

Workflow

Description

Manual analysis of OMERO images using Fiji

Setup

For this workshop, images have already been imported into OMERO. The import instructions can be found at <http://help.openmicroscopy.org/importing-data-5.html>

Install OMERO.insight-ij plugin. Download the IJ/Fiji plugin from <https://www.openmicroscopy.org/omero/downloads/>.



Image J / Fiji

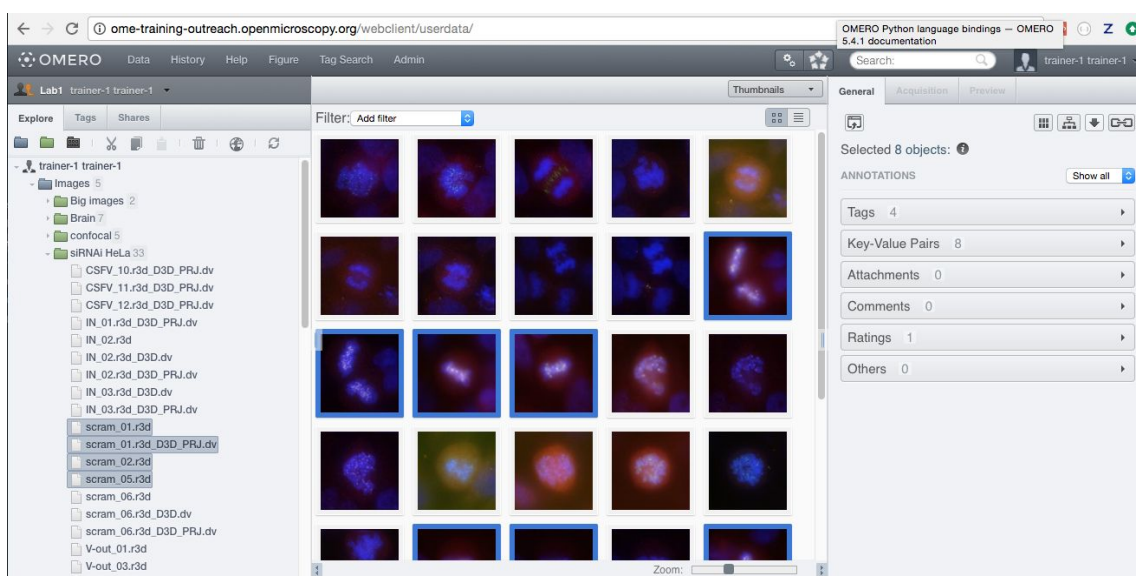
OMERO.insight-ij-5.4.1-ice36-b75.zip


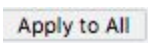
View instructions at [Using Imagej with OMERO.](#)


[Download \(70.23 MB\)](#)

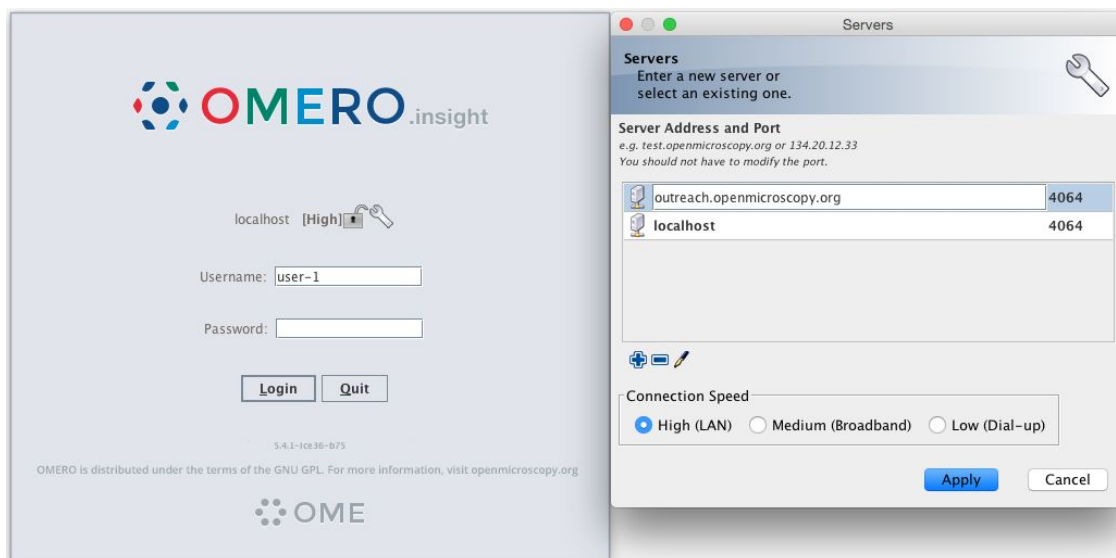
Unzip the downloaded plugin and put it into the *Plugins* folder of your Fiji. The installation instructions can be also found at <http://help.openmicroscopy.org/imagej.html#plugins>

