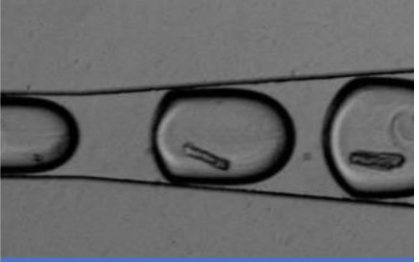


dkfz. DEUTSCHES KREBSFORSCHUNGSZENTRUM IN DER HELMHOLTZ-GEMEINSCHAFT

OMERO and Fiji

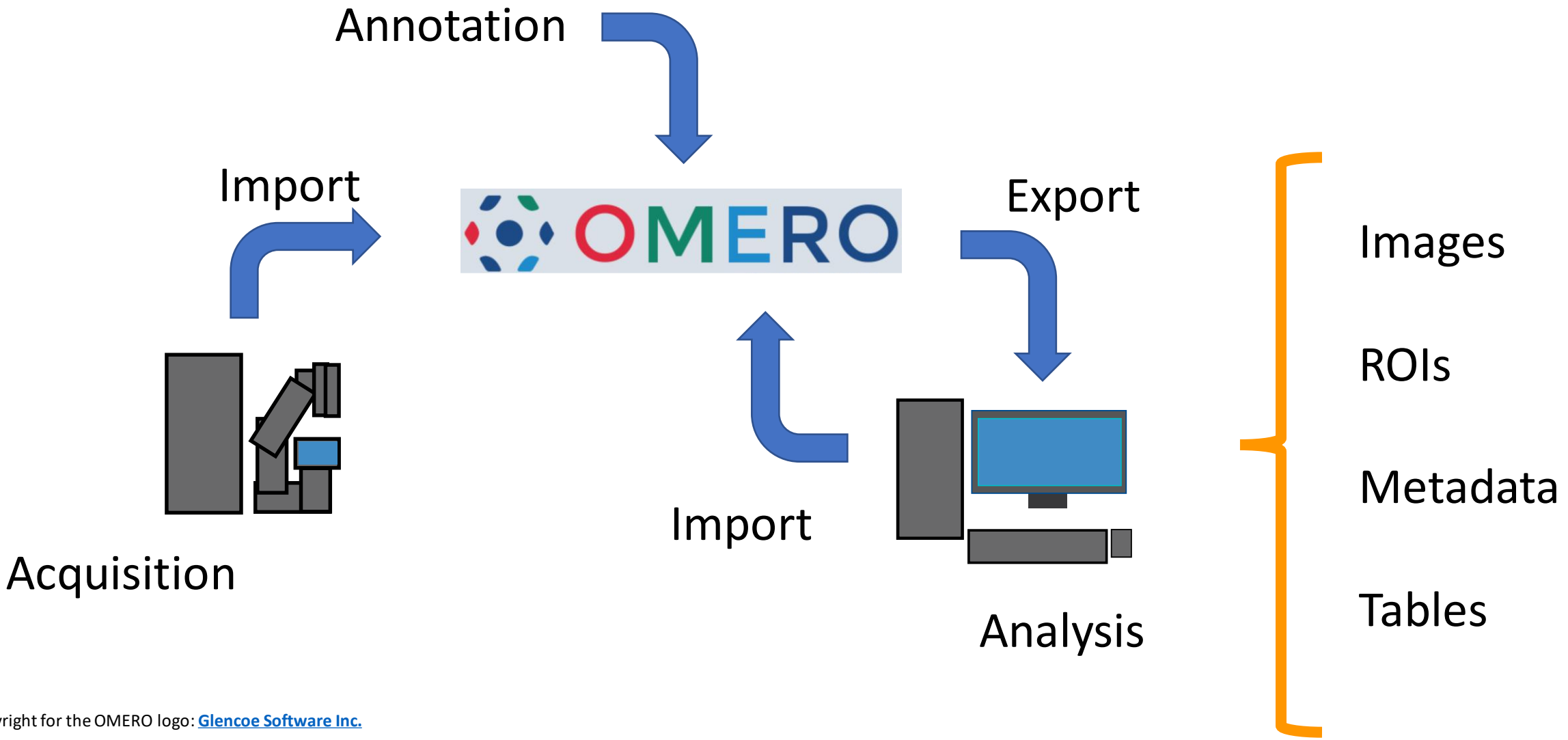
Michele Bortolomeazzi

13/05/2024

I3D:bio

NFDI4 BIOIMAGE

Image analysis workflow with OMERO

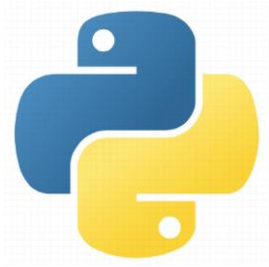


Connections with Image Analysis Software

Non Exhaustive list of tools able to connect to OMERO directly or through plugins:

Fiji

napari



OMERO python API
ezOMERO

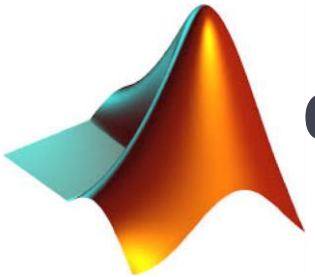
CellProfiler

Orbit

rOMERO Gateway

QuPath

Ilastick



OMERO.matlab toolbox

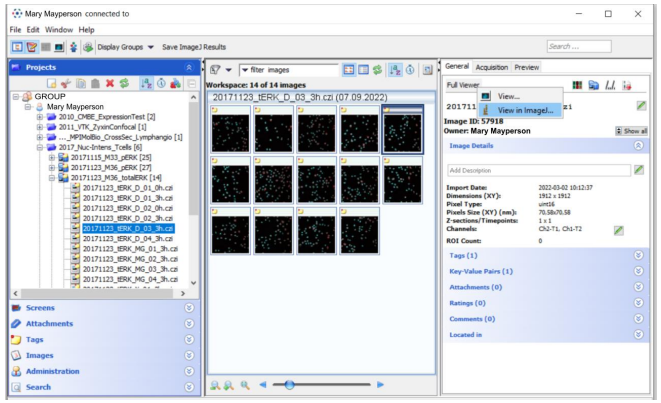
https://www.openmicroscopy.org/omero/features/analyze/
https://omero-guides.readthedocs.io/en/latest/external_tools.html
https://omero-guides.readthedocs.io/en/latest/api_usage.html

Easing OMERO adoption with ezomero. Ratamero et al. bioRxiv 2023.06.29.546930;
doi: https://doi.org/10.1101/2023.06.29.546930

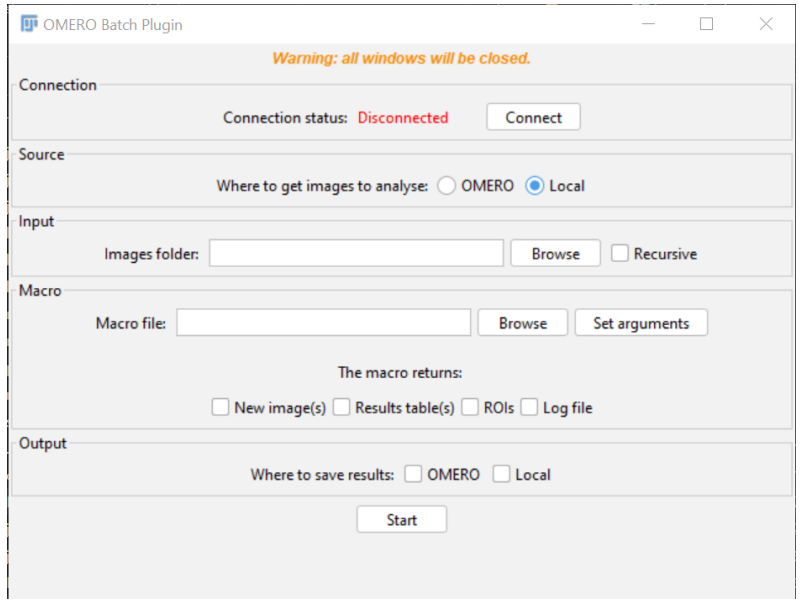
Fiji / ImageJ

Three ways of working with Fiji and OMERO:

