Presentation is available at http://downloads.openmicroscopy.org/presentations/2017/Cambridge-Training/Day1-Cambridge2017-Introduction.pdf

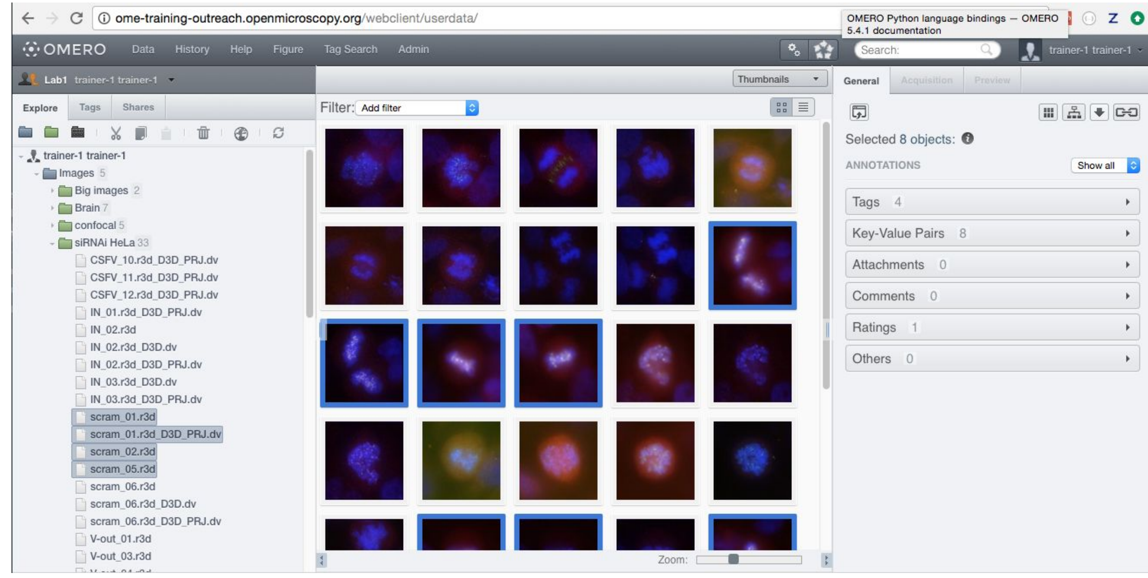
Workflow 1

Description

Determine protein staining intensities and distances in mitotic apparatus in z-stack intensity images, which were stained for the inner-centromere protein INCENP, and compare the results qualitatively and quantitatively. Share the results with colleagues using quick visualisation in OMERO.figure.

Setup

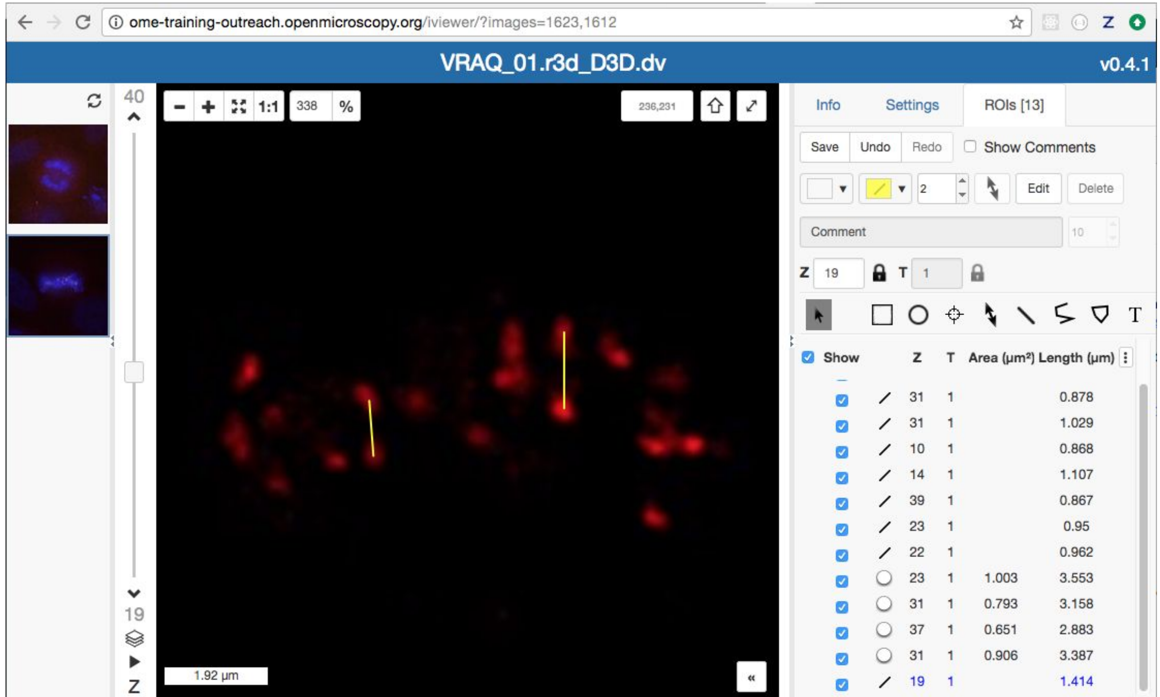
For this workshop, images have already been imported into OMERO. The import instructions can be found at http://help.openmicroscopy.org/importing-data-5.html