1. Login to the webclient at <http://outreach.openmicroscopy.org> with the Username and Password provided. Browse hierarchy of Project “images” and open some Datasets (for example “siRNAi-HeLa”) to find images. Zoom the thumbnails (using the slider below thumbnails) and select multiple images that appear to be in Metaphase (one or two single lines appear in each cell on the image as opposed to one or several round blobs, see screenshot below for the pre-selected images in rows 2 and 3). Use Shift+click or Ctrl+click to multi-select the thumbnails.

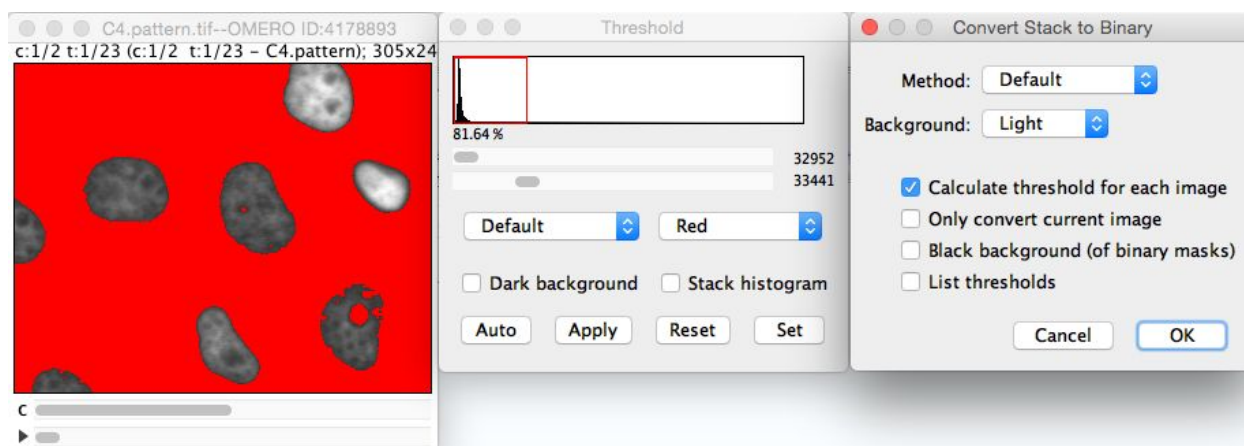


- 2.
3. **Note:** There are several ways how to find your images in OMERO: browsing the Project/Dataset hierarchy, filtering in the central pane, tagging your images and searching for tags, browsing tags hierarchy, **searching** for specific objects (see point 2 below)
4. Search for *mitomycin-A* in the Search field at the top right of webclient. This should find six images. To search for key-value pairs, we can use the search string *key: value*. To narrow down the search, search again for *mitomycin-A:0mM* This should find just the "C4.pattern.tif" image in the Dataset "chromatin-condensation" containing six timelapse images. Click on *Browse* to get back to this image in webclient.
5. Adjust channel names: Click on the *pen:*  icon in the right-hand pane next to Channels. Input "Cy3" (instead of channel "0") and "eGFP" (instead of channel "1"). Click "Apply to all" button:  and confirm by clicking *Continue*.
6. Open the "C4.pattern.tif" Image with OMERO.iviewer, use the Time-slider below the image to move through time and play the video.
7. Start the Fiji app and use the OMERO plugin to browse data in OMERO i.e. *Plugins > OMERO > Connect To OMERO*

8. In the OMERO login dialog, click the wrench icon  and then add the server address (outreach.openmicroscopy.org) in the dialog. Click *Apply*.



