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Image Analysis with OMERO

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Bio-Image Analysis Technology Development – Physics of Life – TU Dresden

From Paper to Pixels - Navigation Through Your Research Data Symposium

@zoccolermarcelo

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General Image Analysis Workflow

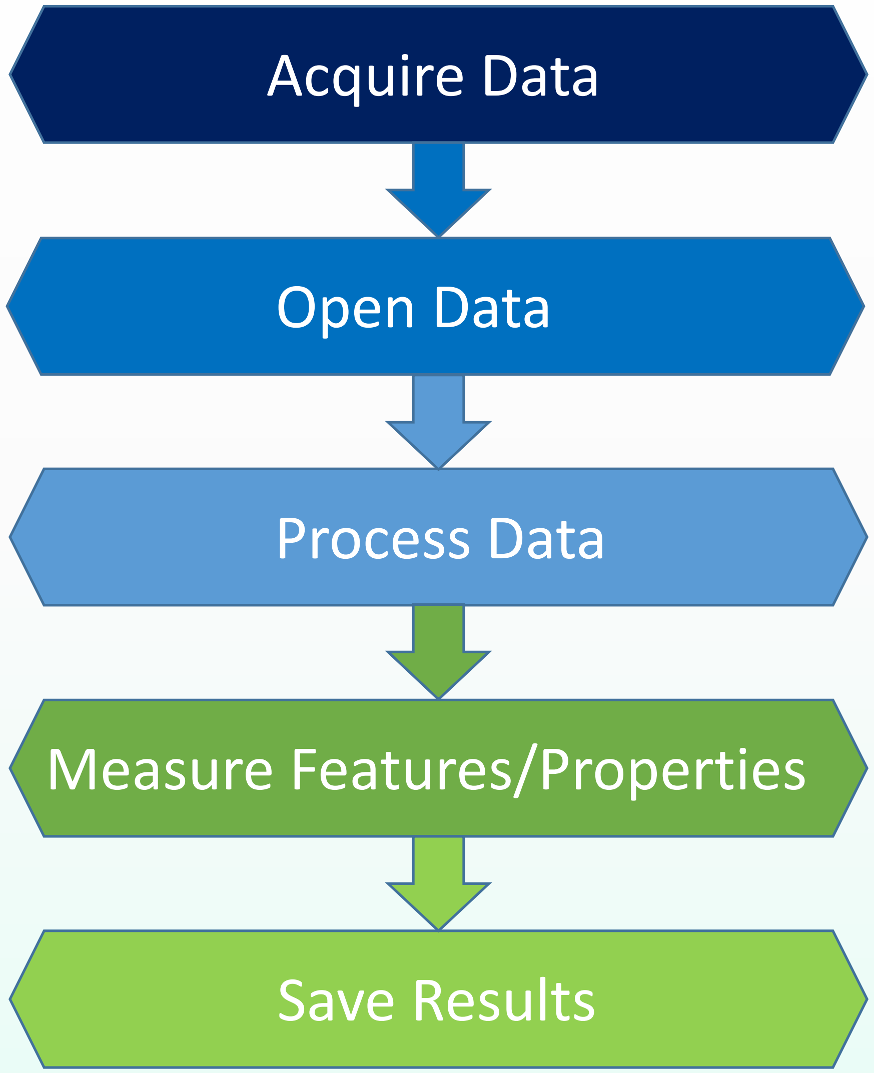
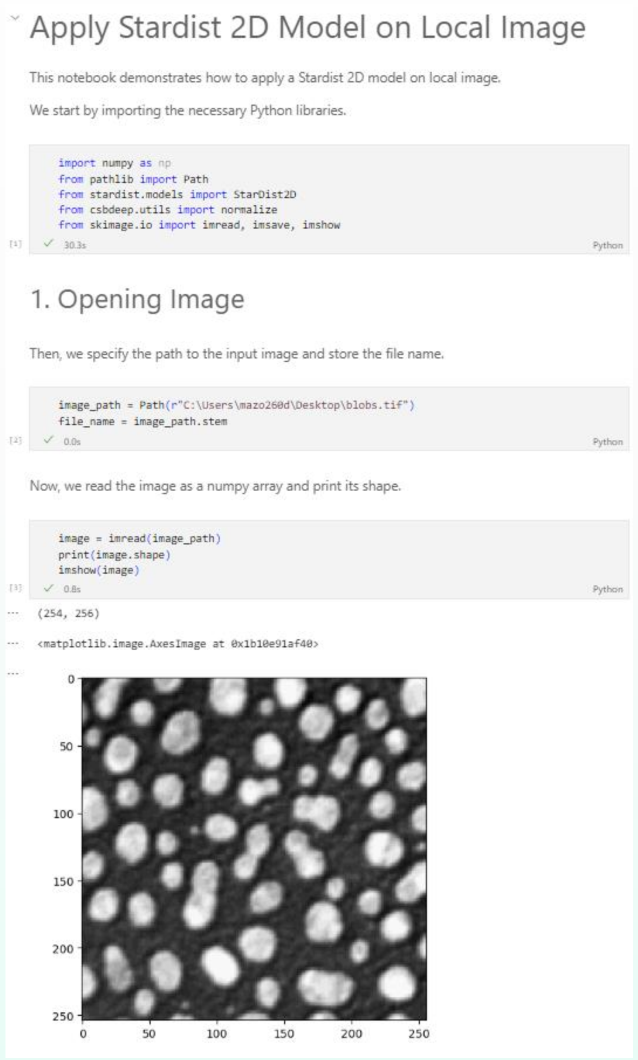


Image Analysis Workflow in Python

Example: Counting nuclei after segmentation with Stardist



Apply Stardist 2D Model on Local Image

This notebook demonstrates how to apply a Stardist 2D model on local image.

We start by importing the necessary Python libraries.

```
[1]
import numpy as np
from pathlib import Path
from stardist.models import StarDist2D
from csbdeep.utils import normalize
from skimage.io import imread, imsave, imshow
✓ 30.3s                                                Python
```

1. Opening Image

Then, we specify the path to the input image and store the file name.

