

Python: File handling & Image Visualization

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SACHSEN



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

Working with files in folders

Key-skill when it comes to automating data analysis tasks

```
[2]: # define the location of the folder to go through
     directory = 'data_banana/'

     # get a list of files in that folder
     file_list = os.listdir(directory)

     file_list
```

It's just a
Python list!

```
[2]: ['banana0002.tif',
      'banana0003.tif',
      'banana0004.tif',
      'banana0005.tif',
      'banana0006.tif',
      'banana0007.tif',

      'banana0026.tif',
      'image_source.txt']
```

There are not
just images
inside

Filtering file lists

To focus on image data, we need to filter the file list

```
[3]: image_file_list = [file for file in file_list if file.endswith(".tif")]
```

```
image_file_list
```

```
[3]: ['banana0002.tif',  
      'banana0003.tif',  
      'banana0004.tif',  
      'banana0005.tif',  
      'banana0006.tif',  
      'banana0007.tif',  
      'banana0026.tif']
```

A for-loop in
a single line

Filtering file lists

To focus on image data, we need to filter the file list

```
[4]: # go through all files in the folder
     for file in file_list:
         # if the filename is of a tif-image, print it out
         if file.endswith(".tif"):
             print(file)
```

banana0002.tif

banana0003.tif

banana0004.tif

banana0005.tif

banana0006.tif

banana0007.tif

banana0026.tif

Filtering file lists

To focus on image data, we need to filter the file list

```
[5]: # go through all files in the folder
for file in file_list:
    # if the filename is of a tif-image, print it out
    if file.endswith(".tif"):
        print(file)

    # store the image
    image = imread(directory + file)

    # show the image
    stackview.imshow(image)
```



Side note: comments

Your code will become longer... Consider structuring it and putting comments and empty lines in between.



```
for file in file_list:
    if file.endswith(".tif"):
        print(file)
        image = imread(directory + file)
        stackview.imshow(image)
```



```
# go through all files in the folder
for file in file_list:
    # if the filename is of a tif-image, print it out
    if file.endswith(".tif"):
        print(file)

    # store the image
    image = imread(directory + file)

    # show the image
    stackview.imshow(image)
```

Image visualization

In Python there are many ways to visualize image data.
We focus on Jupyter Notebook compatible ways for now.

Before visualizing image data, get an idea about the dataset.

```
[2]: image = imread("../day2.1_image_segmentation/data/BMP4blastocystC3-cropped_resampled_8bit.tif")  
image.shape
```

```
[2]: (86, 396, 393)
```

Image.shape
tells you the
dimensions of
an image

Not all image
viewers
support 3D
data

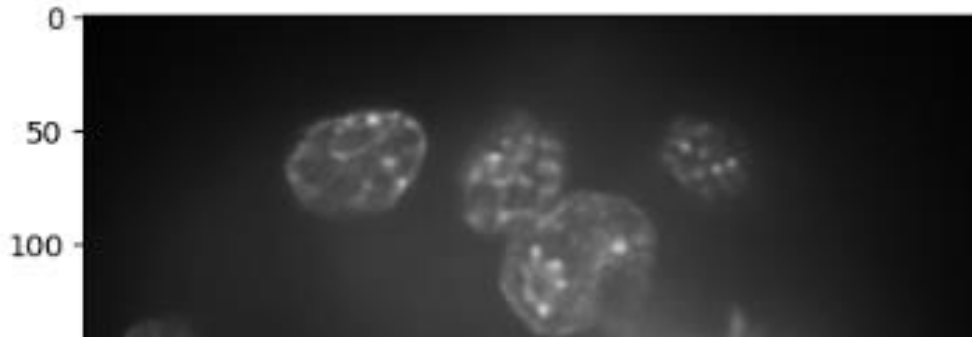
Image visualization

scikit-image's `imshow()` (originating from matlab) can only visualize 2 dimensions.

```
from skimage.io import imread, imshow
```

```
[4]: center_slice = int(image.shape[0] / 2)
      imshow(image[center_slice])
```

```
[4]: <matplotlib.image.AxesImage at 0x2c9b9164820>
```



The first shape-dimension - 2 is the center plane in Z

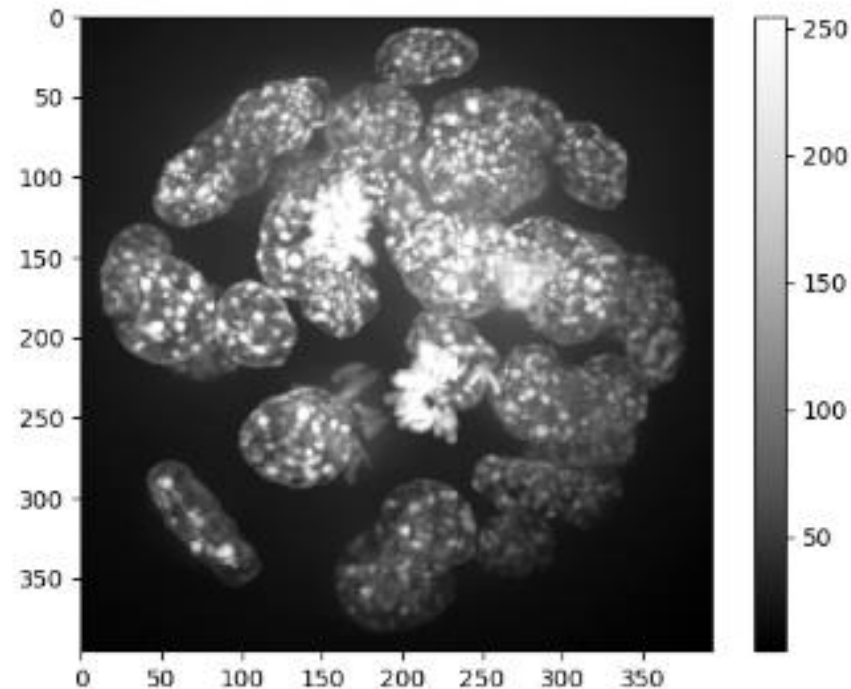
Image data in Python is commonly organized in Z-Y-X dimension order.