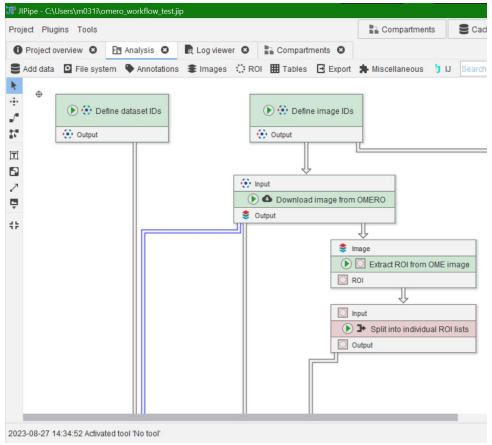
- **OMERO plugin for Fiji**



- **Fiji Macros and OMERO.batch plugin**



- **JIPipe**



https://github.com/GReD-Clermont/omero_batch-plugin

https://omero-guides.readthedocs.io/en/latest/fiji/docs/index.html

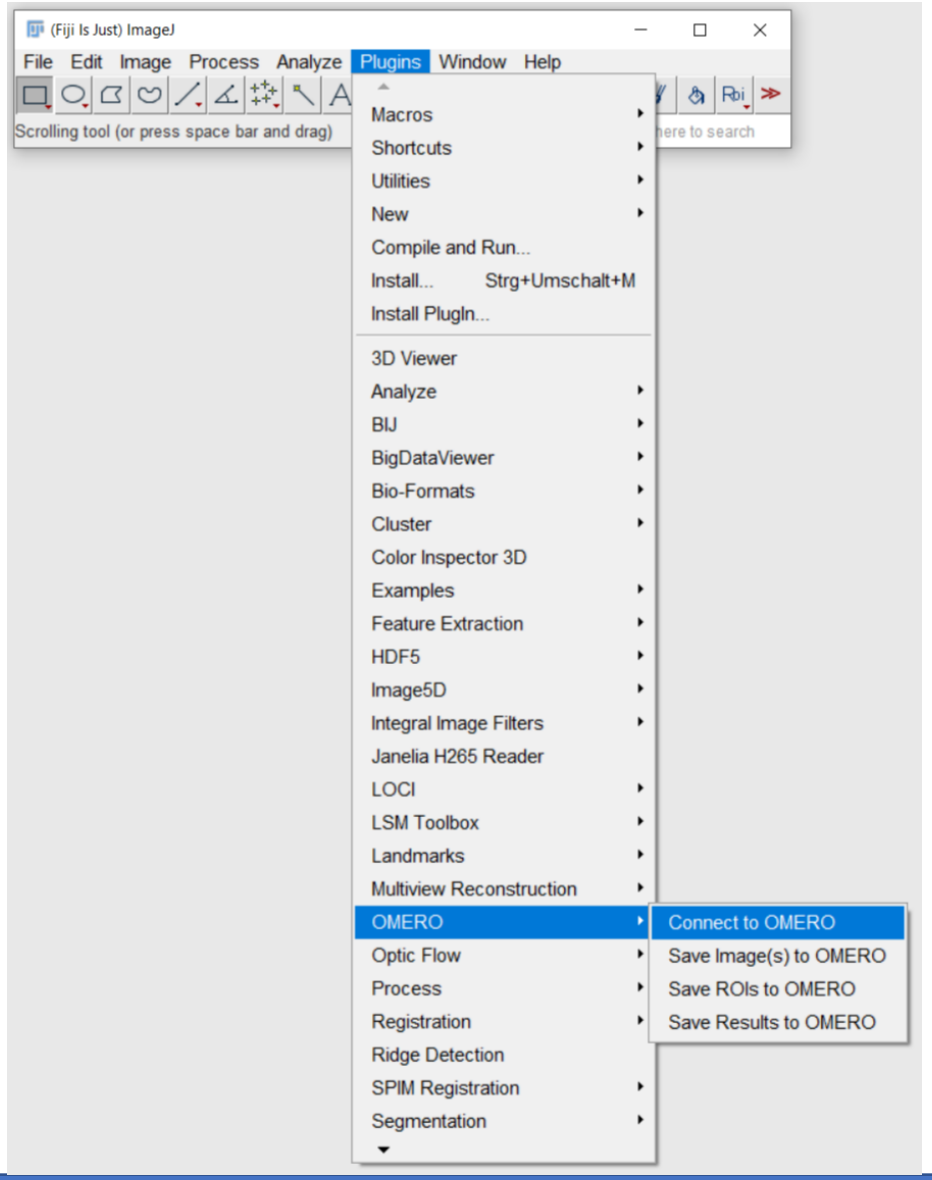
https://jipipe.hki-jena.de/

Opening the OMERO Fiji Plugin

1. Open Fiji and go to
   Plugins → OMERO → Connect to OMERO

2. Log in to OMERO with your user credentials.

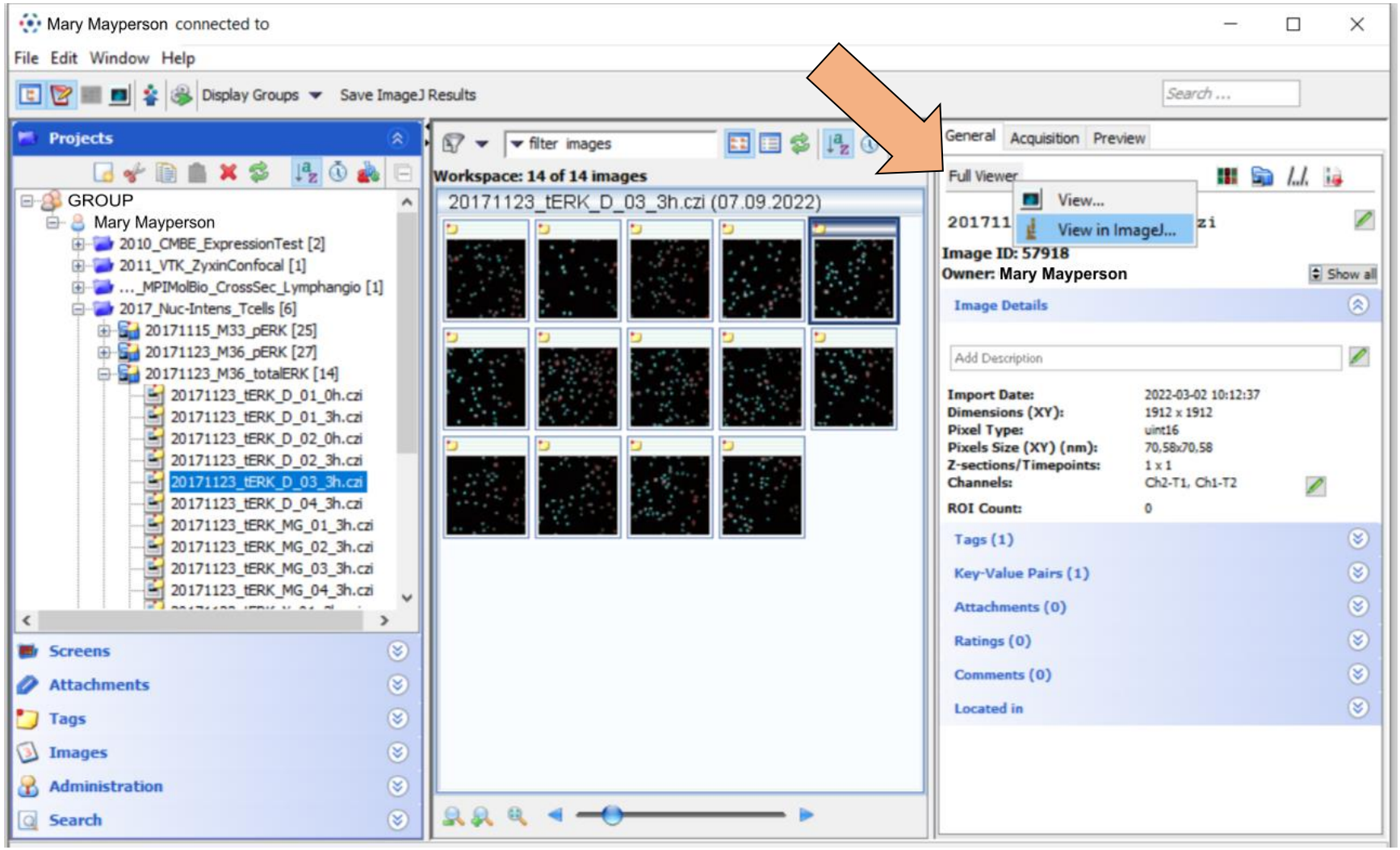
The Fiji-OMERO plugin looks almost precisely like OMERO.insight, but is, in fact, part of the open Fiji application



Schindelin, J., Arganda-Carreras, I., Frise, E. et al. Fiji: an open-source platform for biological-image analysis. Nat Methods 9, 676–682 (2012). https://doi.org/10.1038/nmeth.2019

