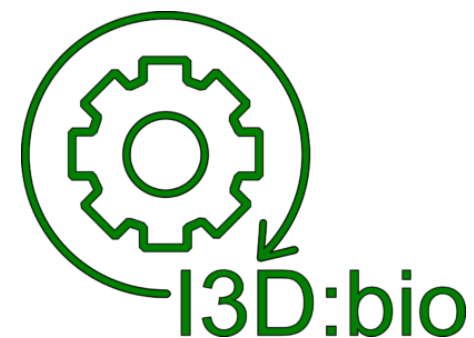


DEUTSCHES
KREBSFORSCHUNGSZENTRUM
IN DER HELMHOLTZ-GEMEINSCHAFT

OMERO and Fiji

Michele Bortolomeazzi

13/05/2024

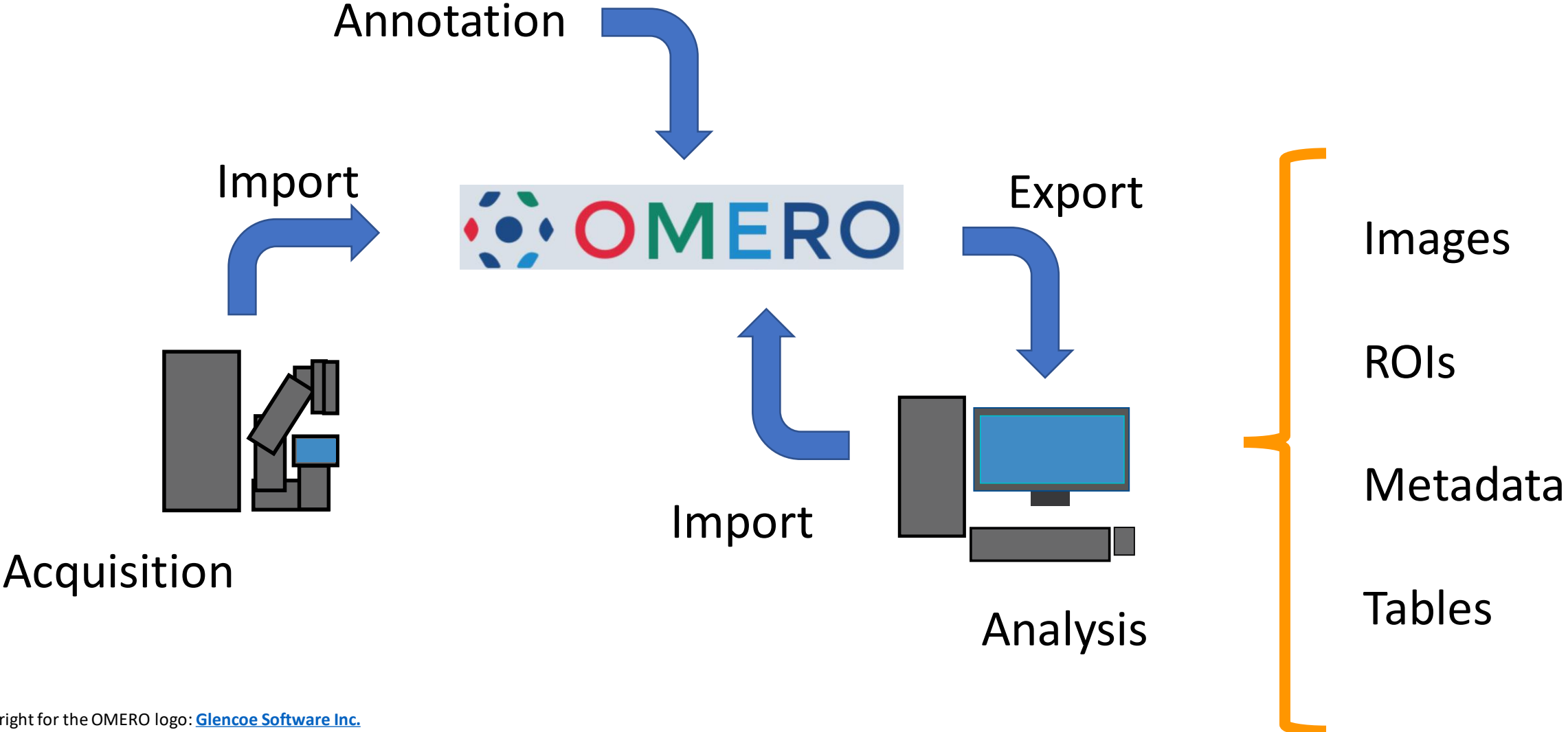


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NFDI4
BIOIMAGE

Image analysis workflow with OMERO



Copyright for the OMERO logo: [Glencoe Software Inc.](http://www.glencoesoftware.com)

Connections with Image Analysis Software

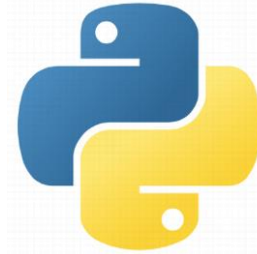
Non Exhaustive list of tools able to connect to OMERO directly or through plugins:



Fiji



napari



OMERO python API
ezOMERO



CellProfiler



Orbit



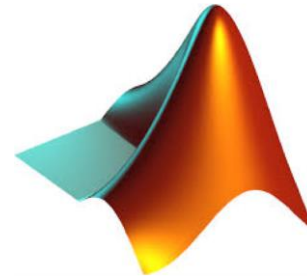
rOMERO Gateway



QuPath



Ilastick

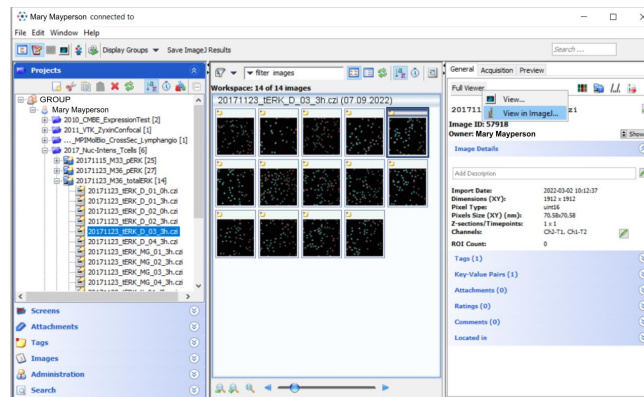


OMERO.matlab toolbox

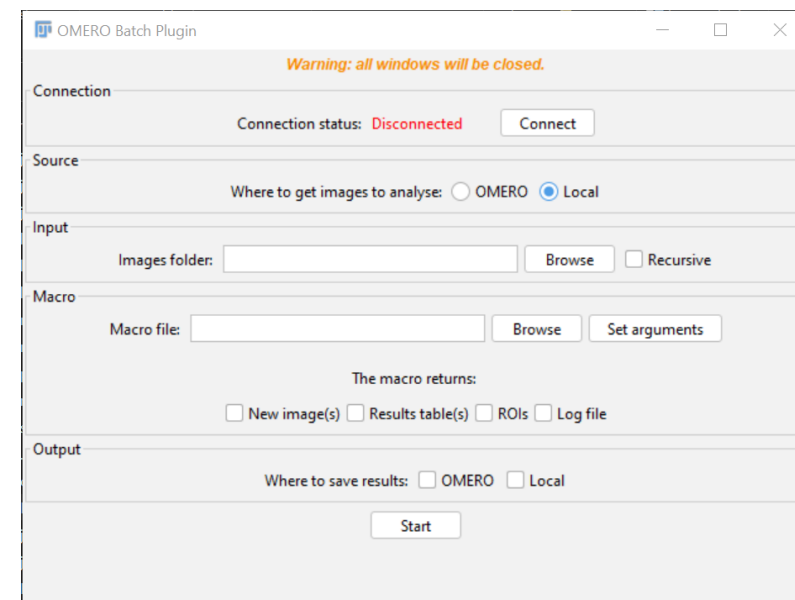
Fiji / ImageJ

Three ways of working with Fiji and OMERO:

