

Turku OMERO Workshop 27/11/2018

The presentation and a PDF version of the workshop are available at <https://downloads.openmicroscopy.org/presentations/2018/TB-Turku/>

Software versions used for this workshop:

- OMERO: 5.4.9
- OMERO.iviewer: 0.6.0
- OMERO.figure: 4.0.1
- OMERO.mapr 0.2.3
- OMERO.parade: 0.1.1
- OMERO.FPBioimage: 0.3.0
- OMERO training scripts: 0.4.1
- OMERO training notebooks: 0.3.0
- Bio-Formats: 5.9.2
- Fiji/ImageJ: 2.0.0-rc-68/1.52e
- CellProfiler: 3.1.3

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- OMERO core concepts
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- Analyse data using OMERO API
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- Analyze metadata using OMERO.parade
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OMERO core concepts

Description:

We introduce some key features of the OMERO platform using the webclient.

Data setup and ownership, usernames

All images for this workshop have been pre-imported for you into the OMERO.server. For training purposes, we prepared 40 users on the OMERO.server. **Each** of these 40 users has **their own set of images**. These sets consist of **images of the same name, size, shape, form and quality for each user**. Thus, the data of each user appears the same, but, in actual fact, are different and totally independent sets. Thus, small discrepancies and differences between the users are completely possible. Further, if one user deletes their own data in OMERO (**please do not delete anything** on our server), this will not have any bearing on the other 39 sets of images belonging to the 39 other users.


The **login names** of the 40 users are “user-x” where x goes from 1 to 40, We have given to each of the 40 users in OMERO a unique first name and surname which we picked from a list of 40 famous scientists e.g. Ada Lovelace or Francis Crick. This is the name (“your” name) which you will see in the top-right corner of the OMERO webclient after you log in with your loginname. In the OMERO webclient the default view shows only your own images. The beginning of the **Browse images** section below explains how to navigate to other users’ data.



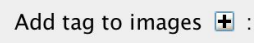


Import setup

For this workshop, images have already been imported into OMERO. To demonstrate the import process, we will import a few more images using the Java Desktop client: OMERO.insight.

1. Import using OMERO.insight
 - a. Requirement: Java version 1.7 or higher must be installed.
 - b. Download OMERO.insight from <https://www.openmicroscopy.org/omero/downloads/>
 - c. The import instructions can be found at <https://help.openmicroscopy.org/importing-data-5.html>
2. Import using the Command Line tool (for your information only)
 - a. Installation instructions are available from <https://docs.openmicroscopy.org/latest/omero/users/cli/installation.html>
 - b. The import instructions can be found at <https://docs.openmicroscopy.org/latest/omero/users/cli/import.html>

Import images

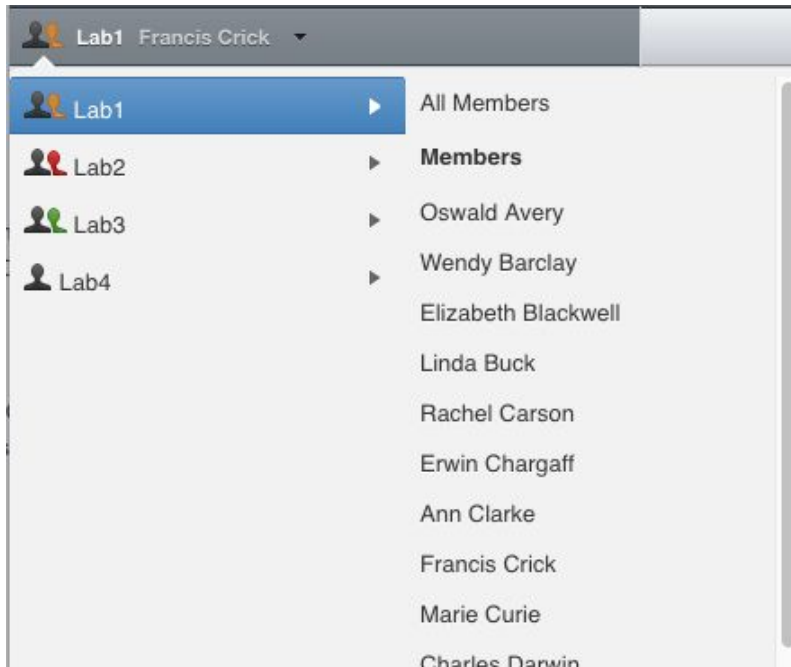
1. Open OMERO.insight and in the login dialog, click onto the spanner icon . This will open a list of servers to which you can connect to. By default, only “localhost” is listed. Click on the *plus* icon to add a new line to the list and type into the line the server address outreach.openmicroscopy.org
2. When done, click *Apply*.
3. Log in using the username and password provided.

4. In OMERO.insight, click on the Importer Icon  in the toolbar.
5. Browse your local hierarchy in the left-hand pane of the importer, select single images or whole folders and add these to the Queue by clicking on the *arrow*  icon.
6. In the *Import Location* window, select the target Project and Dataset (existing or create a new one) to import to.
7. Note: If no Dataset is selected or created, a new Dataset will be automatically created and named after the folder containing the images to be imported.
8. Optional: Go to the *Options* tab
 - a. Click on  : to bring the Tag selection dialog.
 - b. Select the tag(s) on the left-hand side or create a new one.
 - c. Click  to move the tag(s) to the right-hand side.
 - d. Click *Save*.
9. Click on the *Import* button in the bottom-right corner of the Importer window. You should see two progress bars for every image imported, *Upload* and *Processing*.
10. Note: The import of the next image in the queue starts immediately after the *Upload* of the previous one is finished. The *Processing* phase of the import is done on the OMERO.server only, and can be finished while the next image(s) is/are being uploaded to the server.
11. Once imports are finished, go back to the OMERO.insight main window and click the *Refresh* button  above the right-hand pane. This will display the imported images inside the Dataset and/or Project you specified previously in the *Import Location* window.
12. (demo only step) Now, the demonstrator will log out from OMERO.insight and login again, this time as some other user and will show the import process again, this time importing a different set of images. After this import, the two sets of images (belonging to two different users) will be shown in the webclient.

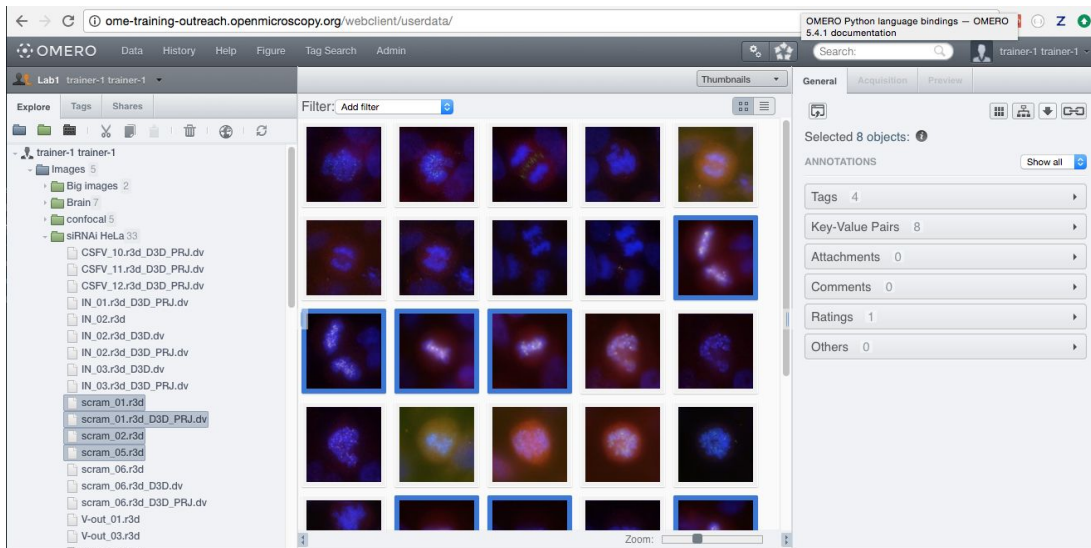
Browse Images

1. In your web browser, go to <https://outreach.openmicroscopy.org/>
2. Log in using the username and password provided.
3. OMERO offers various levels of permissions for groups and users. Depending on the permissions level of each group, a given user might be able to view, annotate or edit data belonging to other users. Permissions are managed by users with admin privileges. For this training session, we will work in a “Read-Annotate” group. This implies that users can view and annotate each other’s data but **cannot** delete other people’s data.

