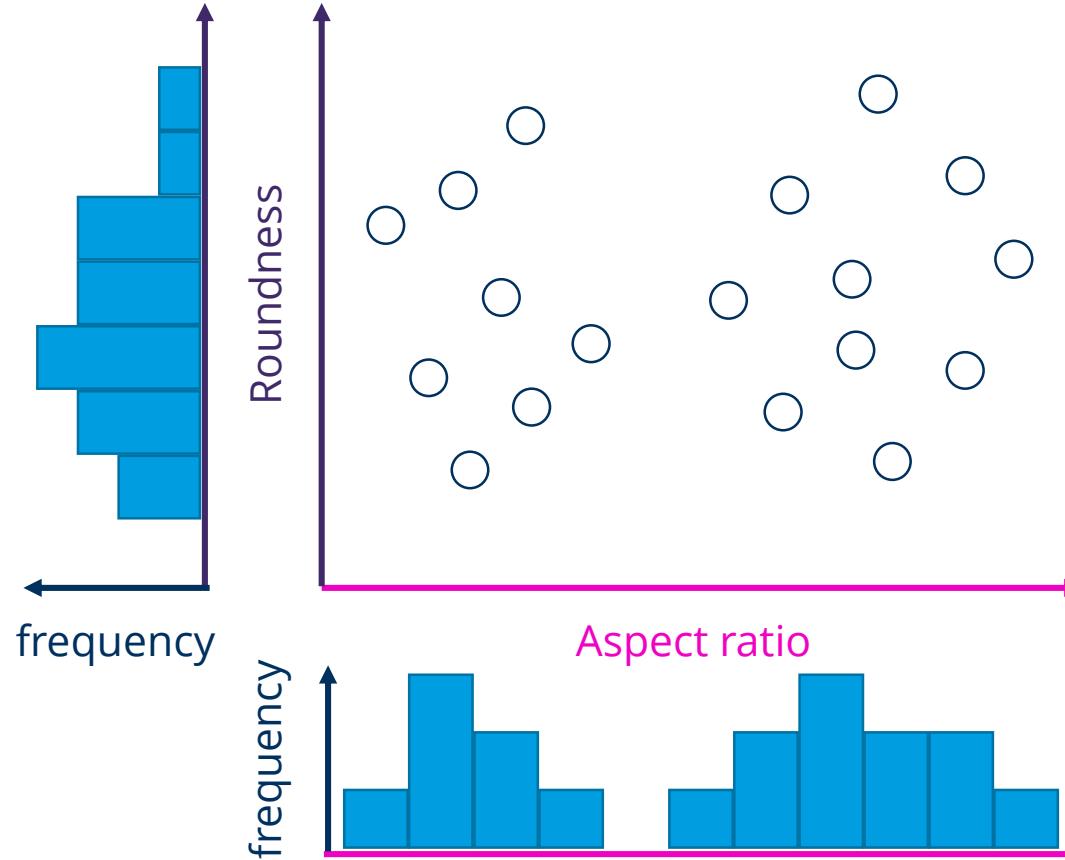


Challenge: Which of these features reflect biological properties?

	label	area	bbox_area	convex_area	equivalent_diameter	max_intensity	mean_intensity	min_intensity	solidity	extent	eret_diameter_max	local_centroid-0	l
1	1	3379	13949	5120	18.61786412639...	613.0	345.6717963894...	259.0	0.6599609375	0....	37.3496987939662	15.77952056821...	18
2	2	2319	7448	3491	16.42230229224...	421.0	297.8434670116...	240.0	0....	0....	38.65229618017...	4....	12
3	3	2304	14415	4281	16.38681751812...	456.0	300.8298611111...	245.0	0....	0....	34.19064199455...	17.73828125	13
4	4	3278	13804	5139	18.43048549951...	467.0	316.1446003660...	249.0	0....	0....	34.84250278036...	15.52287980475...	10
5	5	1501	3315	1681	14.20563625190...	458.0	302.147235176549	236.0	0....	0....	17.97220075561...	6....	6.
6	6	2341	6061	2714	16.47407088948...	594.0	355.4446817599...	261.0	0....	0....	30.67572330035...	16.54250320375...	6.