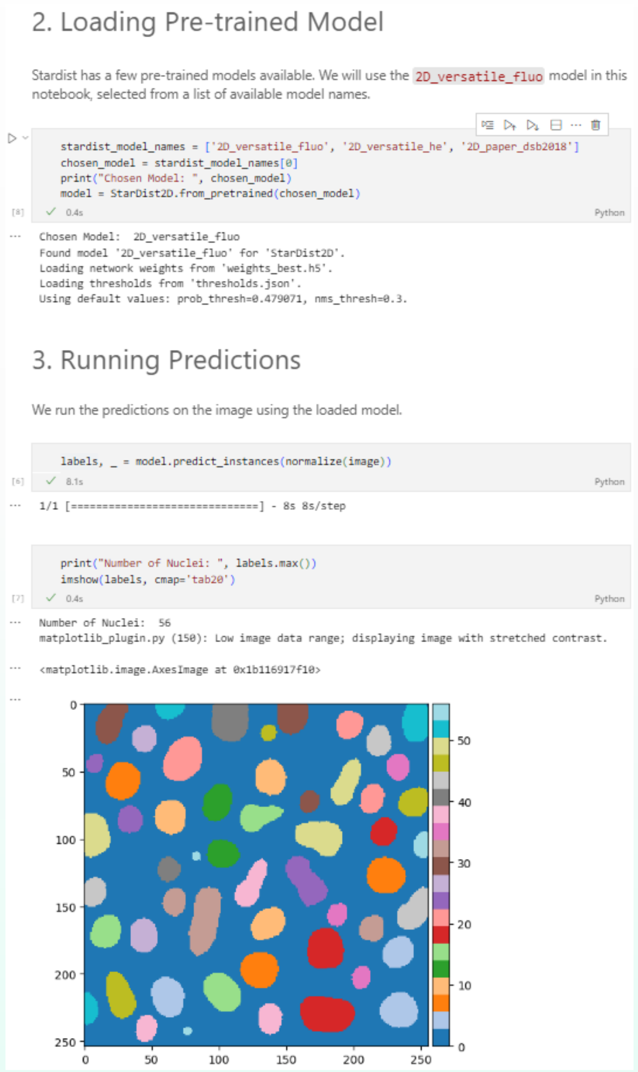
```
[2]
image_path = Path(r"C:\Users\mazo260d\Desktop\blobs.tif")
file_name = image_path.stem
✓ 0.0s                                                 Python
```

Now, we read the image as a numpy array and print its shape.

```
[3]
image = imread(image_path)
print(image.shape)
imshow(image)
✓ 0.8s                                                 Python
```

```
(254, 256)

<matplotlib.image.AxesImage at 0x1b10e91af40>
```



2. Loading Pre-trained Model

Stardist has a few pre-trained models available. We will use the `2D_versatile_fluo` model in this notebook, selected from a list of available model names.

```
[8]
stardist_model_names = ['2D_versatile_fluo', '2D_versatile_he', '2D_paper_dsb2018']
chosen_model = stardist_model_names[0]
print("Chosen Model: ", chosen_model)
model = StarDist2D.from_pretrained(chosen_model)
✓ 0.4s                                                 Python
```

```
Chosen Model:  2D_versatile_fluo
Found model '2D_versatile_fluo' for 'StarDist2D'.
Loading network weights from 'weights_best.h5'.
Loading thresholds from 'thresholds.json'.
Using default values: prob_thresh=0.479071, nms_thresh=0.3.
```

3. Running Predictions

We run the predictions on the image using the loaded model.

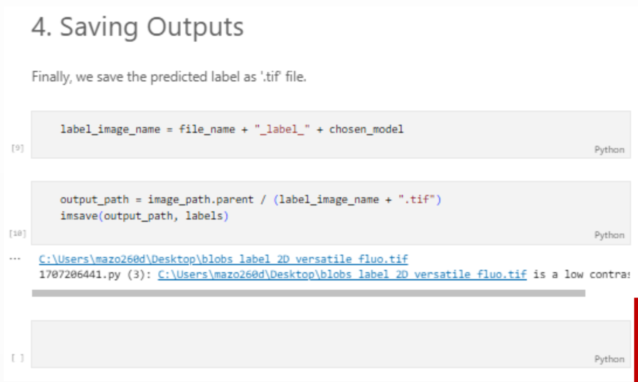
```
[6]
labels, _ = model.predict_instances(normalize(image))
✓ 8.1s                                                 Python
```

```
1/1 [==============================] - 8s 8s/step
```

```
[7]
print("Number of Nuclei: ", labels.max())
imshow(labels, cmap='tab20')
✓ 0.4s                                                 Python
```

```
Number of Nuclei:  56
matplotlib_plugin.py (150): Low image data range; displaying image with stretched contrast.

<matplotlib.image.AxesImage at 0x1b116917f10>
```



4. Saving Outputs

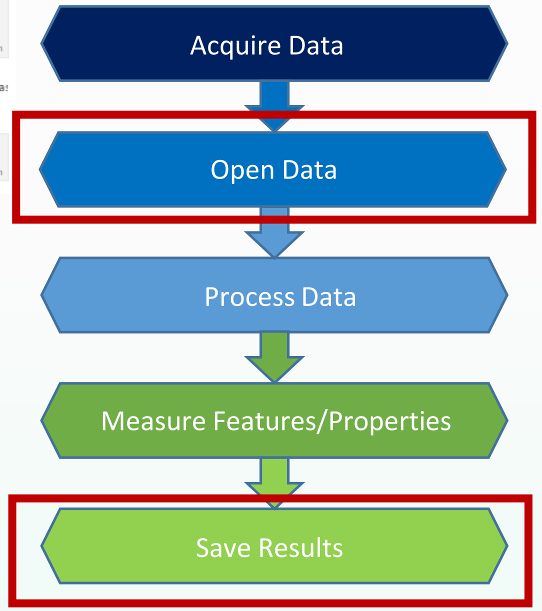
Finally, we save the predicted label as '.tif' file.

```
[9]
label_image_name = file_name + "_label_" + chosen_model
                                                       Python
```

```
[10]
output_path = image_path.parent / (label_image_name + ".tif")
imsave(output_path, labels)
                                                       Python
```

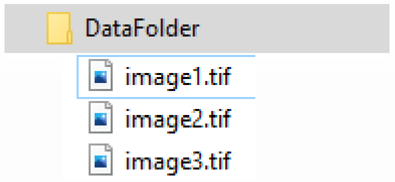
```
C:\Users\mazo260d\Desktop\blobs_label_2D_versatile_fluo.tif
1707206441.py (3): C:\Users\mazo260d\Desktop\blobs_label_2D_versatile_fluo.tif is a low contras
```

```
[ ]
                                                       Python
```