- To see other people's data, click on the *Lab1* group name in the top-left corner of the webclient. Then select the name of the user you wish to see the data from the menu.

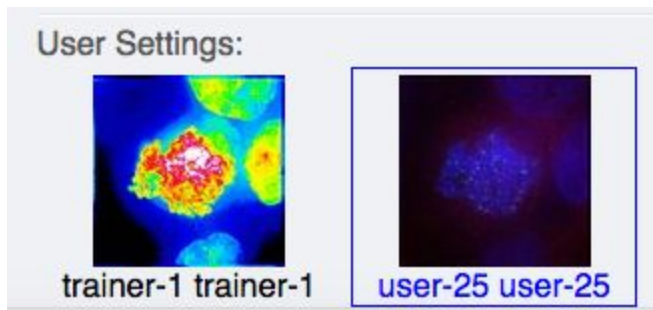


- You can browse 'folders' in the left-hand pane: *Image* folders are called *Datasets* and they are within *Projects*.
- When a Dataset is selected, Image thumbnails are shown in the centre panel.




- These represent imported Images. The original Images are stored on the server and the generated thumbnails allow us to browse them.
- Bio-Formats, <https://www.openmicroscopy.org/bio-formats/>, is used to read the pixel-data and metadata from over **150** different image formats, including multi-z timelapse images with many channels, they are referenced as 5D Images. Large pathology and Medical images are also supported.

9. Select an Image. In the right-hand pane, metadata read by Bio-Formats and stored in a relational database is displayed:
 - a. core metadata in the *General* tab
 - b. additional metadata in the *Acquisition* tab. All the metadata read by Bio-Formats can be downloaded at any time.
10. In the *Preview* tab in the right-hand panel, you can also view the Image.
11. For multi-plane images, sliders allow you to move through Z or Time dimensions.
12. Viewing Images **DOES NOT** download the whole Image to the client. Only the viewed Image plane is rendered from the original Image file on the server and sent back to the client.
13. You can adjust the rendering settings for each channel e.g. turn on/off the channels, adjust color settings, look-up tables, etc..
14. The rendering settings can be saved to the server. This **NEVER** changes the original Image data and can be reverted at any time.
15. The rendering settings can also be copied and pasted between Images. To modify the rendering settings in batch, click on the *Save to All* button to apply the same settings to, for example, all Images in a given Dataset.
16. You can use the settings which other users saved on your Images and apply them for your own Image. These settings are highlighted as thumbnails in the lower part of the *Preview* pane.

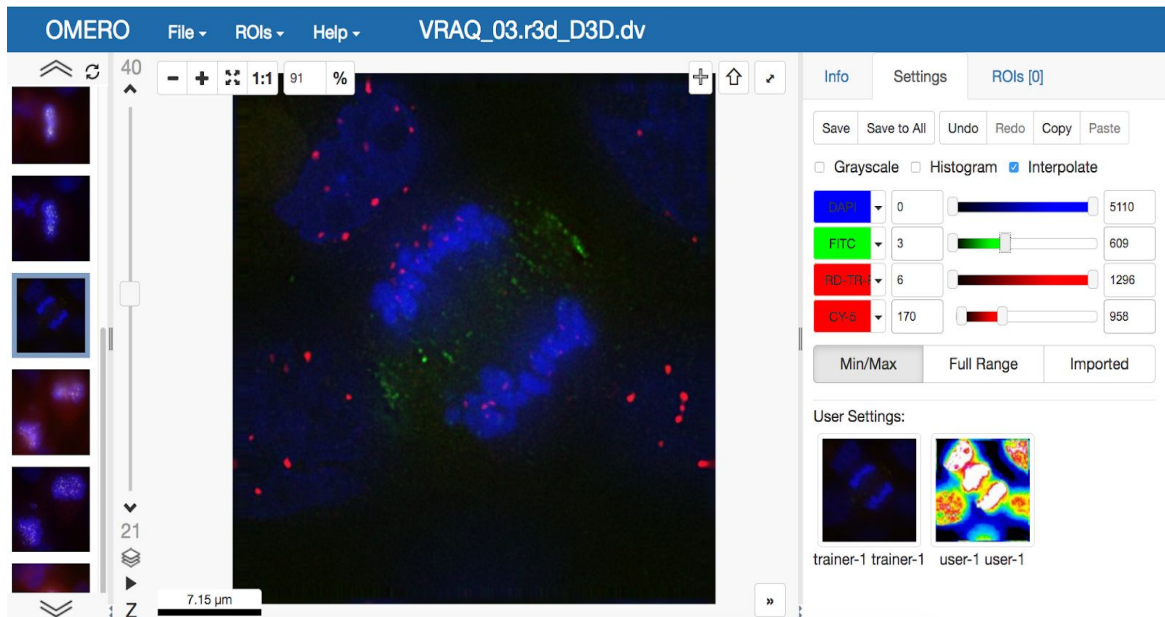



17. Your own settings are highlighted in blue.
18. You can revert to the original settings for an Image or Dataset. For example, using the context menu for a Dataset in the tree, select *Rendering Settings > Set Imported and Save*.

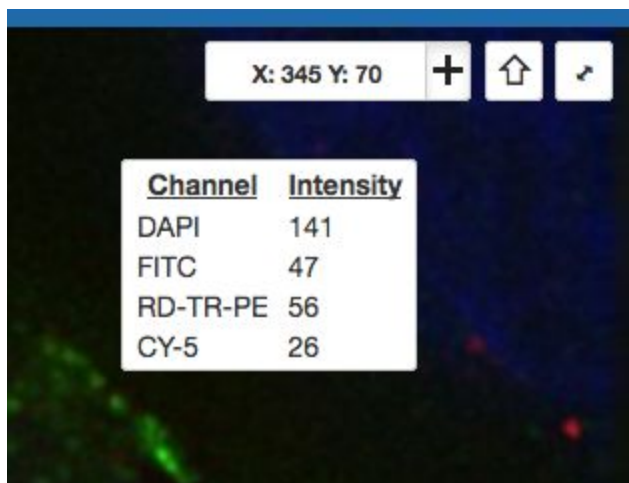
View Images




1. Double-click on an Image thumbnail, or click the *Full Viewer*  button in the right-hand pane to open an Image in a larger viewer called OMERO.iviewer. It is a Web app developed and released independently from the webclient. The OMERO.web framework can be extended with multiple apps to view data in different ways.

- We can adjust the rendering settings and scroll through Z or T.

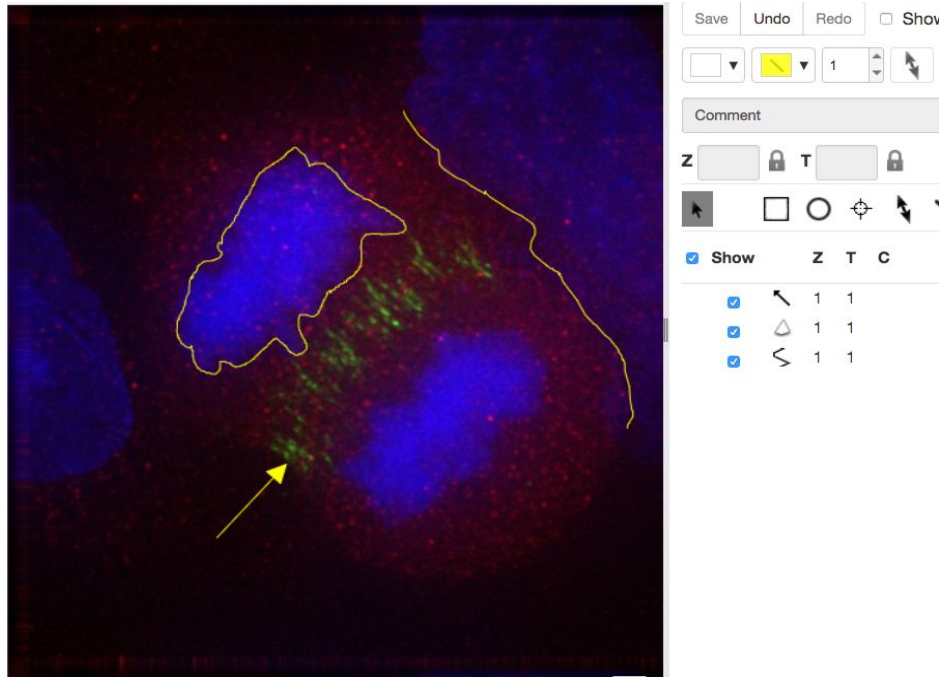


- Click the *Crosshairs* icon  at the top-right of the viewer to enable the pixel intensity display for the mouse pointer. Then mouse over the Image to see the pixel intensities for the channels turned on.



