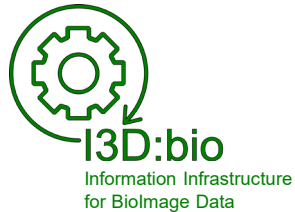


# Research Data Management for Bioimage Data at the HHU

## How to connect Fiji and OMERO?

Tom Boissonnet



Adapted from: Schmidt C., Bortolomeazzi M., Boissonnet T., Fortmann-Grote C. *et al.* (2023). I3D:bio's OMERO training material: Re-usable, adjustable, multi-purpose slides for local user training. Zenodo. DOI: 10.5281/zenodo.8323588  
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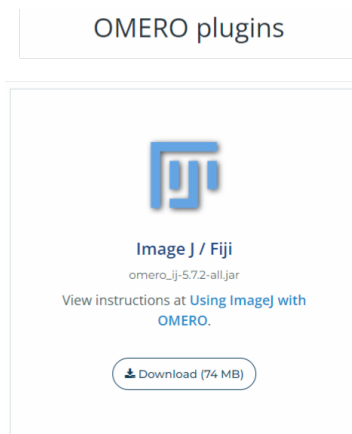


# Connect Fiji and OMERO (1/2)

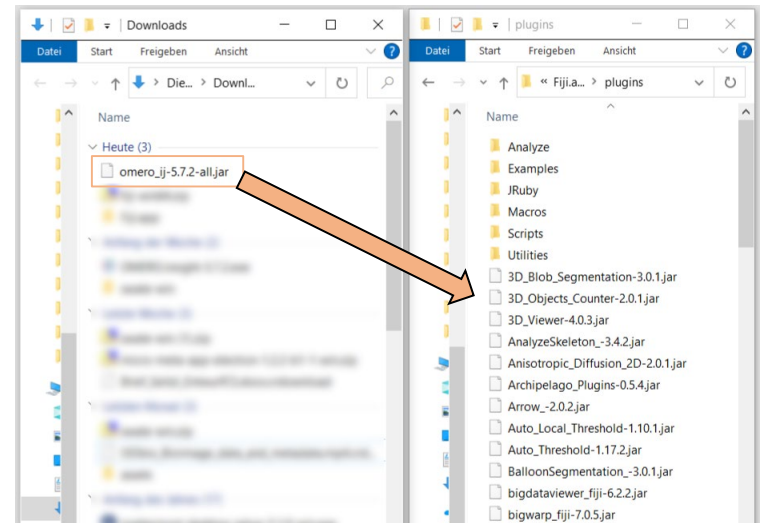
## Prerequisite:

You have downloaded Fiji (<https://fiji.sc>) and have access to the OMERO instance (direct or VPN)

