Presentation is available at

https://downloads.openmicroscopy.org/presentations/2018/Users-Meeting/Workshops/Metadata-Analysis/slides/index.html

This is the second part of the three parts Metadata workshop held at the Annual Users meeting:
- Part 1
  https://downloads.openmicroscopy.org/presentations/2018/Users-Meeting/Workshops/Metadata-Import/import.pdf
- Part 3
  https://downloads.openmicroscopy.org/presentations/2018/Users-Meeting/Workshops/Metadata-Handling/handling.pdf

Raw Data Analysis

Description

In the second part, we show how to analyze a batch of images stored in OMERO using the scripting facility in Fiji, the results of the analysis will be stored as an OMERO.table, then analyze the images using an OMERO script server-side, the results will this time be stored as a map annotation.

The user analyzing the data needs to have admin privileges.

More information about restricted privileges can be found at https://docs.openmicroscopy.org/latest/omero/sysadmins/restricted-admins.html.

Setup
- Fiji has been installed on the local machine with the OMERO.insight-ij plugin. The installation instructions can be found at http://help.openmicroscopy.org/imagej.html#plugins.
- Installing the OMERO.plugin also adds the dependencies required to connect to OMERO using the Script Editor of Fiji.
- The OMERO.script https://github.com/ome/training-scripts/blob/v0.1.0/practical/python/server/batch_roi_export_to_table.py has been uploaded to the server.
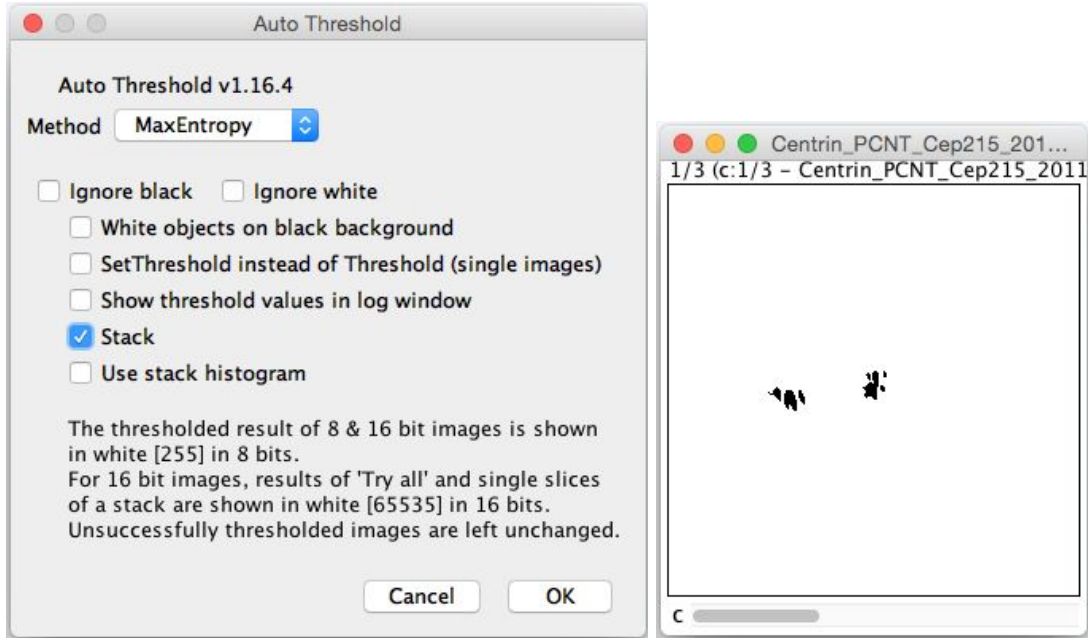
Fiji Manual workflow