- The ROIs tab  includes tools for viewing and drawing ROIs on the Image. These are saved back to the OMERO server. Select the *arrow tool* in the right-hand pane  and draw an arrow on the Image, using Click-Move-Click (not drag), pointing to a feature in the Image and save it using the *Save button*  located in the upper part of the right-hand pane. Draw also a couple of other ROIs on different Z planes.

- a. Dragging on the Image is used for panning the image.
- b. *Shift-drag* rotates the image (using selection tool) or draws “freehand” (polygon, polyline, rectangle and ellipse).

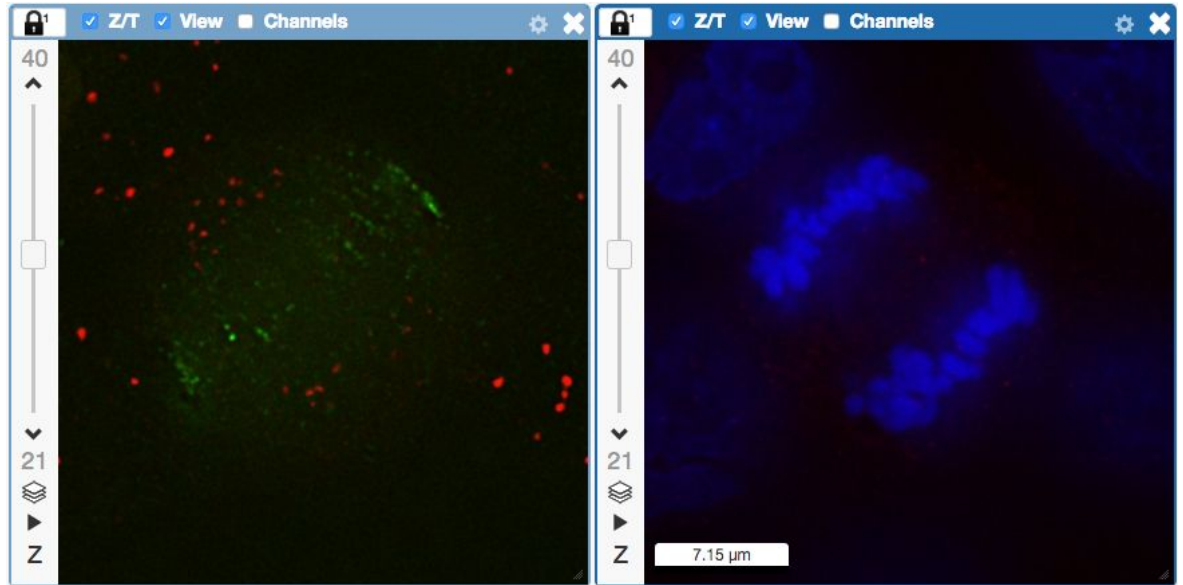


5. Clicking onto the ROIs in the right-hand pane table brings the viewport to the position where the selected ROI is in the middle of it and navigates the viewport to the timepoint the ROI is associated with.


<input checked="" type="checkbox"/> Show	Z	T	C	Comment	
<input checked="" type="checkbox"/>	1	1	1		
<input checked="" type="checkbox"/>	1	1	1		
<input checked="" type="checkbox"/>	1	8	1		
<input checked="" type="checkbox"/>	1	11	1		
<input checked="" type="checkbox"/>	1	15	1		

6. Select several ROIs from different Z planes. You can select in the table (*Shift-click* to select a range) but also in the image itself using *Cmd-drag* to select multiple ROIs.
7. Export the Intensities, areas and line lengths into Excel. Select, in the top-left corner *ROIs > Export (Excel)*. This will export the values from the selected ROIs.
8. Save the viewport by selecting in the top-left corner *File > Save Viewport as PNG*.
9. Compare the two channels of one of the Images in multi-image view:
 - a. Double-click on the thumbnail of the already opened Image in the left-hand pane of the viewer. This will open the Image again in a new window.
 - b. Select one of the windows and in the right-hand pane switch the green channel off.

- c. On the other window, switch the blue channel off.



10. We can synchronize the viewers by adding them both to the same sync Group.

- Click the *sync icon*  in the top-left corner and select the first option in the dropdown menu.
- Repeat for the other viewer.
- Now both Images have the *Z/T* and *View* (zoom and position of viewport)

synchronized.



11. Click on *Info* tab in iviewer now, and find, in the right-hand pane, the link to *Dataset*:



Dataset:

[siRNAi-HeLa](#)

. Click on that link. This will bring you back

to the webclient.


12. Adjust the channel names:

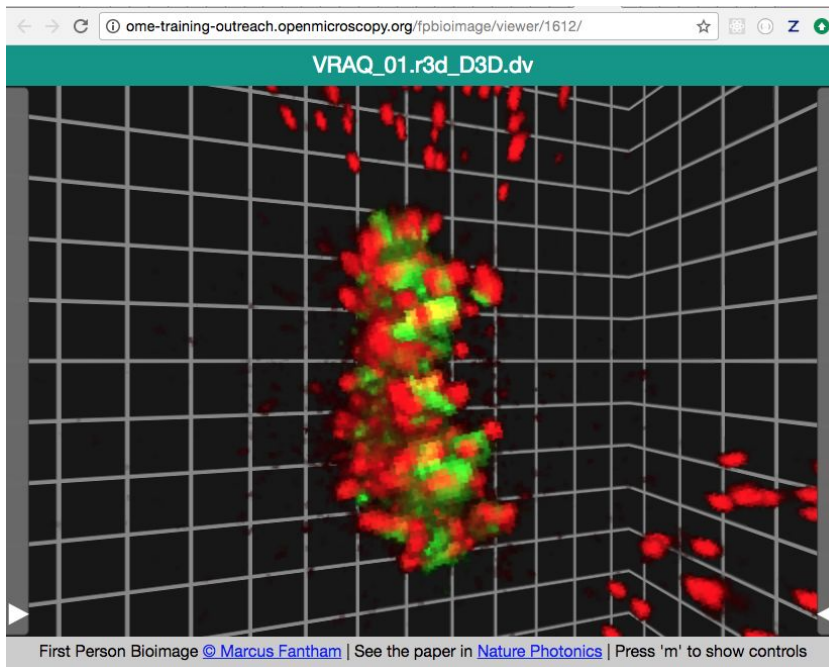
- Select any image inside that Dataset and click on the *pencil*  icon in the right-hand pane next to Channels.
- Input **“DAPI”** instead of channel **“457”** and **“FITC”**, **“GFP-INCENP”** and **“CY-6”** for the other channels.
- Click the *Apply to All* button  and confirm by clicking *Continue*.

13. Open an image from the Dataset **siRNA-HeLa** with multiple Z-sections e.g.

VRAQ_01.r3d_D3D.dv in a 3D viewer: OMERO.FPBioimage.

- First select the Image.
- In the *Preview* tab, switch off all channels except FITC and the GFP-INCENP channel.
- Save the new rendering settings.

- d. Right-click on the image, or use the *Open with...* icon on top of the right-hand pane  to open the image with FPBioimage.
- e. Click *Start* in the new viewer window.
- f. We can see that the centromeres are well aligned on the metaphase plate on the selected Image, whereas the centromeres are located in and around the spheroid on the **IN_02.r3d** Image for example.



14.