Image visualization

Stackview can show 3D images (by applying a projection along Z)

```
import stackview
```

```
stackview.insight(image)
```



shape (86, 396, 393)

dimensions

dtype uint8

size 12.8 MB

min 0

max 255

Basic statistics

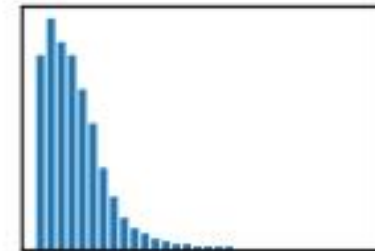


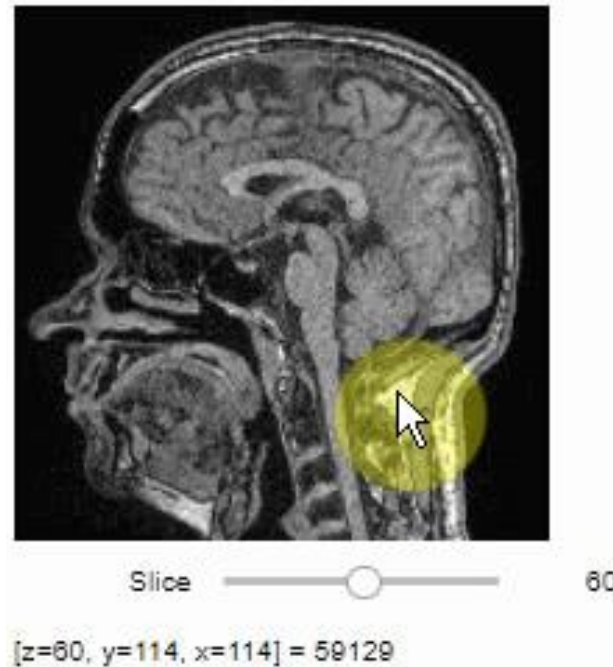
Image visualization

Stackview also has some interactive tools

```
stackview.slice(image)
```



```
stackview.picker(image)
```



```
binary_image = image > 80
```

```
stackview.curtain(image, binary_image)
```

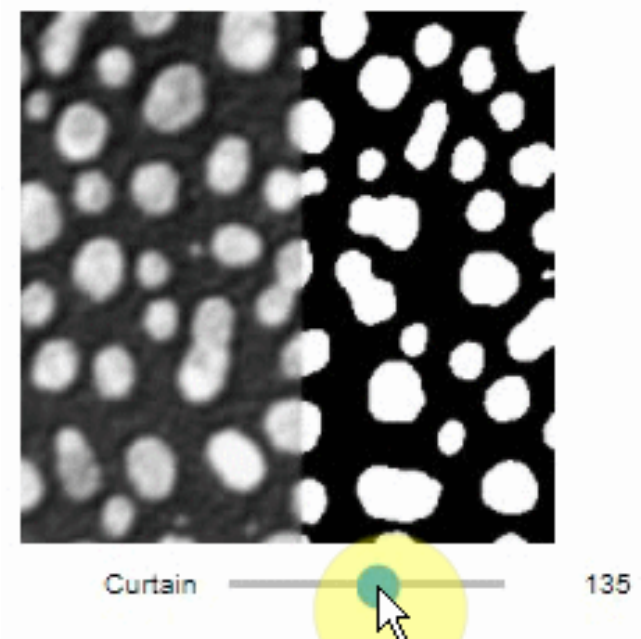


Image visualization

Stackview also has some interactive tools

```
stackview.switch(  
    {  
        "Lysosomes": lysosomes_channel,  
        "Mitochondria": mitochondria_channel,  
        "Nuclei": nuclei_channel  
    },  
    colormap=[  
        "pure_magenta",  
        "pure_green",  
        "pure_blue"  
    ],  
    toggleable=True  
)
```

Identical but less readable:

```
stackview.switch({"Lysosomes":lysosomes_channel,  
"Mitochondria":mitochondria_channel,"Nuclei":nuclei_channel},  
colormap=["pure_magenta", "pure_green", "pure_blue"], toggleable=True)
```