1. Login to the webclient at http://outreach.openmicroscopy.org with the Username and Password provided. Browse hierarchy of Project “images” and Dataset “siRNAi-HeLa” to find images.
2. Several control images in the Dataset have "scram" in their name. Other images are named with siRNAi targets “IN” for INCENP and other names for minor INCENP variants.
3. 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.
4. 

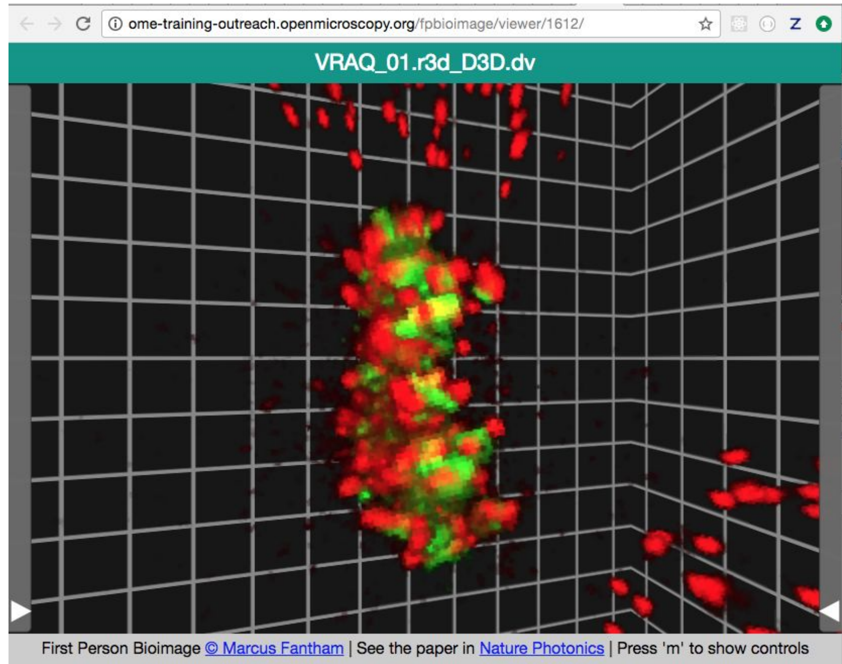
5. In the right-hand panel, expand the *Tags* pane and click to add tags to all the selected images.