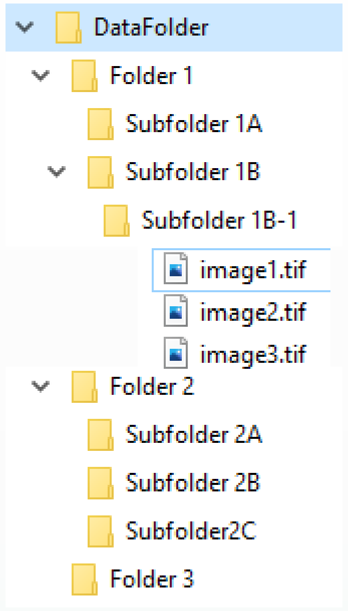


Neat! Let’s run it for another image!
Let me just plug my external HD…

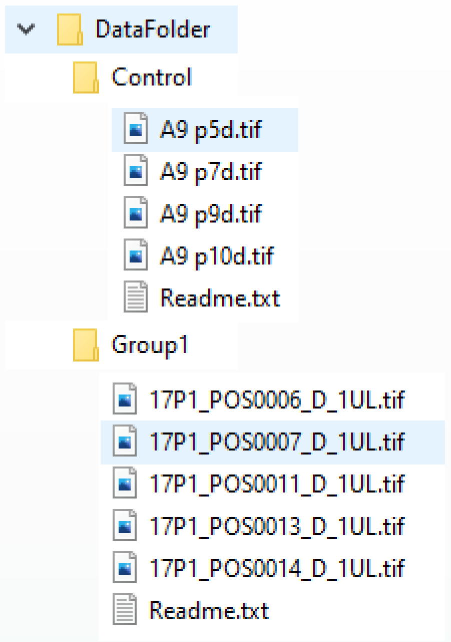
Data Structures



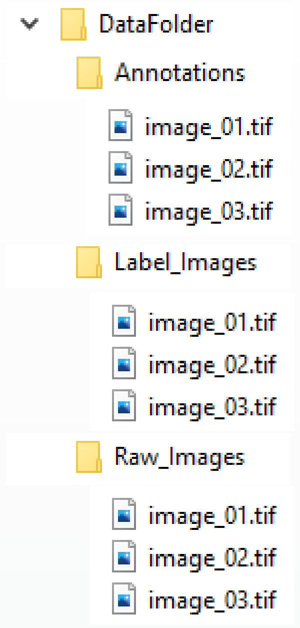
- DataFolder
  - image1.tif
  - image2.tif
  - image3.tif



- DataFolder
  - Folder 1
    - Subfolder 1A
    - Subfolder 1B
      - Subfolder 1B-1
        - image1.tif
        - image2.tif
        - image3.tif
  - Folder 2
    - Subfolder 2A
    - Subfolder 2B
    - Subfolder2C
  - Folder 3



- DataFolder
  - Control
    - A9 p5d.tif
    - A9 p7d.tif
    - A9 p9d.tif
    - A9 p10d.tif
    - Readme.txt
  - Group1
    - 17P1_POS0006_D_1UL.tif
    - 17P1_POS0007_D_1UL.tif
    - 17P1_POS0011_D_1UL.tif
    - 17P1_POS0013_D_1UL.tif
    - 17P1_POS0014_D_1UL.tif
    - Readme.txt



- DataFolder
  - Annotations
    - image_01.tif
    - image_02.tif
    - image_03.tif
  - Label_Images
    - image_01.tif
    - image_02.tif
    - image_03.tif
  - Raw_Images
    - image_01.tif
    - image_02.tif
    - image_03.tif

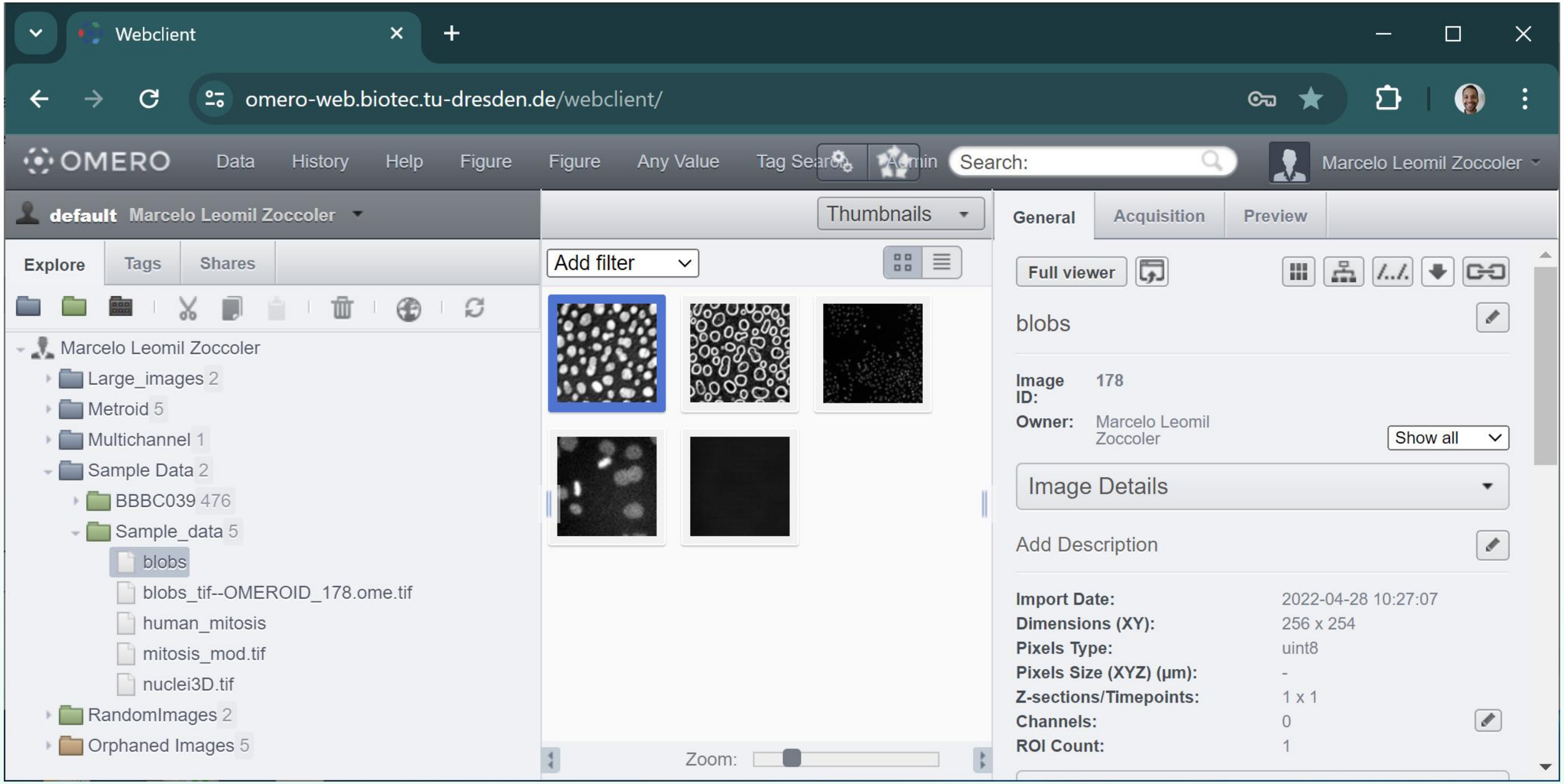
OMERO

Image by eikira from Pixabay (https://pixabay.com/photos/trunk-automobile-vehicle-luggage-1478832/)