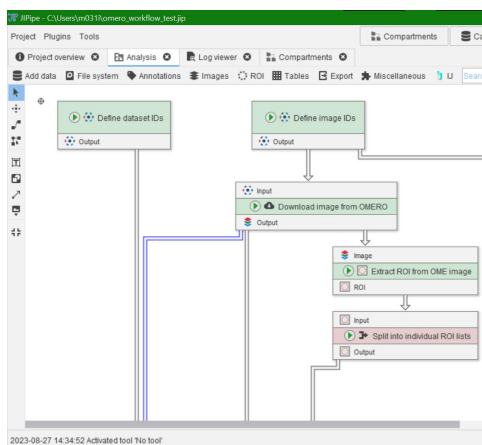
- **OMERO plugin for Fiji**



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



- **JIPipe**



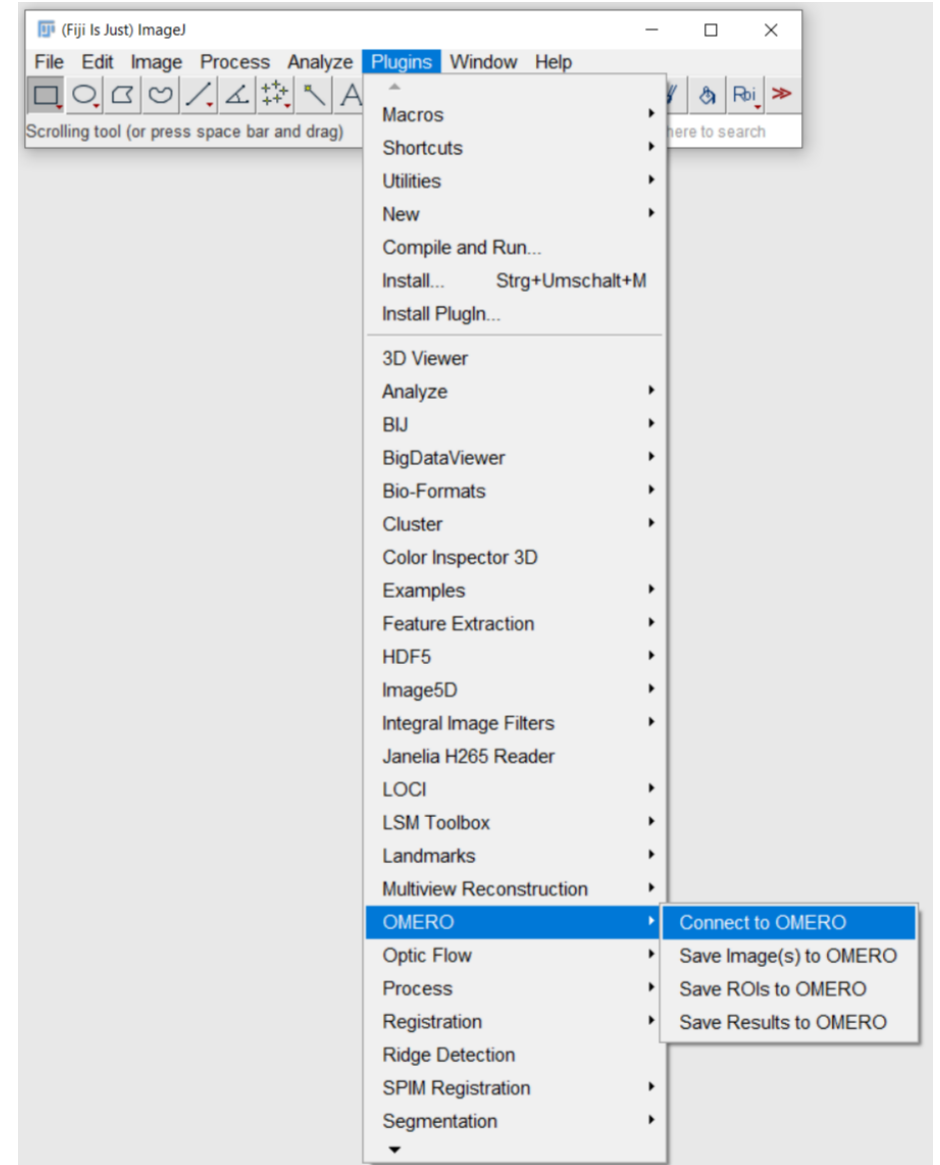
https://github.com/GReD-Clermont/omero_batch-plugin

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

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

Opening the OMERO Fiji Plugin

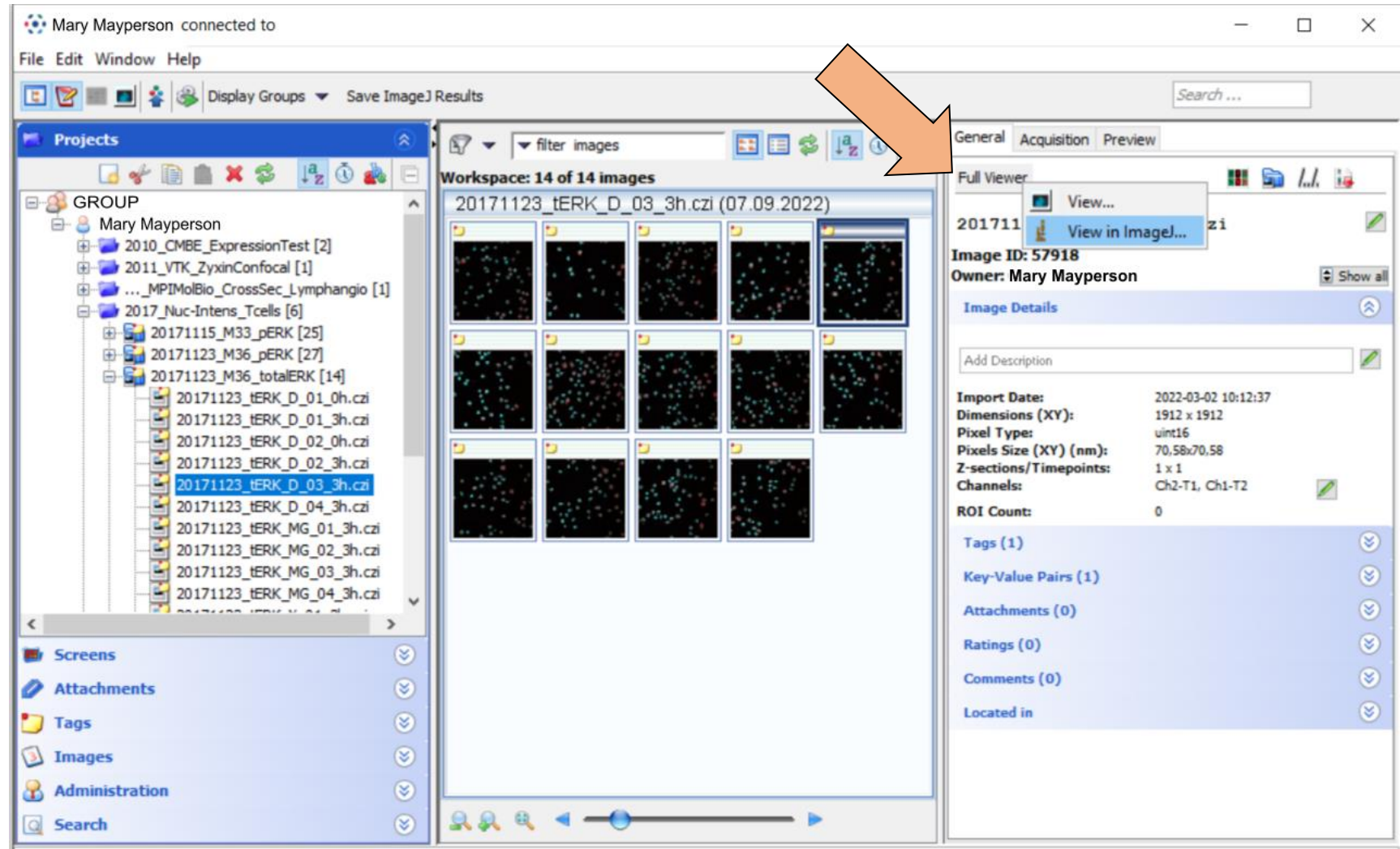
1. Open Fiji and go to *Plugins* → *OMERO* → *Connect to OMERO*
2. Log in to OMERO with your user credentials.