1. Download the OMERO plugin for Fiji from the OME downloads website: <https://www.openmicroscopy.org/omero/downloads>



2. Move the *omero-ij-x.x.x-all.jar* file to the *Plugins* folder of your Fiji application

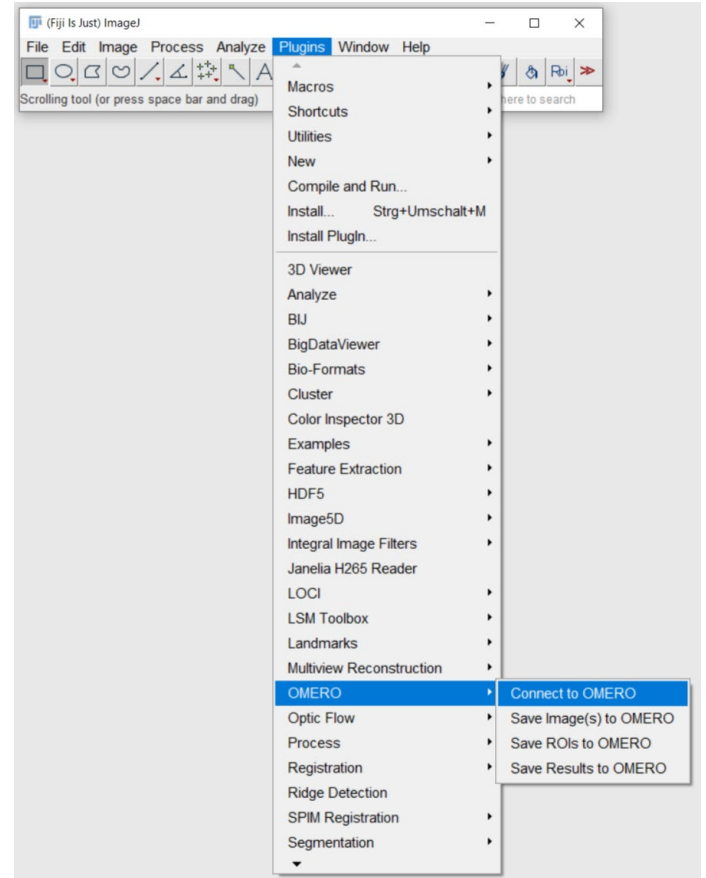


## Connect Fiji and OMERO (2/2)

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

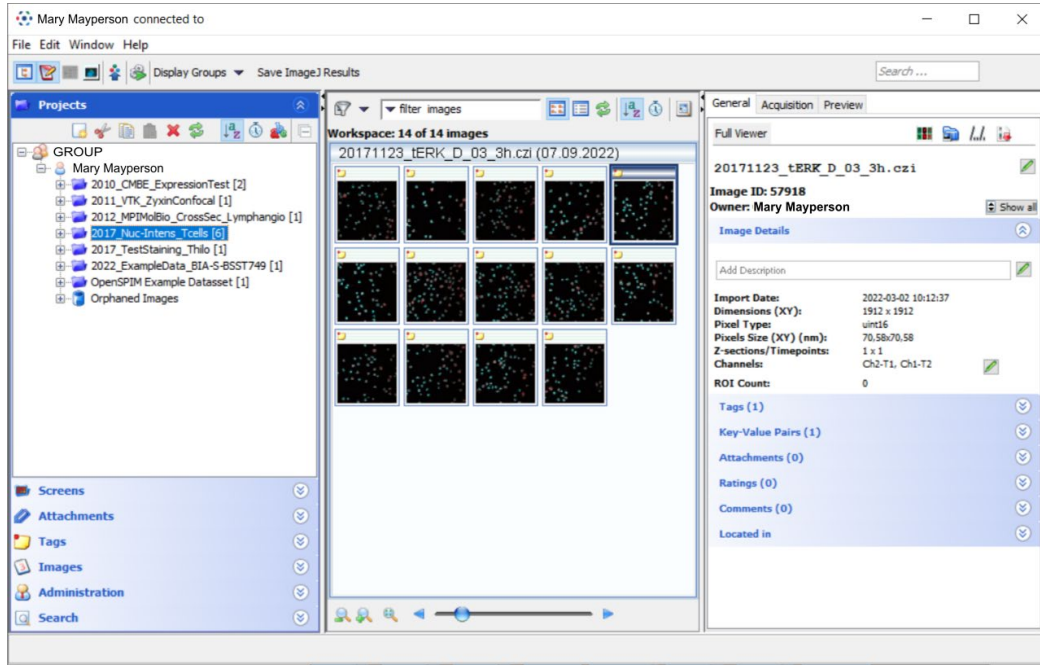


The Fiji-OMERO plugin looks almost precisely like OMERO.insight, but is, in fact, part of the open Fiji application

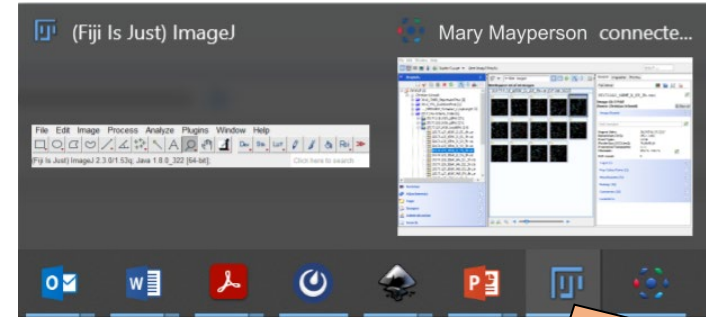


# OMERO plugin for Fiji versus OMERO.insight

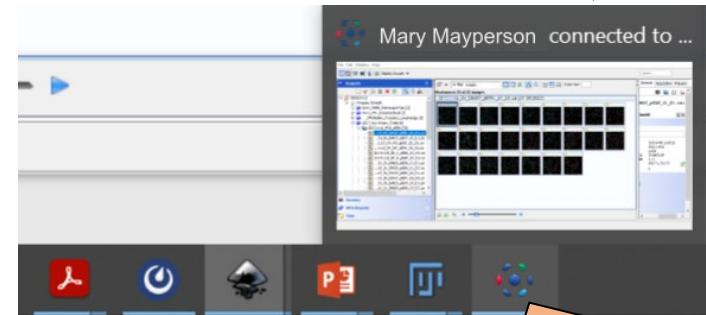
NOTE: The window looks similar to OMERO.insight, but it is a different application. For example, OMERO.insight has no View in ImageJ function [1] nor allows Save ImageJ Results.



You can distinguish the applications by their appearance in the task bar.