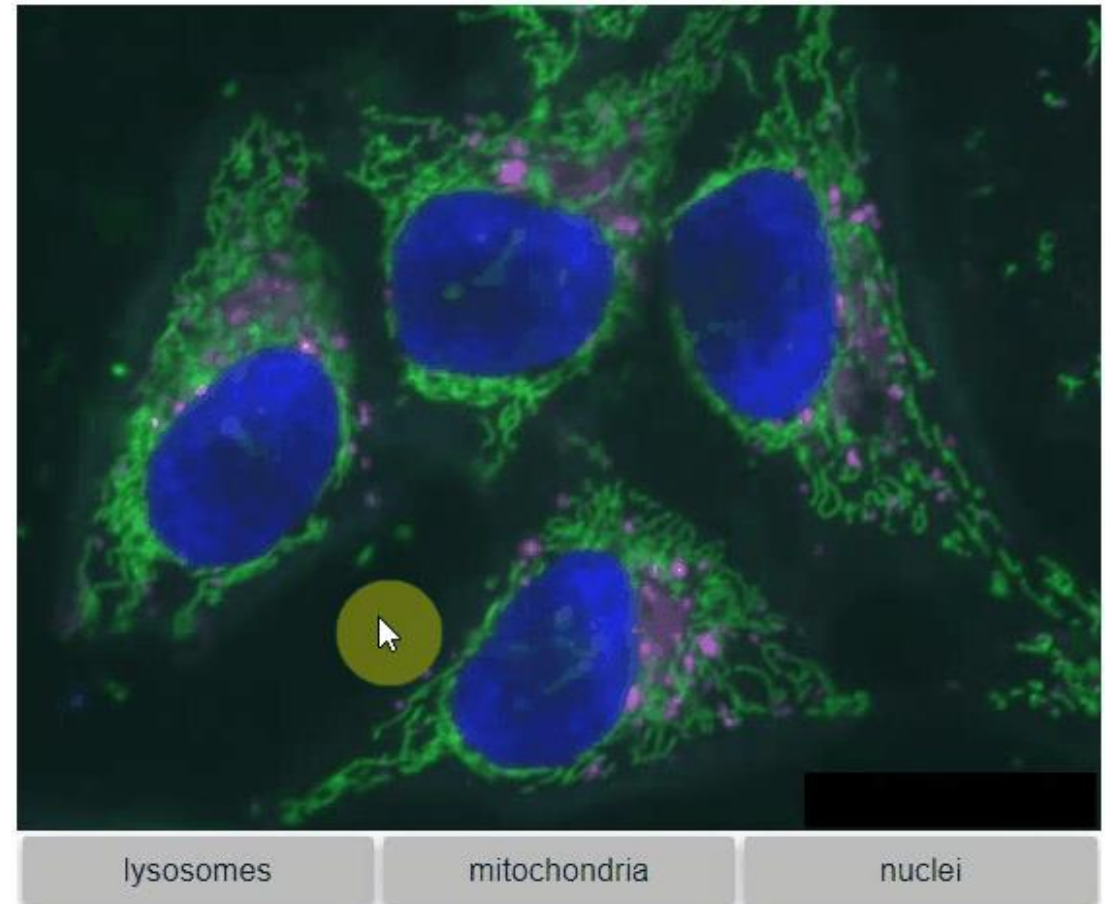
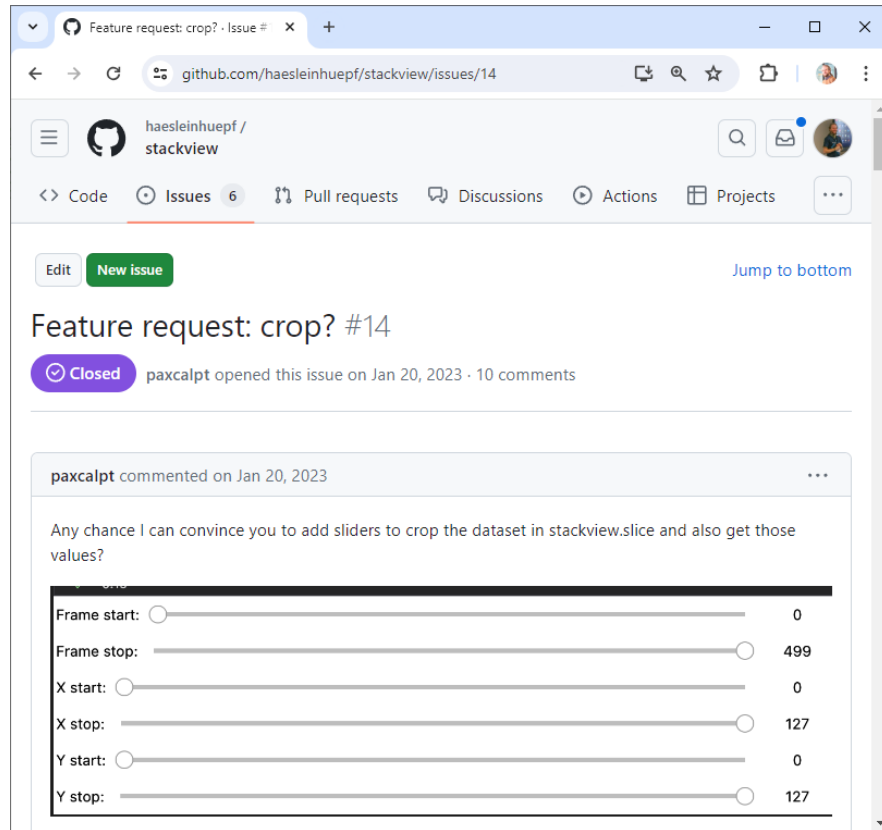
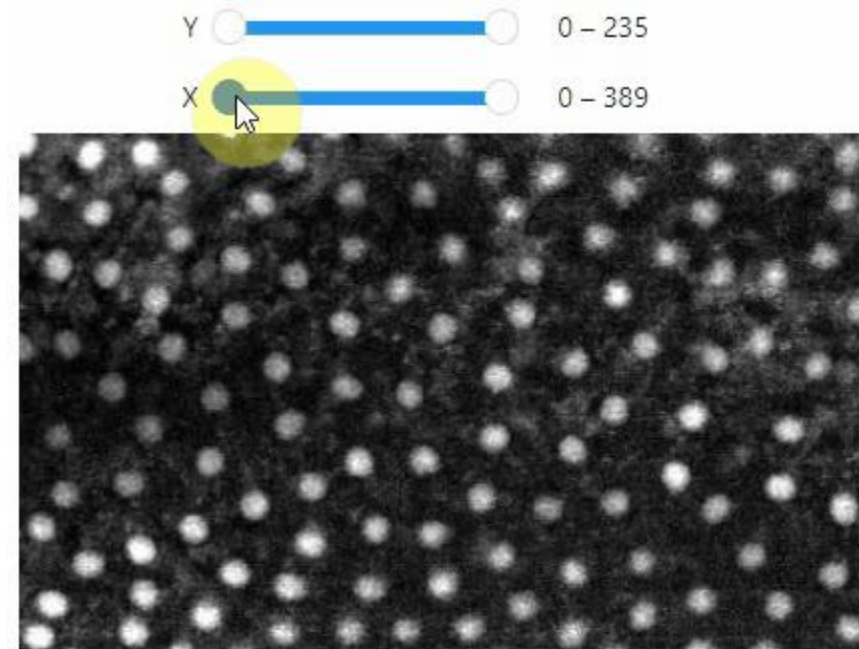


Image Visualization

Stackview is developed for microscopists. If something doesn't work as you expect, communicate it :-)



```
crop_widget = stackview.crop(image_stack, continuous_update=True)
crop_widget
```



Loading domain-specific file formats

Common Python libraries cannot open file formats such as OME-TIF, CZI, LIF, ND2... Solution: aicsimageio

From the Allen Institute for Cell Science

```
from aicsimageio import AICSImage
```

```
aics_image = AICSImage("data/EM_C_6_c0.ome.tif")  
aics_image
```

```
<AICSImage [Reader: OmeTiffReader, Image-is-in-Memory: False]>
```

Loading domain-specific file formats

Aicsimageio supports dimension names and physical voxel sizes.

```
aics_image.shape
```

What's width, height,
depth and time?

```
(1, 1, 256, 256, 256)
```

```
aics_image.dims
```

```
<Dimensions [T: 1, C: 1, Z: 256, Y: 256, X: 256]>
```

Key feature when
working with big
data!