Unsupervised machine learning

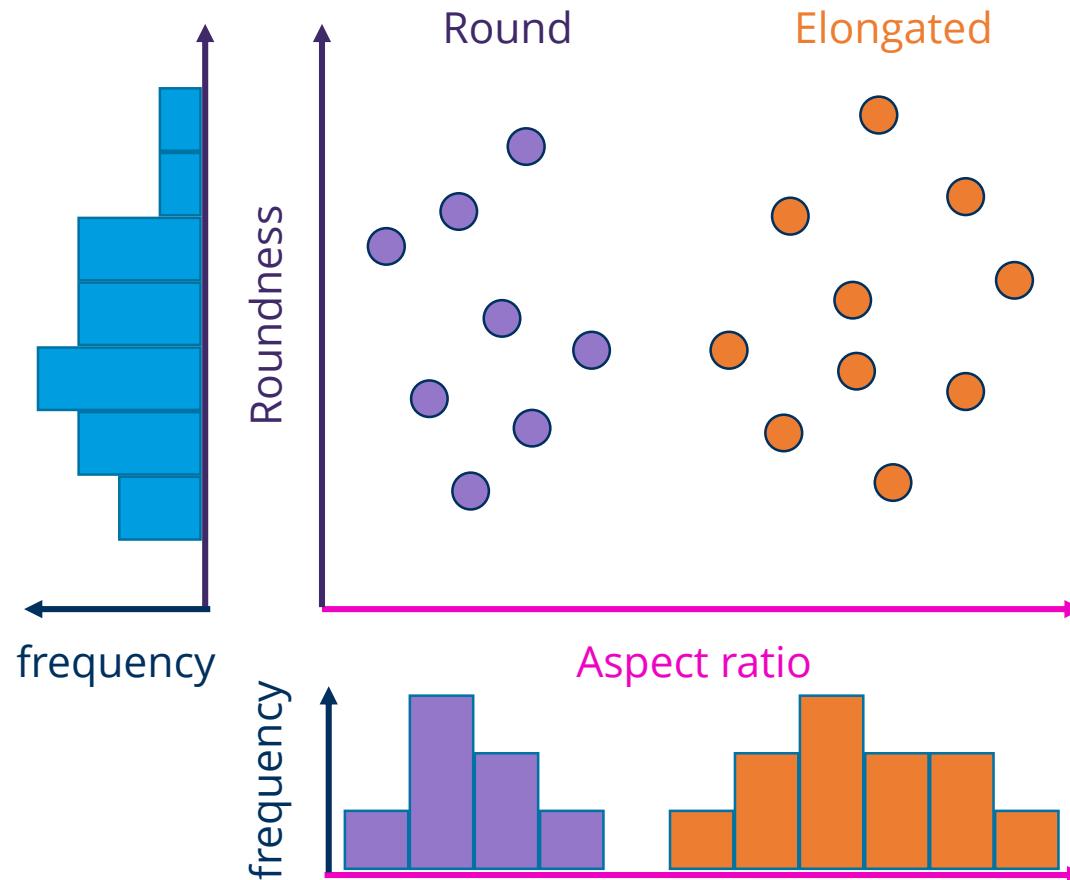
If you don't provide ground truth, the algorithm is *unsupervised*.



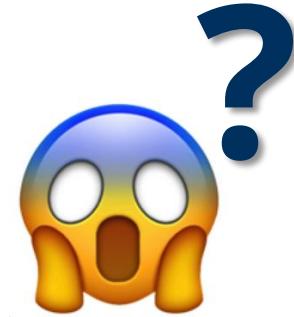
Unsupervised machine learning

If you don't provide ground truth, the algorithm is unsupervised.

Nevertheless, algorithms can tell us something about the data



- Mean intensity
- Standard deviation
- Total intensity
- Textures
- Area / Volume
- Roundness
- Solidity
- Circularity / Sphericity
- Elongation
- Centroid
- Bounding box
- ...



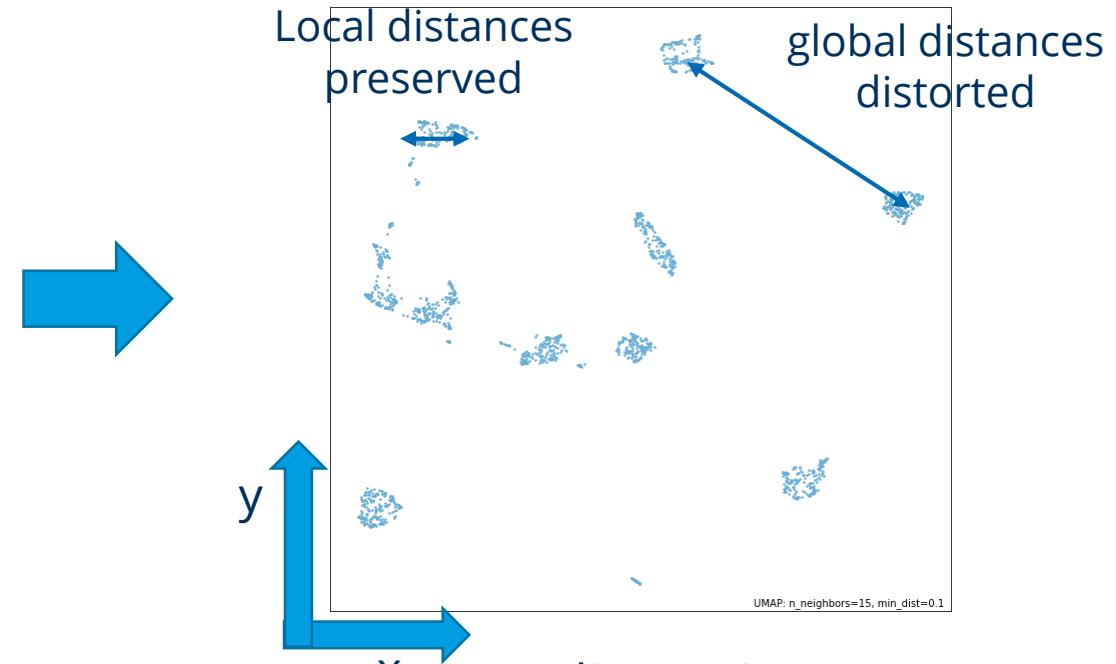
Dimensionality reduction

Challenge: Find a representation (embedding) of your data that represents the data in fewer dimensions