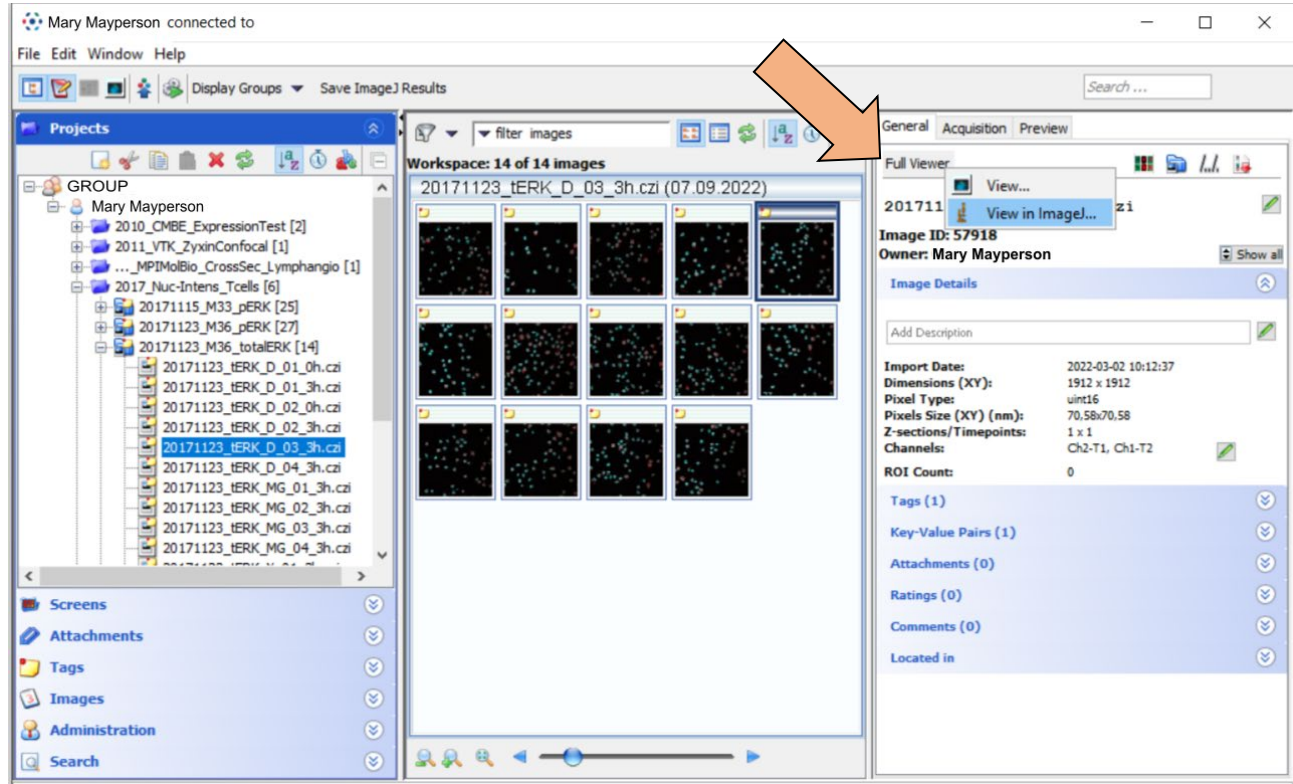
OMERO plugin for Fiji



OMERO.insight

# Select image(s) to open in Fiji (1/2)

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

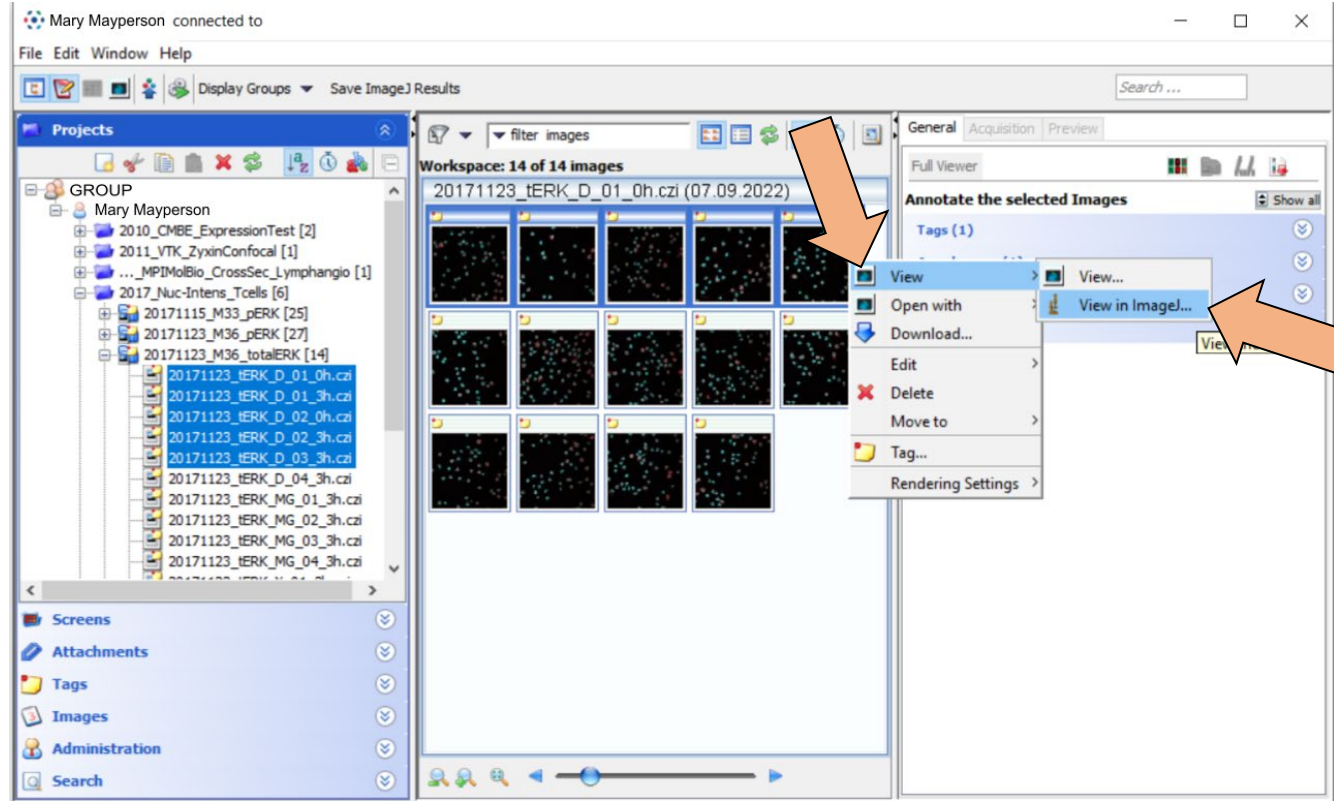


# Select image(s) to open in Fiji (2/2)

OR

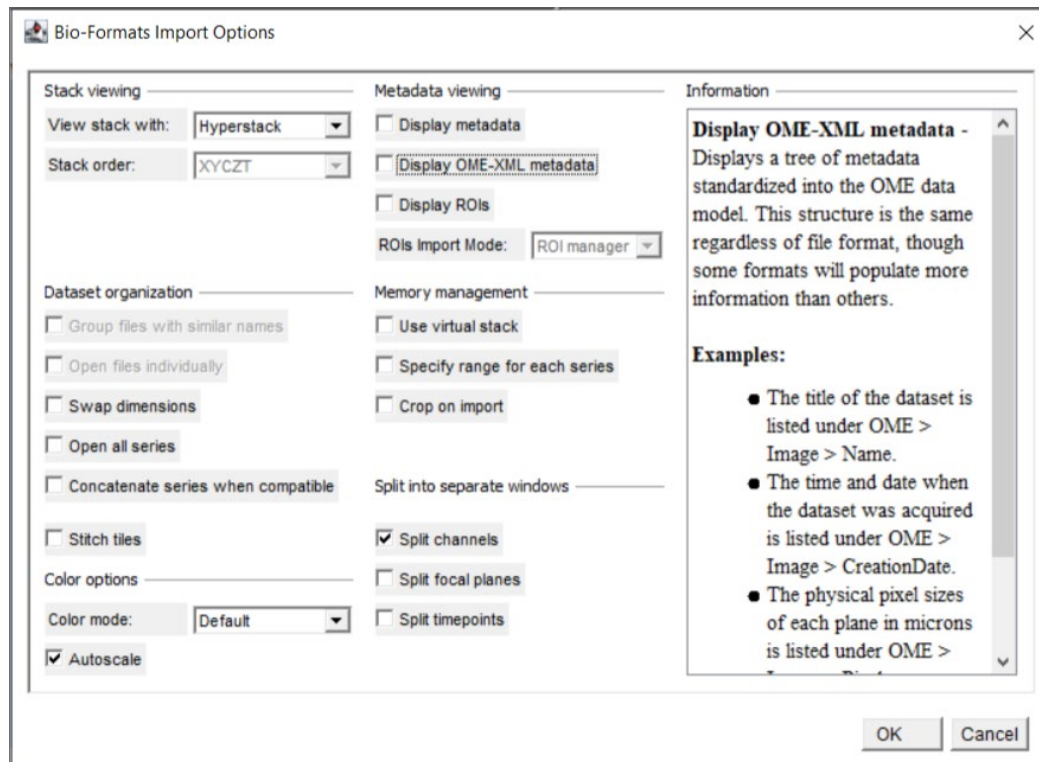
3. Open image(s) by right-click **View** and then **View in ImageJ...**