6. In the “Tags Selection” dialog, you can select available Tags from the list on the left (filtering if needed). Find the “Metaphase” Tag and click on the button to move it into the right-hand list then click “Save”.
7. Add another Tag to the selected images: This time, create a new tag that is likely unique to you (see bottom left of Tag dialog) e.g. could be the name of your favourite gene or simply “Bob likes this”.
8. Click the newly-added *Metaphase* Tag: in the right-hand panel. This will browse by Tags to show all Images with this Tag, be those your images or images of your colleagues. NB: notice at the top-left of the page that we are now browing data belonging to Trainer-1, the owner of the Metaphase tag.
9. Click on *Tag Search:* link at the top of the webclient. Start typing tag names to search for data by tag: .
10. First start typing "Metaphase", then add your own tag to filter by both tags. Filtering by your unique tag will remove the images of your colleagues from the results of the Tag Search, highlighting only your own images.
11. Click *Browse:* for one of the images in the table to return to the Dataset.
12. Above the thumbnails, click *Add Filter* > *Name* and enter “scram” to filter for all control images in this RNAi experiment.
13. Select an image, click the *Preview* tab of the right panel and Turn off channels 1 and 4.
14. Adjust the intensity levels of the green channel which is INCENP, and channel 3 which is Aurora-B. Use *Histogram feature*: to help you with the adjustments. We want to compare levels of these targets across all images in the Dataset. Click *Save to All*.
15. Thumbnails will be updated with new rendering settings, allowing comparison across the Dataset. Thumbnails can be zoomed using the slider below.
16. In the left panel hierarchy, right-click on the *siRNAi-HeLa* Dataset and choose *Rendering Settings… > Set Imported and Save* to return to original settings.
17. Filter images by Name “VRAQ D3D” and select the first image “VRAQ_01.r3d_D3D.dv”.
18. In the *General* tab of the right-hand panel, expand the *Ratings* panel and click the stars to give this image a rating of 5.
19. Filter images by Name “IN 02 D3D”, select the first image “IN_02.r3d_D3D.dv” and add a rating of 5.

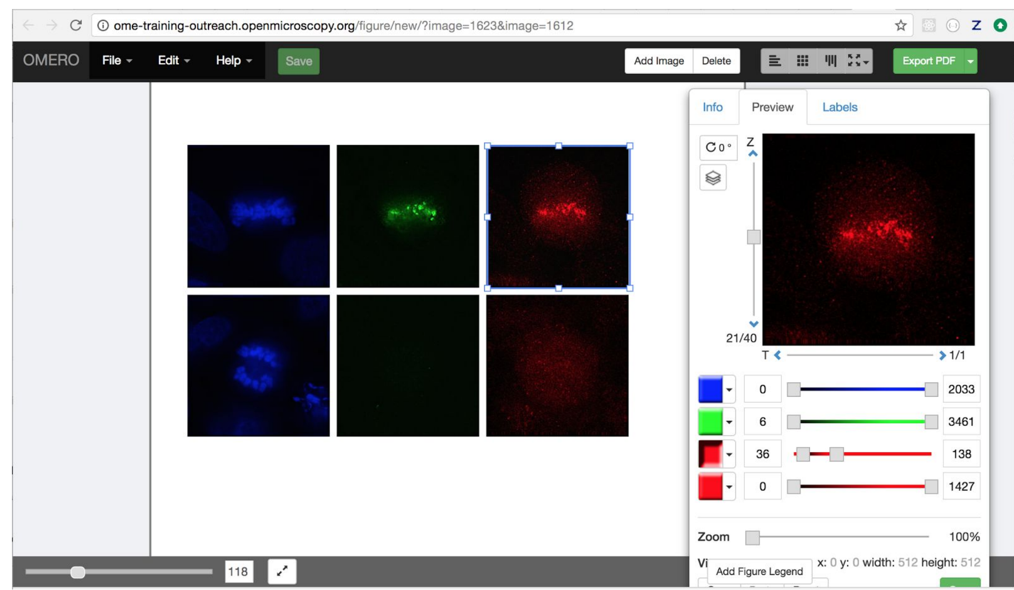
Session 2

20. Now, clear the filtering by Name and instead, filter images by Rating of 5 to show only the 2 images we have just rated.
21. Select both images, right click on the selected images in the tree and choose *Open with... > OMERO.iviewer.* You can also find the *Open with* option at the top of the right panel .
22. In iviewer, we want to measure distance between Centromeres, stained with ACA in the 4th Channel. Turn on ONLY the 4th channel and open the *ROIs* tab to the right.
23. Try to identify centromere pairs, select the *Line* tool and draw a line between the centres of the centromeres. In the ROIs table, in the Comments column, click the 3 dots in the column header and choose to *Show Area/Length*.
24. 

25. Click *Save* to Save ROIs.
26. You can also use Z-Projection which might help find outlying centromeres.
27. Once done with drawing ROIs, click Save again.
28. Click on the *Select tool:*
29. To export statistics, we first need to select all the ROIs using Cmd-drag to select a region in the viewer (if ROIs are spread across multiple Z-sections, we can show them all at once using Z-projection). The selected ROIs will be highlighted blue on the image.
30. To export lengths and intensities of ONLY the 3rd channel (Aurora-B) turn on only this channel on the *Settings* tab of the right-hand pane
31. Check the *Pixel Intensity* checkbox.