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

Selecting images in the OMERO Fiji Plugin

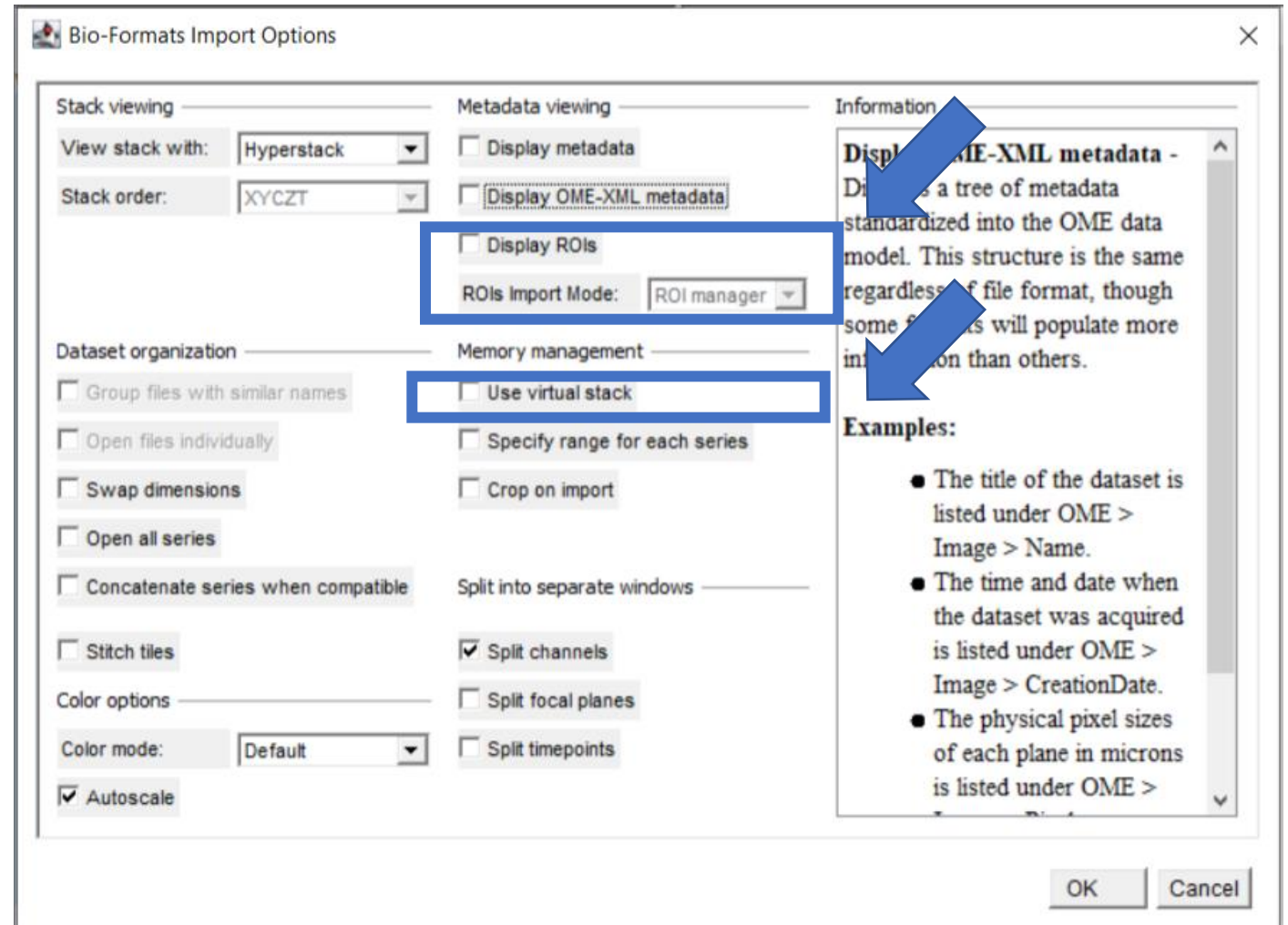
1. Select image(s) from the file tree
2. Open in Fiji by clicking Full Viewer and then View in ImageJ...



Opening images in the OMERO Fiji Plugin

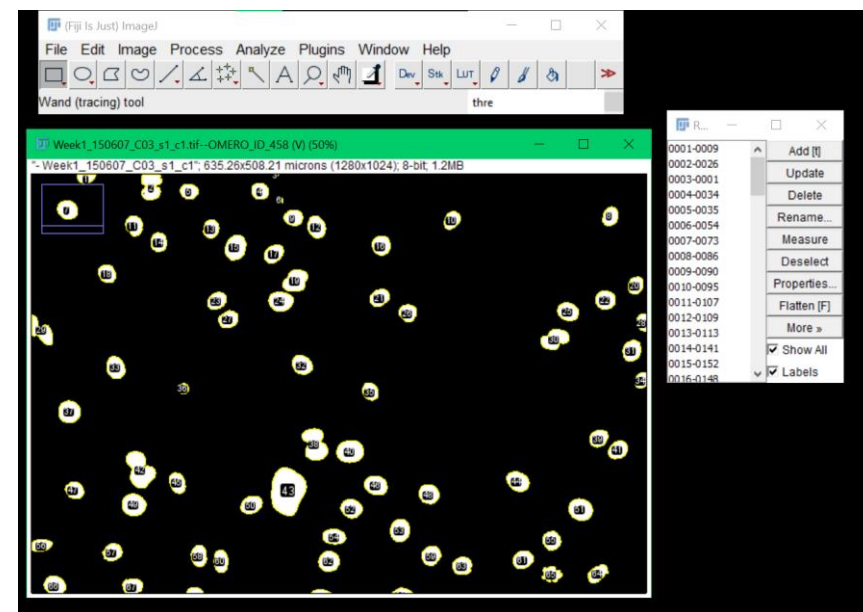
Use your preferred settings to open the image(s) as required for your work, I would recommend:

- 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

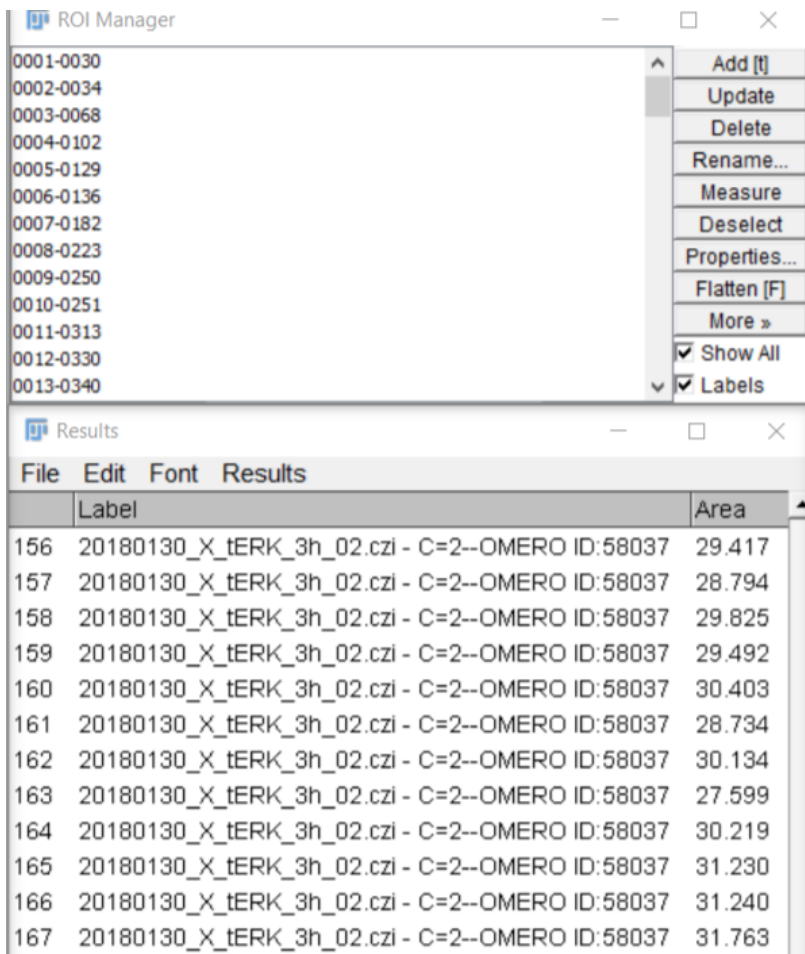
Saving ROIs and Results Back to OMERO

1

The screenshot shows the Fiji software interface. The 'Plugins' menu is open, and the 'OMERO' option is highlighted. The 'OMERO' submenu is also open, showing options: 'Connect to OMERO', 'Save Image(s) to OMERO', 'Save ROIs to OMERO', and 'Save Results to OMERO'. The 'Save ROIs to OMERO' option is selected. In the background, the 'ROI Manager' window is visible, showing a list of ROIs with their labels and areas. The 'Results' window is also visible, showing a table of results. A dialog box is open in the foreground, asking to 'Save Image(s) to OMERO' with options for 'Image from current image' and 'Images from all images'. The 'Save' section has 'ROI' and 'Measurements' checked. The 'Measurements File Name' field is empty.

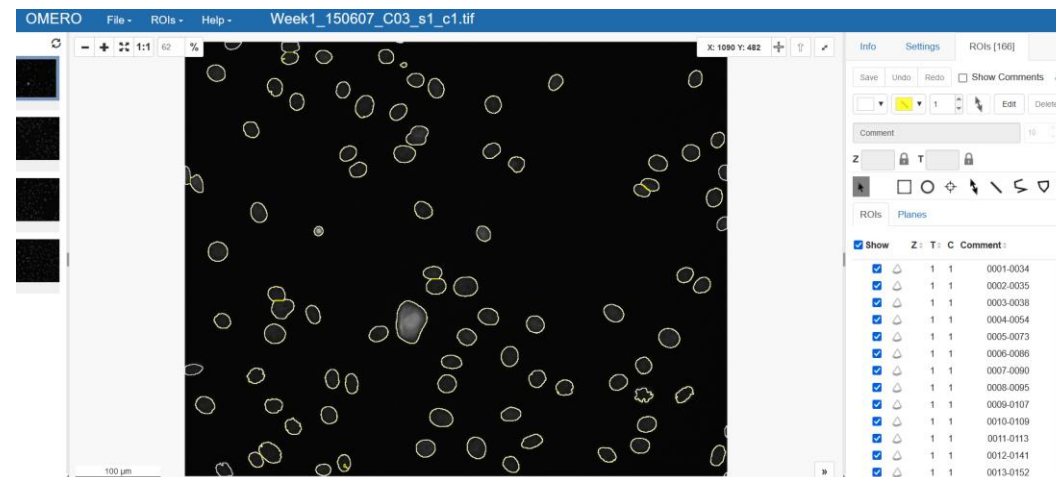
Label	Area
156	29.417
157	28.794
158	29.825
159	29.492
160	30.403
161	28.734
162	30.134
163	27.599
164	30.219
165	31.230
166	31.240
167	31.763

Viewing the ROIs and Results in OMERO

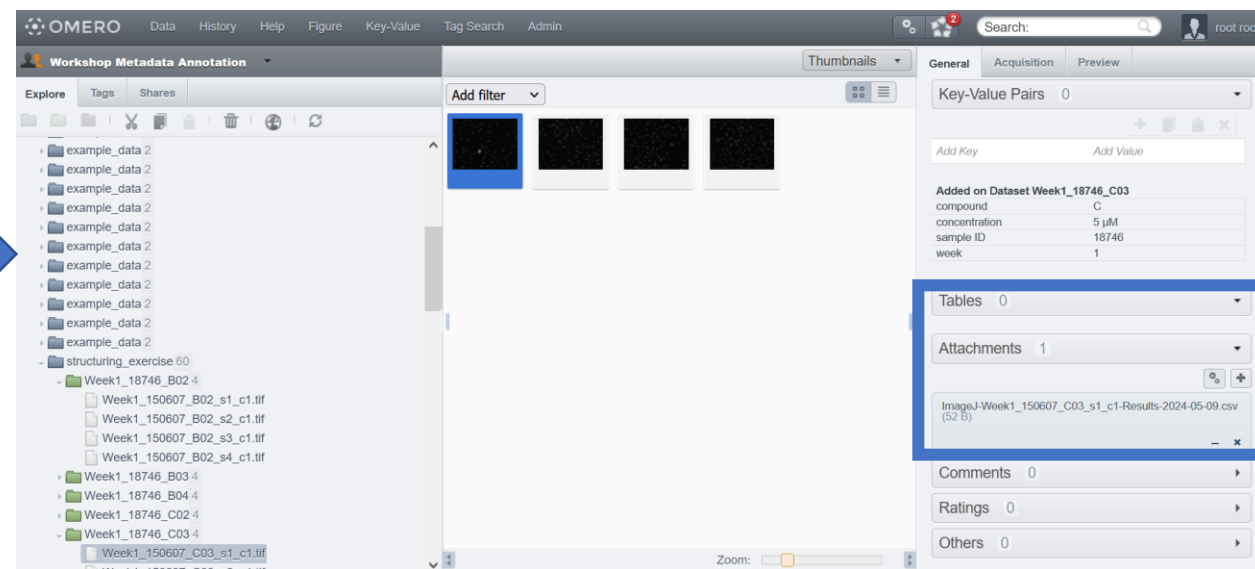


The ROI Manager window shows a list of ROIs with their IDs and a context menu with options: Add [I], Update, Delete, Rename..., Measure, Deselect, Properties..., Flatten [F], More >, Show All, and Labels. The Results window shows a table of results with columns for Label and Area.