*(or double-click)*



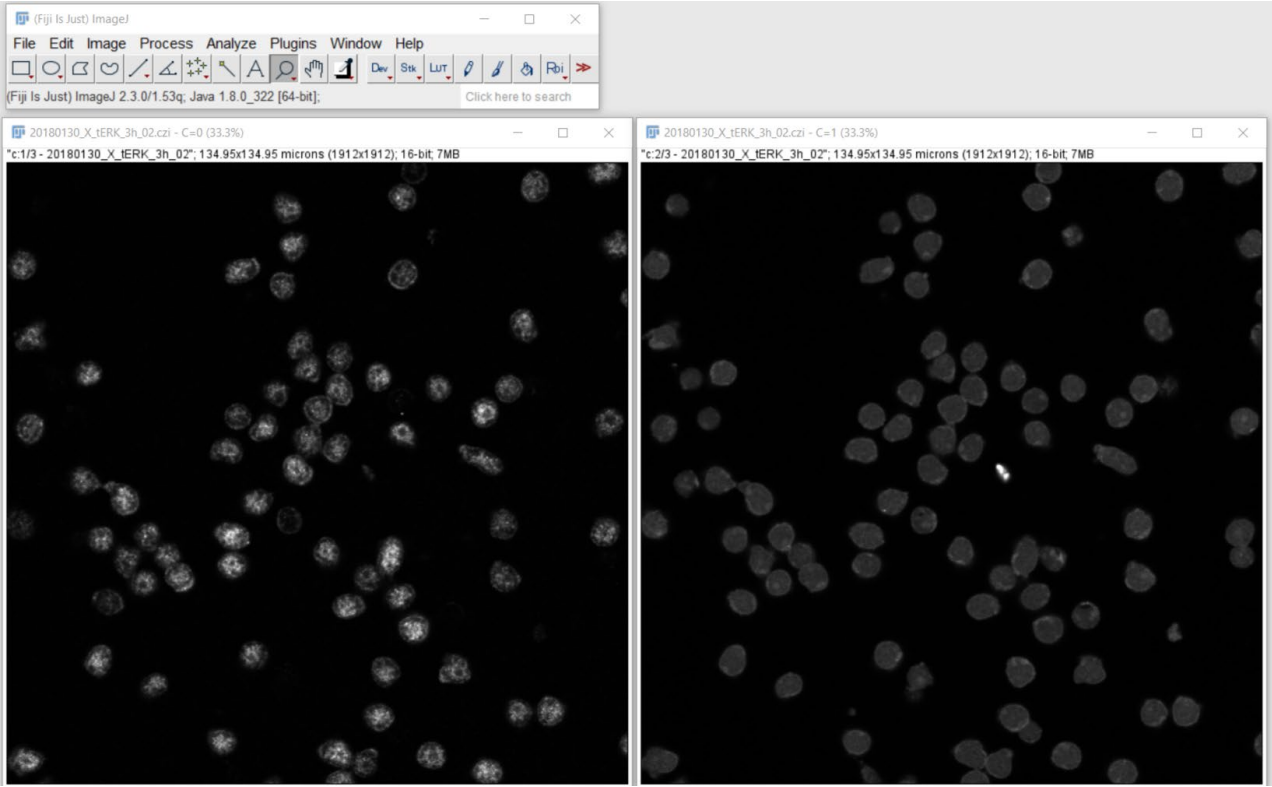
# Choose settings for loading the image(s) in Fiji

Use your preferred settings to open the image(s) as required for your work





# View your images in Fiji and work with the image for processing and analysis

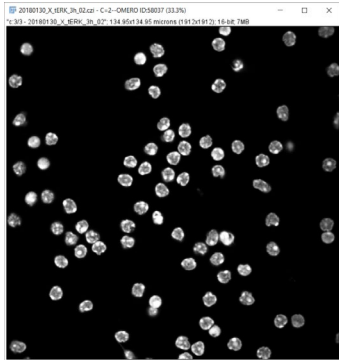




# Example – processing & analysis workflow to segment and count nuclei

Perform your workflow in Fiji

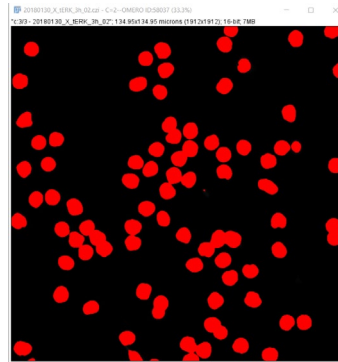
(here: segmentation and cell counting based on nuclear staining with DAPI)



