



BioImage Analysis and Data Processing Workshop 2025 Charles University, Prague

Bio-Image Analysis with napari Plugins

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Re-using material from:

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Morning part:

- Installation and introduction to **napari**
- Loading images from OMERO with **napari-omero**
- Segmentation with Machine Learning using **micro-sam**
- Segmentation with Machine Learning using **napari-apoc**



Afternoon part:

- Feature Extraction and Multichannel Analysis with **napari-skimage-regionprops**
- Object Classification with Machine Learning using **napari-apoc** and **napari-clusters-plotter**
- Scientific Plotting with **seaborn**











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). They should work for most exercises, but we should create a new one for the latest napari-clusters-plotter changes. The instructions are provided here:

- https://biapol.github.io/BioImage-Analysis-and-Data-Processing-Workshop-2025/intro.html

The following slides are meant to clarify what these instructions mean. We will create the course environment together soon after.







Installing napari as a Python package

napari is a <u>Python library</u>

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!

Q # (1) numfocus NumFOCUS README
 ALicense Miniforge https://numfocus.org/donate-to-cond earn more about GitHub Sponso Build miniforge failing downloads 17H Packages This repository holds the minimal installers for Conda and Mamba specific to conda-forge, with the following features pre-configured: No packages published · Packages in the base environment are obtained from the conda-forge channel. Contributors 40 · The conda-forge channel is set as the default (and only) channel ی 🍚 🔂 🌍 🤹 🕲 🕲 We put an emphasis on supporting various CPU architectures (x86 64, ppc64le, and aarch64 including Apple Silicon). Optional support for PyPy in place of standard Python interpreter (aka 🕼 🕲 🙃 🗂 🕼 🕌 "CPython") is provided in the installers with -pypy3- in their filename + 26 contributors Download Languages Miniforge installers are available here: https://github.com/conda-forge/miniforge/releases Shell 89.0% Batchfile 11.0% Miniforge3 Latest installers with Python 3.12 (*) in the base environment: 05 Architecture Minimum Version Download x86 64 (amd64) glibc >= 2.17 Linux Miniforge3-Linux-x86_64 aarch64 (arm64) (**) alibc >= 2.17Linux ppc64le (POWER8/9 alibc >= 2.17Miniforge3-Linux-ppc64l OS X x86 64 macOS >= 10.13 Miniforge3-MacOSX-x86_6 OSX arm64 (Apple Silicon) (***) $macOS \ge 11.0$ Miniforge3-MacOSX-arm6 Windows x86 64 Windows >= 7 Miniforge3-Windows-x86 64

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 Enviroments and install Python (and other) packages, including napari
 https://hackmd.io/@talley/SIB_IObBi#Terms







Installing napari as a Python package



Wait, environments?





Yes, virtual environments!



Images generated by DALL-E, developed by OpenAl. Smiley icons from Flaticon.com







Installing napari as a Python package



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Installing napari

Napari can also be downloaded and installed like other software

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

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













Introduction to napari

- napari Viewer
- Layer Types
- napari Plugins



Napari: 3D viewer for Python

Multi-dimensional image viewer in python

https://napari.org/







Napari: 3D viewer for Python









Napari user interface



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
- Shapes layer: Draw paths or regular shapes





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!



Napari



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





napari plugins





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



https://github.com/napari/napari-animation

segmentation

conv-paint



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



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





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







Plugins and Layers Menus





napari								
e	View	Plugins Window Tools Help						
		Install/Uninstall Plugins						
		Plugin Errors						
		wizard (nanari-animation)						
		nanari blob detection	•					
		pystackreg (napari-pystackreg)						
		regions of interest (napari-roi)						
		napari-stl-exporter	•					
		napari-clusters-plotter	•					
		The Segmentation Game Widget (the-segmentation-game)						
		Red Lionfish widget (RedLionfish)						
		PlatyMatch	•					
		Workflow Optimizer (napari-workflow-optimizer)						
		Workflow Widget (napari-workflow-inspector)						
:.		Assistant (clEsperanto)						
		Surface Annotation Widget (napari-process-points-and-surfaces)						
		Plugin Search (napari-plugin-search)						
		napari-plot-profile	•					
		Mouse Controls (napari-mouse-controls)						
		Layer Details Display (napari-layer-details-display)						
		Folder Browser (napari-folder-browser)						
		curtain (napari-curtain)						
		napari-crop	•					
		Brightness Contrast (napari-brightness-contrast)						
		Assistant (napari-assistant)						
		napari-accelerated-pixel-and-object-classification	•					
		Ortho Viewer Widget (napari-3d-ortho-viewer)						
		convert to 2d timelapse (napari-time-slicer)						
		open in new window (napari-tabu)						
		duplicate current frame (napari_skimage_regionprops2)						
		napari-simpleitk-image-processing	•					
		napari-segment-blobs-and-things-with-membranes	•					
		clEsperanto	•					
		napari-process-points-and-surfaces	•					
-		split stack (napari-assistant)						



devbio-napari plugin bundle

Layers sub-menu items depend on plugin developers

populating them

BioDIP Biopolis Dresden Imaging Platform





The Napari Hub

The plugin you are looking for may be near you!