Add Annotations, Filtering and Searching

1. Let's now add annotations to Images in the webclient. We will add Tags to indicate Metaphase stages of these cells.
2. Select one or more Images of cells which appear to be in metaphase to tag.
3. Choose the *Tag* option in the right-hand General tab and click [+] to launch the Tag dialog.
4. Choose the existing **Metaphase** from the list of Tags (type to filter above the list).
5. Click > to move it to the right column, then click OK.
6. Select an Image from the Dataset and in the right-hand *General* tab, click the harmonica *Key-Value Pairs*.



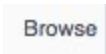
Added by: user-40 user-40

Key	Value
mitomycin-A	0mM
PBS	10mM
incubation	10min
temperature	37
Organism	Homo sapiens

- The *Key-Value Pairs* allow you to add lab-book like additional metadata for the Image. These *Key-Value Pairs* are also specifically searchable.
- It is also possible to add Comment, Attachment and Rating to selected Images.
- Enter **mitomycin-A** into the search box in the top right corner of the webclient

and press Enter.

- The search results will show any objects e.g. Images or Datasets which have anywhere the string **mitomycin-A**. Several images should be found, from 2 different datasets..
- Refine the search now for only *Key-Value Pairs* which have the key **mitomycin-A** and value **0mM** by entering **mitomycin-A:0mM** into the search box and pressing Enter.

Now you should find only 21 images. Click on the *Browse* link  in one search result line of the last image (in the right-hand part of the centre pane) to navigate back to the main webclient.

- Images can also be filtered by Tag or Rating in the centre pane, using the *Add filter* chooser above the thumbnails.
- For example, choose Tag and then select **Metaphase** from the list of Tags to show the images we tagged earlier.
- Review the filtered Images, choose a favourite Image and under the *Rating* section in the right-hand pane, click on the 5th star to add a rating of 5

- Now we can remove the filtering by Tag and instead filter by *Rating of 5* to show only our favourite images.

Analysis with Fiji

Description

Fiji is a free open-source image processing package based on ImageJ.

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 a CSV and OMERO.table,

If analyzing other users data, the user analyzing the data needs to have admin privileges.


More information about restricted privileges can be found at

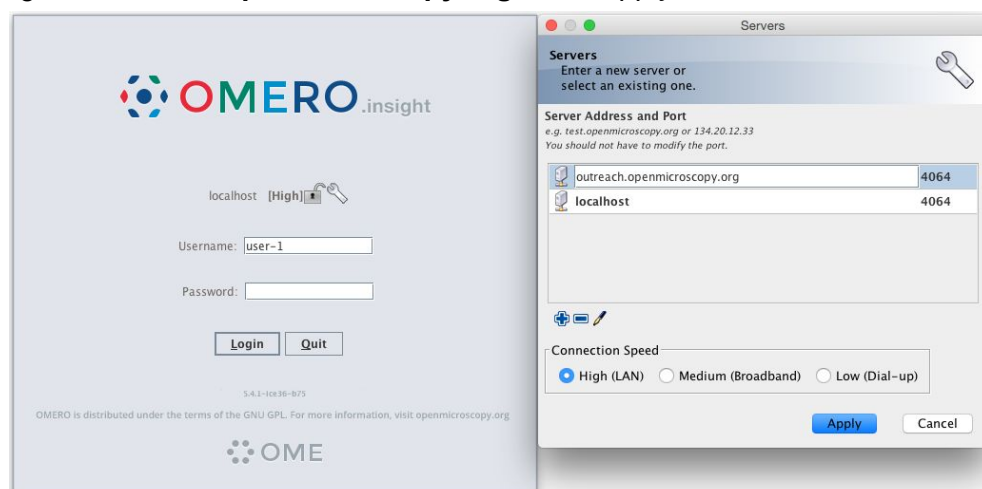
<https://docs.openmicroscopy.org/latest/omero/sysadmins/restricted-admins.html>.

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.

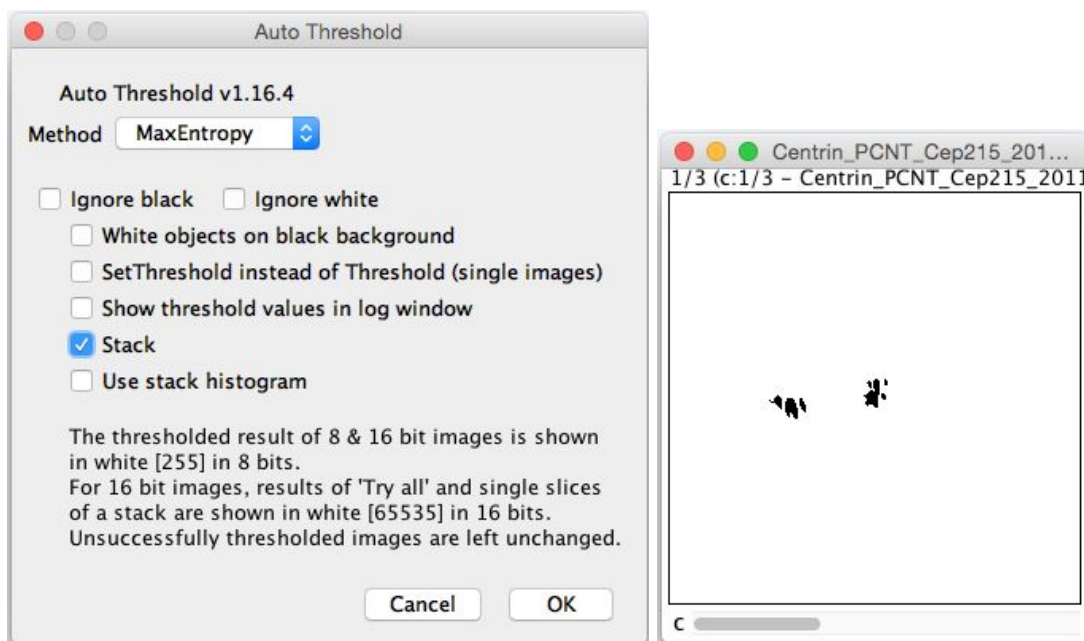
Fiji Manual workflow

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*.

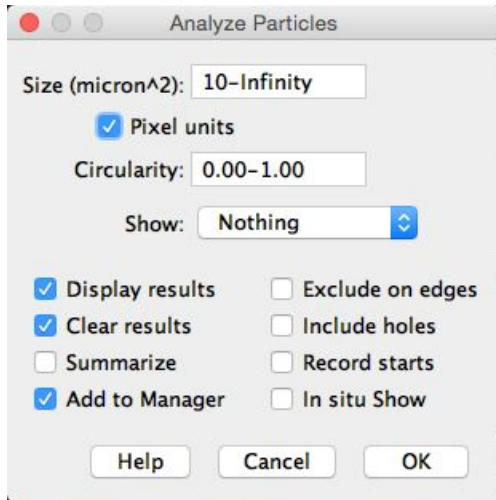


4. Enter your *Username* and *Password* and click *Login*.

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.
 - Make sure to select *View stack with: Hyperstack* in the *Bio-Formats Import Options* dialog.
 - 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:
 - Select *MaxEntropy* for the *Method* parameter.
 - Check the checkbox *Stack*.



9. Click OK.
10. Then open *Analyze > Analyze Particles...*
11. In the dialog
 - Set *Size* to *10-Infinity* and check *Pixel units*.
 - Check the following checkboxes:
 - i. *Display results*
 - ii. *Clear results*
 - iii. *Add to Manager*
 - Click OK then Yes in the popup dialog indicating asking to *Process all 3 images?*



12. To save the thresholded Image back to OMERO with the generated ROIs and the measurements:
 - Select *Plugins > OMERO > Save Image(s) to OMERO*.
 - Create a New Dataset for the image
 - i. Click the *New...* button next to the selection box on the Dataset row.
 - ii. In the dialog that pops up, enter a name and a description (optional).
 - iii. Click *Create*.
 - The newly created Dataset will automatically be selected.
 - Click *Add to the Queue* then *Import*.
 - Go to the webclient 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.