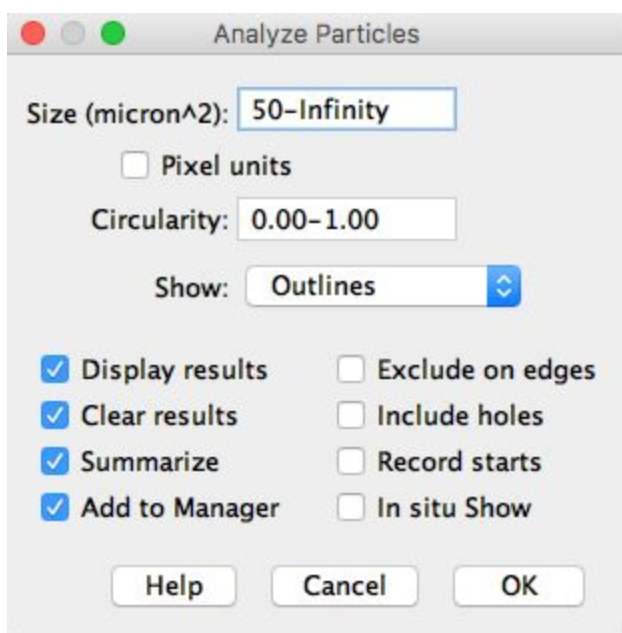
9. Find and open the same image from above “C4.pattern.tif” from Dataset “chromatin-condensation” (double-click on the thumbnail).
10. Select *Image > Adjust > Threshold*
11. The *Threshold* dialog will pop up, click *Apply*.
12. Another window *Convert Stack to Binary* will pop up
13. Select the following parameters:
- Method: Default, Background: Light and Calculate threshold for each Image*
 - Click *OK*.
 - Close the *Threshold* dialog if you want



14. Select *Analyze > Analyze Particles*
15. In the Analyze Particles dialog, select the following parameters
- with *Size: 50-infinity*
 - Show: *Outlines*

c. Check: *Display results*, *Clear results*, *Summarize* and *Add to Manager*.

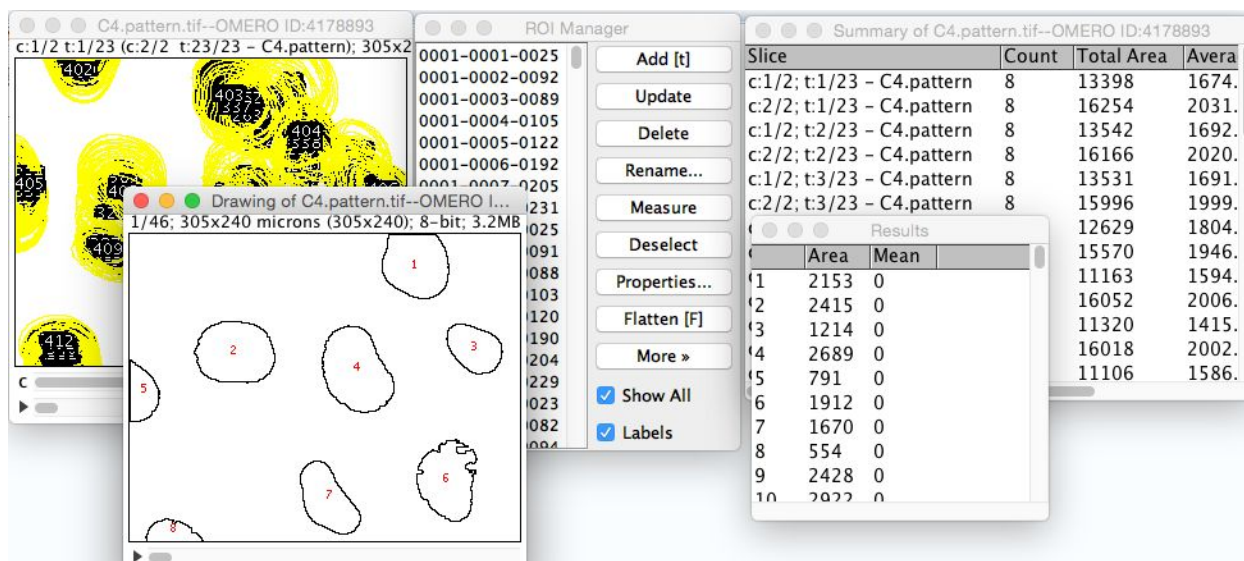
16. Click OK.