Preserve local distances at the expense of global distortions

		x	y	z	...								
	label	area	bbox_area	convex_area	equivalent_diameter	max_intensity	mean_intensity	min_intensity	solidity	extent	eret_diameter_ma	local_centroid-0	l...
1	1	3379	13949	5120	18.61786412639...	613.0	345.617963894...	259.0	0.6599609375	0....	37.3496987939662	15.77952056821...	18...
2	2	2319	7448	3491	16.4223022924...	421.0	297.8434670116...	240.0	0....	0....	38.65229618017...	4....	12...
3	3	2304	14415	4281	16.38681751812...	456.0	300.8298611111...	245.0	0....	0....	34.1906419455...	17.73282125	13...
4	4	3278	13804	5139	18.43048549951...	467.0	316.1446003660...	249.0	0....	0....	34.84250278036...	15.52287980475...	10...
5	5	1501	3315	1681	14.20563625190...	458.0	302.147235176549	236.0	0....	0....	17.97220075561...	6....	6...
6	6	2341	6061	2714	16.47407088948...	594.0	355.4446817599...	261.0	0....	0....	30.67572330035...	16.54250320375...	6...
7	7	1725	3584	1940	14.87979081163...	568.0	343.7866666666...	257.0	0....	0....	17.72004514666...	7.80463768115942	7...
8	8	1502	3840	1753	14.20879025650...	431.0	290.0659121171...	235.0	0....	0....	18.57417562100...	8....	6...
9	9	1602	4080	1894	14.51737058294...	475.0	297.8008739076...	241.0	0....	0....	18.70828693386...	8....	8...
10	10	1395	3600	1624	13.86304166283...	424.0	304.8494623655...	247.0	0....	0.3875	17.60681686165...	7....	7...
11	11	609	1100	697	10.51654029260...	323.0	274.2528735632...	241.0	0....	0....	13.45362404707...	3....	4...
12	12	1686	3757	1894	14.76679738567...	460.0	303.8303677342...	240.0	0....	0....	17.97220075561...	9....	7...
13	13	2157	5184	2531	16.03062694504...	576.0	339.990264255911	270.0	0....	0....	19.54482028569...	8....	8...
14	14	863	2340	1032	11.81237949737...	327.0	272.4449594438...	237.0	0....	0....	16.0312195418814	6....	5...