Label	Area
156 20180130_X_TERK_3h_02.czi - C=2--OMERO ID:58037	29.417
157 20180130_X_TERK_3h_02.czi - C=2--OMERO ID:58037	28.794
158 20180130_X_TERK_3h_02.czi - C=2--OMERO ID:58037	29.825
159 20180130_X_TERK_3h_02.czi - C=2--OMERO ID:58037	29.492
160 20180130_X_TERK_3h_02.czi - C=2--OMERO ID:58037	30.403
161 20180130_X_TERK_3h_02.czi - C=2--OMERO ID:58037	28.734
162 20180130_X_TERK_3h_02.czi - C=2--OMERO ID:58037	30.134
163 20180130_X_TERK_3h_02.czi - C=2--OMERO ID:58037	27.599
164 20180130_X_TERK_3h_02.czi - C=2--OMERO ID:58037	30.219
165 20180130_X_TERK_3h_02.czi - C=2--OMERO ID:58037	31.230
166 20180130_X_TERK_3h_02.czi - C=2--OMERO ID:58037	31.240
167 20180130_X_TERK_3h_02.czi - C=2--OMERO ID:58037	31.763



The OMERO main window displays a microscopy image with numerous white circular ROIs overlaid. The title bar shows 'Week1_150607_C03_s1_c1.tif'. The right sidebar shows the 'ROIs [166]' panel with a list of ROIs and their properties.



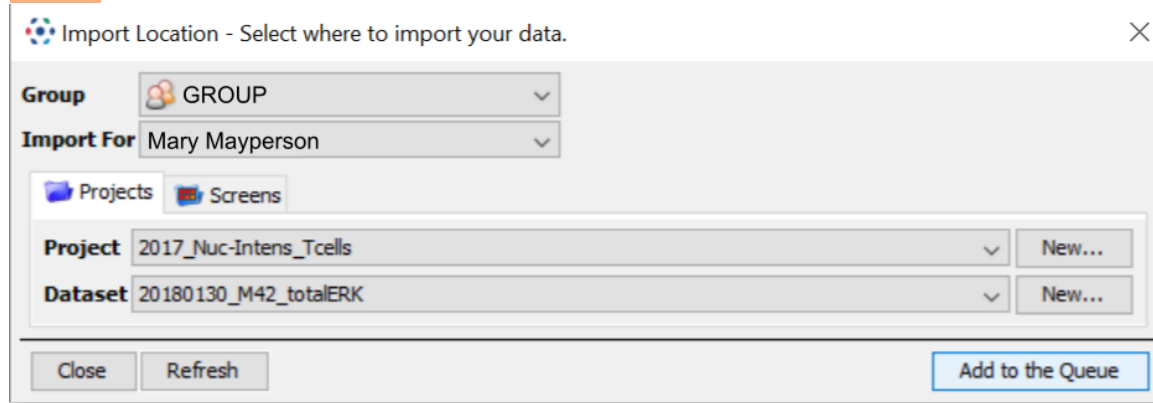
The OMERO Workshop Metadata Annotation window shows a file tree on the left with 'Week1_150607_C03_s1_c1.tif' selected. The right sidebar shows the 'General' tab with 'Key-Value Pairs' and 'Attachments' sections. The 'Attachments' section is highlighted with a blue box, showing a CSV file: 'ImageJ-Week1_150607_C03_s1_c1-Results-2024-05-09.csv (52 B)'.



Uploading images back to OMERO

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

1



Import Location - Select where to import your data.

Group: GROUP

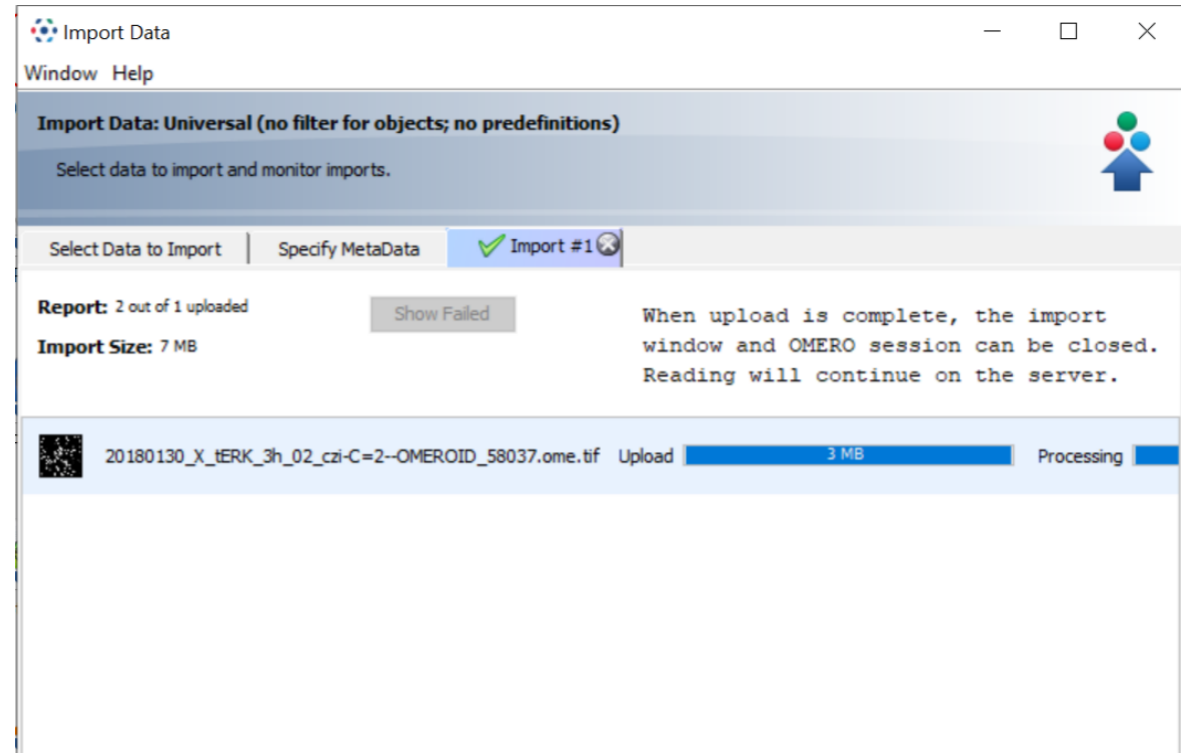
Import For: Mary Mayperson

Project: 2017_Nuc-Intens_Tcells

Dataset: 20180130_M42_totalERK

Close Refresh Add to the Queue

2



Import Data

Window Help

Import Data: Universal (no filter for objects; no predefinitions)

Select data to import and monitor imports.