Lazy loading (virtual stacks)

Allows processing files larger
than computer memory.

```
np_image = aics_image.get_image_data("ZYX", T=0)  
np_image.shape
```

```
(256, 256, 256)
```

Loading domain-specific file formats

Aicsimageio supports dimension names and physical voxel sizes.

```
aics_image.physical_pixel_sizes
```

```
PhysicalPixelSizes(Z=0.16784672897196262, Y=0.16776018346253663, X=0.16776018346253663)
```

```
def get_voxel_size_from_aics_image(aics_image):  
    return (aics_image.physical_pixel_sizes.Z,  
            aics_image.physical_pixel_sizes.Y,  
            aics_image.physical_pixel_sizes.X)
```

Such a helper-function may be different from project to project

```
get_voxel_size_from_aics_image(aics_image)
```

```
(0.16784672897196262, 0.16776018346253663, 0.16776018346253663)
```

Loading domain-specific file formats

Common Python libraries cannot open file formats such as OME-TIF, CZI, LIF, ND2... Solution: aicsimageio

In case your file format is not supported, it gives hints what to install.

```
dzi_image = AICSImage("data/PupalWing.czi")
dzi_image.shape
```

```
dzi_image = AICSImage("data/PupalWing.czi")
dzi_image.shape
```

```
(1, 1, 80, 520, 692)
```

```
np_czi_image = dzi_image.get_image_data("ZYX", T=0)
np_czi_image.shape
```

```
(80, 520, 692)
```

```
get_voxel_size_from_aics_image(dzi_image)
```

```
(1.0, 0.20476190476190476, 0.20476190476190476)
```

```
Attempted file (C:/structure/code/BIDS-training-2024/day1.2_file_handling/data/PupalWing.czi) load with reader: aicsimageio.readers.czi_reader.CziReader failed with error: aicspylibczi is required for this reader. Install with `pip install 'aicspylibczi>=3.1.1' 'fsspec>=2022.7.1'`
```

```
Attempted file (C:/structure/code/BIDS-training-2024/day1.2_file_handling/data/PupalWing.czi) load with reader: aicsimageio.readers.bioformats_reader.BioformatsReader failed with error: bioformats_jar is required for this reader. Install with `pip install bioformats_jar` or `conda install bioformats_jar`
```


Working with files in the cloud

Example nextcloud / owncloud

Install another Python library
into your environment

```
[2]: import ipywidgets as widgets
import nextcloud_client
```

```
conda activate devbio-napari-env
pip install pyncclient
```

Login-form

```
[3]: server_widget = widgets.Text(value='https://speicherwolke.uni-leipzig.de', description='Server')
username_widget = widgets.Text(description='Username:')
password_widget = widgets.Password(description='Password')

widgets.VBox([server_widget, username_widget, password_widget])
```

```
[3]:
```

Server	<input type="text" value="https://speicherwolke.uni-leipzig.d"/>
Username:	<input type="text"/>
Password	<input type="password"/>

Actually logging in

```
[6]: ncc = nextcloud_client.Client(server_widget.value)
ncc.login(username_widget.value, password_widget.value)
```

Working with files in the cloud

Listing files in a remote folder

```
[7]: # enter a folder on the owncloud cloud
remote_folder = "/data/"

for f in ncc.list(remote_folder):
    print (f.path)

/data/blobs.tif
```

Downloading a file

```
[8]: # enter the source file here
remote_source_file = '/data/blobs.tif'
# enter the destination
local_file = 'blobs.tif'

ncc.get_file(remote_path=remote_source_file,
            local_file=local_file)

[8]: True
```

Uploading a file

```
[13]: ncc.put_file(remote_folder, local_file_to_upload)

[13]: True
```

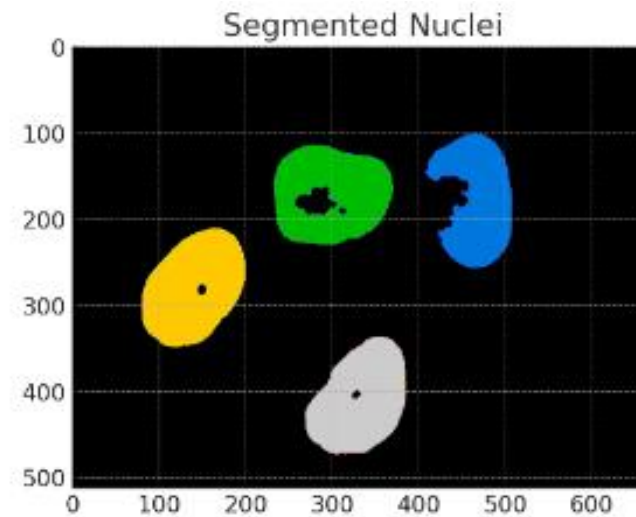
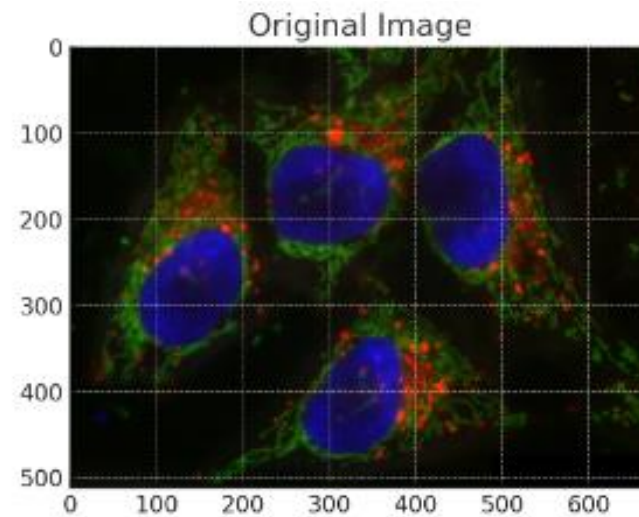
Listing files again

```
[14]: for f in ncc.list(remote_folder):
        print (f.path)

/data/blobs.tif
/data/blobs_labels.tif
```

Bio-image Analysis

My job ...