17.

18. A dialog pops up asking to Process all 46 images? Click Yes

19. ROIs, Results table, and the mask timelapse with outlines will be generated.

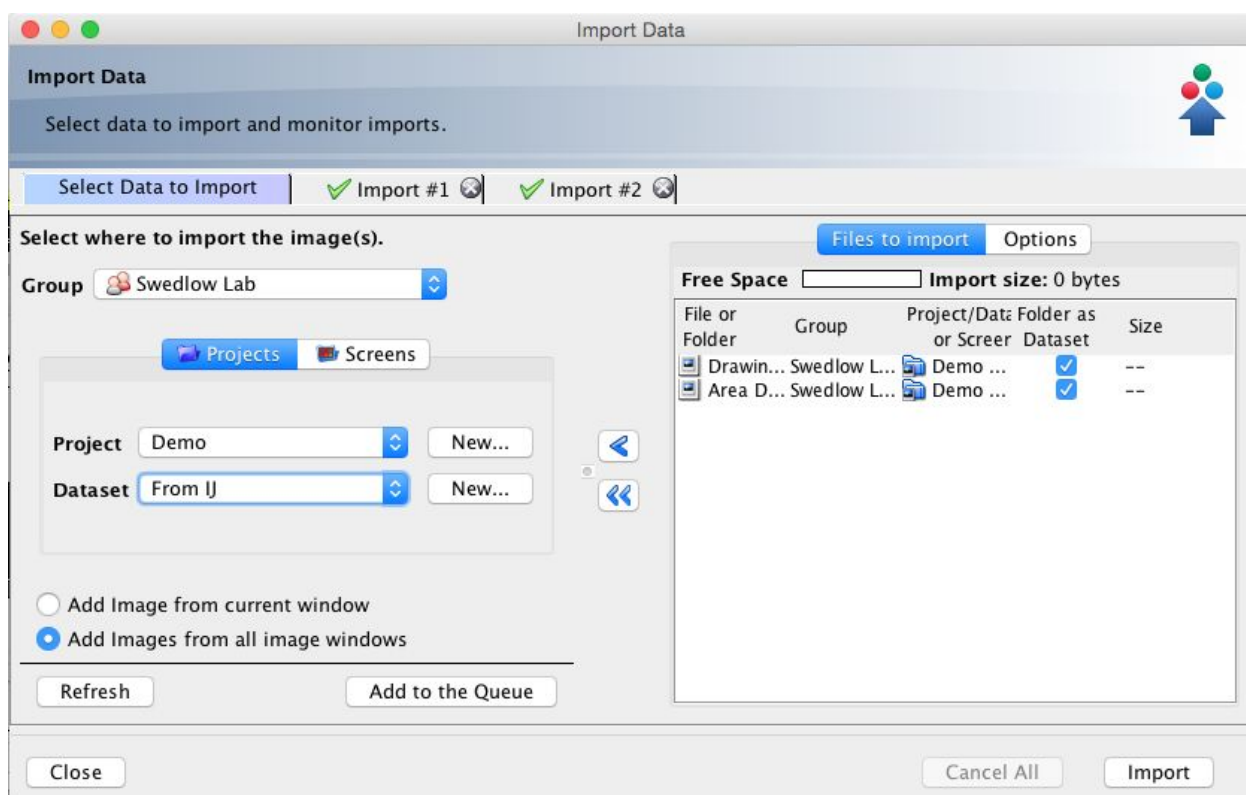


20.