Selecting images in the OMERO Fiji Plugin

1. Select image(s) from the file tree
2. Open in Fiji by clicking
   Full Viewer
   and then
   View in ImageJ…

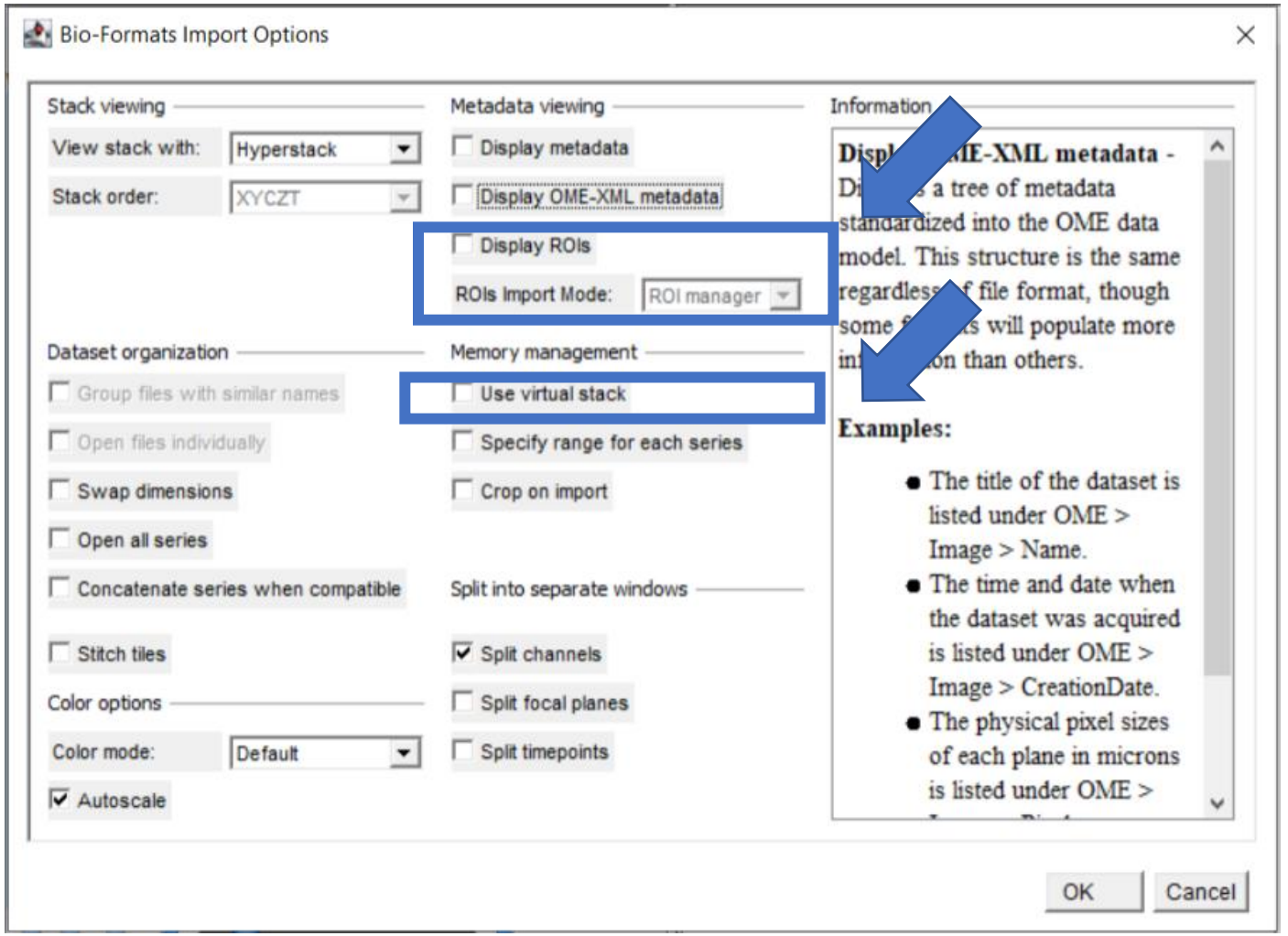


Taken from: Schmidt, C., Bortolomeazzi, M., Boissonnet, T., Fortmann-Grote, C., Dohle, J., Zentis, P., Kandpal, N., Kunis, S., Zobel, T., Weidtkamp-Peters, S., & Ferrando-May, E. (2023). I3D:bio's OMERO training material: Re-usable, adjustable, multi-purpose slides for local user training. Zenodo. https://doi.org/10.5281/zenodo.8323588

Opening images in the OMERO Fiji Plugin

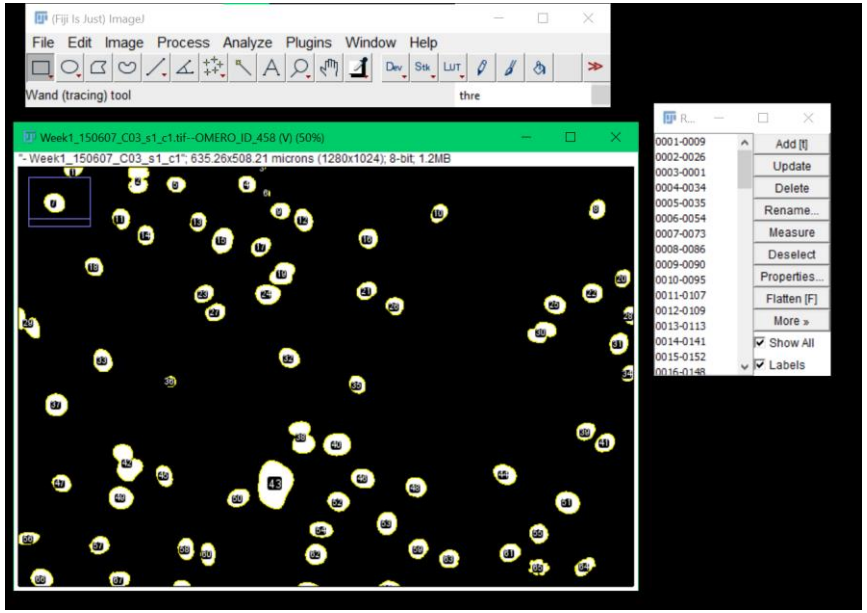
Use your prefered settings to open the image(s) as required for your work, I would recommend:

- Use virtual stack
- Import ROIs to ROI manager if needed



Practical 1: Let‘s count some cells.

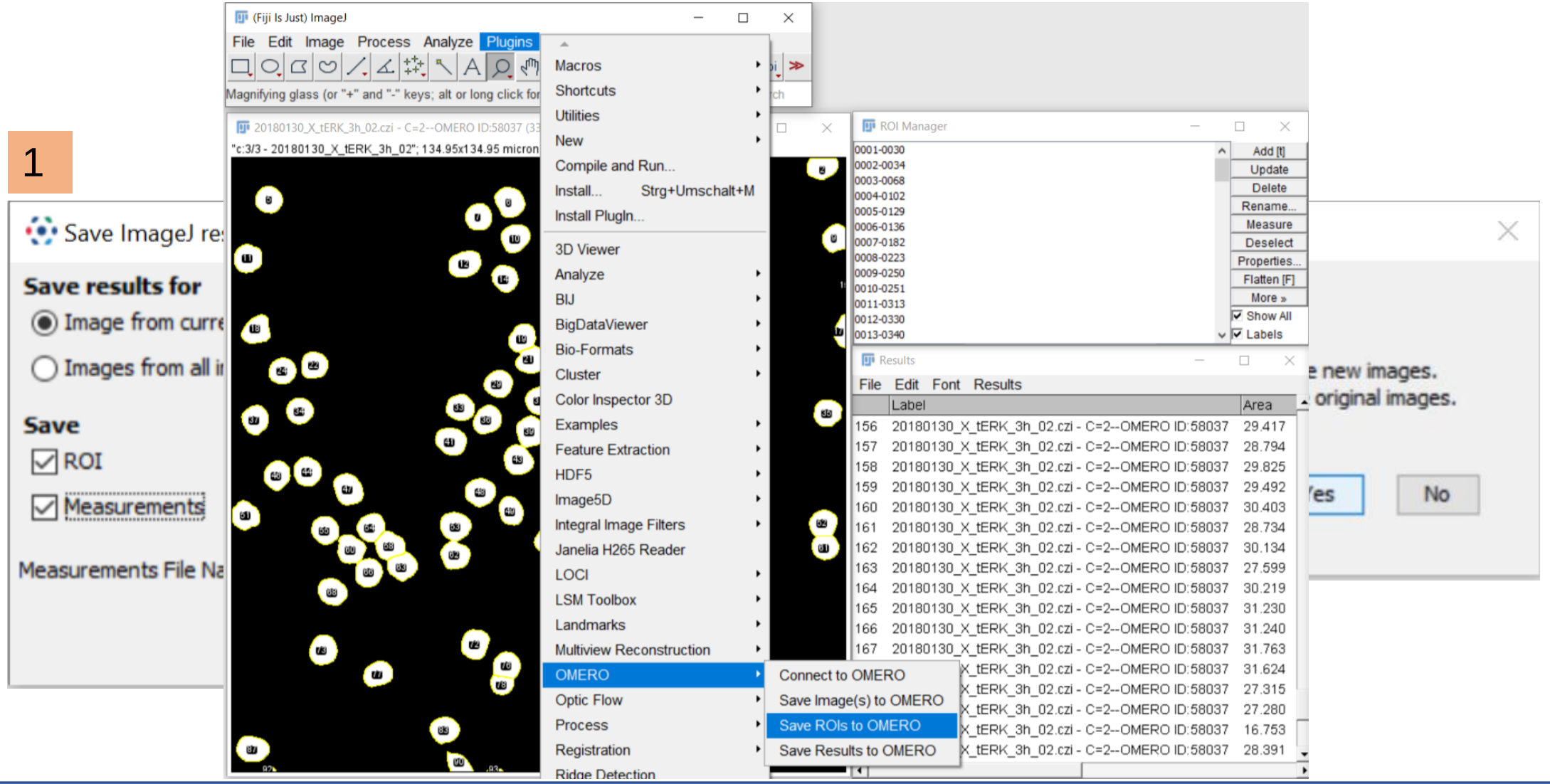
1. Open one image from the „**structuring_exercise**“ project using the OMERO Fiji Plugin.
2. Duplicate the image.
3. Smooth the image (*Process -> Smooth*).
4. Run a Threshold (*Image -> Adjust -> Threshold*).
5. Analyze Particles (*Image -> Analyze Particles, select „Add to Manager“*).
6. Close the segmented image.
7. Run Measure