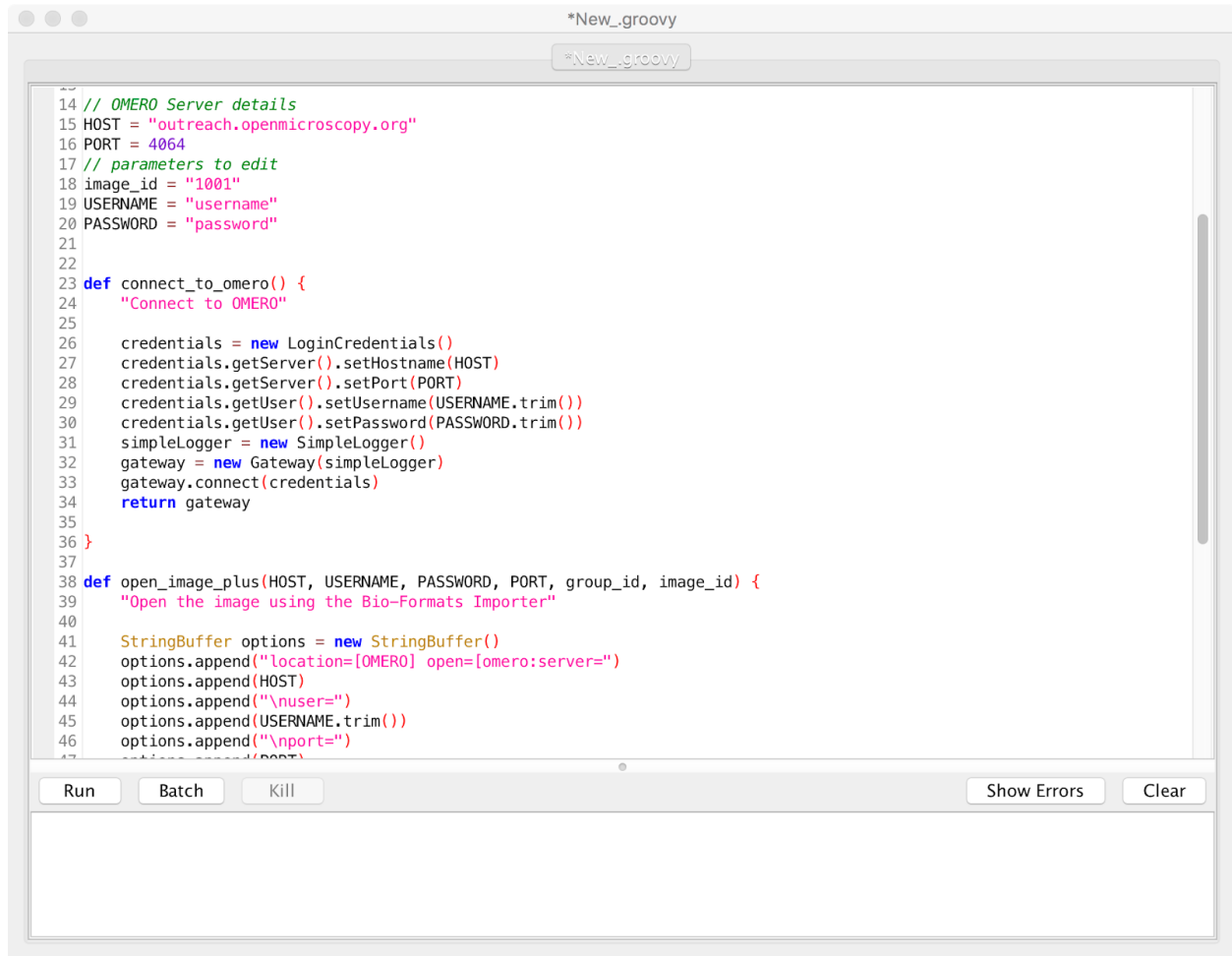
Fiji Scripting workflow

We will now repeat the manual analysis on a Dataset using the scripting facility available in Fiji. Now, we will present 3 simple steps (three scripts) to facilitate this analysis. Later today, in the IDR section, we will show all 3 steps in one larger script.

Step 1: Open an image from OMERO in Fiji using a script

1. Go to <https://outreach.openmicroscopy.org/>.
2. Log in using the credentials provided
3. Make sure you are selecting your own data. Select the Dataset **A-Fiji-dataset**.
4. Select the first image in that Dataset. Note its ID on the right-hand panel, it will be used below.
5. Launch Fiji. (relaunch if opened).
6. Go to *File > New > Script...*
7. A dialog pops up. In the *Language* menu select *Groovy*.

- Copy the content of the script
https://raw.githubusercontent.com/ome/training-scripts/v0.3.0/practical/groovy/open_omero_image_no_download.groovy into the text editor.
- Edit the following parameters: image_id, USERNAME, PASSWORD.



```
14 // OMERO Server details
15 HOST = "outreach.openmicroscopy.org"
16 PORT = 4064
17 // parameters to edit
18 image_id = "1001"
19 USERNAME = "username"
20 PASSWORD = "password"
21
22
23 def connect_to_omero() {
24     "Connect to OMERO"
25
26     credentials = new LoginCredentials()
27     credentials.getServer().setHostname(HOST)
28     credentials.getServer().setPort(PORT)
29     credentials.getUser().setUsername(USERNAME.trim())
30     credentials.getUser().setPassword(PASSWORD.trim())
31     simpleLogger = new SimpleLogger()
32     gateway = new Gateway(simpleLogger)
33     gateway.connect(credentials)
34     return gateway
35 }
36
37
38 def open_image_plus(HOST, USERNAME, PASSWORD, PORT, group_id, image_id) {
39     "Open the image using the Bio-Formats Importer"
40
41     StringBuffer options = new StringBuffer()
42     options.append("location=[OMERO] open=[omero:server=")
43     options.append(HOST)
44     options.append("\nuser=")
45     options.append(USERNAME.trim())
46     options.append("\nport=")
47     options.append(PORT)
48 }
```

- Click *Run*.
- The script will open the specified Image in Fiji.

Step 2: Open all images from one OMERO Dataset in Fiji in sequence, segment them, draw ROIs and save these ROIs back to OMERO using a script.


- Make sure you are selecting your own data. Select the Dataset **A-Fiji-dataset**. Note the Dataset ID from the right-hand pane.
- Launch Fiji. (relaunch if opened).
- Go to *File > New > Script...*
- A dialog pops up. In the *Language* menu select *Groovy*.
- Copy the content of the script
https://raw.githubusercontent.com/ome/training-scripts/v0.3.0/practical/groovy/analyse_dataset_and_save_rois.groovy into the text script editor of Fiji.
- Edit the following parameters: dataset_id, USERNAME, PASSWORD.

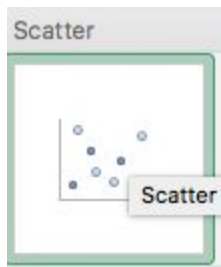
7. The script will process all the Images in the specified Dataset, applying threshold, analyzing particles and saving ROIs back in OMERO i.e. we reproduce in a script the manual steps recorded above.
8. Return to the webclient and open an Image from this Dataset in OMERO.iviewer.
9. Click the *ROIs* tab to see the added ROIs. Note that the ROIs have been assigned a Channel index to indicate which Channel they were derived from.
10. In the *Settings* tab, turning channels on/off will also show/hide ROIs assigned to those channels.
11. Open the image in OMERO.figure for a quick publication by going to *Info* tab in iviewer

and clicking on *OMERO.figure* in the *Open with* line. 

Step 3: Another script in Fiji, which will not only segment the cells and draw ROIs but also will take the measurements from those ROIs from Fiji and export them as CSV saved in OMERO. Further, it will create an OMERO.table out of those measurements.

1. Launch Fiji. (relaunch if opened).
2. Go to *File > New > Script....*
3. A dialog pops up. In the *Language* menu select *Groovy*.
4. Copy the content of the script
https://raw.githubusercontent.com/ome/training-scripts/v0.3.0/practical/groovy/analyse_dataset_save_rois_and_summary_table.groovy into the text script editor of Fiji.
5. Edit the following parameters: *dataset_id*, *USERNAME*, *PASSWORD*.
6. 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. Further, it will create a CSV and OMERO.table to be attached to that Dataset in OMERO.
7. This script was used previously to run on the whole **idr0021** Project and produce the resulting CSV file. We can now download this file from OMERO and open it in Microsoft Excel.
8. In Excel, select the Dataset column and the column *bounding_box* and then click on

Insert > X Y Scatter  *> Scatter* . Depending on the version of Excel you are using, this option might not be available.