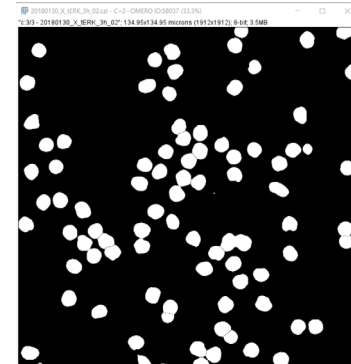
Gaussian blur



Thresholding  
(Huang)



Watershed



Analyze  
Particles

# Example – processing & analysis workflow to segment and count nuclei

Perform your workflow in Fiji  
(here: segmentation and cell counting based on nuclear staining with DAPI)

20180130\_X\_IERK\_3h\_02.czi - C=2--OMERO ID:58037 (33.3%)  
\*c:/3/ - 20180130\_X\_IERK\_3h\_02\*; 134.95x134.95 microns (1912x1912); 8-bit; 3.5MB

ROI Manager

- 0001-0030
- 0002-0034
- 0003-0068
- 0004-0102
- 0005-0129
- 0006-0136
- 0007-0182
- 0008-0223
- 0009-0250
- 0010-0251
- 0011-0313
- 0012-0330
- 0013-0340

Count Masks of 20180130\_X\_IERK\_3h\_02.czi - C=2--OMERO ID:58037 (33.3%)  
134.95x134.95 microns (1912x1912); 16-bit; 7MB

Results

Label	Area
156 20180130_X_IERK_3h_02.czi - C=2--OMERO ID:58037	29.417
157 20180130_X_IERK_3h_02.czi - C=2--OMERO ID:58037	28.794
158 20180130_X_IERK_3h_02.czi - C=2--OMERO ID:58037	29.825
159 20180130_X_IERK_3h_02.czi - C=2--OMERO ID:58037	29.492
160 20180130_X_IERK_3h_02.czi - C=2--OMERO ID:58037	30.403
161 20180130_X_IERK_3h_02.czi - C=2--OMERO ID:58037	28.734
162 20180130_X_IERK_3h_02.czi - C=2--OMERO ID:58037	30.134
163 20180130_X_IERK_3h_02.czi - C=2--OMERO ID:58037	27.599
164 20180130_X_IERK_3h_02.czi - C=2--OMERO ID:58037	30.219
165 20180130_X_IERK_3h_02.czi - C=2--OMERO ID:58037	31.230
166 20180130_X_IERK_3h_02.czi - C=2--OMERO ID:58037	31.240
167 20180130_X_IERK_3h_02.czi - C=2--OMERO ID:58037	31.763
168 20180130_X_IERK_3h_02.czi - C=2--OMERO ID:58037	31.624
169 20180130_X_IERK_3h_02.czi - C=2--OMERO ID:58037	27.315
170 20180130_X_IERK_3h_02.czi - C=2--OMERO ID:58037	27.280
171 20180130_X_IERK_3h_02.czi - C=2--OMERO ID:58037	16.753
172 20180130_X_IERK_3h_02.czi - C=2--OMERO ID:58037	28.391

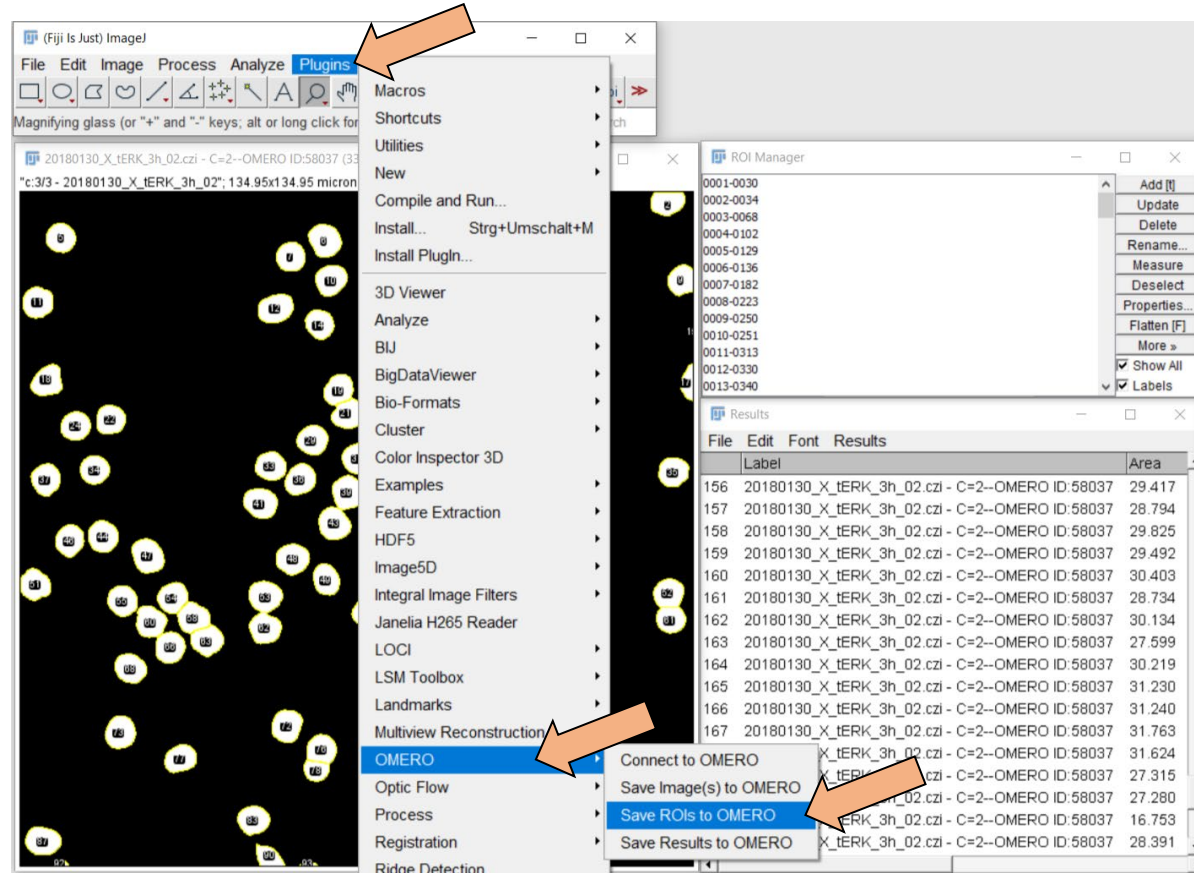
# Save Regions of Interest (ROIs) and Measurement Results to OMERO