32. Right-click on selected ROIs in the viewer and choose *Export Roi Measurements (Excel)*.
33. Open the downloaded table in Excel or similar tool to see the lengths and intensity measurements for the ROIs.
34. We want to check alignment of the metaphase plate in a 3D viewer. The FPBioImage viewer will use the saved rendering settings. Still in OMERO.iviewer, turn on just channels 2 and 4, adjusting levels if needed, and *Save* the rendering settings (do this one-by-one on the two images you have loaded in OMERO.iviewer).
35. Back in the webclient window, select these images (but do this one image at a time) in the tree (left-hand panel), right-click on *Open with… > FPBioImage*. Click *Start* in the new viewer window.
36. We can see that the centromeres are well aligned on the metaphase plate on the "VRAQ…" image, whereas the centromeres are located in and around the spheroid on the "IN…" image.
37. 

38. Again, select both 5-rated images in the webclient, right-click and select *Open with… -> OMERO.figure*.
39. Drag the images to place one above the other, select both and click the Grid layout button . Resize to make the figure smaller. Copy both images and paste twice to make a grid with 2 rows with 3 columns.
40. Select both panels from each column in turn and toggle on a different channel for each column. Adjust Z index and try Z-projections (see screenshot on next page).



41.

42. Select the 2 panels in the left column, click on the Labels tab in the right panel and under *Add Labels* choose *[image-name]* from the drop-down menu. Click on the *position* chooser and choose *Left* from the options. Click *Add*.

43.

44. Save the figure and see that the URL updates to a new URL for this figure which can be shared with colleagues. *File > Open* can be used to open your own figures or those of colleagues.
45. Click *Export PDF* and then when complete, click the *Download* button. Open the downloaded pdf in any suitable program and find the second page of the pdf. Click on the link to the image and see that you are navigated back to OMERO in your browser and the appropriate image, which was used in the Figure, is highlighted.
46. Click *File > Open,* then click on *Owner* button Owner to select a figure of other users (your colleagues). Select and open other users' figures and study them.
47. With another user's figure open, select *File > Save a Copy* to save your own copy of your colleague's figure. Note that you cannot directly save changes on their figure.
48. OMERO.Figure will be revisited in the afternoon in Workflow 2.