Search engine for napari plugins









Inspecting plugin usage and maintenance - GitHub

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🖿 .napari	model and data docs	3 years ago	deep-learning electron-microscopy 3d					
Custom_configs	allow model architecture customization	3 years ago	panoptic-segmentation napari-plugin					
empanada_napari	Updated requirements.txt and Count labe s module.	8 months ago	D Readme					
images	docs atests	3 years ago	婚 BSD-3-Clause license					
🗋 .gitignore	v1.1 release	last year	Activity ■ Custom properties					
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C requirements.txt	Updated setup.cfg and requirements.txt file to include comp.	8 months ago	Releases					
C setup.cfg	Updated setup.cfg and requirements.txt file to include comp.	8 months ago	on Nov 28, 2022					
🗋 toxini	preprint citation	3 years ago	+ 7 releases					
다 README - 한 BSD-3-	Clause license	1 ∷	Packages					
empanada	-napari		Contributors (S)					







Exercise: Create a conda environment for this course

Please follow the instructions in the link below to create a local environment:

https://biapol.github.io/BioImage-Analysis-and-Data-Processing-Workshop-2025/intro.html













Napari – OMERO integration

Demonstration: napari-OMERO plugin



Napari-OMERO usage



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

plugin menu

credentials

server with your TiM







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 Phython: https://biapol.github.io/omero-tools



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Napari-OMERO usage

💭 🥱 omero-web.biotec.tu-dresden.de/webclient/userdata/?expe

OMERO

browser

Add filter 🗸

Coming soon:

- ROI support

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🛞 📄 Python 🛅 Licensed images 🚞 Misc 🛅 campus 🛅 Github

Tags Shares

Adapting Robust Solution
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- Multiscale support: Browse larger-than-RAM samples (*3D limitation remains*)

Zoom:

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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:

→ 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.









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: <u>BBBC038v1</u>, 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.

Artificial intelligence

Machine learning









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





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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.

Stand-alone: Ilastik

www.ilastik.org

Deep learning 2015: UNet 2018: Stardist

Foundational models

2023: Segment anything (SAM) **2024**: SAM 2



Computational demand











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

Random Forest Classifiers

- Pixel Classifier
- napari-apoc plugin


In napari: annotation



In napari: annotation



use <1> for activate the label eraser, use <2> for activate the paint brush, use <3> for activate the fill bucket, use <4> for pick mode



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In napari: annotation

View Window Plugins Tools H





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



use <1> for activate the label eraser, use <3> for activate the fill bucket, use <4> for pick mode, use <5> for pan/zoom modhttps://imagej.nih.gov/ij/images/

と口(おご) #





In napari: Semantic segmentation (training)

Two options:

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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



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In napari: Semantic segmentation (training)







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Semantic segmentation: Choosing the right features

Why not just do this?

ø D	Semanti	c seam	nentation	(APOC)						
Select images (channels) used for training										
blobs										
Classifier file										
PixelClassifier.cl				Select file						
Training Application / Pr	ediction									
Select ground truth annota	tion									
Labels										
▼ Select features										
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- Not all features are equally relevant!
- Calculating the features takes time and computation resources!

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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/







Fit ellipse

For every object, find the optimal ellipse simplifying the object.

Major axis ... long diameter Minor axis ... short diameter

Major and minor axis are perpendicular to each other









napari-skimage-regionprops









Exercise: Object segmentation



- 1. Activate the environment and open napari mamba activate napari25 napari
- 2. Download and open the Blobs sample
- 3. Perform object segmentation on blobs sample dataset

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Select images (channel) used for training blobs Classifier file ObjectSegmenter.cl Training Application Select ground truth an Labels A Select features Tree depth, num. trees Estimated memory cor

Show classifier sta

ø D



Object segmentation (APOC)

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

https://biapol.github.io/BioImage-Analysis-and-Data-Processing-Workshop-2025/interactive_pixel_classification/readme.html









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 napari25
- 3. Run: napari
- 4. Open the following images in napari(located in the <u>data/multichannel</u> folder from the GitHub repository):
 - actin.tif
 - dna.tif
- cell_labels.tif
- dna_labels.tif

- ··· 🕨 🖉 - O 🗑 👌 🖩 🖷
- 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 <u>https://biapol.github.io/BioImage-Analysis-and-Data-Processing-Workshop-2025/multichannel_analysis/readme.html</u>

































































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

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

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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	
7	4	4	1	275	342	18./1205159	284	1/3	96.65818182	23	
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https://github.com/haesleinhuepf/napari-skimage-regionprops

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Outlook: Temporal Features Annotation and Classification



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










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



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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 postprocessing -> Object Classification (APOC)

ø Q		Obiect classification (APOC)				
Image		blobs		▼		
Labels		Result of Object	Segmenter.cl	▼		
Classifier file						
ObjectClassifier.cl				Select file		
Training Application / Prediction						
annotation		Object annotation	on	▼		
Tree depth, num. trees	- 2		— 100	+		
 minimum intensity mean intensity maximum intensity sum intensity standard deviation intensity standard deviation intensity pixel count shape (extension ratio) Show classifier statistics 						
	Tra	ain				

BioDI

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 napari25 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/BioImage-Analysis-and-Data-Processing-Workshop-2025/interactive_object_classification/readme.html



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







A smaller break





Object Classification and <u>Un</u>supervised Machine Learning

- Dimensionality Reduction
- Clustering
- Napari-clusters-plotter plugin



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

...