@zoccolermarcelo

OMERO Server

PoL Physics of Life TU Dresden



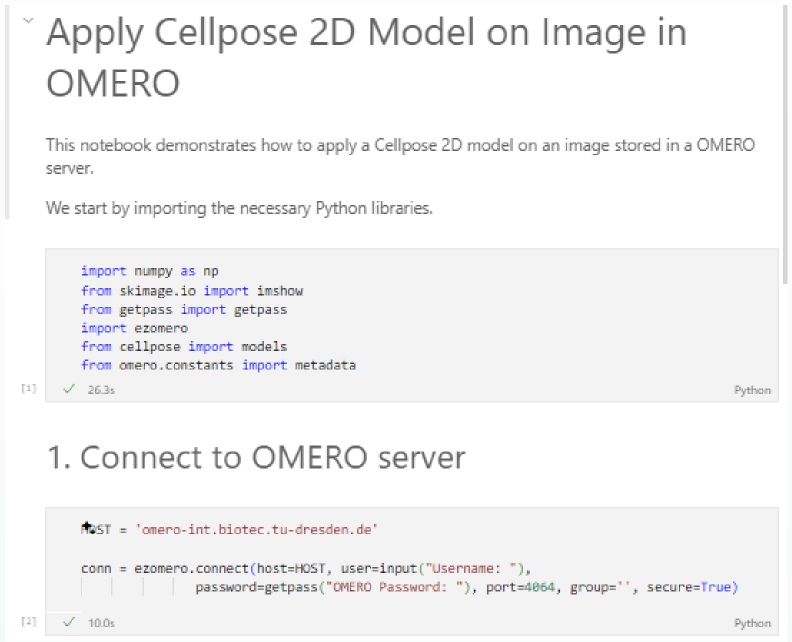
- Marcelo Leomil Zoccoler
  - Large_images 2
  - Metroid 5
  - Multichannel 1
  - Sample Data 2
    - BBBC039 476
    - Sample_data 5
      - blobs
      - blobs_tif--OMEROID_178.ome.tif
      - human_mitosis
      - mitosis_mod.tif
      - nuclei3D.tif
  - RandomImages 2
  - Orphaned Images 5

blobs

Image ID:	178
Owner:	Marcelo Leomil Zoccoler
Import Date:	2022-04-28 10:27:07
Dimensions (XY):	256 x 254
Pixels Type:	uint8
Pixels Size (XYZ) (µm):	-
Z-sections/Timepoints:	1 x 1
Channels:	0
ROI Count:	1

Image Analysis with OMERO

- Example: Counting nuclei after segmentation with Cellpose



Apply Cellpose 2D Model on Image in OMERO

This notebook demonstrates how to apply a Cellpose 2D model on an image stored in a OMERO server.

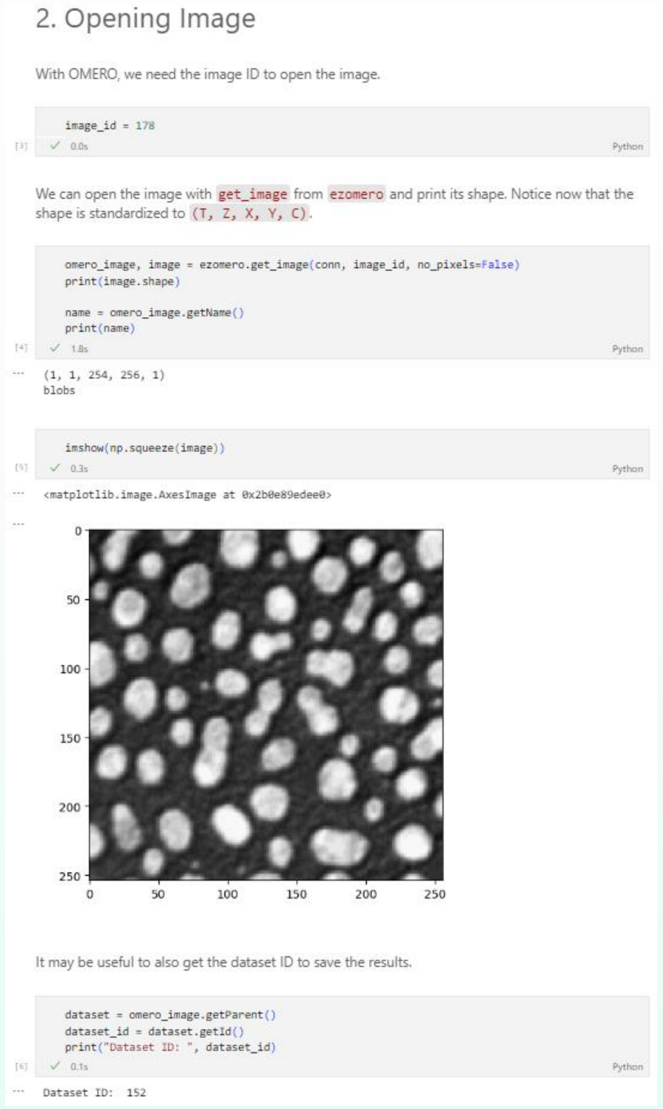
We start by importing the necessary Python libraries.

```python
import numpy as np
from skimage.io import imshow
from getpass import getpass
import ezomero
from cellpose import models
from omero.constants import metadata
```

1. Connect to OMERO server

```python
HOST = 'omero-int.biotec.tu-dresden.de'

conn = ezomero.connect(host=HOST, user=input("Username: "),
                       password=getpass("OMERO Password: "), port=4064, group='', secure=True)
```



2. Opening Image

With OMERO, we need the image ID to open the image.

```python
image_id = 178
```

We can open the image with `get_image` from `ezomero` and print its shape. Notice now that the shape is standardized to `(T, Z, X, Y, C)`.

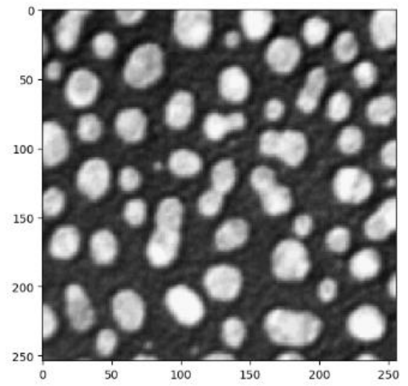
```python
omero_image, image = ezomero.get_image(conn, image_id, no_pixels=False)
print(image.shape)

name = omero_image.getName()
print(name)
```

```
(1, 1, 254, 256, 1)
blobs
```

```python
imshow(np.squeeze(image))
```

```
<matplotlib.image.AxesImage at 0x2b0e89edee0>
```



It may be useful to also get the dataset ID to save the results.

```python
dataset = omero_image.getParent()
dataset_id = dataset.getId()
print("Dataset ID: ", dataset_id)
```

```
Dataset ID:  152
```