Many dimensions

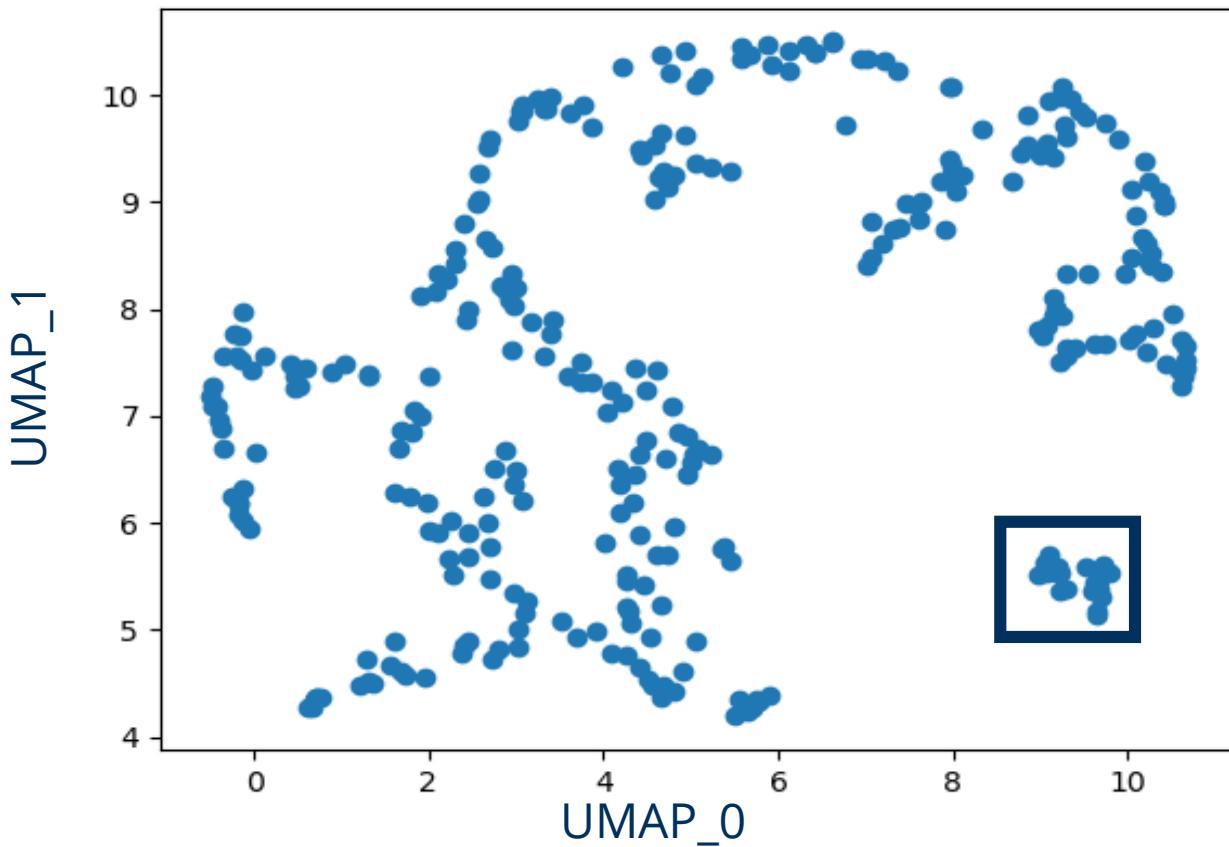


<https://umap-learn.readthedocs.io/en/latest/index.html>

Clustering

Starting point: Feature space or dimensionality reduction reveals “groups” in our data

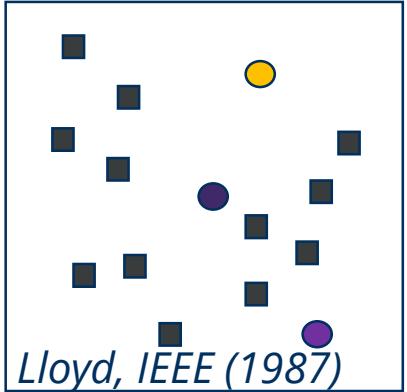
Can we automatically identify these groups?



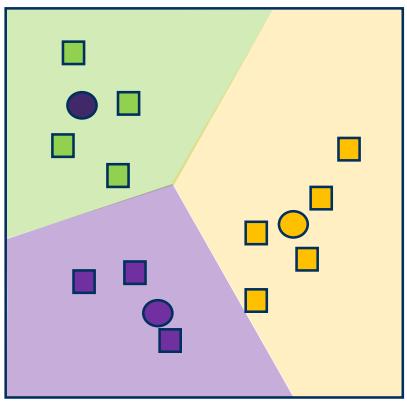
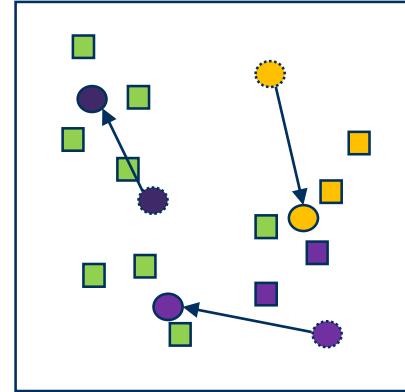
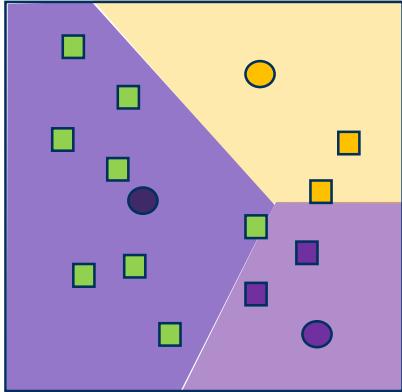
→ **Clustering** allows to stratify data into groups *without previous annotations*

K-means clustering

Strategy: Group data points into n groups so that variance within group is minimal