1. Launch Fiji.
2. To open an image stored in OMERO, Go to *Plugins > OMERO > Connect To OMERO.*
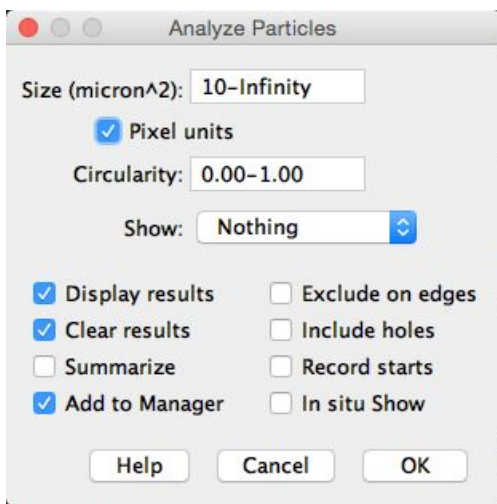
3. In the OMERO login dialog, click the wrench icon and then add the server address in the dialog i.e. **outreach.openmicroscopy.org**. Click *Apply*.
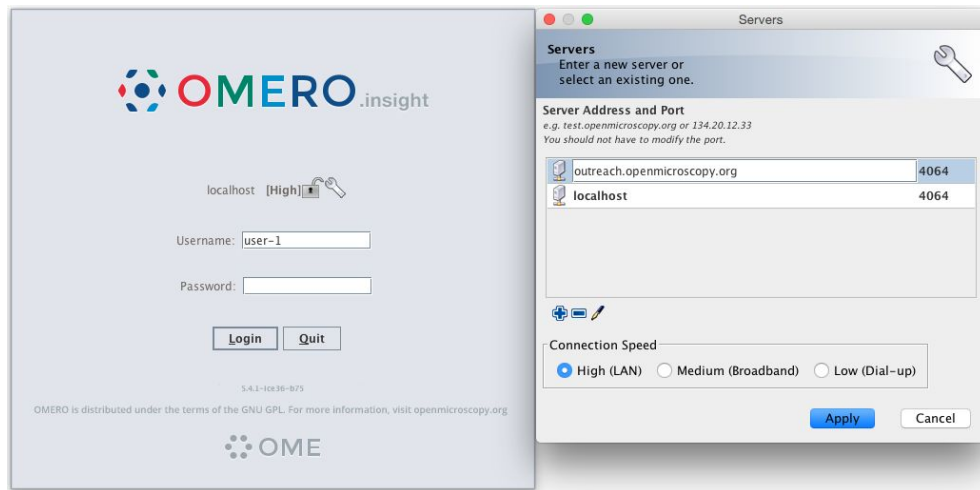
a.

4. Enter your *Username* and *Password* and click *Login*.
5. Browse to Project **idr0021**, open any Dataset and double-click once on an Image to open it in Fiji. Bio-Formats is used to view the Image.
   a. Make sure to select *View stack with: Hyperstack* in the *Bio-Formats Import Options* dialog.
   b. Note that each plane will be transferred from the server to the client machine so this may take a few moments.
6. To open the recorder, go *Plugins > Macros > Record…*, select *JavaScript* to record the actions. The steps will then be used in the Scripting workflow below.
7. Convert floating-point pixel-type to 8-bit using *Image > Type > 8-bit.*
8. Go to *Image > Adjust > Auto Threshold*, to open the *Auto Threshold* dialog:
   a. Select *MaxEntropy* for the *Method* parameter.
   b. Check the checkbox *Stack.*

Auto Threshold

Auto Threshold v1.16.4

Method MaxEntropy

☐ Ignore black   ☐ Ignore white
☐ White objects on black background
☐ SetThreshold instead of Threshold (single images)
☐ Show threshold values in log window
☑ Stack
☐ Use stack histogram

The thresholded result of 8 & 16 bit images is shown
in white [255] in 8 bits.
For 16 bit images, results of 'Try all' and single slices
of a stack are shown in white [65535] in 16 bits.
Unsuccessfully thresholded images are left unchanged.

Cancel   OK

Centrin_PCNT_Cep215_201...
1/3 (c:1/3 – Centrin_PCNT_Cep215_2011

C

9.

10. Click OK.

11. Then open *Analyze > Analyze Particles….*

12. In the dialog

    a. Set *Size* to *10-Infinity* and check *Pixel units.*

    b. Check the following checkboxes:

        i. *Display results*

        ii. *Clear results*

        iii. *Add to Manager*

    c. Click *OK* then *Yes* in the popup dialog indicating asking to *Process all 3 images?*.