Select Data to Import | Specify MetaData | Import #1

Report: 2 out of 1 uploaded

Import Size: 7 MB

Show Failed

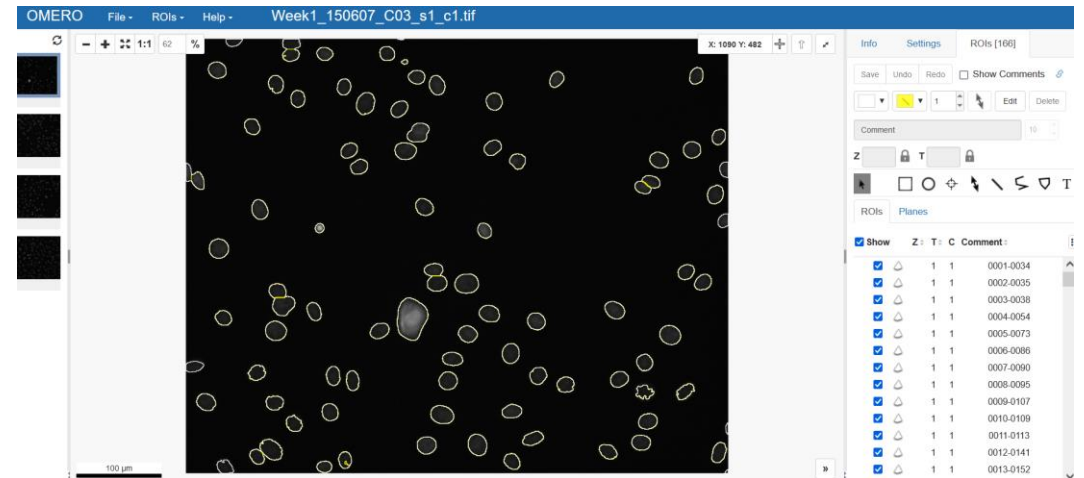
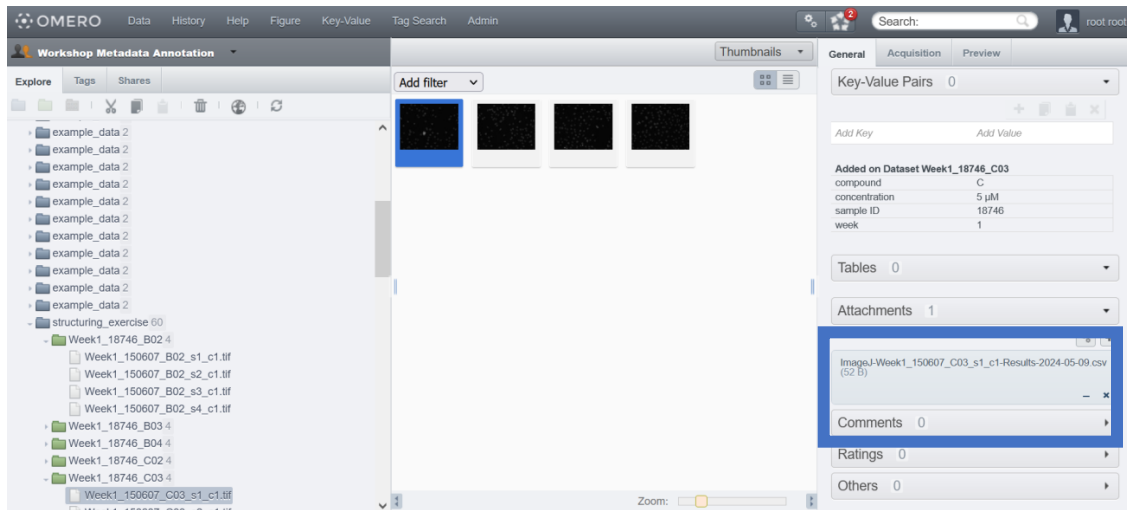
When upload is complete, the import window and OMERO session can be closed. Reading will continue on the server.

20180130_X_tERK_3h_02_czi-C=2--OMEROID_58037.ome.tif Upload 3 MB Processing



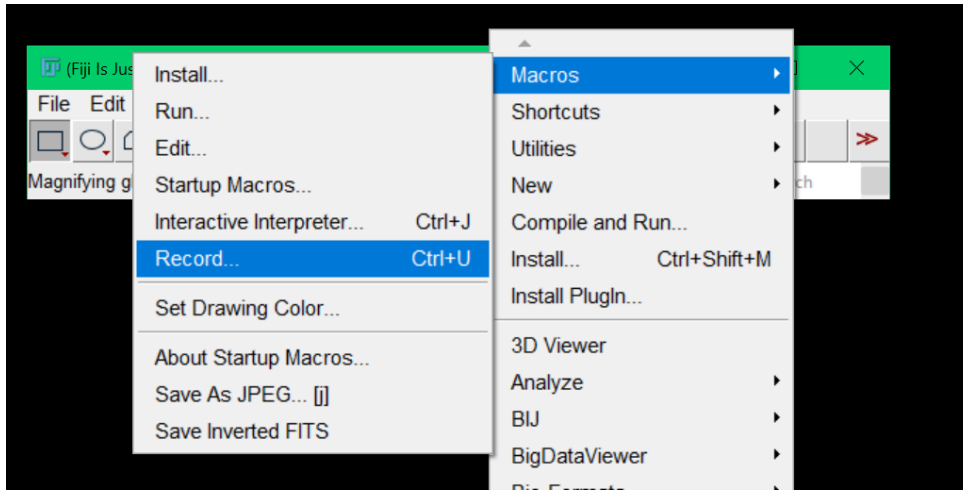
Practical 2: Let's save our results.

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?**

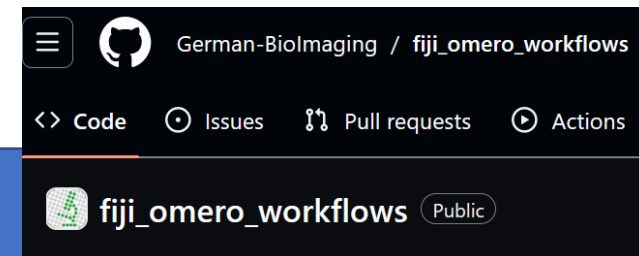
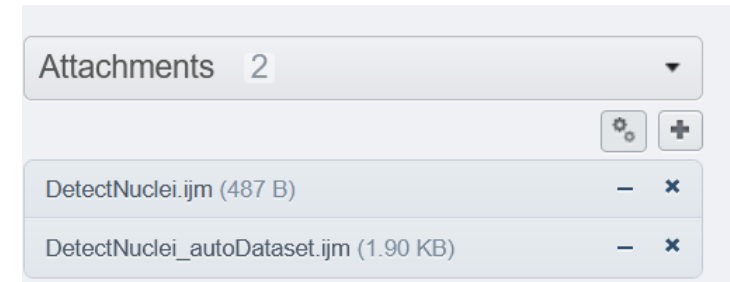
From manual workflows to Macros



```
File Edit Language Templates Run Tools Window Options Help *Analyse_dataset_and_save_rois.i... - □ X
File Explorer Outline < *DetectNuclei.ijm *Analyse_dataset_and_save_rois.ijm
m031i
1 // @String(Label="Username") USERNAME
2 // @String(Label="Password", style="password", persist=false) PASSWORD
3 // @String(Label="Host", value="ws://workshop.openmicroscopy.org/omero-ws") H
4 // @Integer(Label="Port", value=443) PORT
5 // @Integer(Label="Dataset ID", value=2331) dataset_id
6
7 run("OMERO Extensions");
8
9 connected = Ext.connectToOMERO(HOST, PORT, USERNAME, PASSWORD);
10
11 setBatchMode(true);
12 if(connected == "true") {
13     images = Ext.list("images", "dataset", dataset_id);
14     image_ids = split(images, ",");
15
16     for(i=0; i<image_ids.length; i++) {
17         ij_id = Ext.getImage(image_ids[i]);
18         ij_id = parseInt(ij_id);
19         roiManager("reset");
20         run("8-bit");
21         run("Auto Threshold", "method=MaxEntropy stack");
22         run("Analyze Particles...", "size=10-Infinity pixel display clear add
23         run("Set Measurements...", "area mean standard modal min centroid cent
24         roiManager("Measure");
25         nROIs = Ext.saveROIs(image_ids[i], "");
26         print("Image " + image_ids[i] + ": " + nROIs + " ROI(s) saved.");
27         roiManager("reset");
28         close("Results");
29     }
30 }
```



- 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!