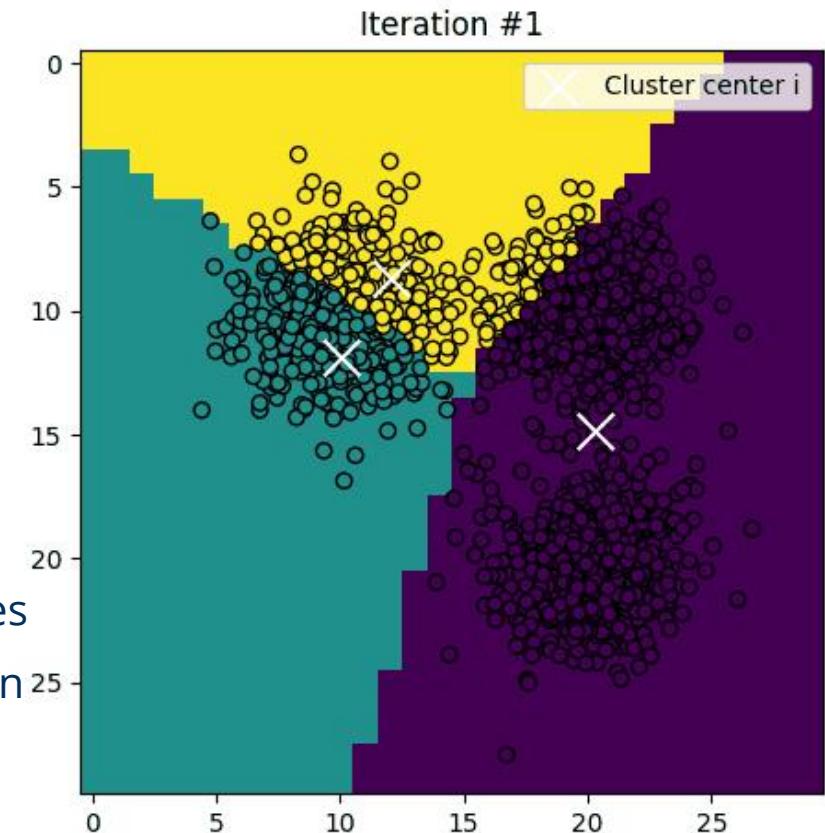


Step1: Random initialization of cluster centers



Strength and weaknesses

- Number of clusters needs to be known
- Clusters can not capture more complex topologies
- Based on Euclidian metrics → every new point can be assigned to a cluster



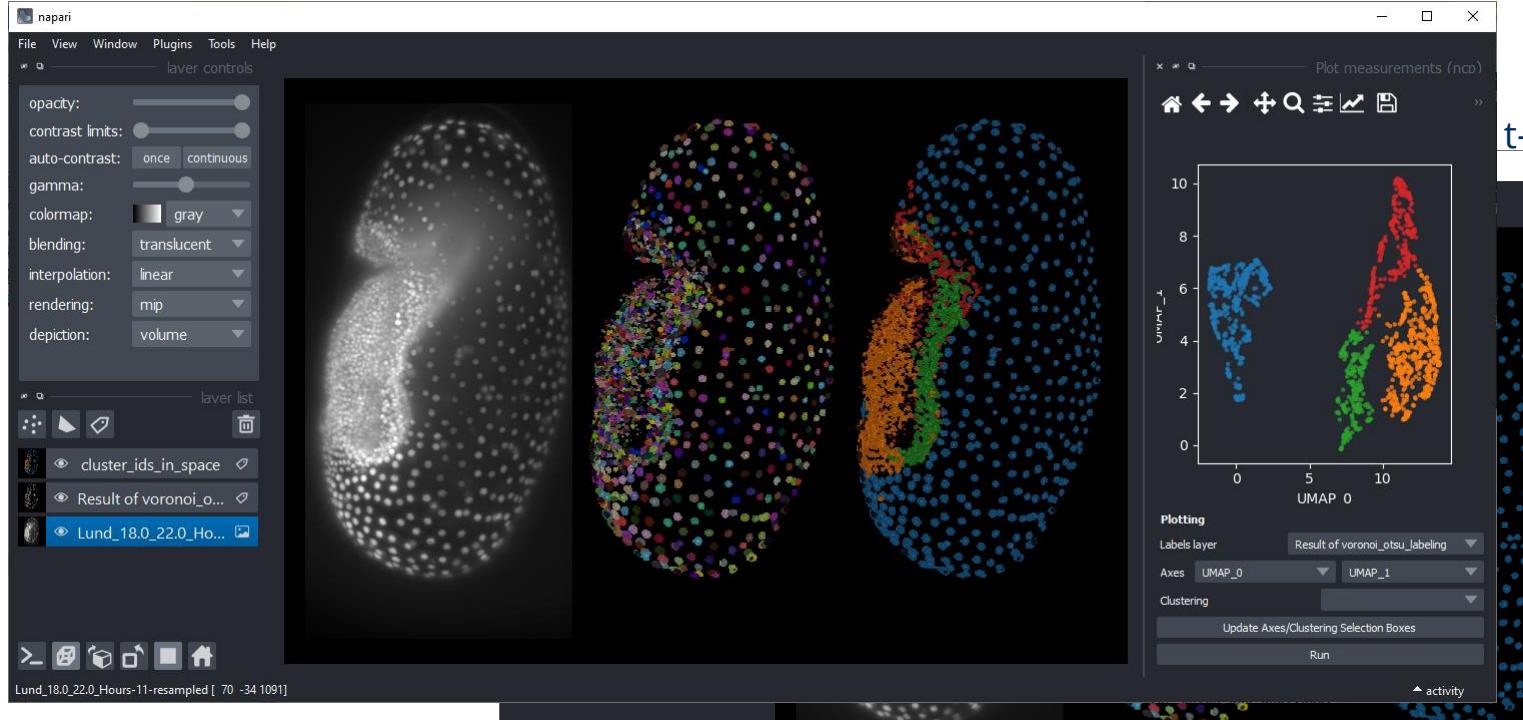


Object Classification and Unsupervised Machine Learning in napari

napari-clusters-plotter plugin

Dimensionality reduction

Uniform manifold approximation and projection (UMAP)