Analyze Particles

Size (micron^2): 10–Infinity
   ☑ Pixel units
Circularity: 0.00–1.00

Show: Nothing

☑ Display results   ☐ Exclude on edges
☑ Clear results   ☐ Include holes
☐ Summarize   ☐ Record starts
☑ Add to Manager   ☐ In situ Show

Help   Cancel   OK

13.

14. To save the thresholded image back to OMERO with the generated ROIs and the
measurements:

    a. select *Plugins > OMERO > Save Image(s) to OMERO.*

    b. Create a New Dataset for the image

   i. Click *New…* button next to the selection box on the Dataset row.

   ii. In the dialog that pops up, enter a name and description (optional).

   iii. Click *Create.*

 c. The newly created dataset will automatically be selected.

 d. Click *Add to the Queue* then *Import*.

 e. Go to OMERO.web and check that the measurements have been saved in a CSV file and attached to the images. The attachment can then be downloaded at any time.

Fiji Scripting workflow

1. We will now repeat the manual analysis on a Dataset using the scripting facility available in Fiji.
2. Go to http://outreach.openmicroscopy.org/.
3. Log in
4. Select the Project **idr0021** and first Dataset **CDK5RAP2-C**.
5. Note its ID on the right-hand panel, it will be used below.
6. Launch Fiji. (relaunch if opened).
7. Go to *File > New > Script….*
8. A dialog pops up. In the *Language* menu select *Python.*
9. Copy the content of the script https://github.com/ome/training-scripts/blob/v0.1.0/practical/jython/analyse_particles_for_another_user.jy into the text editor.
10. Edit the following parameters: dataset_id, USERNAME, PASSWORD.

 a.

11. Click *Run.*

12. The script will process all Images in the specified Dataset, applying threshold, analyzing particles and saving ROIs back to the Images in OMERO i.e. we reproduce in a script the manual steps recorded above.

Inspecting and exporting ROIs in OMERO.iviewer

1. Return to OMERO.web and open an Image from this Dataset in OMERO.iviewer.
2. Click the *ROIs* tab to see the added ROIs. Note that the ROIs have been assigned a Channel index to indicate which Channel they were derived from.
3. In the *Settings* tab, turning channels on/off will also show/hide ROIs assigned to those channels.
4. On the left-hand side in OMERO.iviewer, double-click on the image currently opened. This will switch to the multi-view mode.
5. Turn the Red channel on for the first image, and the Green for the second one.
6. Lock the windows by clicking on the lock icon 🔓 . Zoom in and out.
7. Try opening multiple viewers and browsing through a Dataset. Then close all but the first image to return to the single view mode.
8. Go to the *Info* tab and note the link indicating that the image can be opened with Polygons and Polylines in OMERO.figure.
9. In the *Info* tab, note the name of the Dataset. The Channel with the same name is the one we want to analyze for this image.
10. Turn on just this Channel (channel 3 for **CDK5RAP2-C** Dataset).
11. Select all ROIs either from the right-hand pane table or selecting them in the view.
12. In the header, go to *ROIs > Export (CSV)*. Download the output and open it in Excel to show data for ROI shapes.

Analysis workflow Server side

1. We will now analyse ROIs created on the Images above, getting the pixel intensity and area (points) of ROIs and creating a summary of the ROIs on each image.
2. Go to http://outreach.openmicroscopy.org/ and login.
3. Select Project **idr0021** and first Dataset **CDK5RAP2-C**.
4. Click the *Script* button in the top-right of the page ⚙ .
5. Under omero > export_scripts > Batch_ROI_Export… we can see a new script released with OMERO 5.4.6 to export ROIs from multiple images.
6. We will use a customised version of this script.
7. In the script menu, go back… to the top of the menu, then select *users_meeting > batch roi export to table….*
8. In the dialog that pops up, click on *View Script* to view the Python code.
9. Search for "idr" to find the code block that selects the filter_channel based on Dataset and Channel names.

10. Scroll to the bottom of the script to see where the input parameters are defined, such as *Data_Type* and *IDs*. Notice how these appear in the script dialog and are auto-populated with the currently-selected Datasets or Images.