9. This will create a scatter plot. Right-click into the Chart now and select *Change chart type > Statistical > Box and Whisker*.

Fiji-TrackMate example

In this example we open all images from a dataset in OMERO in Fiji and use TrackMate plugin of Fiji. For more details about TrackMate, go to <https://imagej.net/TrackMate>. We first find the tracks of the moving objects. Afterwards, these tracks (polygon ROIs), as well as the segmented objects ROIs will be saved to the same images in OMERO. Also, an OMERO.table will be produced from the measurement results of TrackMate and saved as an attachment to the Project containing the images in OMERO.

To determine the parameters suitable to analyse the images, we will first go through a manual TrackMate workflow, then later we will use the parameters in a scripted TrackMate workflow.

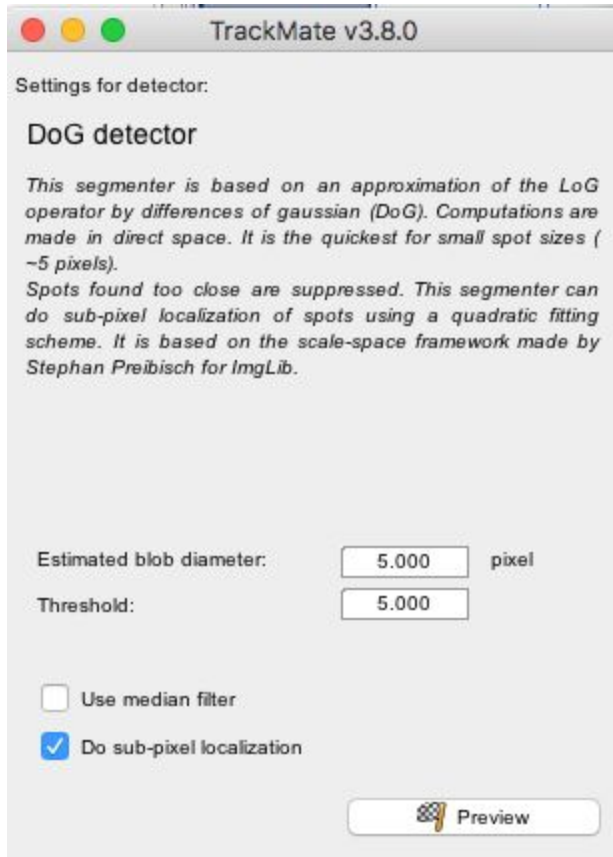
Setup

- 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.

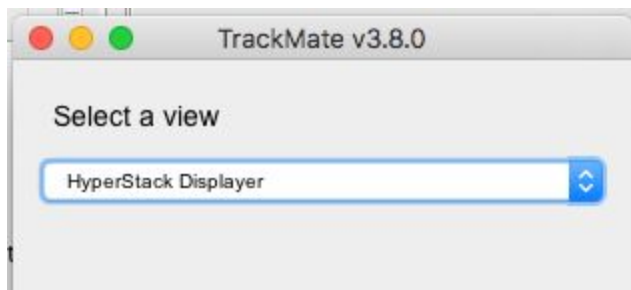
Manual

Each of you can work on your own data. We will use this manual workflow to explain the steps of the script below.

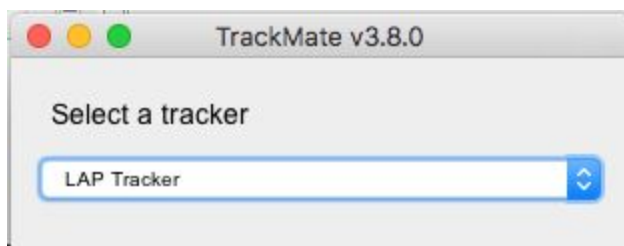
1. Launch Fiji
2. Go to *Plugins > OMERO > Connect to OMERO*
3. Enter the username and password provided for you.
4. Browse the Dataset **artificial-trackmate**
5. Double-click on the **FakeTracks.tif** Image to open it in Fiji. Make sure it is opened using the Hyperstack viewer (This option is dependent on what you did last time when using your Fiji. If you last time used Fiji for running the scripts from Day 1 of this workshop, you should be fine.)
6. Go to *Plugins > Tracking > TrackMate*.
7. Click *Next* in the first dialog that pops up.
8. A new dialog pop ups indicating to select a *detector*. Select the *DoG detector*. Click *Next*.
 - a. Set the *Estimated blob diameter* to 5.0
 - b. Set the *Threshold* to 5.0



9. Click the *Preview* button to find spots. 4 spots should be found
10. Click the *Next* button few times, until you get to the *Select a view* dialog.
11. Select the *HyperStack Displayer* view

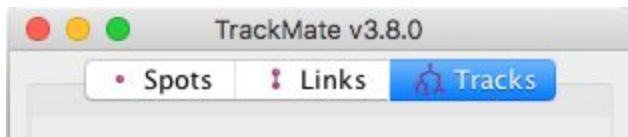


12. Click the *Next* button twice, until you get to the *Select a tracker* window. Select the *LAP Tracker*

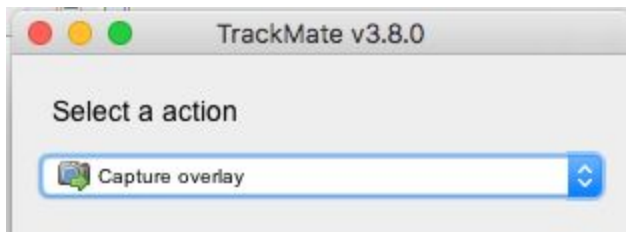


13. Click the *Next* button few times (5), until a dialog with three tabs pops up, see screenshot below.

14. Select the Tracks tab to display the Tracks.



15. Click Next until you get to *Select a action* dialog.
 - a. Select the option *Capture overlay*
 - b. Click *Execute*.

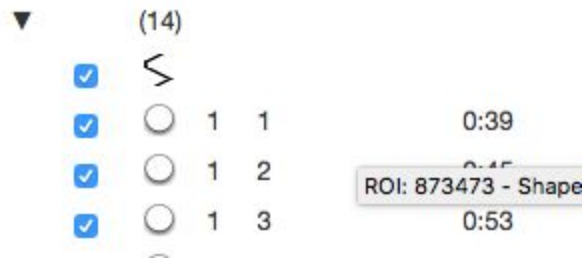


16. Click OK in the following dialog, leaving the defaults (the whole stack will be captured).
17. New image appears. Select it. This new Image can then be imported as on OME-TIFF using the option *Plugins > OMERO > Save Image(s) to OMERO*.

Programatically

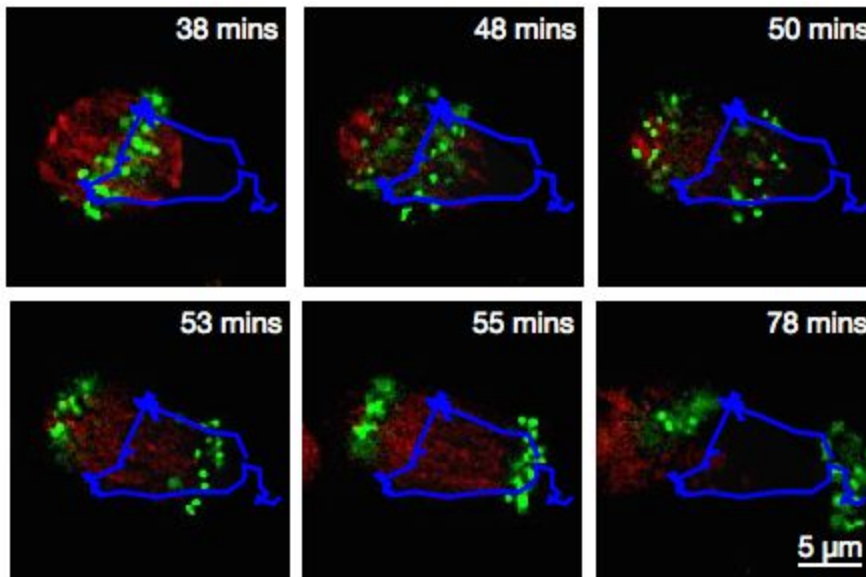
This option saves the ROIs to the server, we do not generate a new Image.