Workflow 2

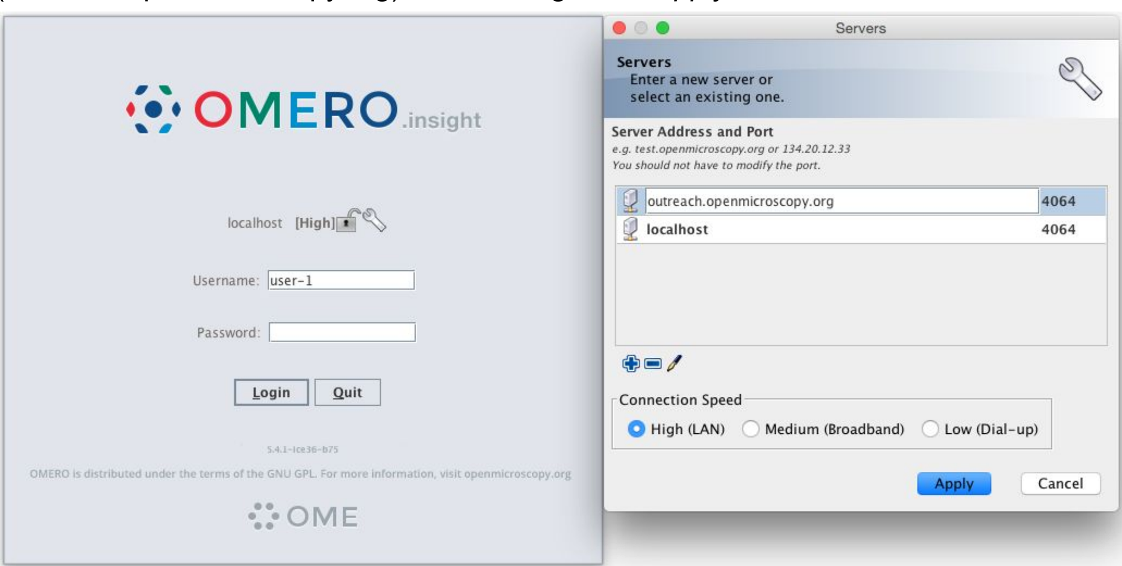
Session 3: Description

Manual analysis of OMERO images using Fiji

Setup

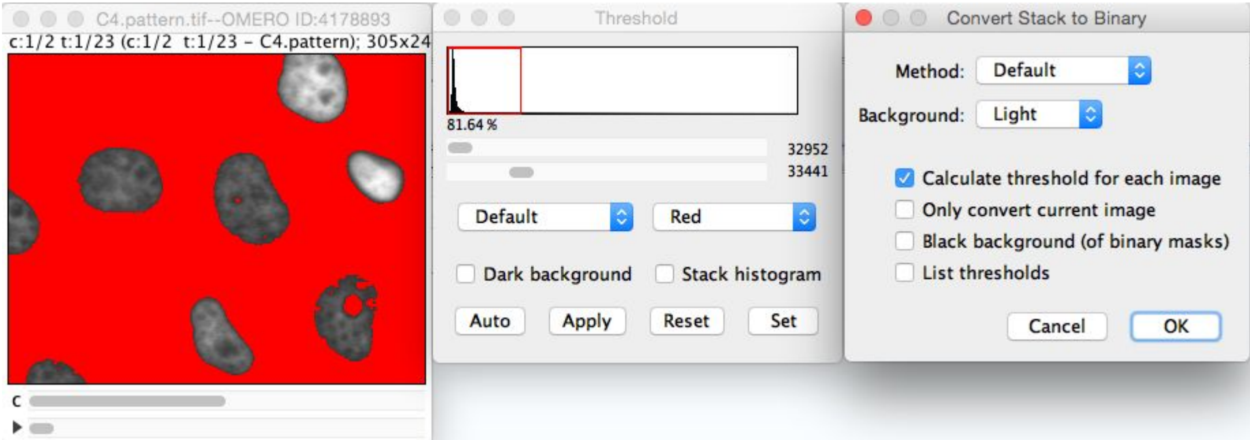
For this workshop, Fiji has already been installed with the OMERO.insight-ij plugin. The installation instructions can be found at http://help.openmicroscopy.org/imagej.html#plugins

1. Login to webclient as before.
2. 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.
3. 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: Apply to All and confirm by clicking *Continue*.
4. Open the "C4.pattern.tif" Image with OMERO.iviewer, use the Time-slider below the image to move through time and play the video.
5. Start the Fiji app and use the OMERO plugin to browse data in OMERO i.e. *Plugins > OMERO > Connect To OMERO*
6. In the OMERO login dialog, click the wrench icon and then add the server address (outreach.openmicroscopy.org) in the dialog. Click *Apply*.

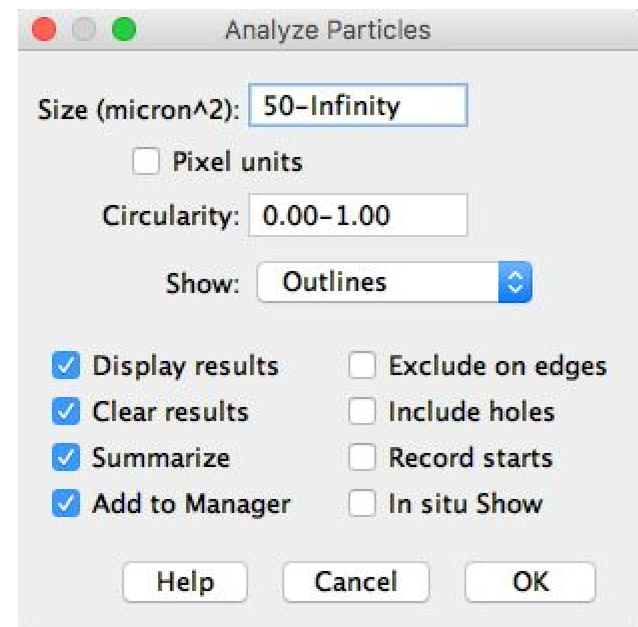


7. Find and open the same image from above "C4.pattern.tif" from Dataset "chromatin-condensation" (double-click on the thumbnail).