Save to OMERO using the plugin

*Plugins*

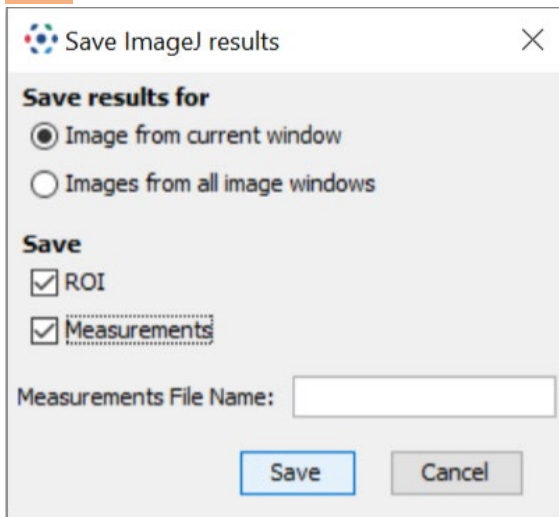
→ OMERO

→ Save ROIs to OMERO

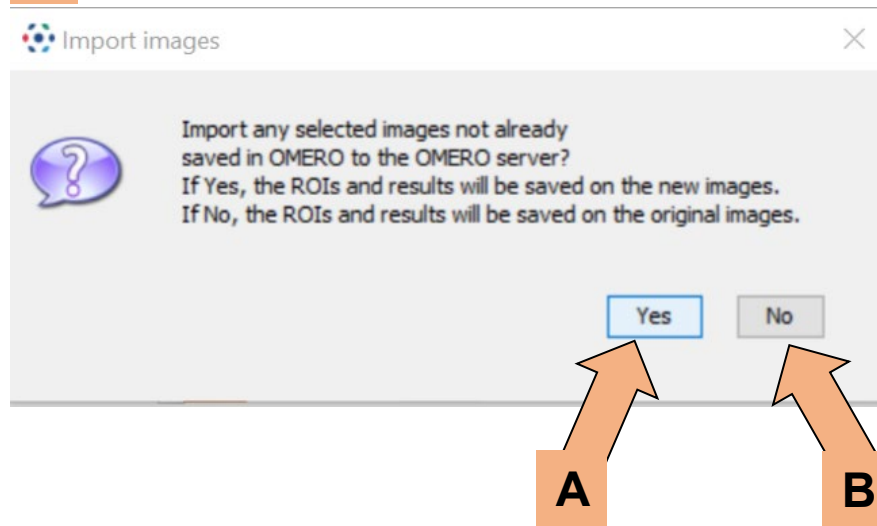


# Choose settings for saving in OMERO

1



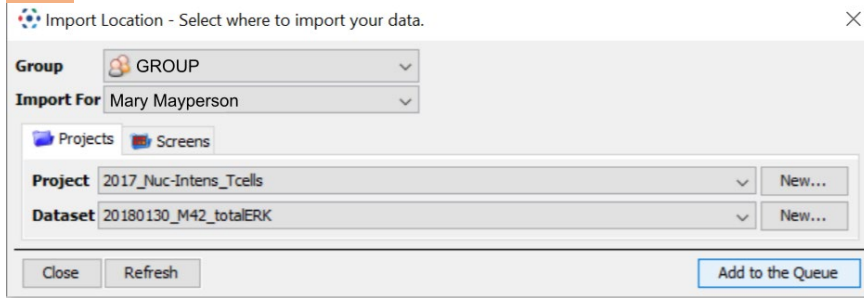
2



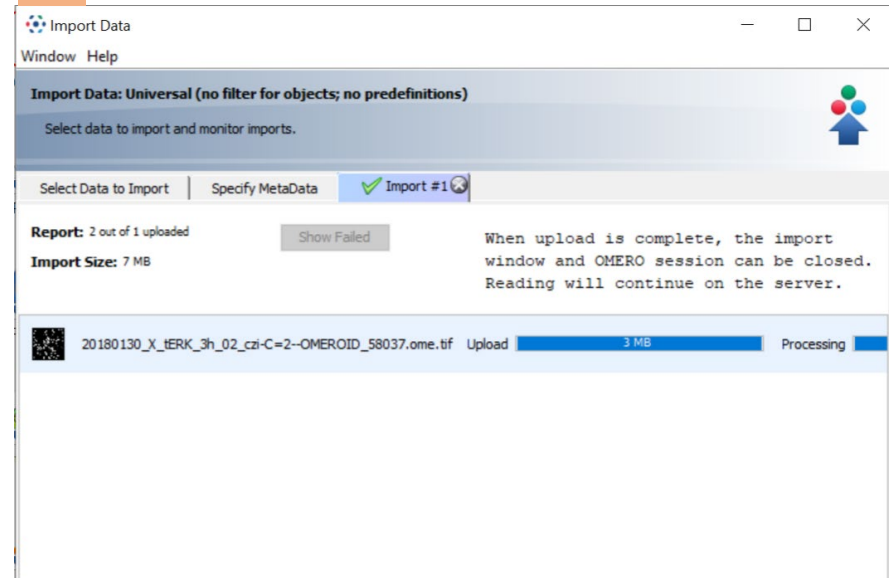
# A – Upload the mask image to OMERO as a new image