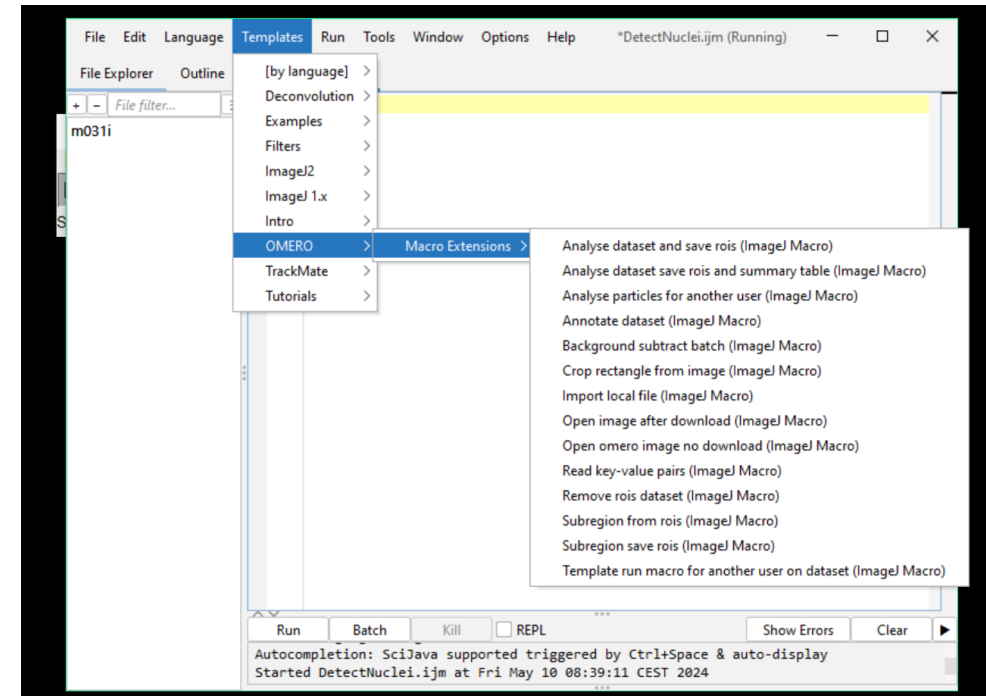
Working with OMERO in FiJI Macros

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     ij_id = Ext.getImage(image_id);
17
18     
19
20 }
21
22
23 Ext.disconnect();
24
```

Add your image processing
code here

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

Disconnect from OMERO

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

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

Make the user input the credentials and address for OMERO

```
6
7 run("OMERO Extensions");
```

Activate the OMERO plugin

```
8
9 connected = Ext.connectToOMERO(HOST, PORT, USERNAME, PASSWORD);
```

Connect to OMERO

```
10
11 if(connected == "true") {
```

Check if the connection worked

```
12
13
14 images = Ext.list("images", "dataset", dataset_id);
15 image_ids = split(images, ",");
```

Get Ids of all the images in the dataset

```
16
17
18 for(i=0; i<image_ids.length; i++) {
19     ij_id = Ext.getImage(image_ids[i]);
20     ij_id = parseInt(ij_id);
```

Get the image from OMERO

```
21
22     roiManager("reset");
23     close("Results");
24     selectImage(ij_id);
25     close();
```

Add your image processing code here

Close image and remove all ROIs for the next cycle

Repeat for every image in the dataset

```
26
27 }
28
29 table_name = "Summary_from_Fiji";
30 Ext.saveTable(table_name, "Dataset", dataset_id);
```

Attach the results to the dataset