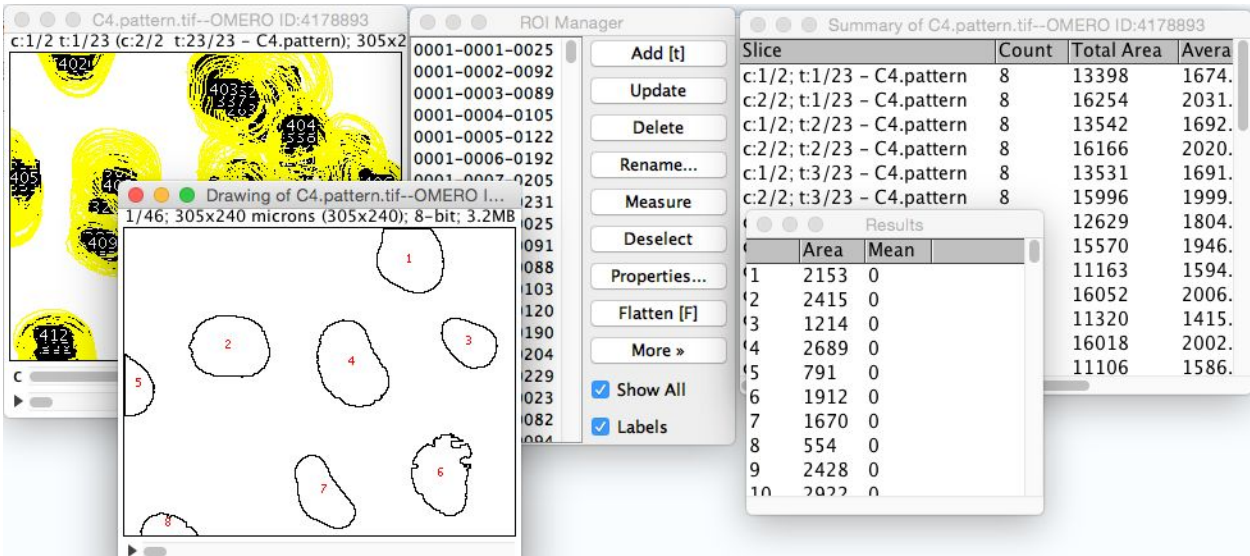
8. Select *Image > Adjust > Threshold*
9. The *Threshold* dialog will pop up, click *Apply.*
10. Another window *Convert Stack to Binary* will pop up
11. Select the following parameters:
    a. *Method: Default*, *Background: Light* and *Calculate threshold for each Image*
    b. Click *OK*.
    c. Close the *Threshold* dialog if you want