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

1



2



# A – View the imported mask image and the analysis results (e.g., in OMERO.web)

The screenshot shows the OMERO.web webclient interface. On the left, the 'Explore' panel displays a file tree with various image files. An orange callout box with an arrow points to a file named '20180130\_X\_tERK\_3h\_02\_czi-C=2-OMERO', stating 'ROI image uploaded as new file (new ID!)'. The main area shows a grid of image thumbnails. One thumbnail, representing the ROI image, is highlighted with a blue border. An orange callout box with an arrow points to this thumbnail, stating 'Results uploaded as attachment in csv-format'. On the right, the 'Image Details' panel shows metadata for the selected image, including 'Image ID: 68712', 'Owner: Mary Mayperson', 'Import Date: 2022-09-07 11:14:01', and 'Attachments: 1'. The attachment list includes 'ImageJ-20180130\_X\_tERK\_3h\_02\_czi-C=2-OMEROID\_58037-Results-2022-09-07.csv (18.30 KB)'.

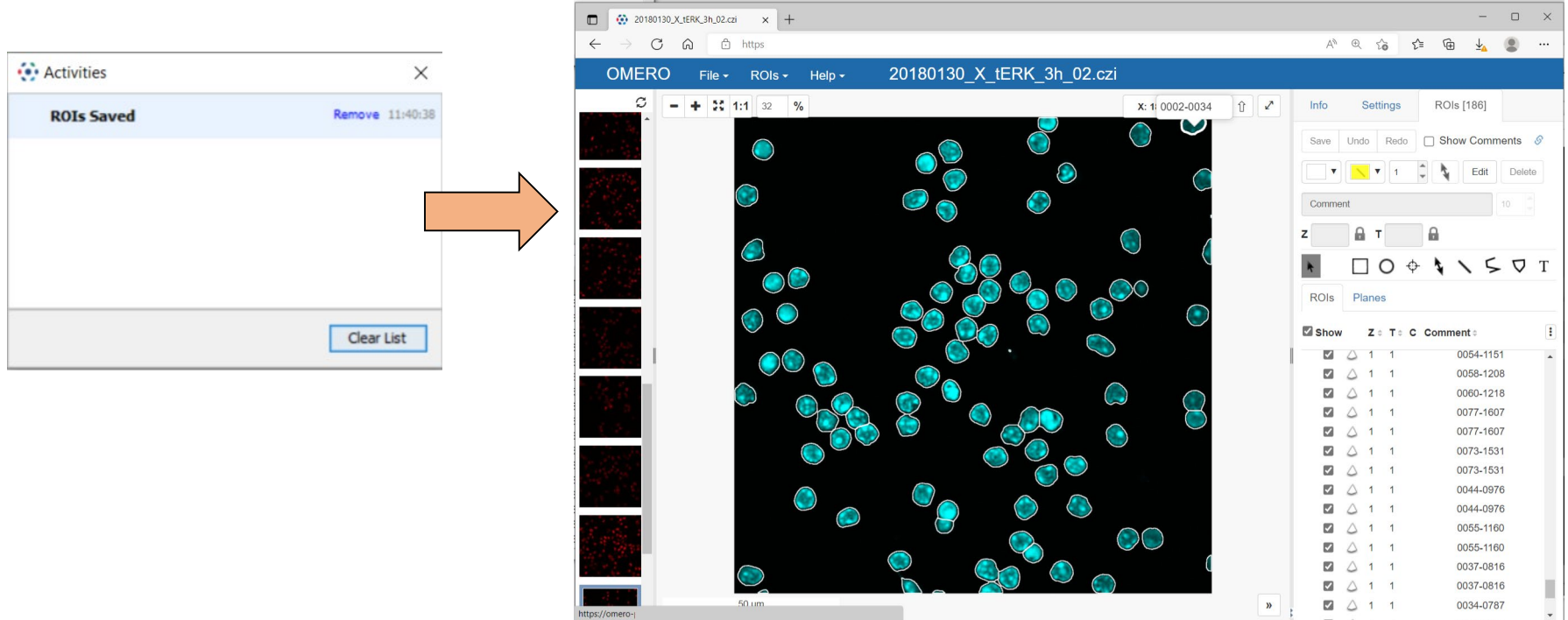
ROI image uploaded as new file (new ID!)

Results uploaded as attachment in csv-format



## B – Add the ROI to the original image in OMERO

Review the ROIs on the original image with OMERO.iviewer



The image shows the OMERO.iviewer interface. On the left, an 'Activities' dialog box titled 'ROIs Saved' is open, showing a list of saved ROIs and a 'Clear List' button. An orange arrow points from this dialog to the main OMERO.iviewer window. The main window displays a large image of cells with numerous cyan-colored ROIs overlaid. The interface includes a top menu bar with 'File', 'ROIs', and 'Help', and a right-hand panel with 'Info', 'Settings', and 'ROIs [186]' tabs. The 'ROIs' tab is active, showing a list of ROIs with columns for 'Show', 'Z', 'T', 'C', and 'Comment'.

Show	Z	T	C	Comment
<input checked="" type="checkbox"/>	1	1		0054-1151
<input checked="" type="checkbox"/>	1	1		0058-1208
<input checked="" type="checkbox"/>	1	1		0060-1218
<input checked="" type="checkbox"/>	1	1		0077-1607
<input checked="" type="checkbox"/>	1	1		0077-1607
<input checked="" type="checkbox"/>	1	1		0073-1531
<input checked="" type="checkbox"/>	1	1		0073-1531
<input checked="" type="checkbox"/>	1	1		0044-0976
<input checked="" type="checkbox"/>	1	1		0044-0976
<input checked="" type="checkbox"/>	1	1		0055-1160
<input checked="" type="checkbox"/>	1	1		0055-1160
<input checked="" type="checkbox"/>	1	1		0037-0816
<input checked="" type="checkbox"/>	1	1		0037-0816
<input checked="" type="checkbox"/>	1	1		0034-0787



## A and B combined

In the OMERO.iviewer you can copy the ROI from the segmentation image to the original image manually, too.