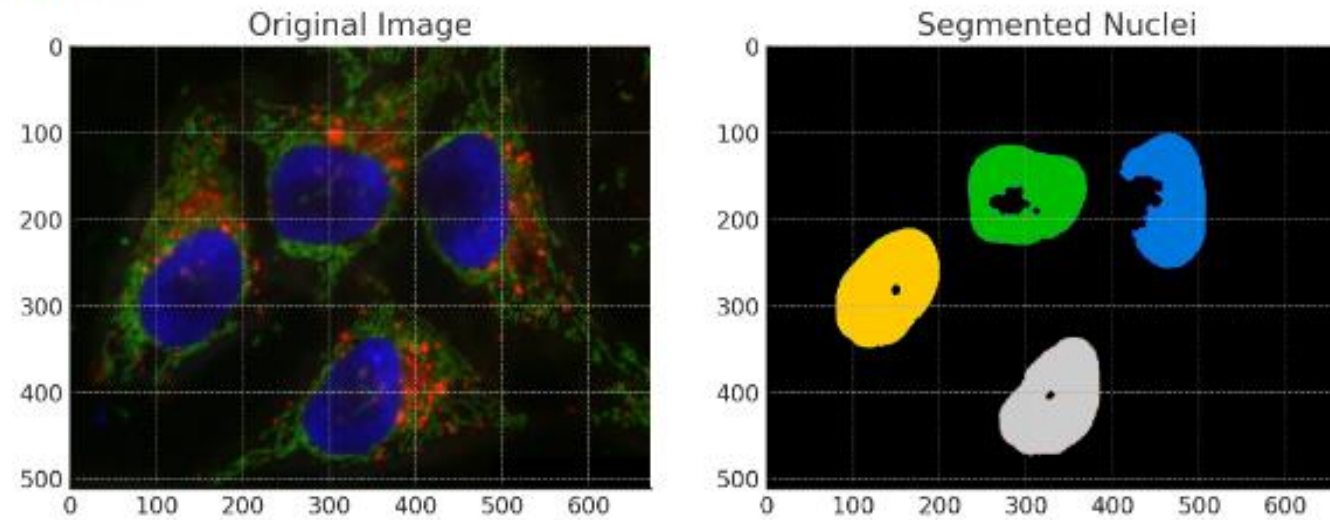


Bio-image Analysis using Large-Language Models

My job is changing, since we have ChatGPT

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

ChatGPT



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



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

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

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

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

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

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

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

Coding assistance: BiA-Bob

- Open-source alternative to OpenAI's Data Analyst
- No need to upload your [image] data
- Extensible through plugins

```
[1]: from bia_bob import bob  
     bob.__version__
```

```
[1]: '0.3.0'
```

```
[ ]:
```



With Christian
Tischer (EMBL
Heidelberg)
@tischitischer

Coding assistance: BiA-Bob

Use the `%bob ...` syntax to ask for small things.

In [2]:

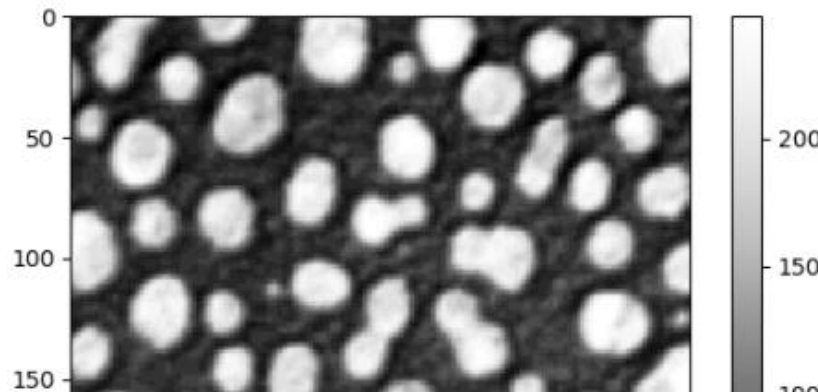
```
%bob Load the blobs.tif image file and show it.
```

In [3]:

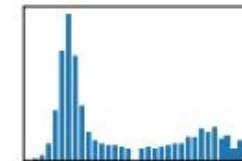
```
from skimage.io import imread
import stackview

image = imread('blobs.tif')
stackview.insight(image)
```

Out[3]:



shape	(254, 256)
dtype	uint8
size	63.5 kB
min	8
max	248



Coding assistance: BiA-Bob

- Bob adds a new cell to your code. If you ask it 3 times, it will make 3 cells.
- Clean up from time to time!

Click on
the bar
here and
enter dd

```
[3]: %bob load blobs.tif and show it
```

We need to load an image named `blobs.tif` from the disk and display it.

```
[ ]: from skimage.io import imread
import stackview

# Load the image from disk
image = imread('blobs.tif')

# Display the image
stackview.insight(image)
```

```
[ ]: from skimage.io import imread
import stackview

# Load the image file
image = imread('blobs.tif')

# Display the image
stackview.insight(image)
```

```
[ ]: from skimage.io import imread
import stackview

# Load image from disk
image_blobs = imread('blobs.tif')

# Display the image
stackview.insight(image_blobs)
```

Coding assistance: BiA-Bob

Use the `%%bob ...` syntax to ask for complex analysis tasks.

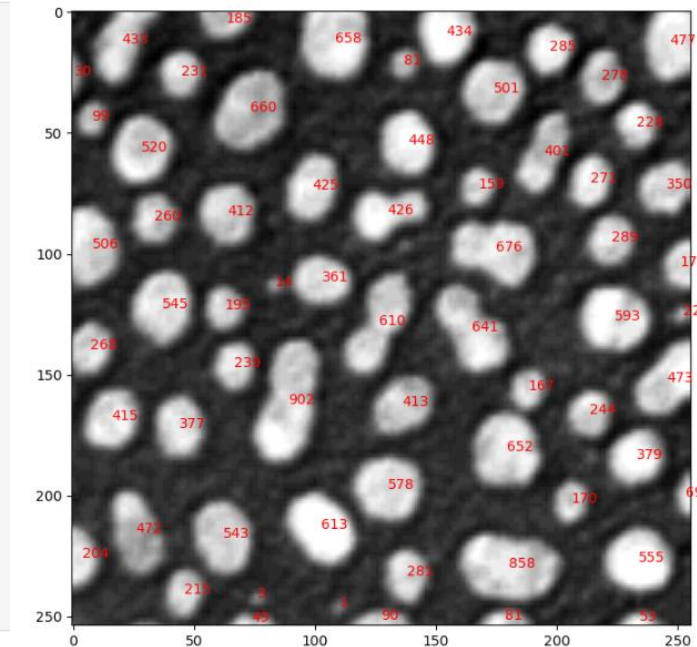
In [4]:

```
%%bob
```

```
Load the blobs.tif grayscale image from disc.  
Segment the nuclei-like objects in it using  
Otsu's method and connected component labeling.  
Measure area of the segmented labels.  
Print the area on top of the labels.
```