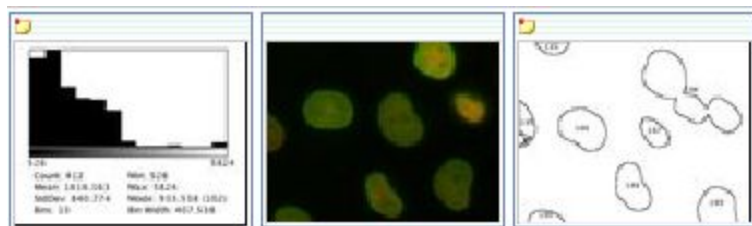
21. Select the *Results* table and Menu *Results* > *Distribution* > *OK*

22. To Import images to OMERO, select: *Plugins* > *OMERO* > *Save Image(s) to OMERO*.

23. In the selection dialog, Choose Project and Dataset (You can click on *New* button to create new Project and Dataset or import into existing ones). Check *Add Images from all image windows*. Click *Add to Queue* then *Import*.



24.

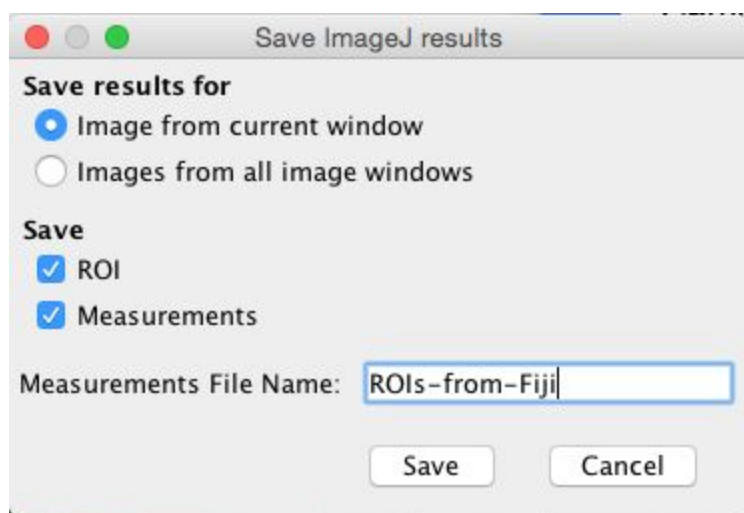


25.

26. In Fiji, select the original image showing ROIs. *Plugins > OMERO > Save ROIs to OMERO.*

27. We want to Save ROI and Measurements, which will attach the results as a CSV attachment (to open for example in Excel) to the image in OMERO.

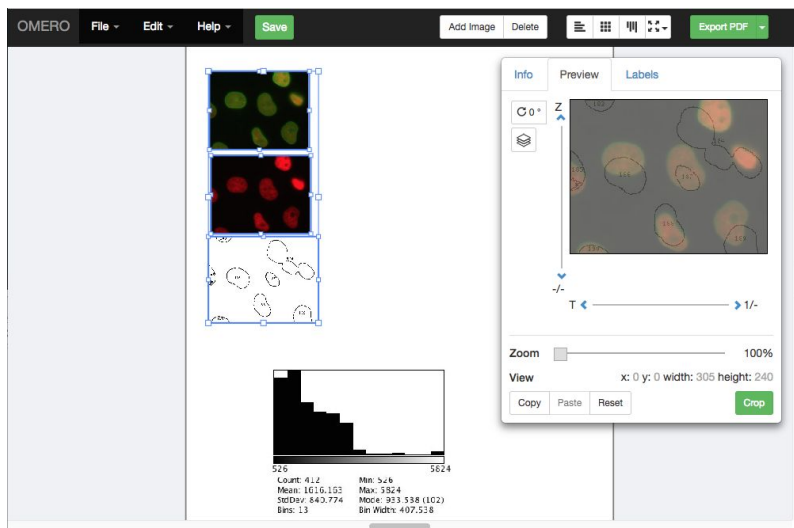
28. Enter a File Name and click *Save* (*Note: There is also a manual workflow which you could have used to attach the Excel sheets resulting from Workflow1 to OMERO.*)



29. .
30. Return to the webclient and open the original image in OMERO.iviewer by double-clicking on it and inspect the newly created ROIs. Notice that you have ROIs for each channel on all timeplanes.