1. Go to <https://outreach.openmicroscopy.org/webclient/>.
2. Enter the username and password provided.
3. Make sure you are working with **your own data**.
4. Create a Project: **trackmate**.
5. Drag and Drop the Dataset **dv-iain-multiTZ** into the Project: **trackmate**. Note the ID of the Project.
6. Launch Fiji, open *File > New > Script...* and select *Python* as language.
7. Copy the content of the script <https://raw.githubusercontent.com/ome/training-scripts/v0.3.0/practical/jython/tracker.jy> into the script window of Fiji.
8. Edit the credentials to connect to the server and change the ID of the Project in the script window of Fiji, replacing it with the ID of your Project **trackmate**.
9. Study the script step-by-step.
10. Click *Run*.
11. After the script has finished, go to the images in the webclient and open the second image in OMERO.iviewer.
12. Click on the ROI tab and observe that you now have ROIs under which there are Shapes. Each ROI is a collection of shapes. The ROI corresponds to a Track in Trackmate. There is always one polyline shape in each ROI which represents the track. The other, elliptical shapes in the same ROI represent the tracked spots.



13. Play the timelapse video in OMERO.iviewer.

14. Go to the *Info* tab, and in the *Open with:* line click on *OMERO.figure*. In *OMERO.figure*, add the Tracks and ellipses to the panel by selecting the appropriate ROIs in the *Labels* tab of *OMERO.figure*.



Orbit workflow: Manual training and segmentation

Description:

Orbit Image Analysis is a free open-source software with the focus to quantify big images like whole slide scans. It offers sophisticated image analysis algorithms. Of those, tissue quantification using machine learning techniques, object / cell segmentation, and object classification are the basic ones. For more details, go to <http://www.orbit.bio/>.

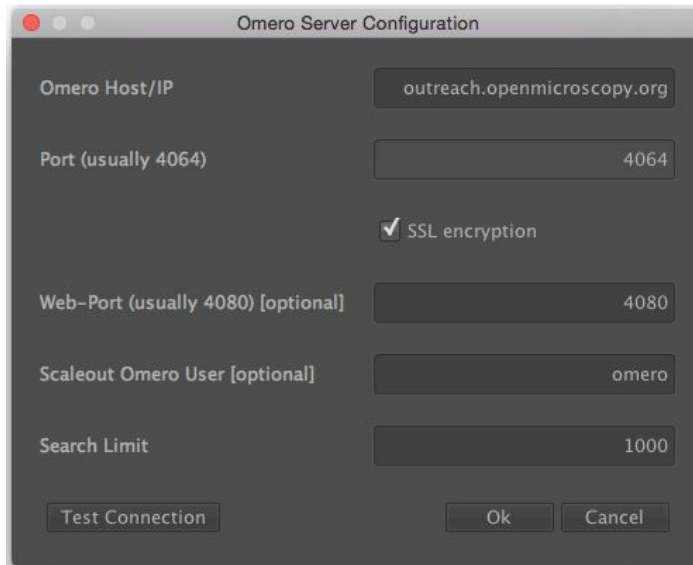
Setup

- Orbit has been installed on the local machine.

1. Launch Orbit, click Yes in the first dialog box:



2. Then enter server details in the next dialog. Only the Host/IP field is essential here and should be set to **outreach.openmicroscopy.org**.

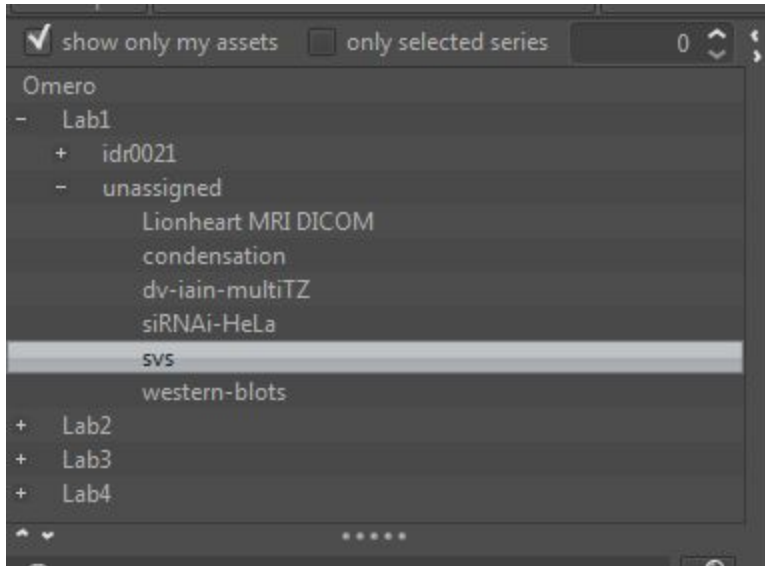


3. Then login to OMERO with the username and password provided:



4. Orbit will show data from OMERO in the left-hand panel. Click *show only my assets* to filter by data you own.
5. Select the group **Lab1**.

6. Datasets not within a Project are listed under *unassigned*:



7. Select the **svs** Dataset. Image thumbnails will be shown in the panel below.
8. Double-click on the **77928.svs [Series 1]** thumbnail or drag and drop it into the centre panel and maximise the viewer window to fill the centre panel.

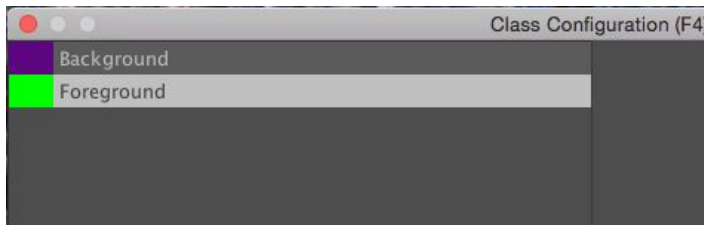


9. We want to train a model to recognize cell nuclei and use this for segmentation.



10. Click the *Model* tab and then the *Classes* button
11. In the dialog, remove the *Celltype 1* class by selecting it and click the *remove class* button.
12. The select the *Celltype 2* class, and rename it by typing *Foreground* in the name field and clicking *rename class*.

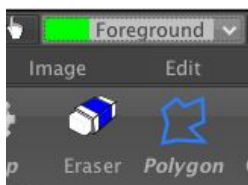
13. You should now have two classes named *Background* and *Foreground*:



14. Click *OK* to close the dialog.

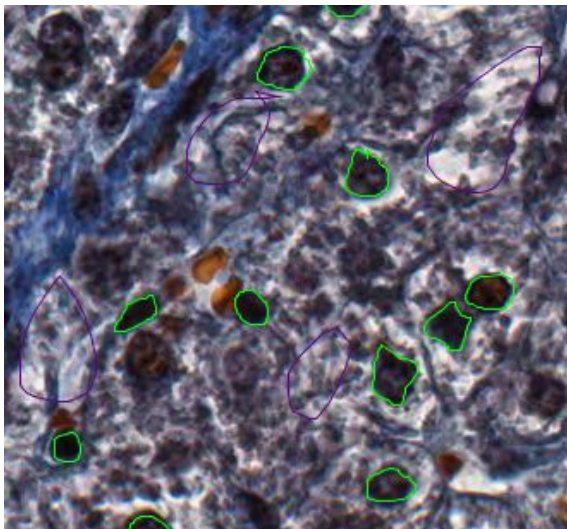
15. Now we will construct the model by defining regions of *Foreground* and *Background* on the Image.

16. Click the *Object Detection* tab, select the *Polygon* tool and choose the *Foreground* class from the chooser at the top-left of the screen.



17. You can now draw around a number of cell nuclei on the Image. The more accurately you draw and the higher number of objects you define will improve the performance of the segmentation, but about a dozen should be sufficient.