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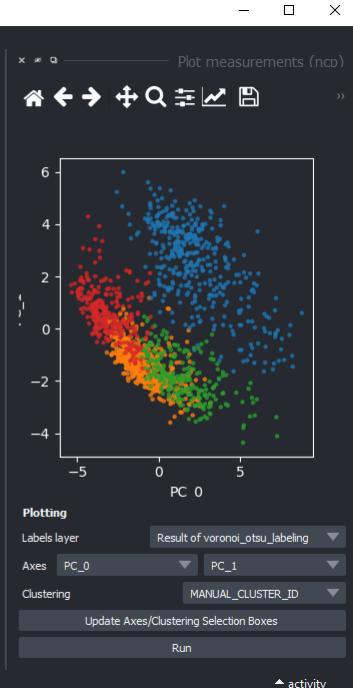
<https://github.com/BiAPoL/napari-clusters-plotter>

Image Data Source: Daniela Vorkel, Myers lab, MPI-CBG/CSBD Dresden

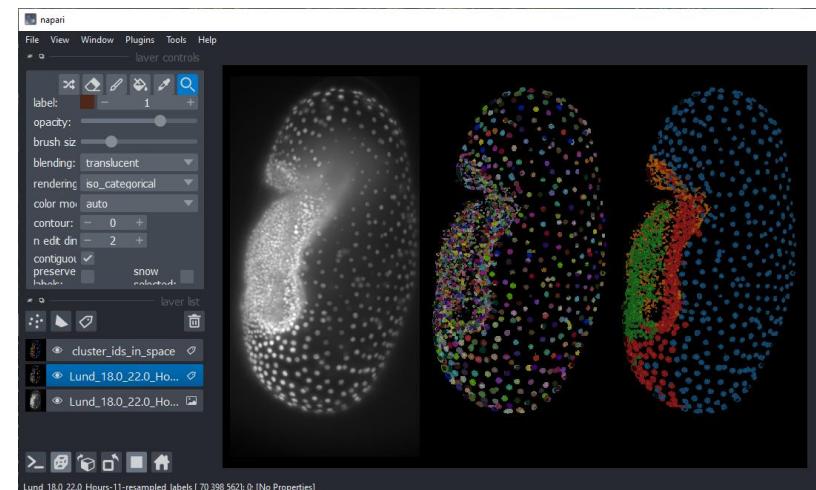
t-distributed stochastic neighbor embedding (t-SNE)



Principal component analysis (PCA)



Clustering



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

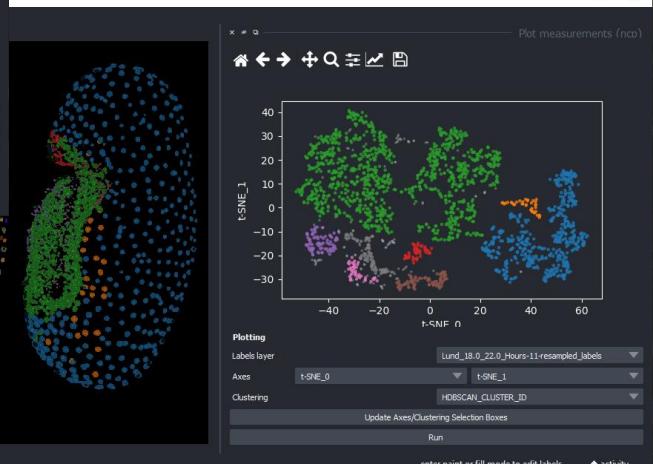
Image Data Source: Daniela Vorkel, Myers lab, MPI-CBG/CSBD Dresden

K-means clustering



Agglomerative clustering

Hierarchical Density-Based
Spatial Clustering of
Applications with Noise
(HDBSCAN)