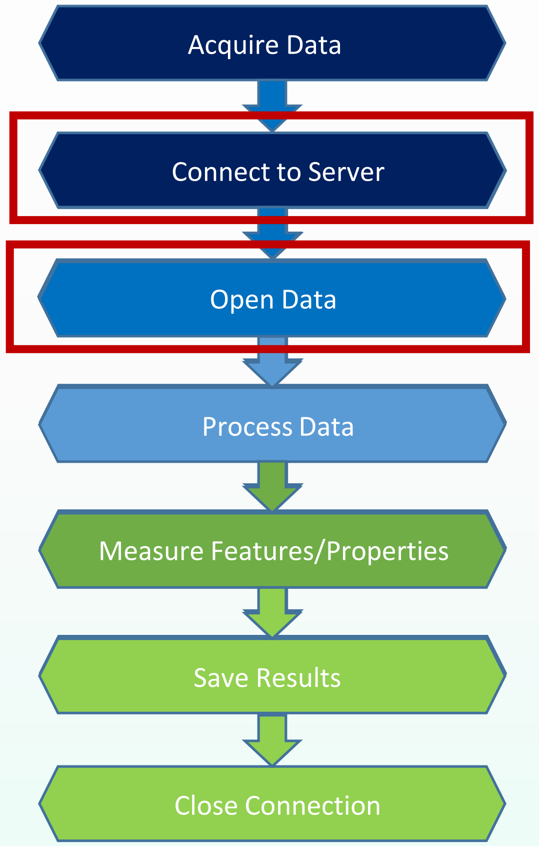
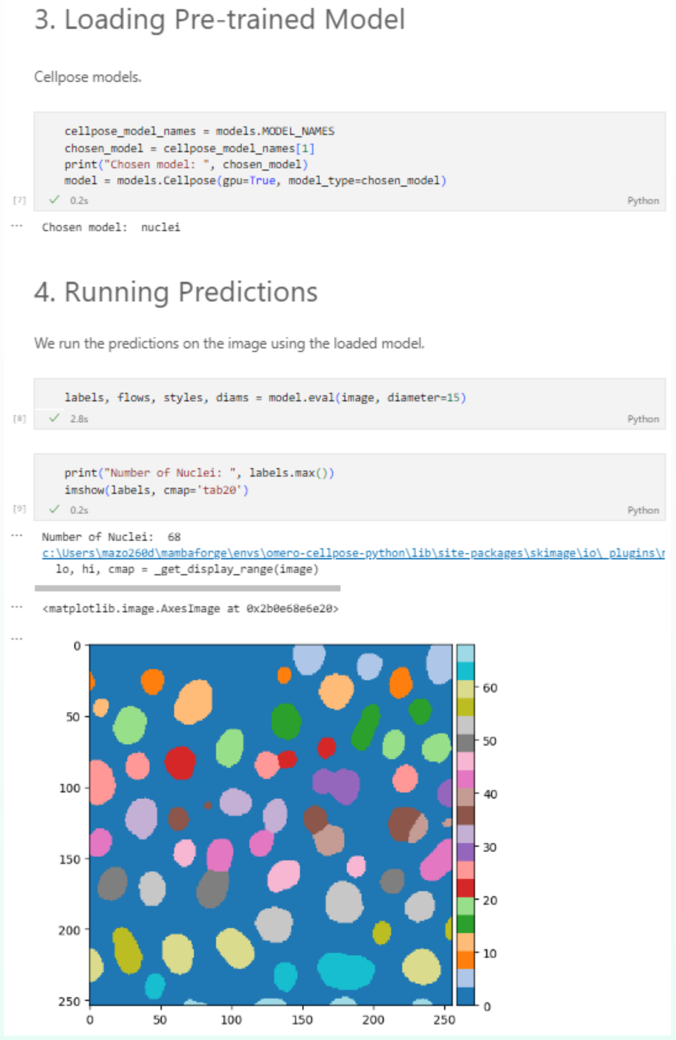


Image Analysis with OMERO

- Example: Counting nuclei after segmentation with Cellpose



3. Loading Pre-trained Model

Cellpose models.

```
cellpose_model_names = models.MODEL_NAMES
chosen_model = cellpose_model_names[1]
print("Chosen model: ", chosen_model)
model = models.Cellpose(gpu=True, model_type=chosen_model)
```

[7] ✓ 0.2s Python

```
Chosen model:  nuclei
```

4. Running Predictions

We run the predictions on the image using the loaded model.

```
labels, flows, styles, diams = model.eval(image, diameter=15)
```

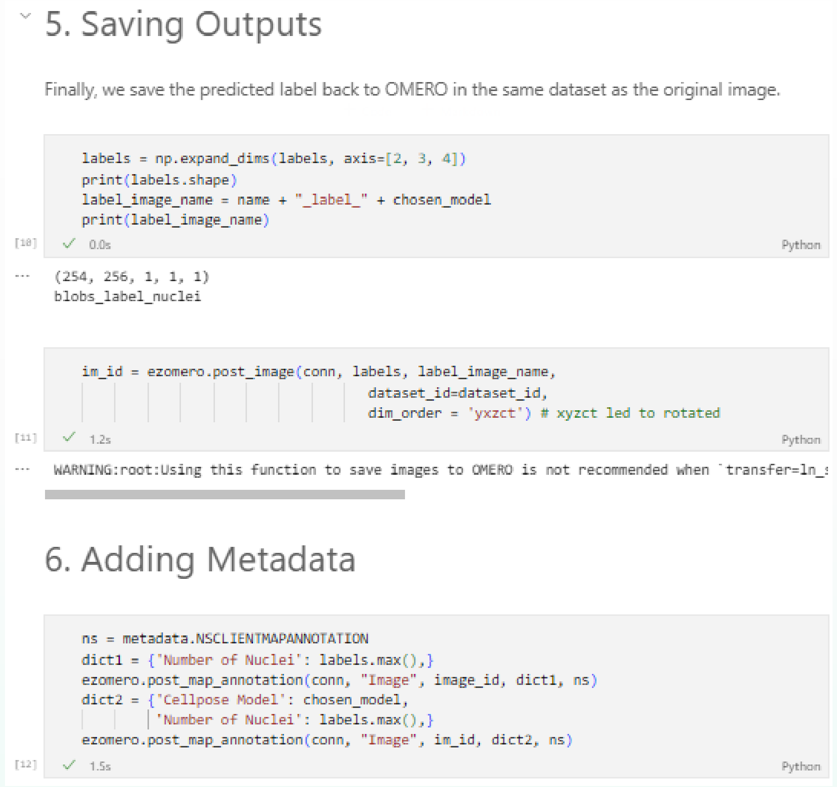
[8] ✓ 2.8s Python

```
print("Number of Nuclei: ", labels.max())
imshow(labels, cmap='tab20')
```

[9] ✓ 0.2s Python

```
Number of Nuclei:  68
c:\Users\mazo260d\mambaforge\envs\omero-cellpose-python\lib\site-packages\skimage\io\_plugins\r
  lo, hi, cmap = _get_display_range(image)
```

```
<matplotlib.image.AxesImage at 0x2b0e68e6e20>
```



5. Saving Outputs

Finally, we save the predicted label back to OMERO in the same dataset as the original image.

```
labels = np.expand_dims(labels, axis=[2, 3, 4])
print(labels.shape)
label_image_name = name + "_label_" + chosen_model
print(label_image_name)
```