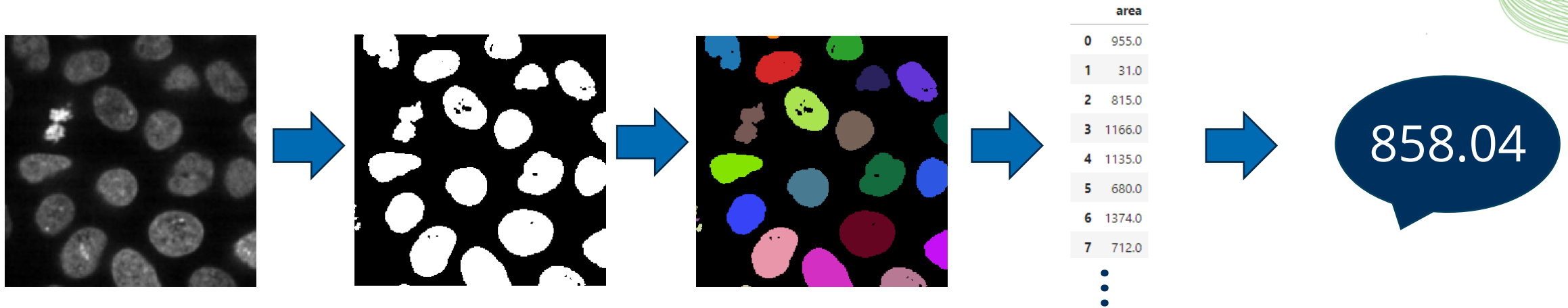
In [5]:

```
from skimage.io import imread  
from skimage.filters import threshold_otsu  
from skimage.measure import label, regionprops  
import matplotlib.pyplot as plt  
import stackview  
  
# Load the image  
image = imread('blobs.tif')  
  
# Apply Otsu's thresholding  
threshold = threshold_otsu(image)  
binary_image = image > threshold  
  
# Perform connected component labeling  
labels = label(binary_image)  
  
# Measure area of the segmented labels  
props = regionprops(labels)  
areas = [prop.area for prop in props]  
  
# Create an image with labels and area values on top  
fig, ax = plt.subplots(figsize=(8, 8))  
ax.imshow(image, cmap='gray')  
  
for prop in props:  
    y, x = prop.centroid  
    area = prop.area  
    ax.text(x, y, f'{area}', color='red')  
  
stackview.curtain(image, labels)
```



Benchmarking LLMs for Bio-image Analysis

Use case: segment the image and measure the average area of objects.



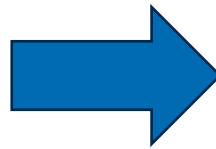
Unit-test pass-rate (n=10):

	reference	gpt-4-turbo-2024-04-09	Claude-3-opus-20240229	gpt-4-1106-preview	gpt-3.5-turbo-1106	gemini-pro	codellama
workflow_segmentation_measurement_summary	1.0	0.9	1.0	0.8	0.5	0.5	0.1

Benchmarking LLMs for Bio-image Analysis

Use-case: correlation matrix

	a	b	c	d	e
0	1.600000	0.100000	1.600000	1.700000	1.700000
1	2.300000	0.200000	2.300000	2.400000	2.400000
2	2.600000	0.300000	2.600000	2.400000	2.400000
3	3.700000	0.300000	3.700000	3.600000	3.600000
4	3.400000	0.400000	3.400000	3.500000	3.500000
5	3.900000	0.400000	3.900000	3.900000	3.900000
6	4.300000	0.400000	4.300000	4.400000	4.400000
7	4.300000	0.500000	4.300000	4.200000	4.200000
8	4.000000	0.500000	4.000000	4.100000	4.100000
9	5.100000	0.500000	5.100000	5.000000	5.000000
10	5.200000	0.600000	5.200000	5.100000	5.100000
11	5.300000	0.600000	5.300000	5.400000	5.400000
12	5.500000	0.600000	5.400000	5.600000	5.600000








	a	b	c	d	e
a	1.000000	0.949504	0.999775	0.995800	0.995800
b	0.949504	1.000000	0.949594	0.946039	0.946039
c	0.999775	0.949594	1.000000	0.995001	0.995001
d	0.995800	0.946039	0.995001	1.000000	1.000000
e	0.995800	0.946039	0.995001	1.000000	1.000000

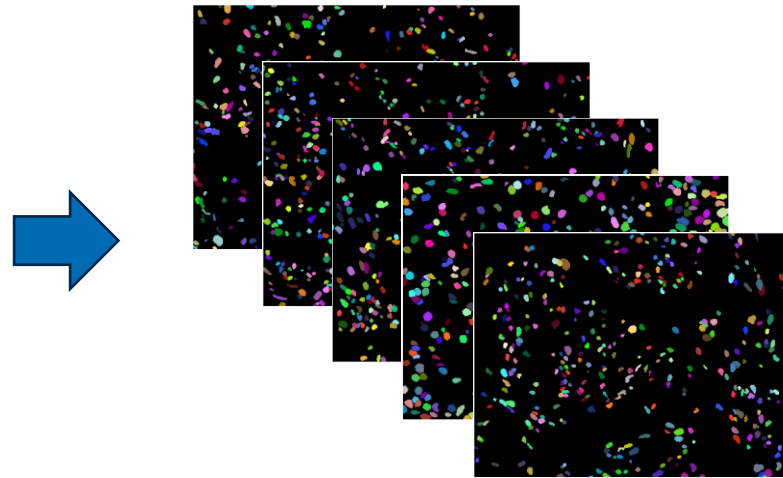
Unit-test pass-rate (n=10):

	reference	gpt-4-turbo-2024-04-09	Claude-3-opus-20240229	gpt-4-1106-preview	gpt-3.5-turbo-1106	gemini-pro	codellama
pair_wise_correlation_matrix	1.0	1.0	1.0	0.9	1.0	0.5	0.1

Benchmarking LLMs for Bio-image Analysis

Use case: Count segmented objects in a folder of segmentation results.

 Ganglioneuroblastoma_0.tif
 Ganglioneuroblastoma_1.tif
 Ganglioneuroblastoma_2.tif
 Ganglioneuroblastoma_3.tif
 Ganglioneuroblastoma_4.tif



300
398
368
378
363

Unit-test pass-rate (n=10):

	reference	gpt-4-turbo-2024-04-09	Claude-3-opus-20240229	gpt-4-1106-preview	gpt-3.5-turbo-1106	gemini-pro	codellama
workflow_batch_process_folder_count_labels	1.0	0.1	0.0	0.3	0.0	0.0	0.0