Please leave the results and ROI manager Open for the next practical

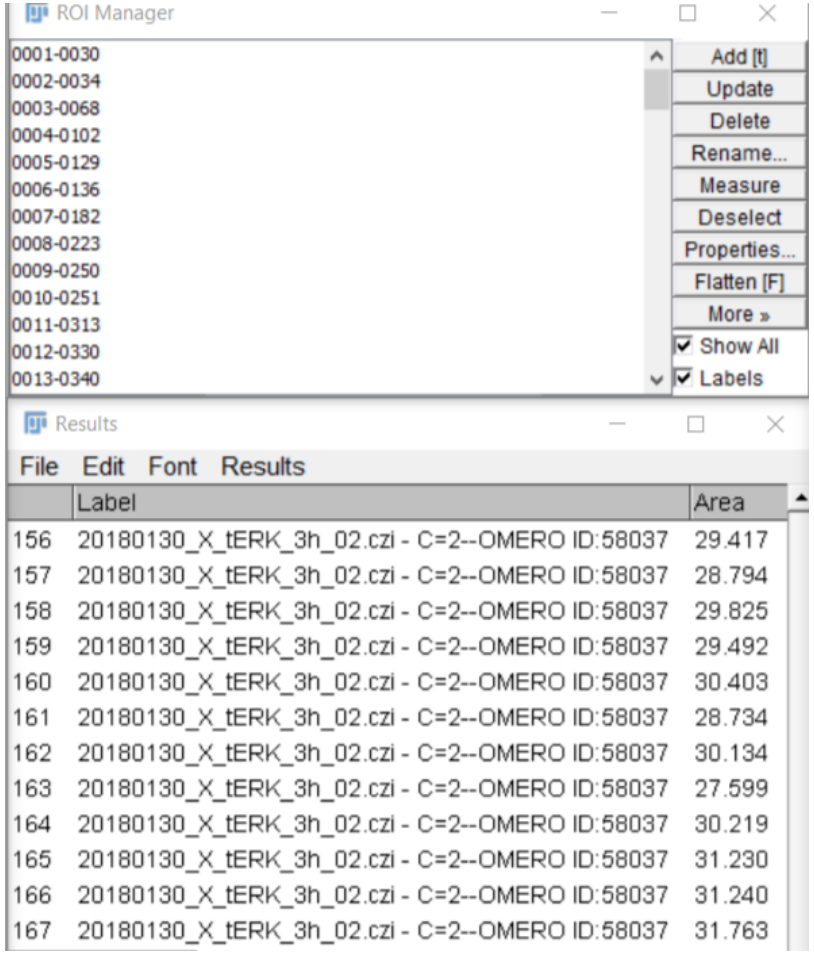
Saving ROIs and Results Back to OMERO

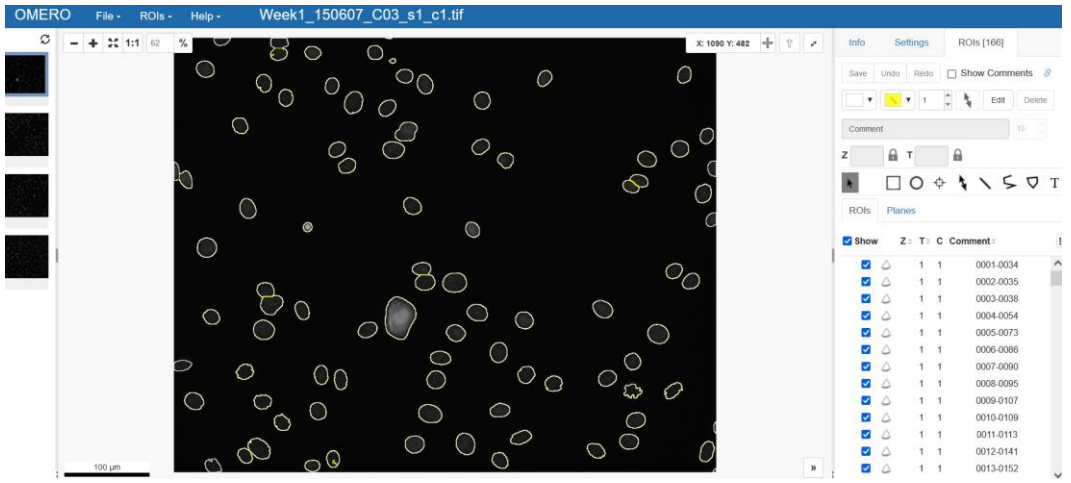
1

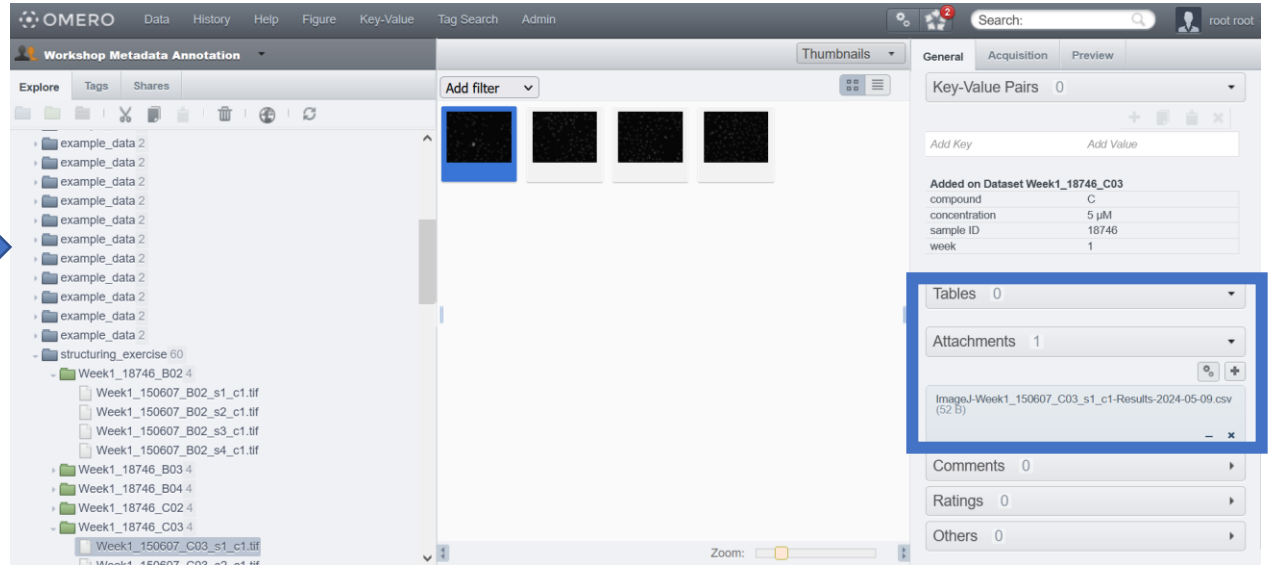


Taken from: Schmidt, C., Bortolomeazzi, M., Boissonnet, T., Fortmann-Grote, C., Dohle, J., Zentis, P., Kandpal, N., Kunis, S., Zobel, T., Weidtkamp-Peters, S., & Ferrando-May, E. (2023). I3D:bio's OMERO training material: Re-usable, adjustable, multi-purpose slides for local user training. Zenodo. https://doi.org/10.5281/zenodo.8323588

Viewing the ROIs and Results in OMERO





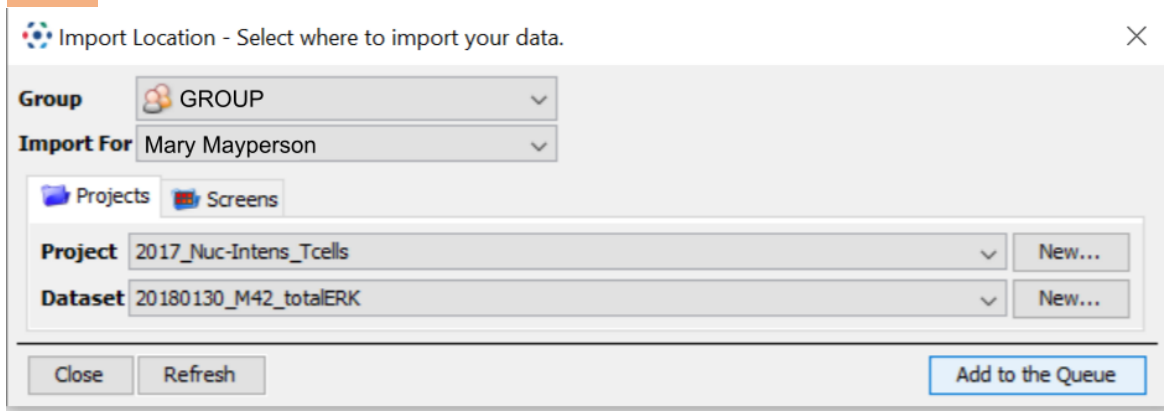


Taken from: Schmidt, C., Bortolomeazzi, M., Boissonnet, T., Fortmann-Grote, C., Dohle, J., Zentis, P., Kandpal, N., Kunis, S., Zobel, T., Weidtkamp-Peters, S., & Ferrando-May, E. (2023). I3D:bio's OMERO training material: Re-usable, adjustable, multi-purpose slides for local user training. Zenodo. https://doi.org/10.5281/zenodo.8323588