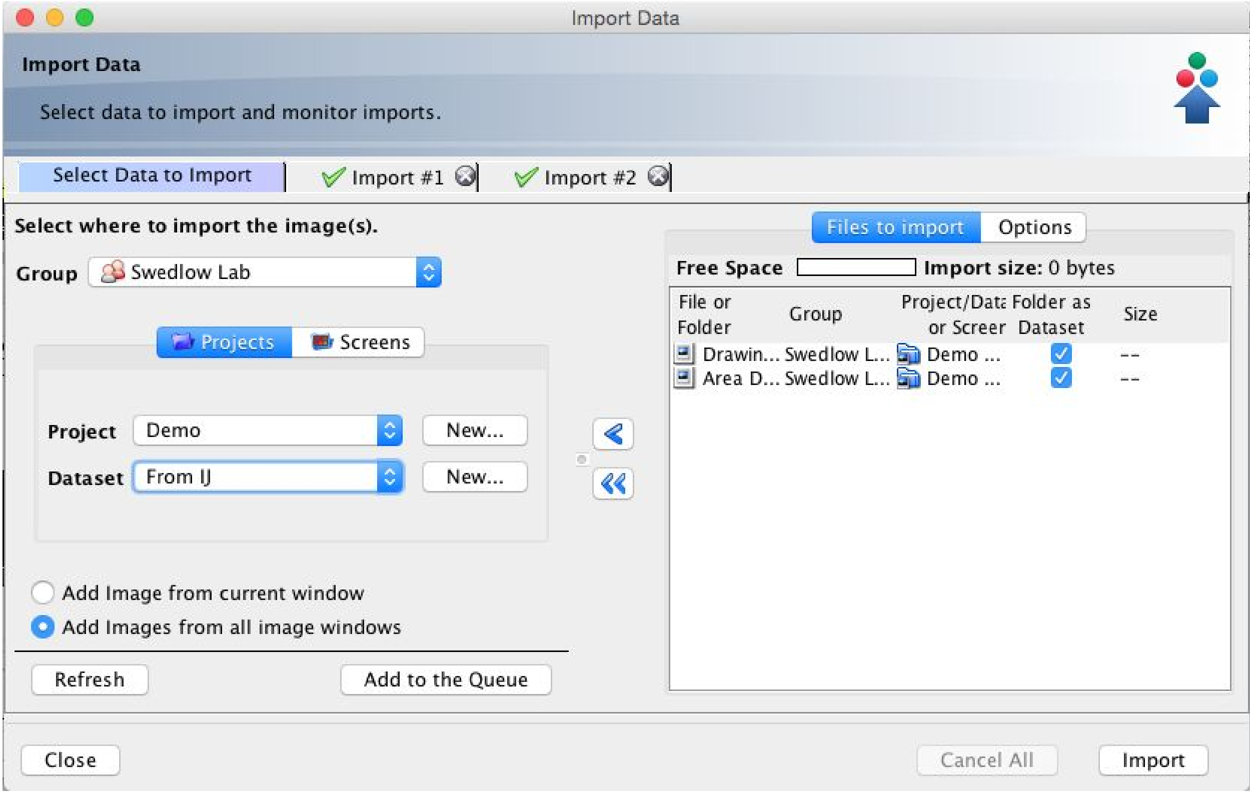


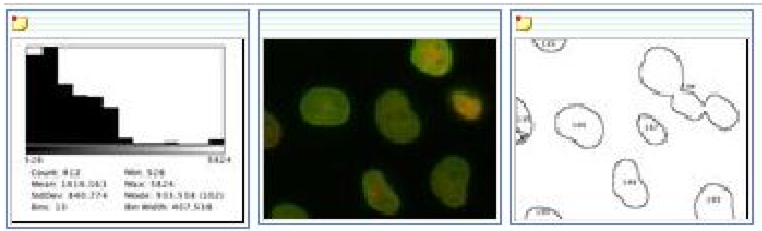
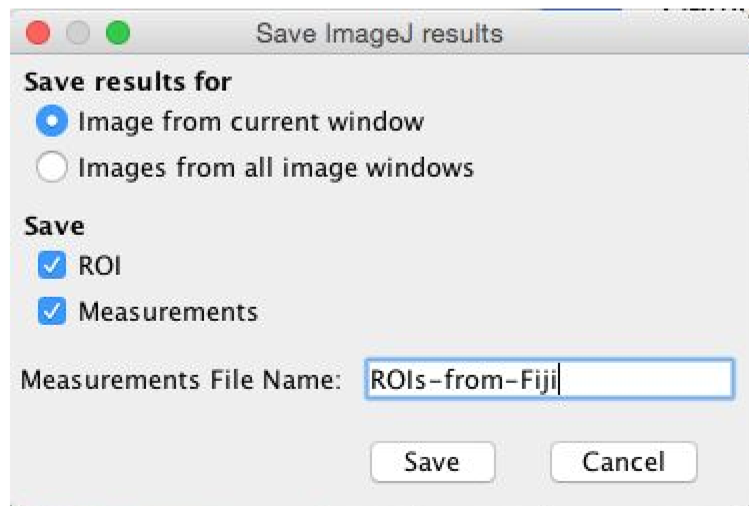
12. Select *Analyze > Analyze Particles*
13. In the Analyze Particles dialog, select the following parameters
    a. with *Size: 50-infinity*
    b. Show: *Outlines*
    c. Check: *Display results, Clear results, Summarize* and *Add to Manager*.
14. Click *OK*.



15.

16. A dialog pops up asking to Process all 46 images? Click *Yes*
17. ROIs, Results table, and the mask timelapse with outlines will be generated.
18. 

19. Select the *Results* table and Menu *Results* > *Distribution* > *OK*
20. To Import images to OMERO, select: *Plugins* > *OMERO* > *Save Image(s)* to OMERO.
21. 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*.
22. 