The image shows two side-by-side screenshots of the OMERO.iviewer interface, illustrating a manual workflow for copying ROIs. The left screenshot shows a segmentation image with blue ROIs. An orange arrow points to the 'Copy' menu item, with the text '2. Copy to clipboard'. Another orange arrow points to the 'ROIs' table, with the text '1. Select all ROIs (Ctrl+A)'. The right screenshot shows the same image with the ROIs pasted as cyan. An orange arrow points to the 'Paste' menu item, with the text '3. Paste to the selected original image'. The 'ROIs' table in the left screenshot contains the following data:

Checked	Up	Down	Comment
<input checked="" type="checkbox"/>	1	1	0087-1840
<input checked="" type="checkbox"/>	1	1	0087-1840
<input checked="" type="checkbox"/>	1	1	0088-1850
<input checked="" type="checkbox"/>	1	1	0088-1850
<input checked="" type="checkbox"/>	1	1	0089-1873
<input checked="" type="checkbox"/>	1	1	0089-1873
<input checked="" type="checkbox"/>	1	1	0090-1880
<input checked="" type="checkbox"/>	1	1	0090-1880
<input checked="" type="checkbox"/>	1	1	0091-1888
<input checked="" type="checkbox"/>	1	1	0091-1888
<input checked="" type="checkbox"/>	1	1	0092-1904
<input checked="" type="checkbox"/>	1	1	0092-1904
<input checked="" type="checkbox"/>	1	1	0093-1905
<input checked="" type="checkbox"/>	1	1	0093-1905

# Batch processing, macros and scripts for Fiji and OMERO

Running image analysis pipelines with Fiji over several images from OMERO in batch is possible, too.

*Recommended:* OMERO Macro Extension & OMERO batch plugin

F1000Research  
F1000Research 2022, 11:392 Last updated: 06 JUL 2023

Check for updates

SOFTWARE TOOL ARTICLE

## Easing batch image processing from OMERO: a new toolbox for ImageJ [version 1; peer review: 2 approved]

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<sup>2</sup>Université Côte d'Azur, CNRS, IPMC, Valbonne, France  
<sup>3</sup>Polytech Nice Sophia, Campus SophiaTech, Sophia Antipolis, France  
<sup>4</sup>Univ. Lille, CNRS, Inserm, CHU Lille, Institut Pasteur de Lille, U1019 - UMS 9017 - CIIIL - Center for Infection and Immunity of Lille, Lille, 59000, France

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

*Example:*

```
File Edit Language Templates Run Tools Window Options T access... - □ X
Macro.ijm  accessOMERO_Christian_Try.ijm
Outline <
File Explorer
+ - File f:
15 //This feature will enable the consecutive macro functions
16 run("OMERO Extensions");
17
18
19 //Connect to OMERO with the given variables
20 Ext.connectToOMERO(host, port, username, pwd);
21 pwd = "mockpassword"
22
23 images = Ext.list("images", "dataset", dataset_id);
24 imageIds_array = split(images, ","); //retrieves the indivi
25 // The for-Loop defines which action is performed with eac
26 // One image after another is processed with the function
27 // The last index of the array is logically one less than
28 //<lengthOf(imageIds_array) i<2
29 for(i=0; i<lengthOf(imageIds_array); i++){
30 imageplusID = Ext.getImage(imageIds_array[i]);
31 img_nuclearintensitymeasure();
32 //save the ROI to OMERO
33 nROIS = Ext.saveROIs(imageIds_array[i]);
34 // delete the ROI manager table after the ROI was save
35 roiManager("Delete");
36 print("finished for " + imageplusID); //this is imple
37
38
39 }
40 Ext.disconnect()
```


# JiPipe visual macro programming with a connection to OMERO

Batch processing in Fiji/ImageJ and the connection to OMERO can now be established with a graphical user interface (GUI) in the software JiPipe:

## Correspondence

<https://doi.org/10.1038/s41592-022-01744-4>

## JIPipe: visual batch processing for ImageJ

 Check for updates

The growth in microscopy adoption has led to a concomitant upsurge in the development of software tools for the automated analysis of image data. Pillars among these tools are ImageJ<sup>1</sup> and its Fiji<sup>2</sup> distribution, which have been serving the imaging community for decades and continue to gain public support to keep up with the quantification

needs of the newest and most-demanding microscopy techniques. The hallmark of ImageJ is its intuitive graphical user interface, which provides access to its many tools. On the other hand, the creation of reproducible batch-processing workflows is only possible using a macro language. As programming skills are uncommon among experimentalists<sup>3</sup>, the need for scripting contributes to an

already-existing communication gap between life and computer scientists. Visual programming languages that replace the writing of text commands with the design of a flowchart offer a solution. Existing tools contribute to this effort by providing a visual way to build pipelines or by simplifying the scripting procedure (Supplementary Information, section 1). Our newly developed visual programming



# Extended resources on using Fiji and OMERO

Official OMERO guide:

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

A workshop on image analysis with Fiji and OMERO:

<https://learning.rc.virginia.edu/notes/fiji-omero/>

Workshop recordings by the Open Microscopy Environment Consortium on YouTube, including scripting in Fiji:

[https://www.youtube.com/watch?v=W5EDx3yKA\\_o](https://www.youtube.com/watch?v=W5EDx3yKA_o)

(<https://www.youtube.com/watch?v=dOtnEO-nmlg>)

Image Analysis Lecture by Robert Haase (TU Dresden):

<https://www.youtube.com/playlist?list=PL5ESQNfM5lc7SAMstEu082ivW4BDMvd0U>

Help for Image Analysis or OMERO-related issues - Image.sc forum:

<https://image.sc>