[10] ✓ 0.0s Python

```
(254, 256, 1, 1, 1)
blobs_label_nuclei
```

```
im_id = ezomero.post_image(conn, labels, label_image_name,
                           dataset_id=dataset_id,
                           dim_order = 'yxzct') # xyzct led to rotated
```

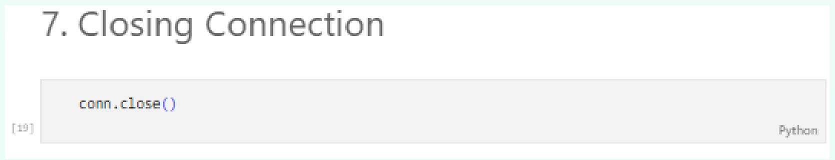
[11] ✓ 1.2s Python

```
WARNING:root:Using this function to save images to OMERO is not recommended when `transfer=ln_s
```

6. Adding Metadata

```
ns = metadata.NSCLIENTMAPANNOTATION
dict1 = {'Number of Nuclei': labels.max(),}
ezomero.post_map_annotation(conn, "Image", image_id, dict1, ns)
dict2 = {'Cellpose Model': chosen_model,
         'Number of Nuclei': labels.max(),}
ezomero.post_map_annotation(conn, "Image", im_id, dict2, ns)
```

[12] ✓ 1.5s Python



7. Closing Connection

```
conn.close()
```

[19] Python

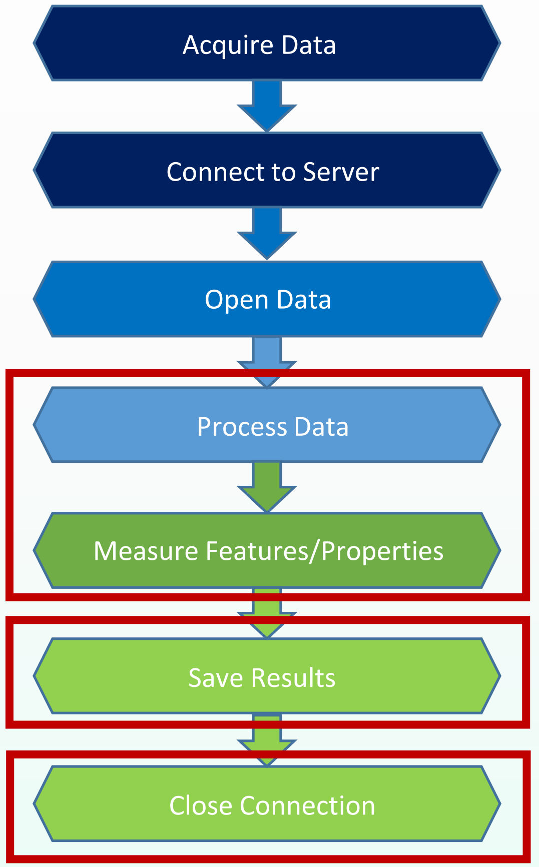
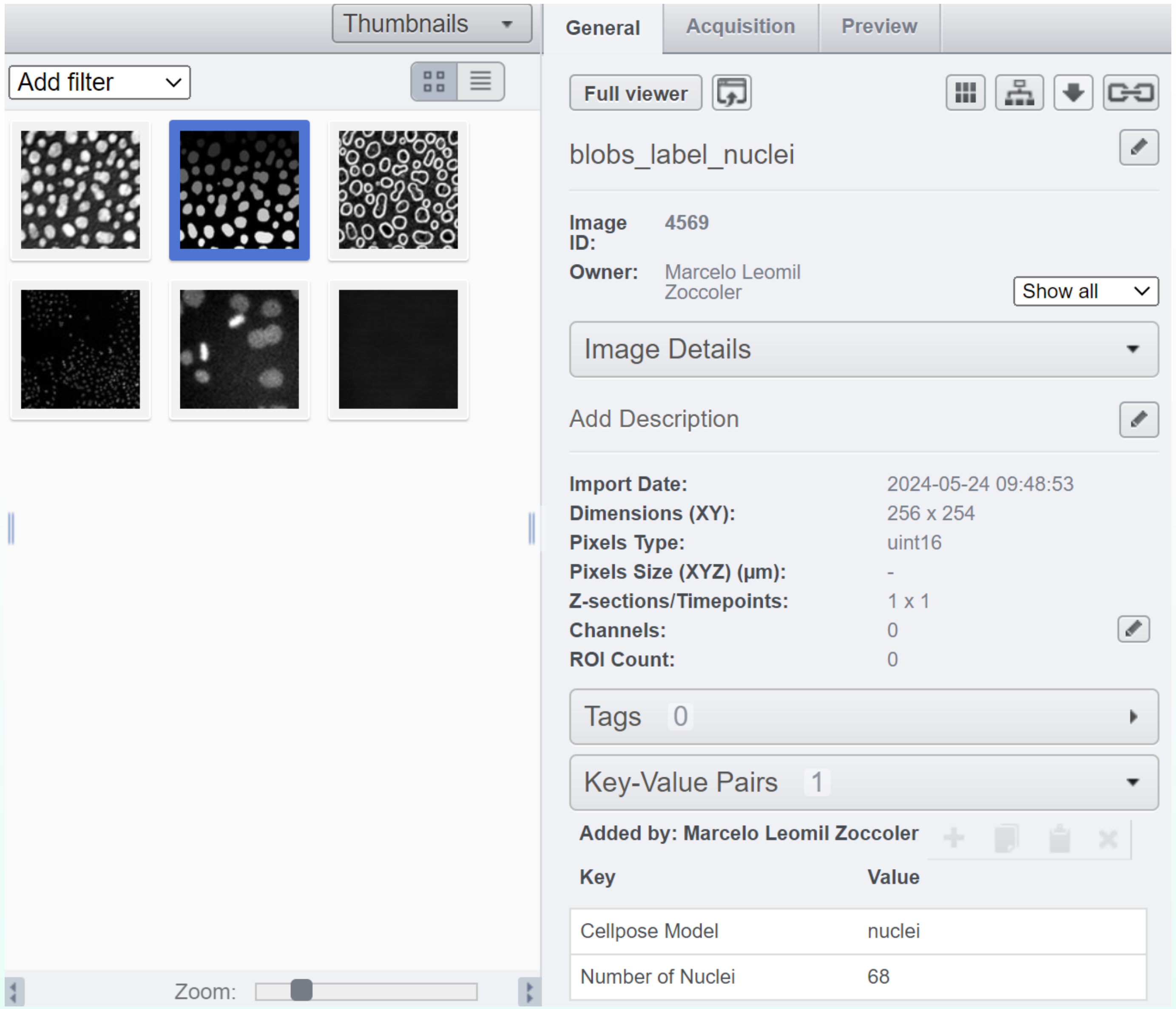