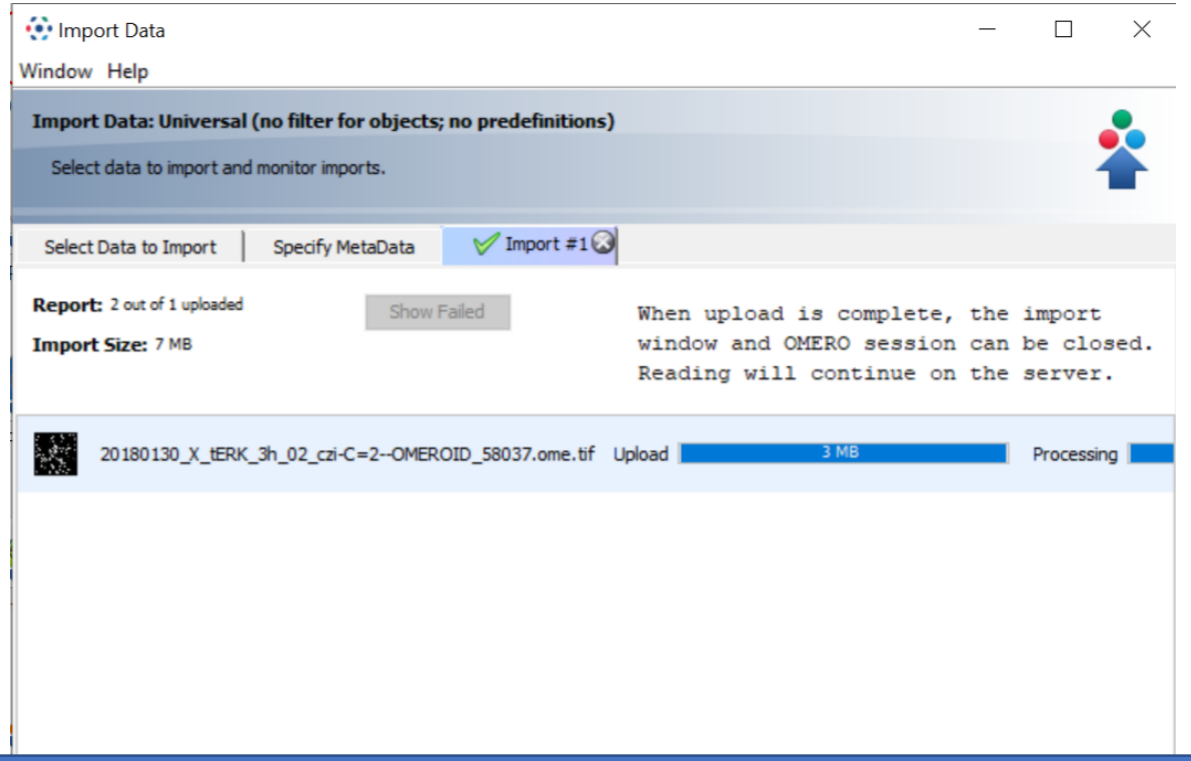
Uploading images back to OMERO

Choose upload destination (Group, User, Project, Dataset) and upload

1



2



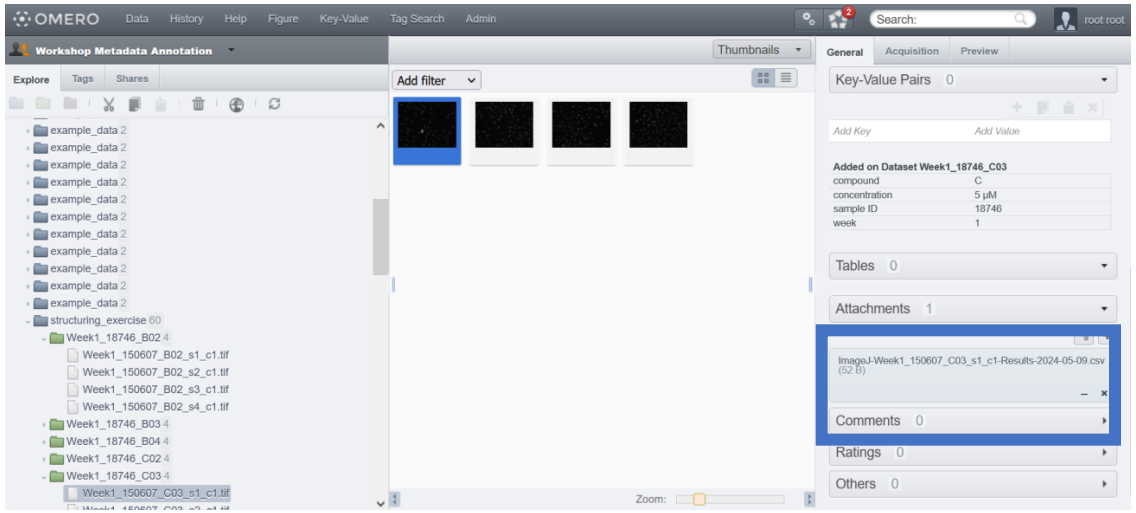
Taken from: Schmidt, C., Bortolomeazzi, M., Boissonnet, T., Fortmann-Grote, C., Dohle, J., Zentis, P., Kandpal, N., Kunis, S., Zobel, T., Weidtkamp-Peters, S., & Ferrando-May, E. (2023). I3D:bio's OMERO training material: Re-usable, adjustable, multi-purpose slides for local user training. Zenodo. https://doi.org/10.5281/zenodo.8323588

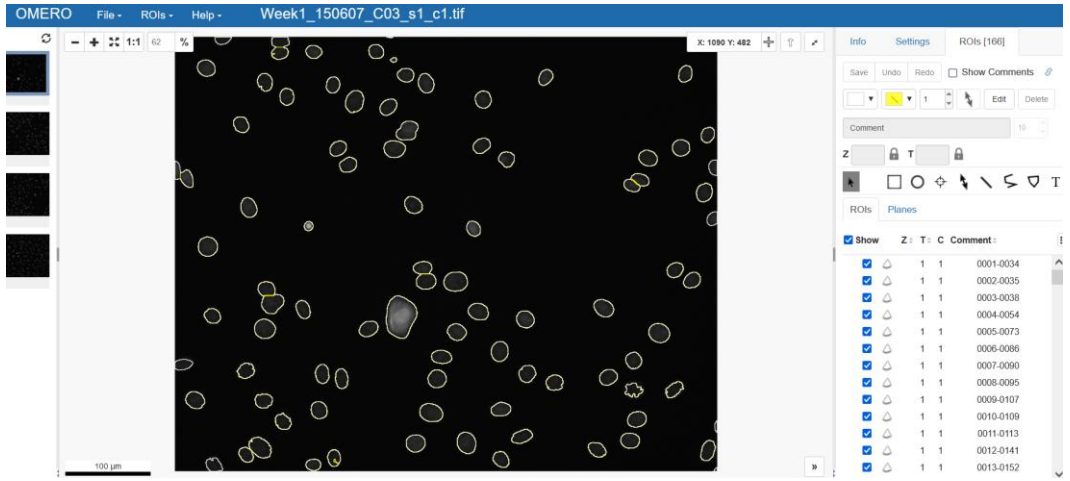
Practical 2: Let‘s save our results.

1. Save the ROIs and measurements back to OMERO.

2. Find and download the measurements from OMERO.web (Select the correct image and look under „Attachments“)

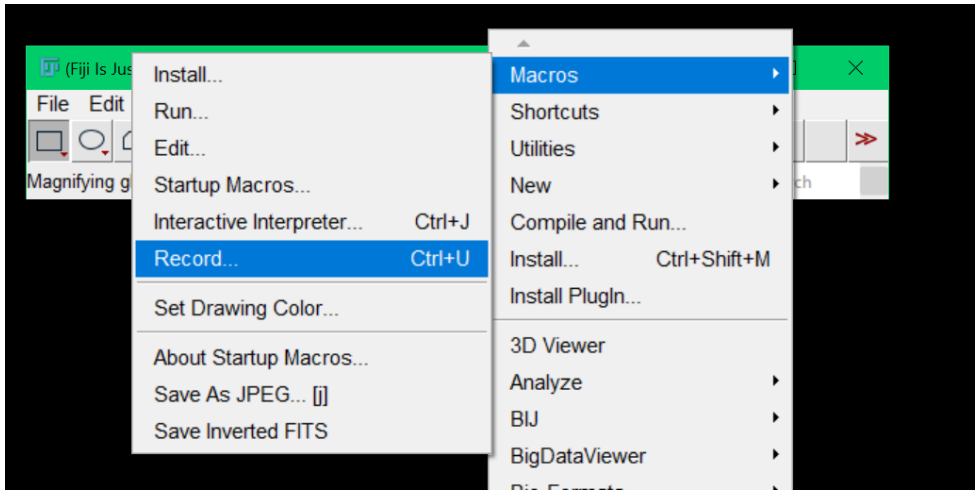
3. Find and examine the ROIs in OMERO.web (Double click the image and go the the „ROI“ tab).

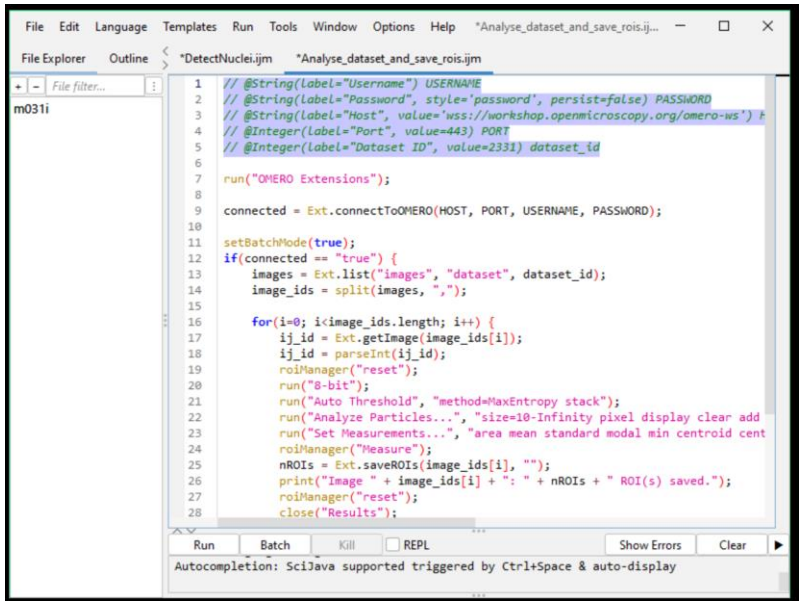




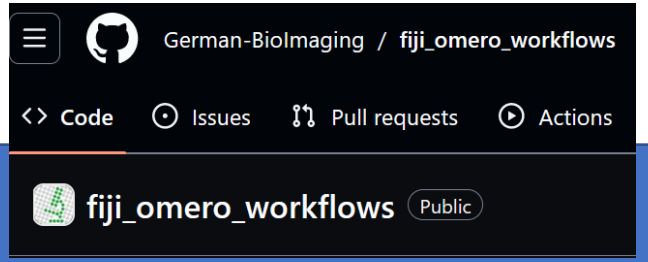
How do I make this workflow reproducible?