Data Exploration

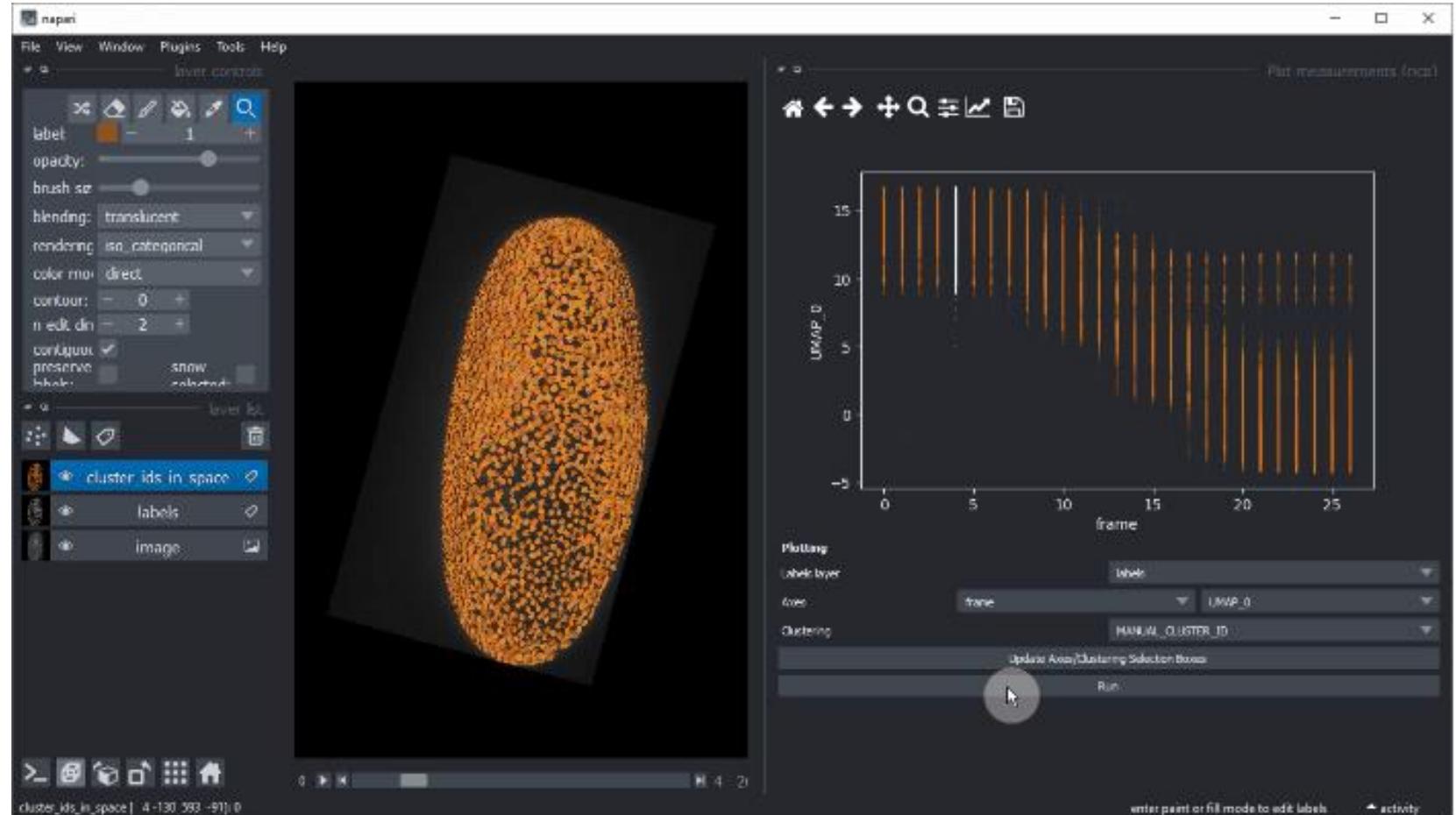
... using interactive clustering



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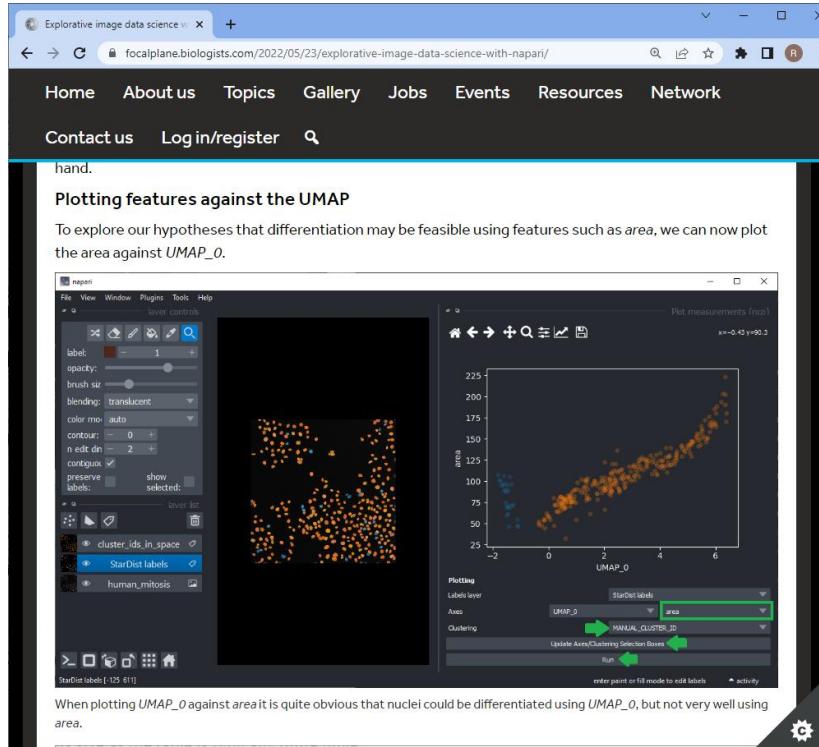