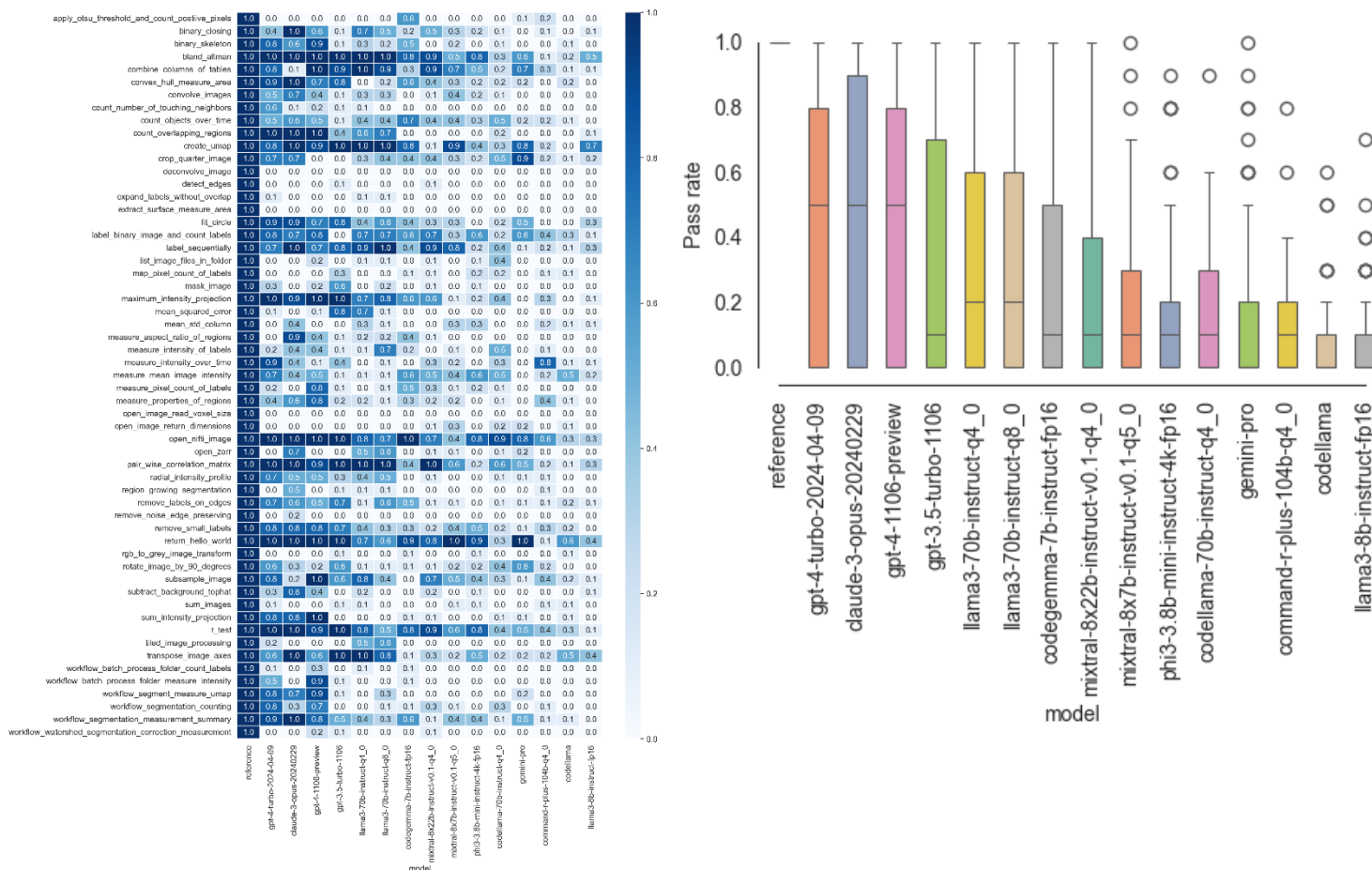
Coding assistance: BiA-Bob

Keep your feed on the ground with *Bob*. Bob can do crazy things, but you are responsible for what it does with your data.

Do not enter personal / private information. What you enter will be sent to the server of an american company.

Benchmarking LLMs for Bio-image Analysis

Summary: 57 use-cases (yet), 15 LLMs (yet), n=10



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Benchmarking Large Language Models for Bio-Image Analysis

Code Generation

Robert Haase, Christian Tischer, Jean-Karim Hériché, Nico Scherf

doi: <https://doi.org/10.1101/2024.04.19.590278>

This article is a preprint and has not been certified by peer review [what does this mean?].

Abstract Full Text Info/History Metrics Preview PDF

Exercises

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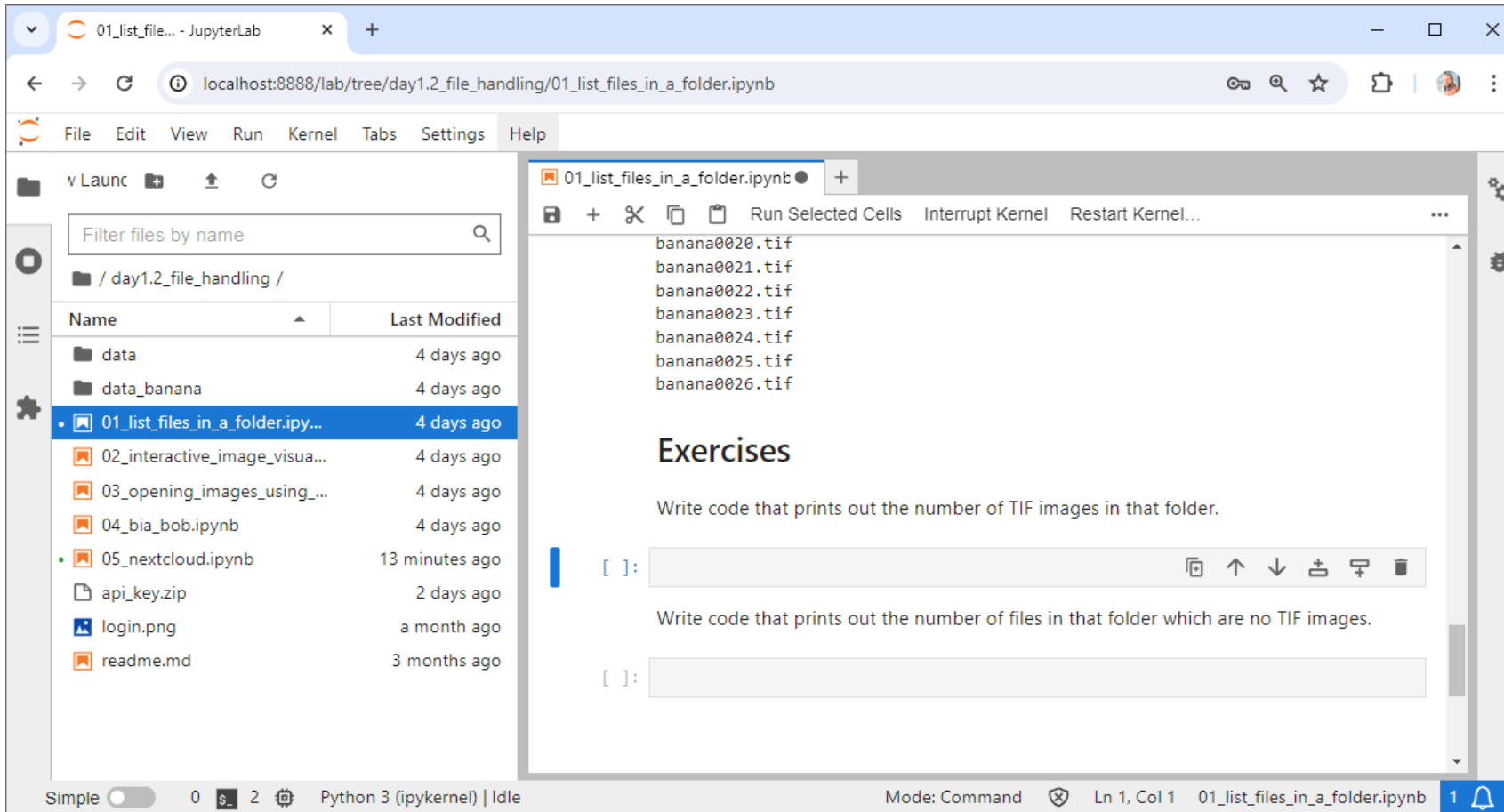
SACHSEN