From manual workflows to Macros





- Attached to your data in OMERO
- Added to a repository in github/gitlab and then linked from your data in omero



Practical 2: Running the macro

1. Download the „**DetectNuclei.ijm**“ macro attached to the „**structuring_exercise**“ Project
2. Drag the macro into Fiji
3. Open an image from the OMERO Fiji Plugin
4. Run the Macro

But I still need to interact with OMERO manually!

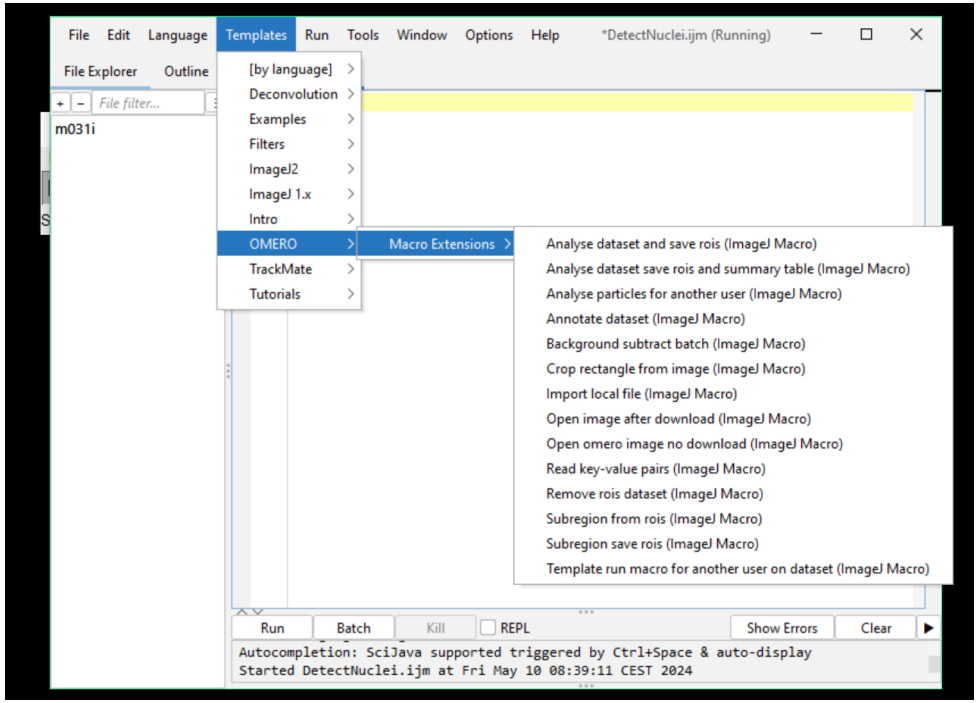
Working with OMERO in FiJi Macros

OMERO Macro Extensions plugin

Plugin for simple access to OMERO objects from FiJi macros.

https://github.com/GReD-Clermont/omero_macro-extensions

Check out the template menu for many examples.



Already installed in the Fiji app provided for the workshop

Getting an image from the macro

```
1  // @String(label="Username") USERNAME
2  // @String(label="Password", style='password', persist=false) PASSWORD
3  // @String(label="Host", value='omero-training.gerbi-gmb.de') HOST
4  // @Integer(label="Port", value=4064) PORT
5  // @Integer(label="Image ID", value=2331) image_id
6
7
8  run("OMERO Extensions");
9
10
11 connected = Ext.connectToOMERO(HOST, PORT, USERNAME, PASSWORD);
12
13
14 if(connected == "true") {
15
16
17     ij_id = Ext.getImage(image_id);
18
19
20 }
21
22
23 Ext.disconnect();
```

Make the user input the credentials and address for OMERO

Activate the OMERO plugin

Connect to OMERO

Check if the connection worked

Get the image

Add your image processing code here

Disconnect from OMERO

Tom Boissonet

Practical 3: Run the macro

1. Download the „**DetectNuclei_OMERO.ijm**“ macro attached to the „**structuring_exercise**“ Project

2. Drag the macro into Fiji

3. Run the Macro

Now the macro is fully integrated with OMERO!

I need to process a lot of images!

Running the macro over a dataset

```
// @String(label="Username") USERNAME
// @String(label="Password", style='password', persist=false) PASSWORD
// @String(label="Host", value='wss://workshop.openmicroscopy.org/omero-ws') HOST
// @Integer(label="Port", value=443) PORT
// @Integer(label="Dataset ID", value=2331) dataset_id

run("OMERO Extensions");

connected = Ext.connectToOMERO(HOST, PORT, USERNAME, PASSWORD);

if(connected == "true") {


   images = Ext.list("images", "dataset", dataset_id);
   image_ids = split(images, ",");


   for(i=0; i<image_ids.length; i++) {
        ij_id = Ext.getImage(image_ids[i]);
        ij_id = parseInt(ij_id);

        roiManager("reset");
        close("Results");
        selectImage(ij_id);
        close();

    }

table_name = "Summary_from_Fiji";
Ext.saveTable(table_name, "Dataset", dataset_id);
}

Ext.disconnect();
```

Make the user input the credentials and address for OMERO

Activate the OMERO plugin

Connect to OMERO

Check if the connection worked

Get Ids of all the images in the dataset

Get the image from OMERO

Add your image processing code here

Close image and remove all ROIs for the next cycle

Repeat for every image in the dataset

Attach the results to the dataset

Disconnect from OMERO

Practical 4: Run the macro on a dataset

1. Download the „**DetectNuclei_autoDataset.ijm**“ macro attached to the „**structuring_exercise**“ Project

2. Drag the macro into Fiji

3. Have a look at the macro, where is the image processing code? How many times will it be executed?

4. Run the Macro

Exploring tables in OMERO

2024-05-11_15-52-15_Summary_from_Fiji

Download as CSV: Whole Table
Show current page as: CSV | JSON

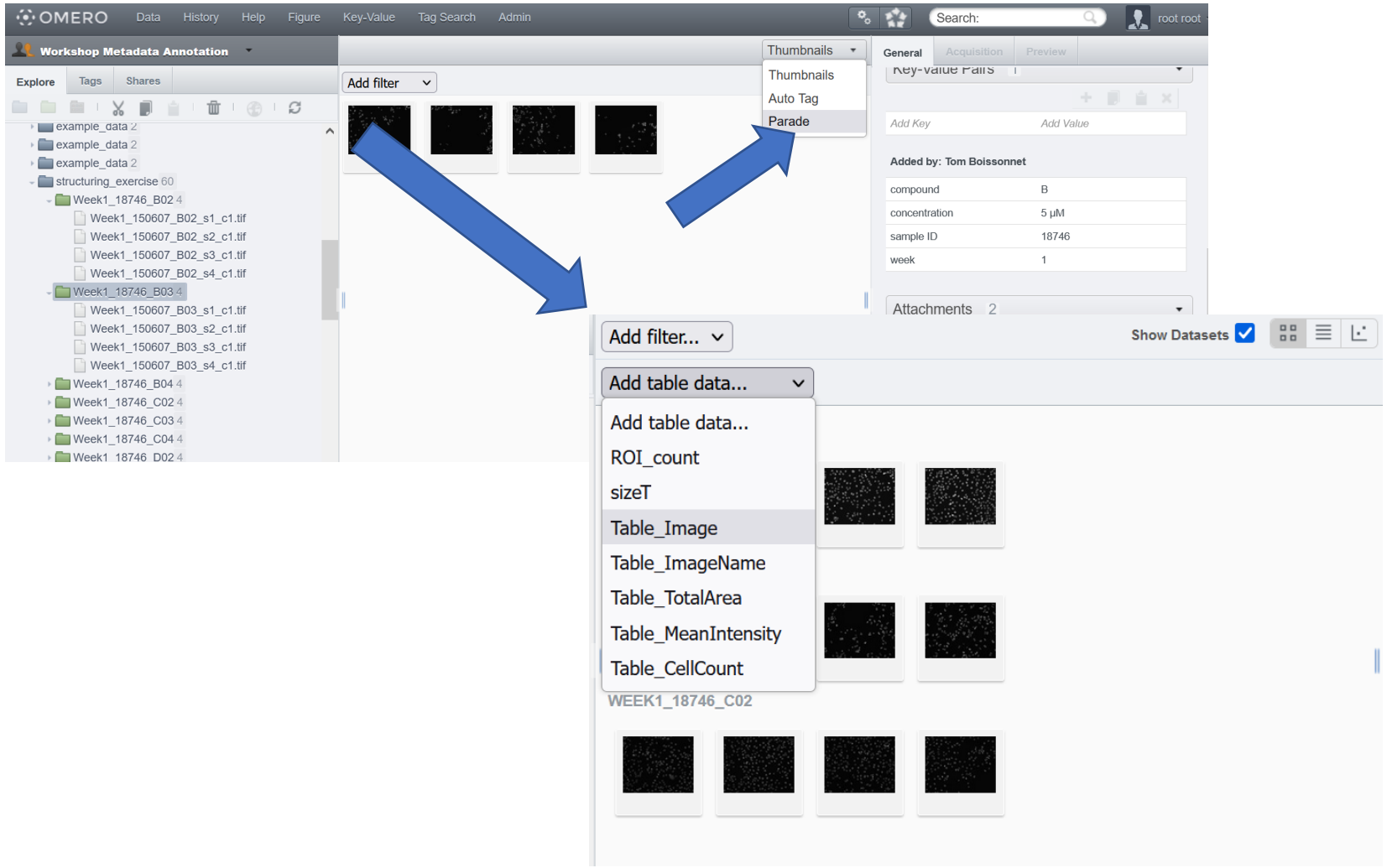
To filter rows you can use a query based on named columns. For example, to filter for rows where **TotalArea** is greater than **322845.6777206989** add ?query=TotalArea>322845.6777206989 to the URL.
For a more complex example, try ?query=(TotalArea>322845.6777206989)&(TotalArea<322845.6777206989)

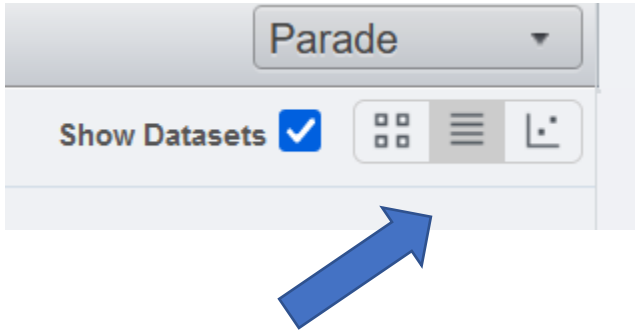
Table rows: **4**.