Image Analysis with OMERO



blobs_label_nuclei

Image ID:	4569
Owner:	Marcelo Leomil Zoccoler

Image Details

Add Description

Import Date:	2024-05-24 09:48:53
Dimensions (XY):	256 x 254
Pixels Type:	uint16
Pixels Size (XYZ) (µm):	-
Z-sections/Timepoints:	1 x 1
Channels:	0
ROI Count:	0

Tags 0

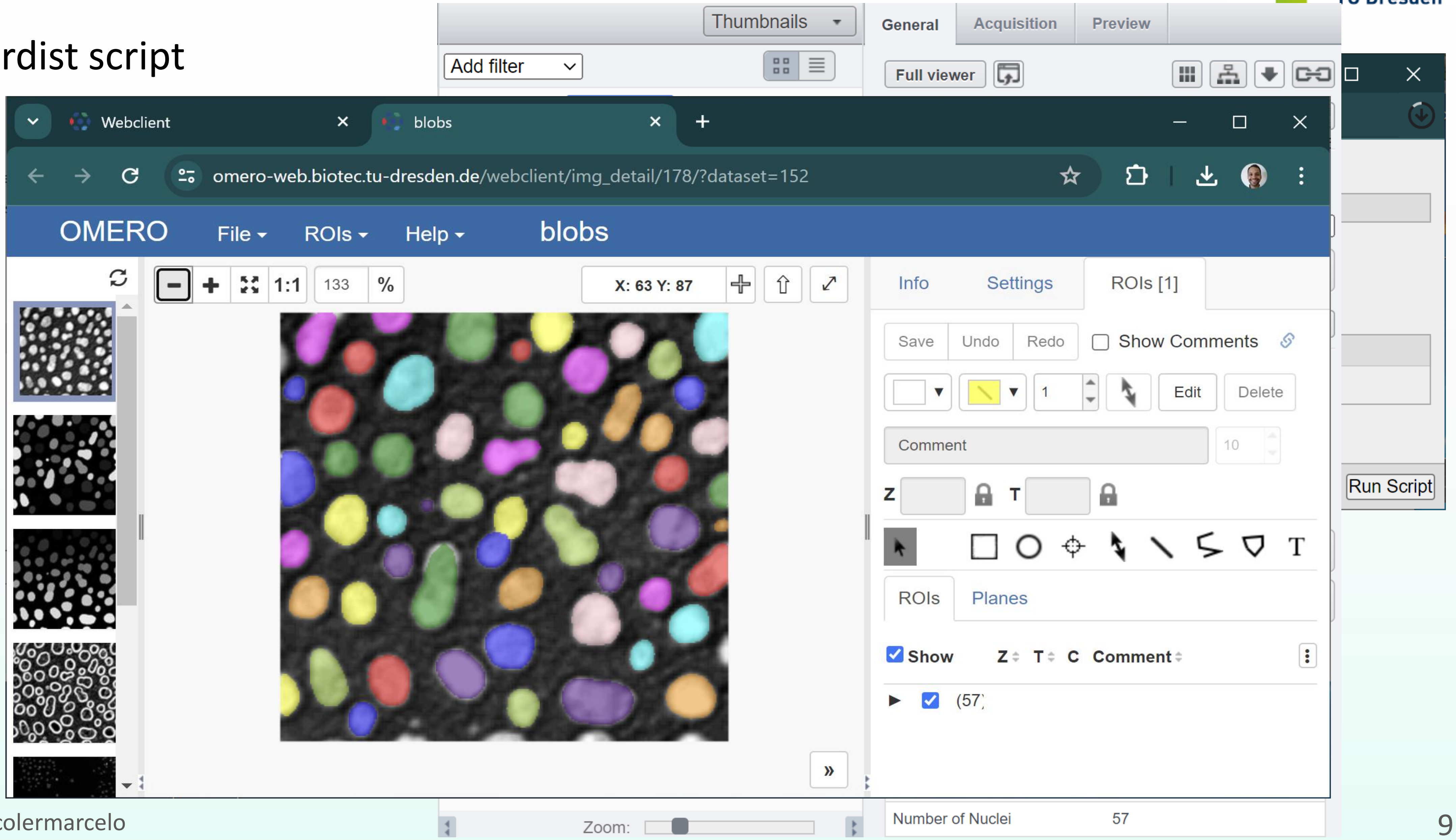
Key-Value Pairs 1

Added by: Marcelo Leomil Zoccoler

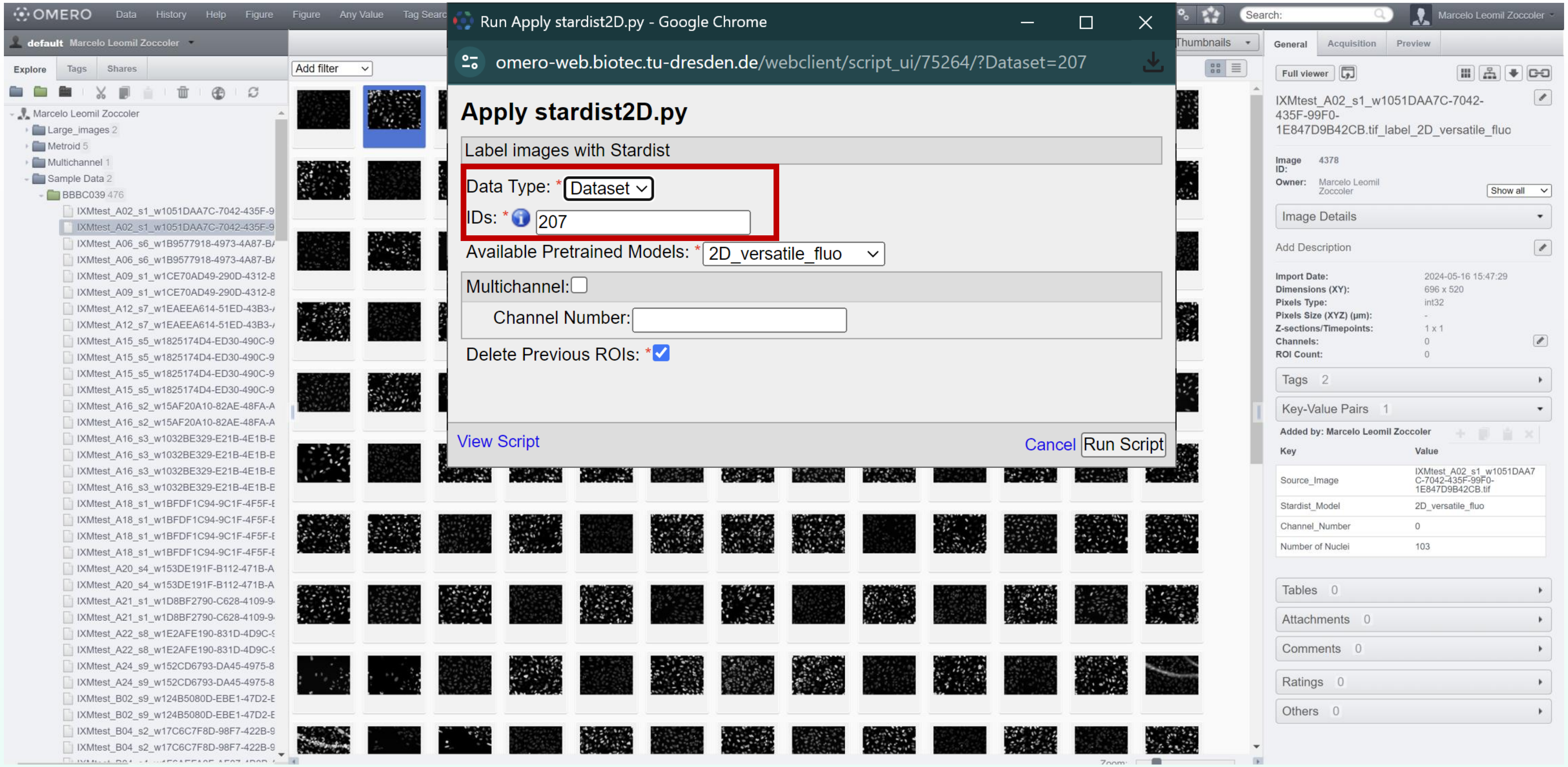
Key	Value
Cellpose Model	nuclei
Number of Nuclei	68