```
31 }
32
33 Ext.disconnect();
```

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

The screenshot shows the OMERO interface with the 'Parade' feature being configured. A dropdown menu is open over the 'Parade' button, showing options like 'Thumbnails', 'Auto Tag', and 'Parade'. A blue arrow points from the 'Parade' button to a detailed view of the 'Parade' settings, which includes a list of filters and a 'Show Datasets' checkbox.

Key-value Pairs

compound	B
concentration	5 μ M
sample ID	18746
week	1

Added by: Tom Boissonnet

Attachments 2

Show Datasets

Table Image

Table_ImageName

Table_TotalArea

Table_MeanIntensity

Table_CellCount

WEEK1_18746_C02

Parade

Show Datasets

Filtering data with OMERO.parade

OMERO Data History Help Figure Key-Value Tag Search Admin

Search: root root

Workshop Metadata Annotation

Parade

General Acquisition Preview

module3_data

Dataset ID: 2059
Owner: Michele Botolomeazzi

Dataset Details

Add Description

Creation Date: 2024-05-11 14:35:44

Tags 0

Key-Value Pairs 0

Add Key Add Value

Attachments 1

2024-05-11_16-13-04_Summary_from_Fiji (74.63 KB)

Comments 0

Ratings 0










Others 0

Explore Tags Shares

Add filter... Add table data...

Show Datasets

module3_data

Name	Table_MeanIntensity	Table_CellCount
 01-A-01.ome.tif	20.723646394375	413
 02-A-02.ome.tif	14.55166748046875	424
 03-A-03.ome.tif	17.776748046875	328
 04-A-04.ome.tif	5.810168015625	315
 05-A-05.ome.tif	12.14799560546875	344
 06-A-06.ome.tif	11.938857421875	307
 07-A-07.ome.tif	11.09012939453125	289
 08-A-08.ome.tif	15.63352294921875	305
 09-A-09.ome.tif	9.7438134765625	298

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



Viewing data with OMERO.parade

The screenshot displays the OMERO.parade interface. On the left, a file explorer shows a list of images, with '26-C-02.ome.tif' selected. The main area features a scatter plot with 'Table_CellCount' on the y-axis (ranging from 193.00 to 442.00) and 'Table_MeanIntensity' on the x-axis (ranging from 4.33 to 20.72). A tooltip for a data point provides the following information:

- Image Name: 26-C-02.ome.tif
- Table_MeanIntensity: 9.6486474609375
- Table_CellCount: 314

On the right, the 'General' tab of the image details panel is visible, showing the following information for '26-C-02.ome.tif':

- Image ID: 10768
- Owner: Michele Botolomeazzi
- Import Date: 2024-05-11 14:35:44
- Dimensions (XY): 640 x 640
- Pixels Type: uint8
- Z-sections/Timepoints: 1 x 1
- Channels: 0, 1
- ROI Count: 0

<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 within the project, how could I select the images/datasets I need?

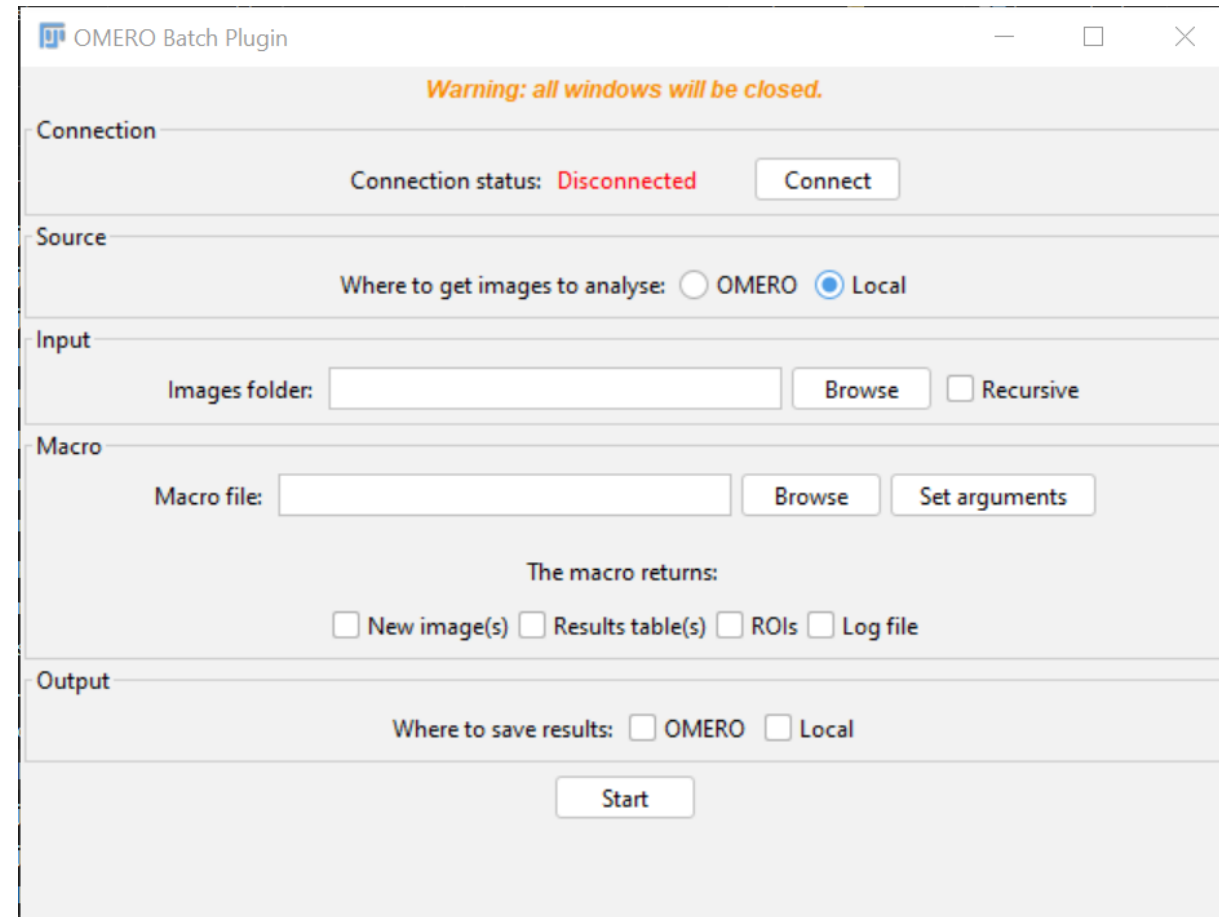
**Can't I just use the macros
I already have?**

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, Zoghلامي 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**

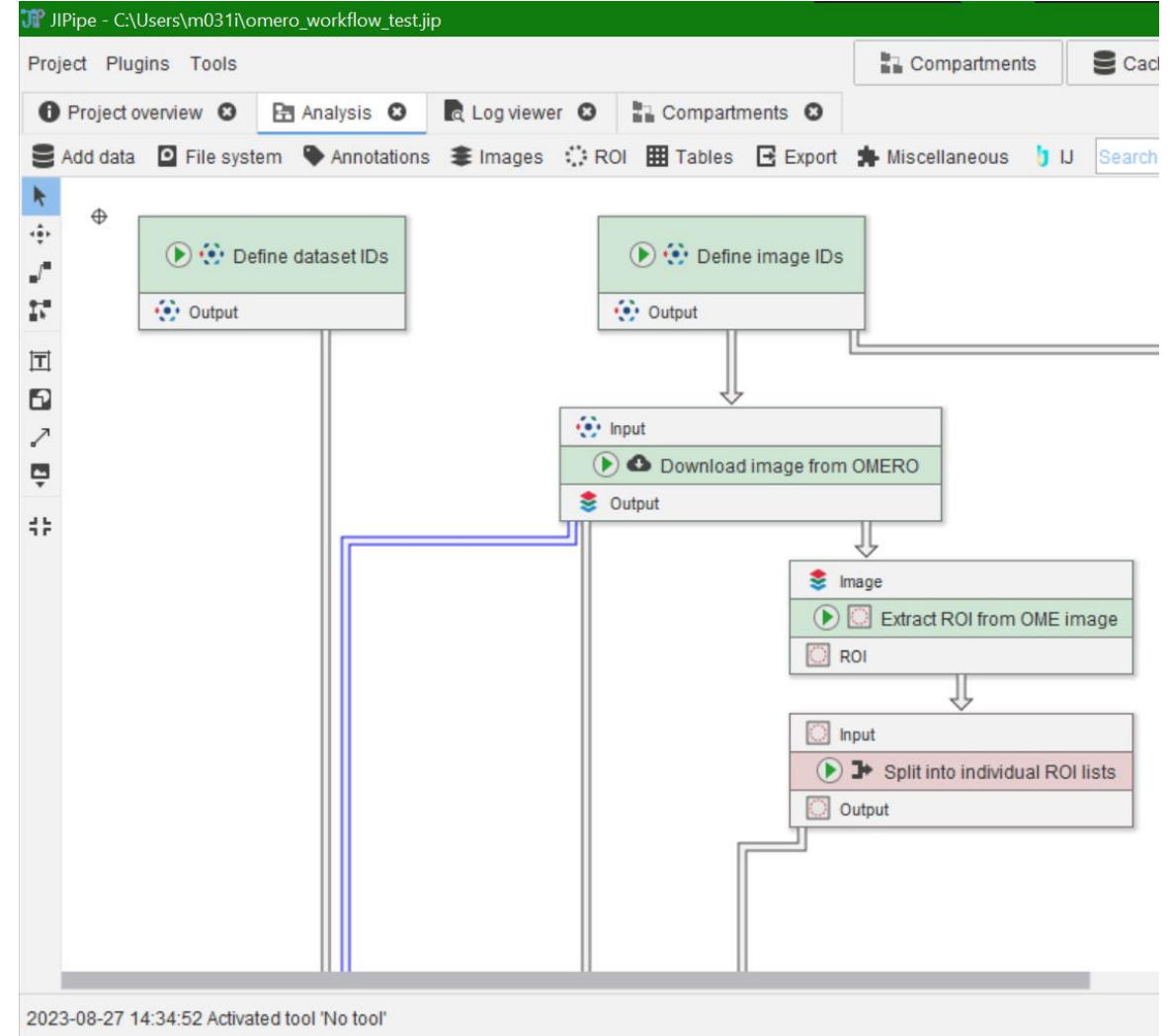
JiPipe: visual batch processing with Fiji

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.



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 Training material:**
[Image Data Management with OMERO at the DKFZ - Overview](https://zenodo.org/records/8323588)
<https://zenodo.org/records/8323588>
- **Official OMERO user guides**
<https://omero-guides.readthedocs.io/en/latest/>

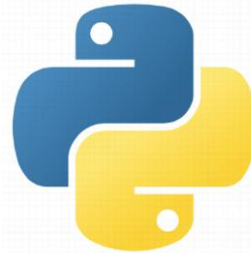
Summary

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

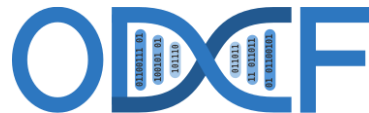
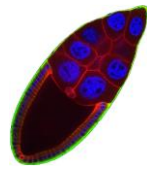
- Felix Bestvater
- Ivo Buchhalter
- Claudia Galuschka
- Alik Huseynov
- Jan-Philipp Mallm
- Elisa May
- Jordi Pujol
- Christian Schmidt

All our pilot users



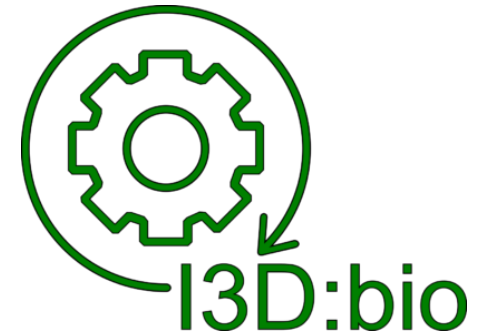
Core Facilities at DKFZ

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



DFG Projects

- I3D:bio
- NFDI4BIOIMAGE



NFDI4
BIOIMAGE

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