Diese Maßnahme wird gefördert durch die Bundesregierung
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der Grundlage des von den Abgeordneten des Sächsischen
Landtags beschlossenen Haushaltes.

Exercise: File lists and folder

Apply your knowledge about Python lists to list of files.



The screenshot shows a JupyterLab environment. On the left is a file browser pane for the directory `/ day1.2_file_handling /`. It contains a table of files and folders:

Name	Last Modified
data	4 days ago
data_banana	4 days ago
01_list_files_in_a_folder.ipynb	4 days ago
02_interactive_image_visua...	4 days ago
03_opening_images_using_...	4 days ago
04_bia_bob.ipynb	4 days ago
05_nextcloud.ipynb	13 minutes ago
api_key.zip	2 days ago
login.png	a month ago
readme.md	3 months ago

The main area shows the code editor for `01_list_files_in_a_folder.ipynb`. It contains a list of files:

```
banana0020.tif  
banana0021.tif  
banana0022.tif  
banana0023.tif  
banana0024.tif  
banana0025.tif  
banana0026.tif
```

Below the list, under the heading "Exercises", are two tasks:

- Write code that prints out the number of TIF images in that folder.
- Write code that prints out the number of files in that folder which are no TIF images.

The bottom status bar indicates the environment is Python 3 (ipykernel) and the current cell is at Line 1, Column 1.

Exercise: Loading and visualizing image files

02_interacti... - JupyterLab

localhost:8888/lab/tree/day1.2_file_handling/02_interactive_image_visualizati...

File Edit View Run Kernel Tabs Settings Help

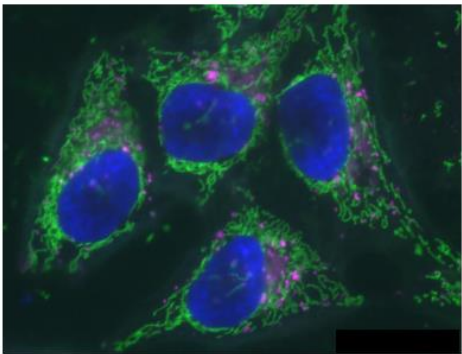
v Launc

Filter files by name

/ day1.2_file_handling /

Name	Last Modified
data	9 minutes ago
data_banana	4 days ago
01_list_files_i...	an hour ago
02_interactiv...	24 minutes ago
03_opening...	2 minutes ago
04_nextclou...	an hour ago
05_bia-bob.i...	2 minutes ago
api_key.zip	2 days ago
login.png	a month ago
readme.md	3 months ago

[14]:



Lysosomes Mitochondria Nuclei

Exercise 1

Modify the code above to visualize the HeLa nuclei in red and the mitochondria in cyan.

Exercise 2

Use the picker tool to get an idea about the intensity in the nuclei.

[]:

Simple 0 \$ 6 Python 3 (ipykernel...) Mode: Com... Ln 1, C... 02_interactive_image_visualizati... 1

03_opening_i... (2) - JupyterLab

localhost:8888/lab/tree/day1.2_file_handling/03_opening_images_using_aicsi...

File Edit View Run Kernel Tabs Settings Help

v Launc

Filter files by name

/ day1.2_file_handling /

Name	Last Modified
data	12 minutes ago
data_banana	4 days ago
01_list_files_i...	an hour ago
02_interactiv...	a minute ago
03_opening...	5 minutes ago
04_nextclou...	an hour ago
05_bia-bob.i...	4 minutes ago
api_key.zip	2 days ago
login.png	a month ago
readme.md	3 months ago

In case additionally the [aicspylibczi](#) library is installed one can also open CZI files using AICSImageIO (example dataset kindly provided by kindly provided by Romina Piscitello-Gómez, MPI CBG).

```
[12]: czi_image = AICSImage("data/Pupalwing.czi")
      czi_image.shape
[12]: (1, 1, 80, 520, 692)
[13]: np_czi_image = czi_image.get_image_data("ZYX", T=
      np_czi_image.shape
[13]: (80, 520, 692)
[14]: get_voxel_size_from_aics_image(czi_image)
[14]: (1.0, 0.20476190476190476, 0.20476190476190476)
```

Exercise

Open a file from a recent project. Try out CZI, LIF, ND2 and others. Check the [aicsimageio documentation](#) for additional installation instructions.

[]:

Simple 0 \$ 6 Python 3 (ipykernel...) Mode: Com... Ln 1, C... 03_opening_images_using_aicsima... 1

Optional exercise: BiA-Bob

localhost:8888/lab/tree/Untitled1.ipynb

Open previous tabs? You can restore your previous session from the Firefox application menu ≡, under History. [Show me how](#)

File Edit View Run Kernel Tabs Settings Help

Filter files by name

Name	Last Modified
Desktop	7 days ago
Documents	6 days ago
Downloads	5 minutes ago
Music	6 days ago
Pictures	6 days ago
Public	6 days ago
Templates	6 days ago
Videos	6 days ago
blobs.tif	5 minutes ago
ObjectSe...	6 days ago
test	2 hours ago
test.ipynb	6 days ago
Untitled.i...	6 days ago
Untitled1....	53 seconds ago

```
[1]: import os
os.environ['OPENAI_API_KEY']='sk-

[5]: from bia_bob import bob

[6]: %bob load blobs.tif and show it
```

shape (254, 256)
dtype uint8
size 63.5 kB
min 8
max 248

0 50 100 150 200

200 150 100 50 0