<https://github.com/BiAPoL/napari-clusters-plotter>

Image Data Source: Daniela Vorkel, Myers lab, MPI-CBG/CSBD Dresden

Further reading

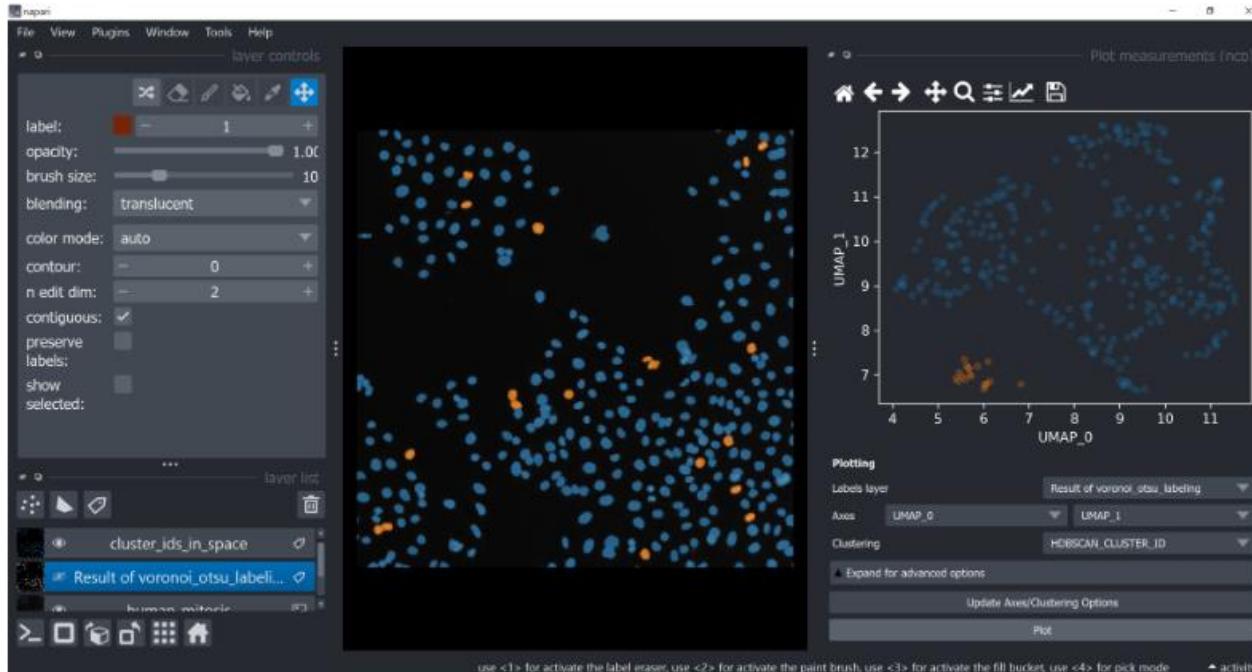
Blog posts on the Focalplane about Unsupervised Machine Learning



<https://focalplane.biologists.com/2022/05/23/explorative-image-data-science-with-napari/>

Exercise: unsupervised object classification

Use Napari to classify objects (nuclei) without ground-truth



https://biapol.github.io/QM_Course_Bio_Image_Analysis_with_napari_2025/interactive_unsupervised_object_classification/readme.html



Further resources:

Napari-hub:

www.napari-hub.org

BiaPoL image analysis course materials:

<https://github.com/BiAPoL/Bio-image Analysis with Python>

Bio-image analysis materials:

<https://bioimagebook.github.io/README.html>

<https://haesleinhuepf.github.io/BiolmageAnalysisNotebooks/intro.html>

Image Science Community Forum:

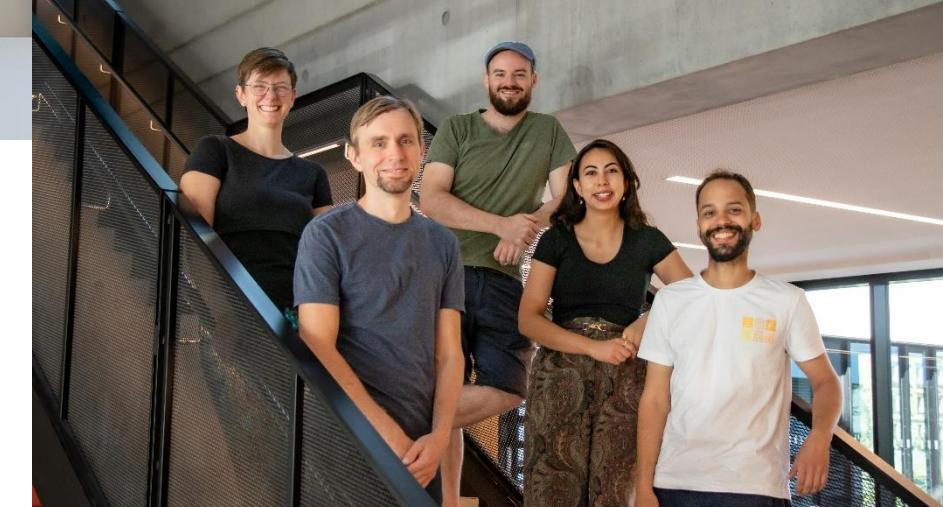
<https://forum.image.sc/>

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BiAPoL team

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