OMERO.figure

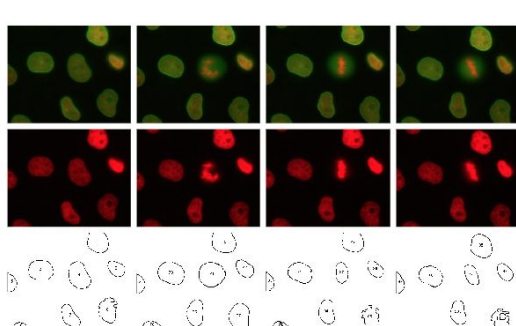
1. Select the original C4.pattern.tif image, the ROIs image and distribution plot. Click on *Open with... OMERO.figure*.
2. Copy and paste the original image using the Edit menu or keyboard shortcuts to duplicate it.
3. Adjust the rendering settings in this image in the right panel, to turn off the Green channel and enhance the Red.
4. Arrange these 2 images in a column with the ROIs image below, resizing as needed.



- 5.
6. Select the 3 panels and use the *Align > Width & Height* toolbar to ensure these panels are all the same size.

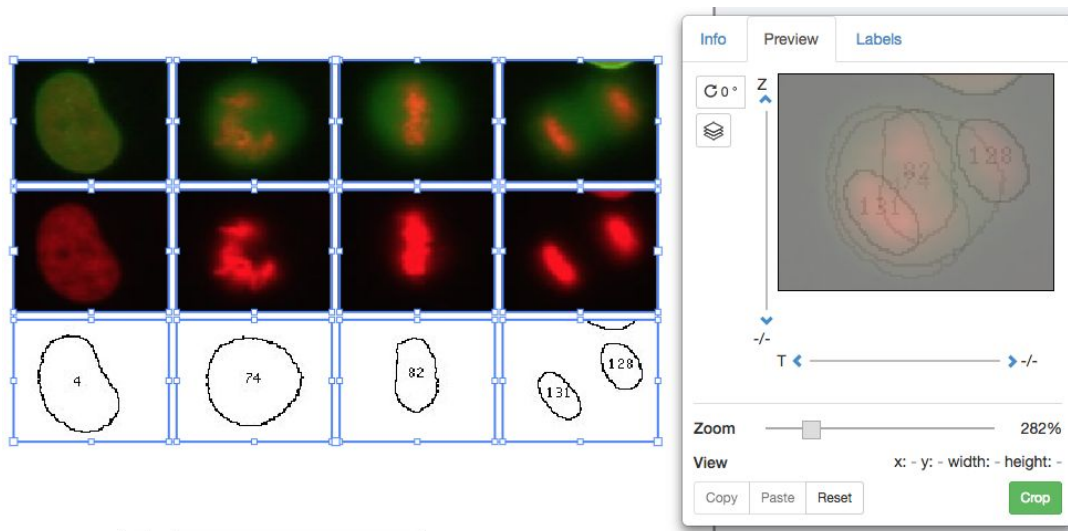



- 7.
8. Then click the *Grid* layout button in the same toolbar to snap the panels to a grid.
9. Copy the 3 panels and paste several times to create 3 new columns of 3 panels.
10. Select just the upper 2 image panels from each new column in turn and in the right panel, drag the T-slider to set the time to index, e.g. 5 (20 mins), then 6 (25 mins) then 9 (40 mins).



- 11.

12. NB: the ROIs image is actually a Z-stack so we have to choose the frame using the Z-slider. There are 46 planes in the Z stack, from 2 channels x 23 time points. To pick time-frames that correspond to the 4 images above, set the Z-index for the 4 ROI images to 1, 9, 11 and 17 respectively.
13. Select all the 12 panels in the grid and use the Zoom slider to zoom in.
14. Pan the images by dragging in the right-hand panel Preview image.



15. 
16. Select just the first row of images, click on the Labels tab in the right-hand panel. Under *Add Labels* choose *Time (hrs:mins:secs)* from the drop-down menu. Choose label color (white), position (top-left) and font-size (12) then click *Add*.



17. Click *Save* to save your figure.
18. To export your figure as PDF, click the *Export PDF* button at the top-right of the screen and wait for the PDF to be created on the server and the *Download* button to appear. Click to download the PDF and import to a PDF editor. You can post-process the PDF in Inkscape or Adobe Illustrator/Photoshop for example.