11.
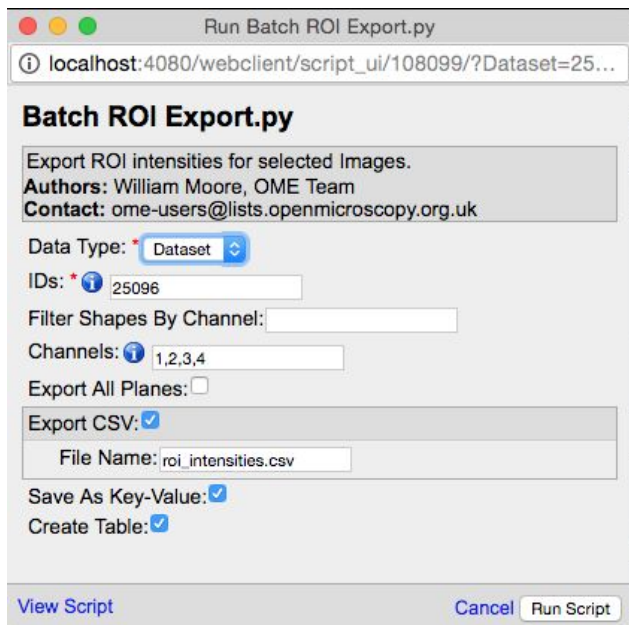
12. The script will process all the Images in the selected Dataset and data can be:
    a. Exported as CSV file, with one table row per Shape/Channel.
    b. Added as Key-Value pairs (Map Annotations) to Images. ROIs summarised as min/max/mean per Image.
    c. Added as an OMERO Table to the parent Project, with one row per Image.
13. Uncheck the *Create Table* option, since we have previously created a Table by running this script on ALL the Datasets in the **idr0021** Project.
14. Click *Run Script.*
15. The status of the processing is displayed in the Activities dialog 📷.
16. When the script has completed, it will show in the the Activities dialog and allow you to download the CSV file. Download this and open in e.g. Excel to see the output data for all the shapes.
17. You will also be able to view the Key-Value Pairs (Map Annotations) in the right-hand panel of each Image that summarise all the ROIs on each image.

Notebook R

For this section, we will use a notebook since the setup is a bit more complex. It is necessary to install several dependencies. We use JupyterLab, a dedicated computational environment.
In the following example, we will generate a plot in R and some basic statistics from the output of the *batch roi export to table* script. The plot will be uploaded back to OMERO.
1. Go to https://idr-analysis.openmicroscopy.org/training/hub/tmplogin
2. In the Files > notebooks > R folder, select the notebook *idr0021_rois.ipynb.*
3. Set the 'username' and 'password' in the first code cell.

4. Use the play button to move through the cells (for code cells wait until the * changes to a number, i.e. the execution finished).
5. Run the first code cell to connect to the server.
6. The first step is to find the idr0021 project, the attached table and load the image and max_points data directly as R dataframe. (Right-click -> Enable Scrolling for output, to collapse the long output)
7. Prepare the R dataframe: Reformat the image column to only contain the image ID. Iterate through the datasets to get the Image <-> Protein (dataset name) relation. Estimate the toroid parameter from the area (max_points).
8. Then plot the data. First the datasets (Proteins) are ordered from lowest to highest mean diameter (to match the figure in the paper).
9. Check if there is a significant difference between the Proteins with respect to the toroid diameter. This is done as one-way analysis of variance. The value of 2e-16 indicates that there is a difference. One-way analysis of variance is questionable because it assumes normal distributed data and similar variances between the groups (this is not given, see plot). Therefore also do a 'Mann-Whitney' test to check the differences between each possible pairwise combination of Proteins. -> There is a significant difference between most of the groups, not for some of the groups (e. g. CDK5RAP2-C vs CEP192-M vs TUBG1-N , which can be seen on the plot as well).
The results show a similar picture as the paper, but the details are different, because we used a simpler approach to estimate the toroid diameter, whereas the authors of the paper used a more accurate method to measure the toroid diameter.
10. Finally the plot and results are saved back to OMERO and attached to the dataset.