23. 
24. In Fiji, select the original image showing ROIs. *Plugins* > *OMERO* > *Save ROIs* to OMERO.
25. 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.
26. 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.)
27. 

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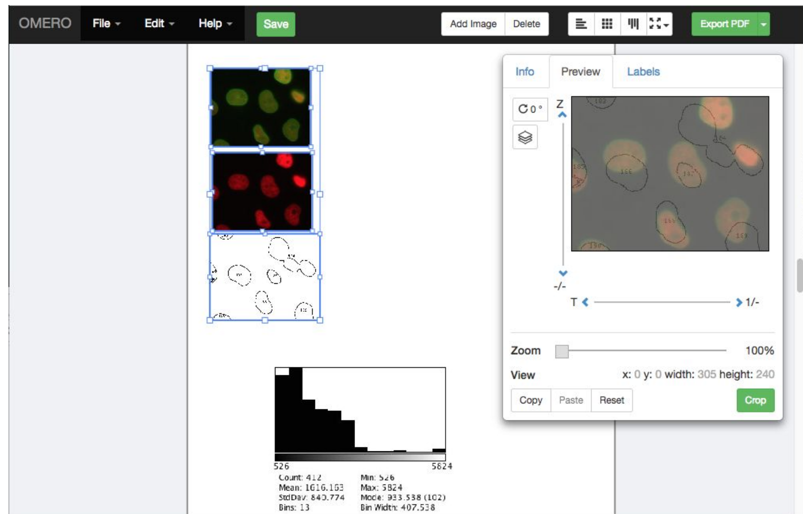
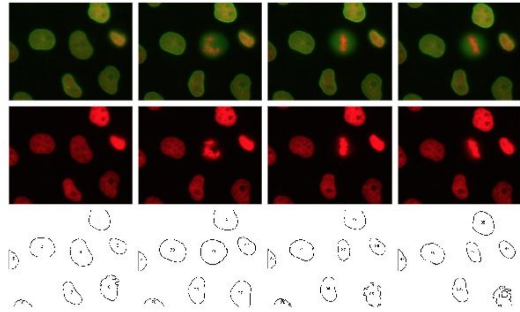
28. Return to the webclient and open the original image in OMERO.iviewer and inspect the newly created ROIs. Notice that you have ROIs for each channel on all timeplanes.

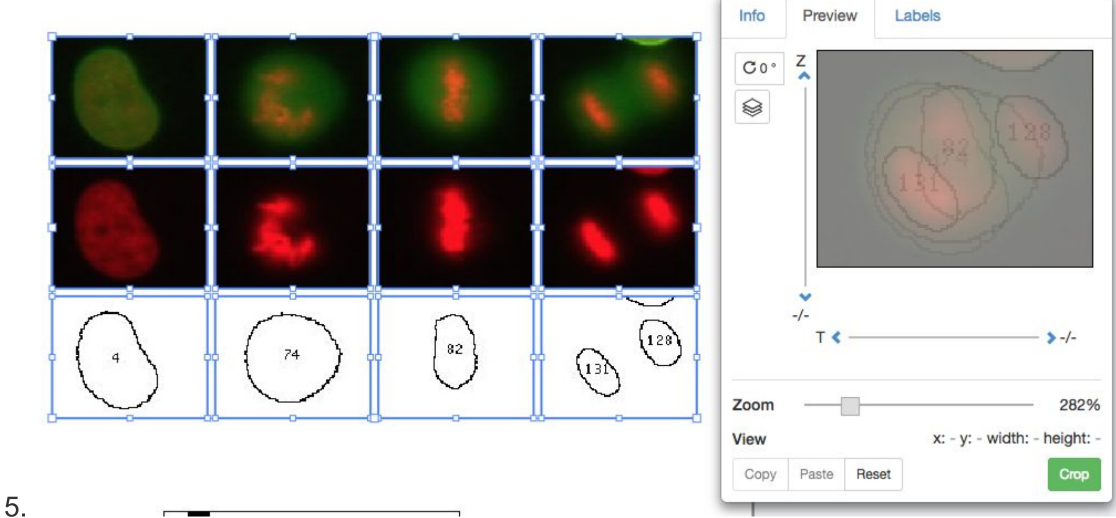
Session 4 - OMERO.figure

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.