X

mazoc.bsky.social

Challenge: Find a representation (embedding) of your data that represents the data in fewer dimensions Preserve local distances at the expense of global distortions

	label	area	bbox_area	convex_area	quivalent_diamete	max_intensity	mean_intensity	min_intensity	solidity	extent	eret_diameter_ma	local_centroid-0	
1		3379	13949	5120	18.61786412639	613.0	345.6717963894	259.0	0.6599609375	0	37.3496987939662	15.77952056821	
2		2319	7448	3491	16.42230229224	421.0	297.8434670116	240.0	0	0	38.65229618017	4	
3		2304	14415	4281	16.38681751812	456.0	300.8298611111	245.0	0	0	34.19064199455	17.73828125	
4		3278	13804	5139	18.43048549951	467.0	316.1446003660	249.0	0	0	34.84250278036	15.52287980475	
5		1501	3315	1681	14.20563625190	458.0	302.147235176549	236.0	0	0	17.97220075561	6	
6	6	2341	6061	2714	16.47407088948	594.0	355.4446817599	261.0	0	0	30.67572330035	16.54250320375	
7		1725	3584	1940	14.87979081163	568.0	343.7866666666		0	0	17.72004514666	7.80463768115942	
8	8	1502	3840	1753	14.20879025650	431.0	290.0659121171	235.0	0	0	18.57417562100	8	(
9	9	1602	4080	1894	14.51737058294	475.0	297.8008739076	241.0	0	0	18.70828693386	8	
10		1395	3600	1624	13.86304166283	424.0	304.8494623655	247.0	0	0.3875	17.60681686165		
11		609	1100	697	10.51654029260	323.0	274.2528735632	241.0	0	0	13.45362404707		
12		1686	3757	1894	14.76679738567	460.0	303.8303677342	240.0	0	0	17.97220075561	9	
13			5184		16.03062694504	576.0	339.990264255911	270.0	0	0	19.54482028569	8	
14		863	2340	1032	11.81237949737		272.4449594438	237.0	0	0	16.0312195418814	6	

Many dimensions



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

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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









Data Exploration

... using interactive feature visualization...





Laura Žigutytė

@zigutyte





Ryan Savill Johannes @RyanSavill4 Soltwedel

Marcelo Zoccoler

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







Dimensionality Reduction and Clustering

... as well as interactive dimensionality reduction and clustering...





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



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Marcelo

Zoccoler



Data Exploration

... and introspection!



"How are clusters in my data influenced by individual features?"

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



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Marcelo

Zoccoler



Data Exploration

Works for all sorts of layer types!





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









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Johannes Soltwedel

Marcelo Zoccoler





Scientific Plotting with Seaborn

- seaborn



Seaborn gallery



https://seaborn.pydata.org/examples/index.html







Exercise: unsupervised object classification

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



https://biapol.github.io/BioImage-Analysis-and-Data-Processing-Workshop-2025/interactive_unsupervised_object_classification/readme.html







Exercise: scientific plotting with seaborn

- 1. Activate the environment mamba activate napari25
- 2. Navigate to your local repository cd BioImage-Analysis-and-Data-Processing-Workshop-2025
- 3. Start Jupyter Lab jupyter lab
- 4. Read and run the notebook, which can be found in docs > seaborn > seaborn_demo.ipynb

visualizations.

Let's load all the packages we need here:

import seaborn as sns from pathlib import Path import pandas as pd

Loading the data

As a first step, we need to load the measurements from napari. Alternatively, you can download some from the course repository <u>here</u>. For this, we need to compile a list of all .csv files we take into account.

root = './measurements'

file_paths = [file_path for file_path in Path(root).iterdir() if file_path.suffix == '.csv']
file_paths

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[WindowsPath('measurements/17P1_POS0006_D_1UL_features.csv'), WindowsPath('measurements/17P1_POS0007_D_1UL_features.csv'), WindowsPath('measurements/17P1_POS0011_D_1UL_features.csv'), WindowsPath('measurements/20P1_POS0005_D_1UL_features.csv'), WindowsPath('measurements/20P1_POS0007_D_1UL_features.csv'), WindowsPath('measurements/20P1_POS0008_D_1UL_features.csv'), WindowsPath('measurements/20P1_POS0008_D_1UL_features.csv'), WindowsPath('measurements/20P1_POS0010_D_1UL_features.csv'), WindowsPath('measurements/20P1_POS0010_D_1UL_features.csv'), WindowsPath('measurements/A9_p5d_features.csv'), WindowsPath('measurements/A9_p7d_features.csv'),







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/BioImageAnalysisNotebooks/intro.html

Image Science Community Forum: https://forum.image.sc/







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https://physics-of-life.tu-dresden.de/bia

Thank you!