Image	ImageName	TotalArea	MeanIntensity	CellCount
343	Week1_150607_B02_s3_c1.tif	322845.6777206989	796.5457763671875	185.0
347	Week1_150607_B02_s4_c1.tif	322845.6777206989	901.1114013671875	267.0
491	Week1_150607_B02_s2_c1.tif	322845.6777206989	921.122509765625	253.0
576	Week1_150607_B02_s1_c1.tif	322845.6777206989	965.2109375	285.0

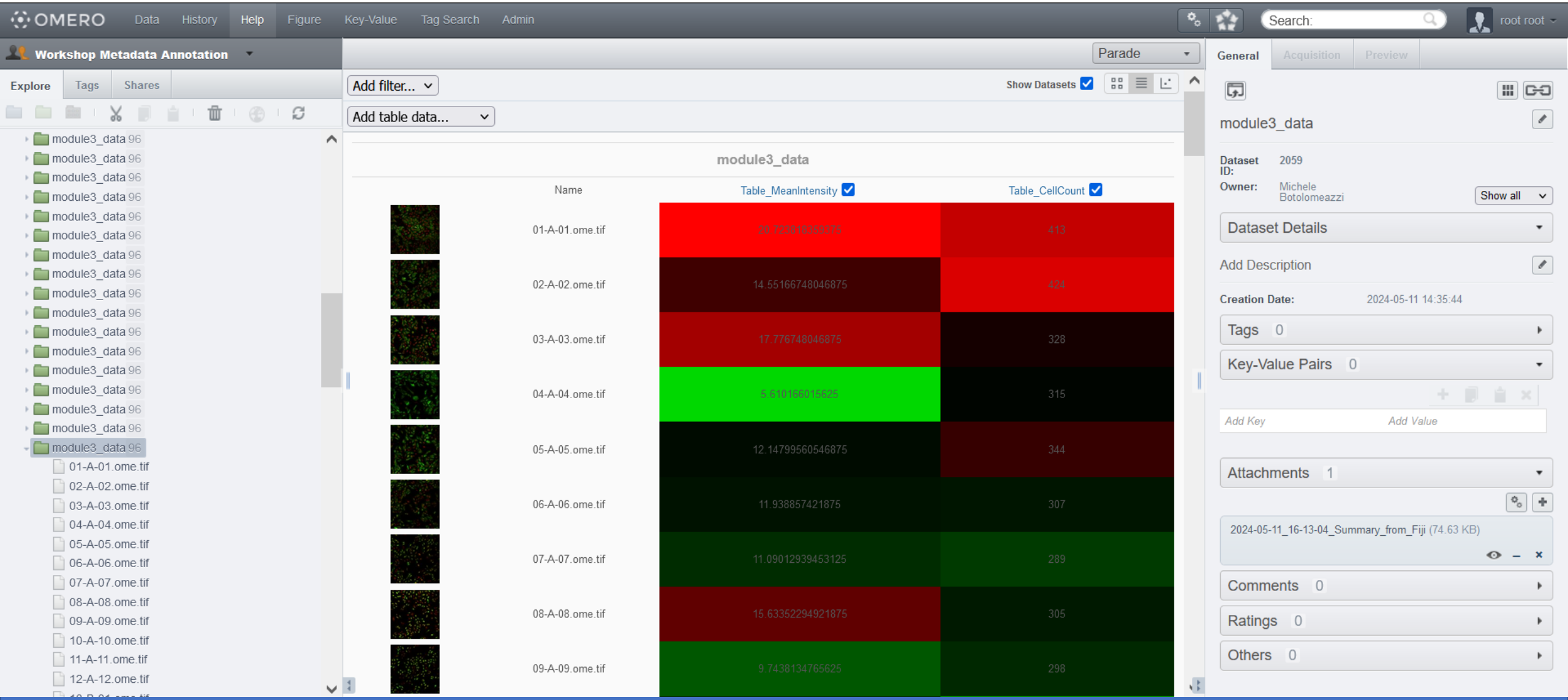
https://wiki-biop.epfl.ch/data-management/omero/omero-tables#tables-on-dataset-level

OMERO.Parade setup



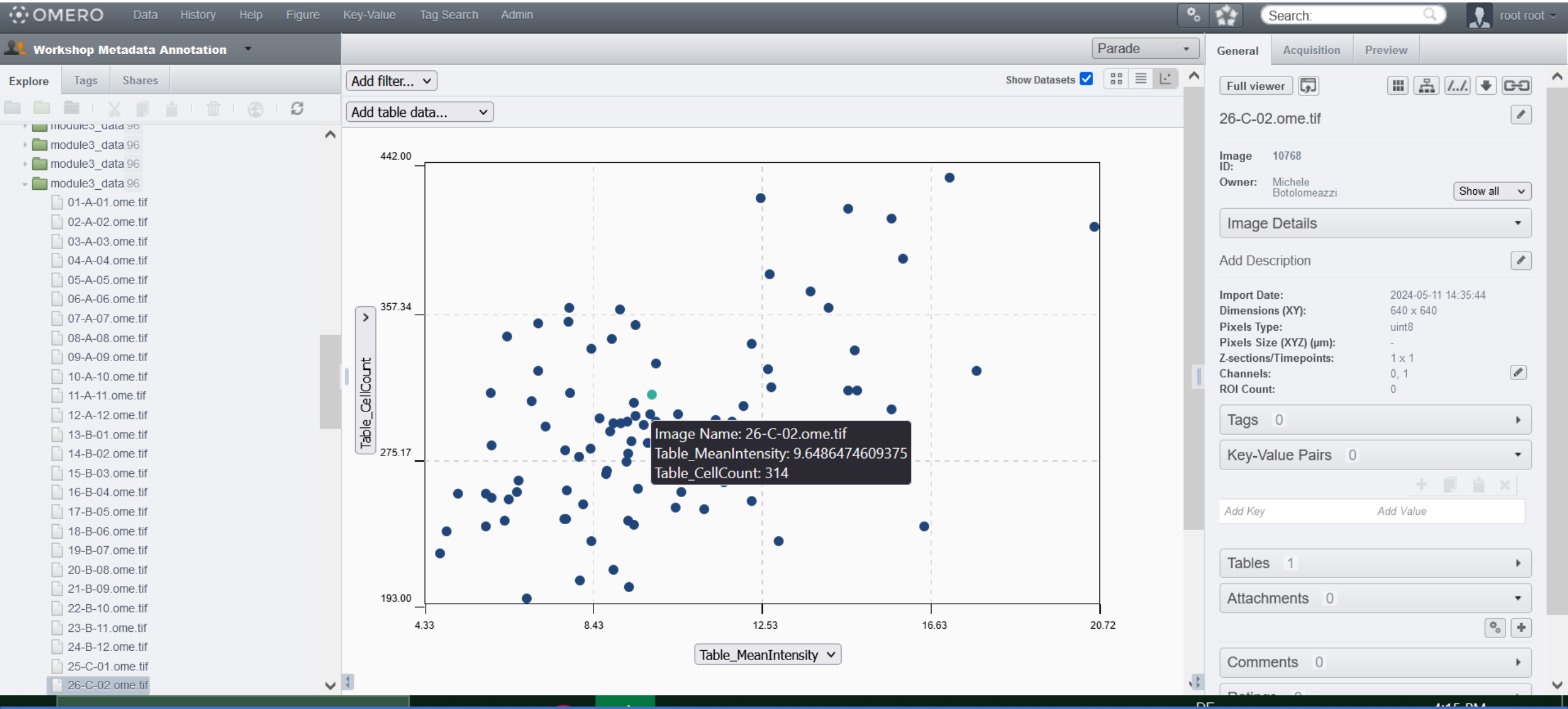


Filtering data with OMERO.parade



https://wiki-biop.epfl.ch/data-management/omero/omero-tables#tables-on-dataset-level

Viewing data with OMERO.parade



https://wiki-biop.epfl.ch/data-management/omero/omero-tables#tables-on-dataset-level

Practical 5: Projects and Beyond

Try to answer the following questions (no code needed):

- How would you make the macro work on all datasets in a project?

- I want the macro to process only specific datasets or images whitin the project, how could I select the images/datasets I need?