OMERO Scripts

- Stardist script



Batch Processing with OMERO



Marcelo Leomil Zoccoler
- Large_images 2
- Metroid 5
- Multichannel 1
- Sample Data 2
  - BBBC039 476
    - IXMtest_A02_s1_w1051DAA7C-7042-435F-9
    - IXMtest_A02_s1_w1051DAA7C-7042-435F-9
    - IXMtest_A06_s6_w1B9577918-4973-4A87-BA
    - IXMtest_A06_s6_w1B9577918-4973-4A87-BA
    - IXMtest_A09_s1_w1CE70AD49-290D-4312-8
    - IXMtest_A09_s1_w1CE70AD49-290D-4312-8
    - IXMtest_A12_s7_w1EAEEA614-51ED-43B3-A
    - IXMtest_A12_s7_w1EAEEA614-51ED-43B3-A
    - IXMtest_A15_s5_w1825174D4-ED30-490C-9
    - IXMtest_A15_s5_w1825174D4-ED30-490C-9
    - IXMtest_A15_s5_w1825174D4-ED30-490C-9
    - IXMtest_A15_s5_w1825174D4-ED30-490C-9
    - IXMtest_A16_s2_w15AF20A10-82AE-48FA-A
    - IXMtest_A16_s2_w15AF20A10-82AE-48FA-A
    - IXMtest_A16_s3_w1032BE329-E21B-4E1B-E
    - IXMtest_A16_s3_w1032BE329-E21B-4E1B-E
    - IXMtest_A16_s3_w1032BE329-E21B-4E1B-E
    - IXMtest_A16_s3_w1032BE329-E21B-4E1B-E
    - IXMtest_A18_s1_w1BFDF1C94-9C1F-4F5F-E
    - IXMtest_A18_s1_w1BFDF1C94-9C1F-4F5F-E
    - IXMtest_A18_s1_w1BFDF1C94-9C1F-4F5F-E
    - IXMtest_A18_s1_w1BFDF1C94-9C1F-4F5F-E
    - IXMtest_A20_s4_w153DE191F-B112-471B-A
    - IXMtest_A20_s4_w153DE191F-B112-471B-A
    - IXMtest_A21_s1_w1D8BF2790-C628-4109-9
    - IXMtest_A21_s1_w1D8BF2790-C628-4109-9
    - IXMtest_A22_s8_w1E2AFE190-831D-4D9C-9
    - IXMtest_A22_s8_w1E2AFE190-831D-4D9C-9
    - IXMtest_A24_s9_w152CD6793-DA45-4975-8
    - IXMtest_A24_s9_w152CD6793-DA45-4975-8
    - IXMtest_B02_s9_w124B5080D-EBE1-47D2-E
    - IXMtest_B02_s9_w124B5080D-EBE1-47D2-E
    - IXMtest_B04_s2_w17C6C7F8D-98F7-422B-9
    - IXMtest_B04_s2_w17C6C7F8D-98F7-422B-9

Run Apply stardist2D.py - Google Chrome

omero-web.biotec.tu-dresden.de/webclient/script_ui/75264/?Dataset=207

Apply stardist2D.py

Label images with Stardist

Data Type: * Dataset

IDs: * 207

Available Pretrained Models: * 2D_versatile_fluo

Multichannel: ☐

Channel Number:

Delete Previous ROIs: * ✓

View Script   Cancel   Run Script

IXMtest_A02_s1_w1051DAA7C-7042-435F-99F0-1E847D9B42CB.tif_label_2D_versatile_fluo

Image ID:	4378
Owner:	Marcelo Leomil Zoccoler

Show all

Image Details

Add Description

Import Date:	2024-05-16 15:47:29
Dimensions (XY):	696 x 520
Pixels Type:	int32
Pixels Size (XYZ) (µm):	-
Z-sections/Timepoints:	1 x 1
Channels:	0
ROI Count:	0

Tags 2

Key-Value Pairs 1

Added by: Marcelo Leomil Zoccoler

Key	Value
Source_Image	IXMtest_A02_s1_w1051DAA7C-7042-435F-99F0-1E847D9B42CB.tif
Stardist_Model	2D_versatile_fluo
Channel_Number	0
Number of Nuclei	103

Tables 0

Attachments 0

Comments 0

Ratings 0

Others 0

@zoccolermarcelo

Considerations

- Fixed 2-level hierarchy forces researchers to structure their data from start
- Extra levels of complexity could get achieved by adding tags
- Data, metadata and results from analysis can be all in the same place and linked (FAIR)
- OMERO scripts are very handy for light simple algorithms
- Complex algorithms that require lots of computational resources, like deep-learning, would need special servers and would be best loaded and processed in HPCs or powerful workstations
- Tiled processing for large images is not straight-forward (bandwidth limitations for writing whole large images)
- ezomero library eases a lot programming with OMERO compared to default Python bindings, but it does not have some functionalities yet (like write tiles)

Useful Links for Python Developers

- OMERO guide Python: https://github.com/ome/omero-guide-python
- OMERO Python Language Bindings: https://docs.openmicroscopy.org/omero/5.4.5/developers/Python.html
- ezomero: https://thejacksonlaboratory.github.io/ezomero/

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- Stefan Hahmann

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- Till Korten
- Mara Lampert
- Svetlana Iarovenko
- Ryan George Savill
- Laura Zigutyte
- Somashekhar Kulkarni

CMCB TECHNOLOGY PLATFORM
Center for Molecular and Cellular Bioengineering TU Dresden

Networks

BioDIP Biopolis Dresden Imaging Platform

NFDI4 BIOIMAGE

image.sc

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Funding

DFG public

Chan Zuckerberg Initiative