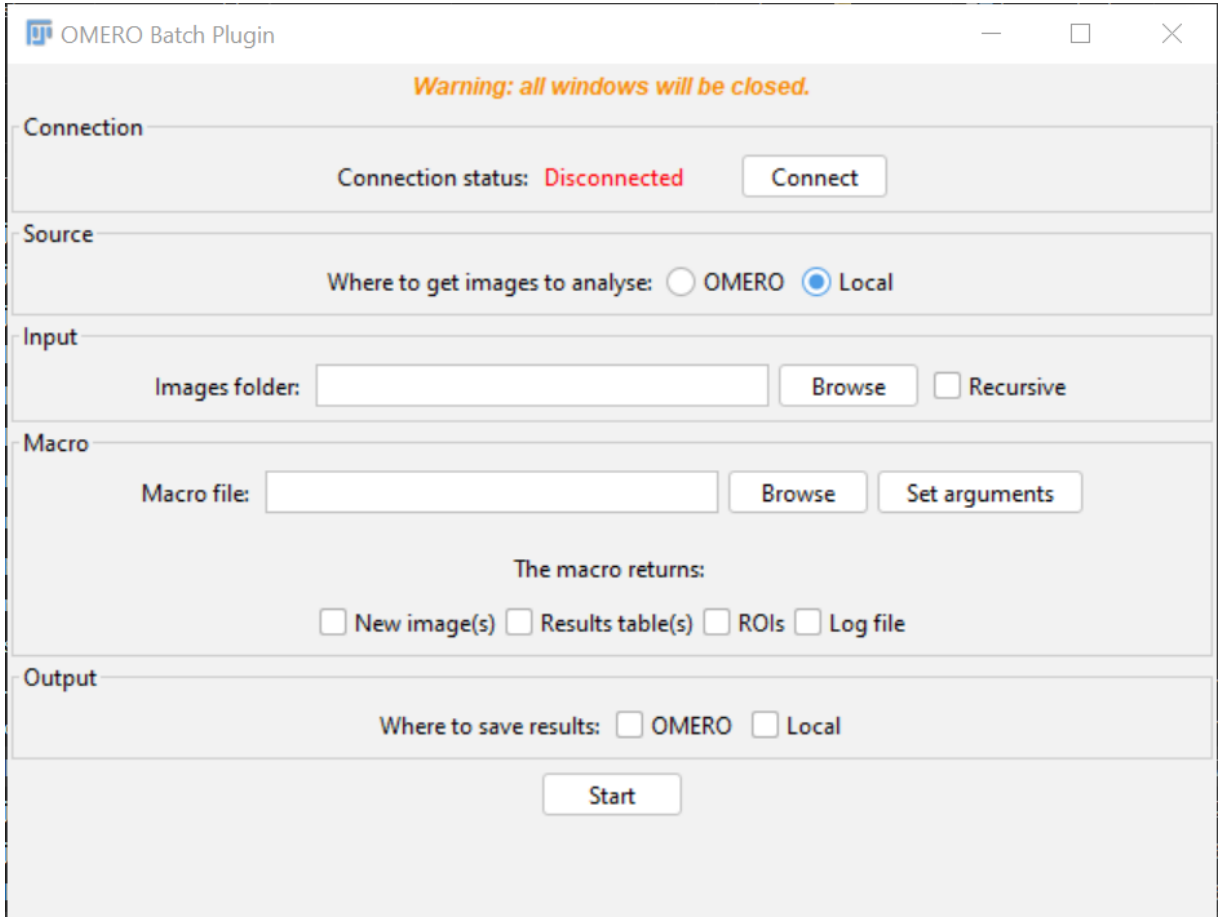
Can‘t I just use the macros I already have?

OMERO Batch Plugin for FiJi

OMERO Batch Plugin for FiJi

Runs a macro on a dataset of images (local or in OMERO) and saves the results (locally or in OMERO). Very efficient way to build OMERO based workflows from pre-existing macros.

https://github.com/GReD-Clermont/omero_batch-plugin



Pouchin P, Zoghlami R, Valarcher R *et al.* Easing batch image processing from OMERO: a new toolbox for ImageJ [version 2; peer review: 2 approved]. *F1000Research* 2022, **11**:392 (https://doi.org/10.12688/f1000research.110385.2)

I would still prefer a graphical interface

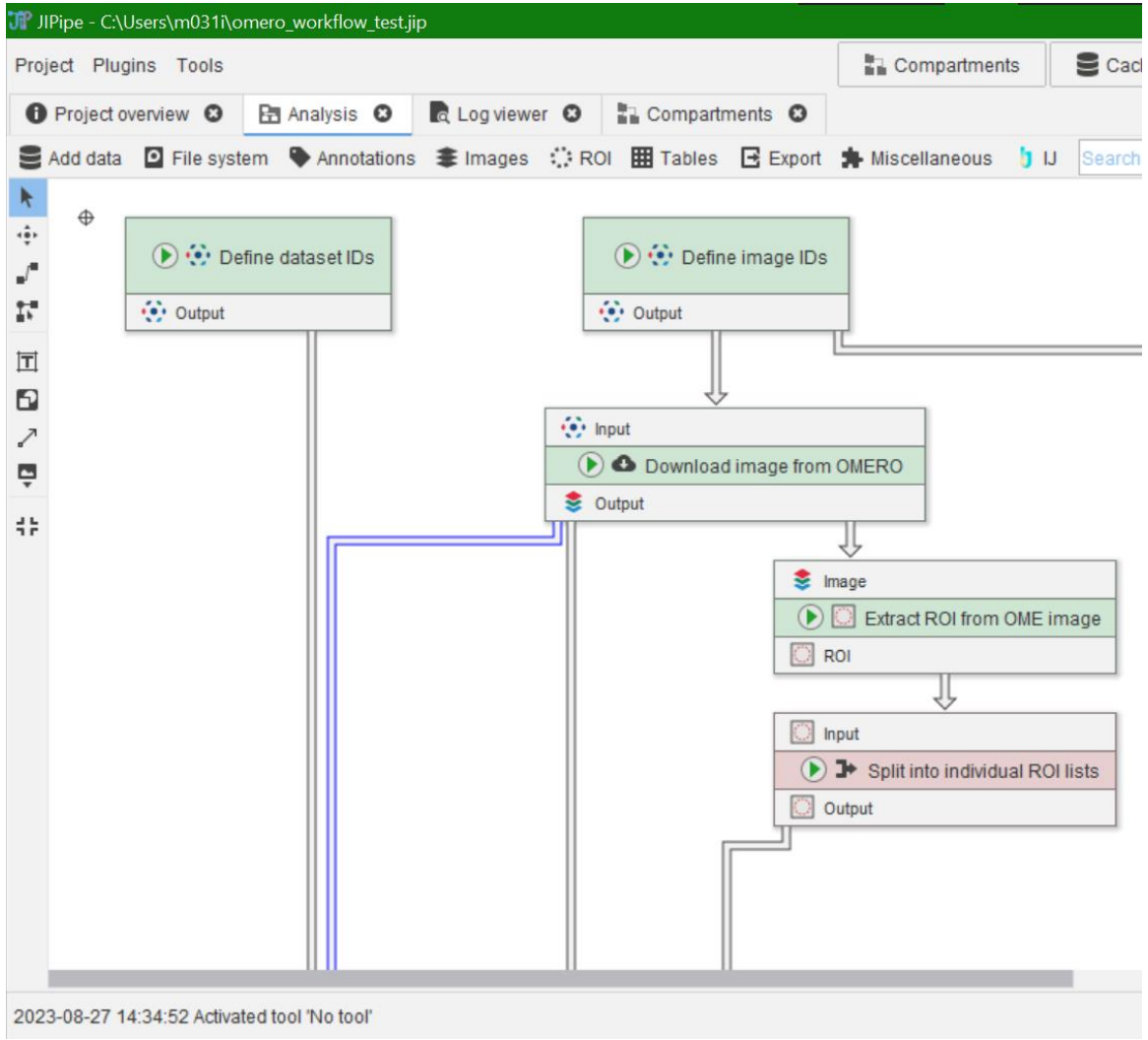
JiPipe: visual batch processing with Fiji

Plugin for visual macro programming, it can connect to OMERO and allows the export/import of:

- Images
- ROIs
- Tables

Very flexible, can support complex pipelines.

Very nice UI, and documentation.



Gerst, R., Cseresnyés, Z. & Figge, M.T. JIPipe: visual batch processing for ImageJ. *Nat Methods* **20**, 168–169 (2023). https://doi.org/10.1038/s41592-022-01744-4

Resources

Fiji

- **Documentation on all macro Functions**
https://wsr.imagej.net/developer/macro/functions.html

- **OMERO and Fiji macro tutorial**
https://omero-guides.readthedocs.io/en/latest/fiji/docs/threshold_scripting_macro_language.html

- **Fiji + Python Bioimage analysis tutorials**
https://www.youtube.com/watch?v=e-2DbkUwKk4&list=PL5ESQNfM5lc7SAMstEu082ivW4BDMvd0U

OMERO

- **BIOP guide for all things OMERO:**
https://wiki-biop.epfl.ch/en/data-management/omero

- **OMERO Traning material:**
Image Data Management with OMERO at the DKFZ - Overview
https://zenodo.org/records/8323588

- **Official OMERO user guides**
https://omero-guides.readthedocs.io/en/latest/

Summary

Different ways to use OMERO and Fiji for workflows that are:

- Reproducible
- Automated

For any questions please get in touch

I would like more…

OMERO python API
ezOMERO

Acknowledgements

OMERO Team at DKFZ

- Felix Bestvater
- Ivo Buchhalter
- Claudia Galuschka
- Alik Huseynov
- Jan-Philipp Mallm
- Elisa May
- Jordi Pujol
- Christian Schmidt

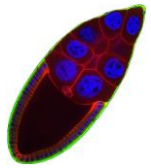
All our pilot users

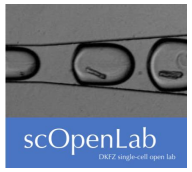
Core Facilities at DKFZ

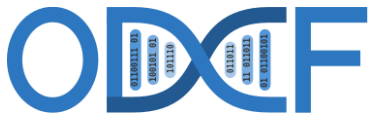
- Light Microscopy Facility
- IT Core Facility
- Omics IT and Data Management
- Single-Cell Open Lab

DFG Projects

- I3D:bio
- NFDI4BIOIMAGE







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