18. Now switch to the *Background* class and draw around several background regions:



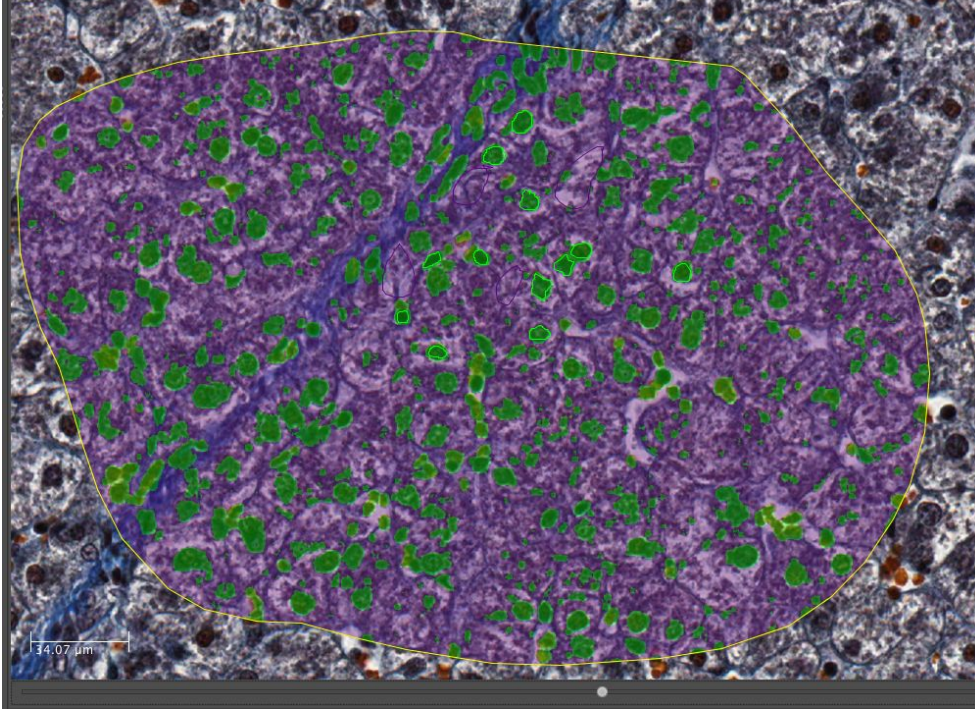
19. We can then train the model by clicking the *Train* button or press F7. You will see a progress bar in the right-hand panel.

20. To see how this model classifies objects within a region, click the *Define ROI* button and draw around a region of the image. Then click *Classify*. If no ROI is drawn, Orbit will

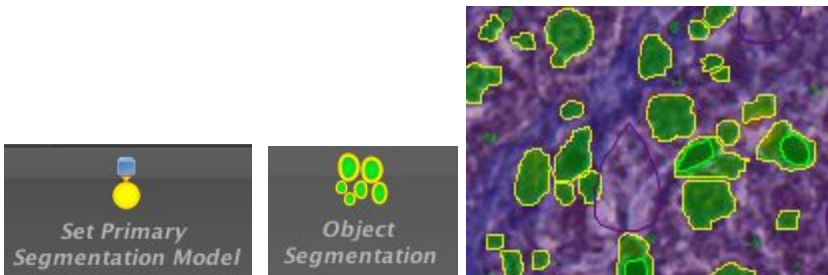
attempt to classify the whole Image which can be very time-consuming.



21. Once the classification is complete, a notification window pops up. Close it and view the results on the Image by dragging the slider below the Image to the right:



22. To segment the Image using this classification, click *Set Primary Segmentation Model* and then *Object Segmentation*.



23. Click the *Model* tab and *Save Model On Server*, enter a name to save the model to OMERO. Note that you can also use *Save Model as...* to save the model to your local drive.

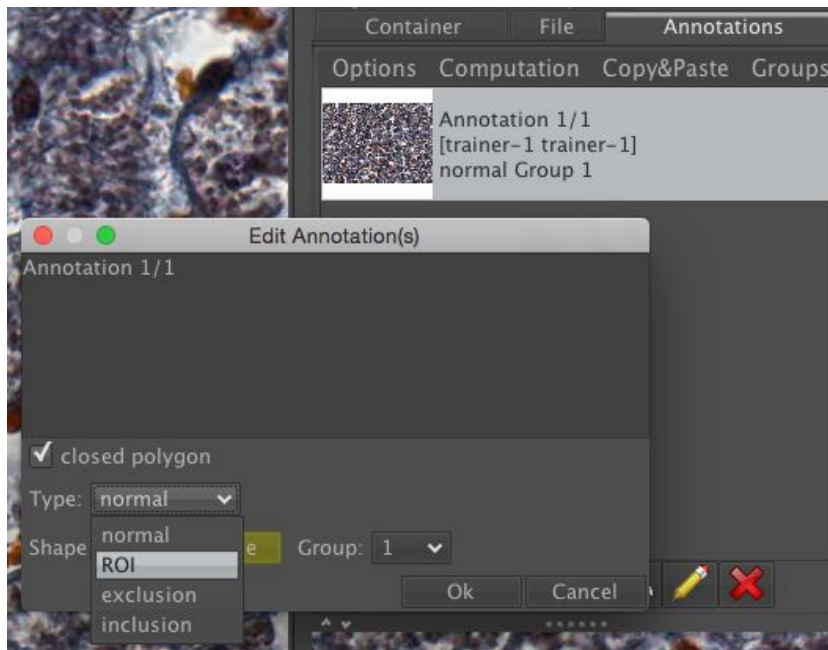
Orbit workflow: Scripted segmentation and saving to OMERO

Description: We will use the model created in the last step above to repeat the segmentation, using a script which allows us to save the results back to OMERO. This will use a saved ROI Annotation instead of a temporary ROI as in the manual workflow.

1. Re-open the same image **77928.svs [Series 1]** to clear the ROIs and in the right-hand panel select the *Annotations* tab.
2. Pan the Image to a region you wish to analyse, select the *Add Polygon* button and draw

around a region. 

3. Select this *Annotation* from the list in the right panel and click *Edit* (pencil icon).
4. In the dialog, set the *Type* to *ROI*.



5. Click *Ok*. This will save the ROI as an annotation on this image in OMERO.
6. Click on *Tools > Script Editor* to open a scripting window.
7. Copy the script from training-scripts:
<https://raw.githubusercontent.com/ome/training-scripts/v0.3.0/practical/orbit/segmentation.groovy> and replace the existing code in the script window.
8. Update the username and password
9. The script will load the Orbit model and the ROI that we saved to OMERO, segment the image within the ROI and save the segmented shapes as Polygons to OMERO.
10. Click *Run*.
11. When complete, you can use OMERO.iviewer to see the ROIs created in OMERO.

Using the OMERO Java API to create Annotations

1. We can use the Orbit's script editor as a convenient way to explore the OMERO Java API.
2. Orbit interacts with OMERO (as above) using the Java class
<https://github.com/mstritt/image-provider-omero/blob/master/src/main/java/com/actelion/research/orbit/imageprovider/ImageProviderOmero.java>

3. We will use the “**Create a Map Annotation**” example from <https://docs.openmicroscopy.org/latest/omero/developers/Java.html> to add an annotation to the Image we have just analysed.
4. We will reuse the segmentation script that we have just run, but instead of saving Polygons we will create a Map Annotation to summarise some parameters of the analysis.
5. Find the “**Create a Map Annotation**” section on the OMERO Java documentation page above.
6. Copy this code and go to the Orbit script editor, with the segmentation script.
7. Edit the script to remove the `for` loop that saves Polygons `for (Shape shape: res.shapeList) {`, replacing it with the code we have just copied.
8. We can update the Keys and Values to e.g.:
 - Analysis: Orbit
 - ROIs: Roi count
 - Model: Model name
9. We also need to link the Annotation to an Image instead of a Project, using the `omeroImageId`.
10. For example:

```

57
58 List<NamedValue> result = new ArrayList<NamedValue>();
59 result.add(new NamedValue("Analysis", "Orbit"));
60 result.add(new NamedValue("ROIs", "" + res.shapeList.size()));
61 result.add(new NamedValue("Model", model.getName()));
62 MapAnnotationData data = new MapAnnotationData();
63 data.setContent(result);
64 //Use the following namespace if you want the annotation to be editable
65 //in the webclient and insight
66 data.setNameSpace(MapAnnotationData.NS_CLIENT_CREATED);
67 DataManagerFacility fac = gateway.getFacility(DataManagerFacility.class);
68 fac.attachAnnotation(ctx, data, new ImageData(new ImageI(omeroImageId, false)));
69


```

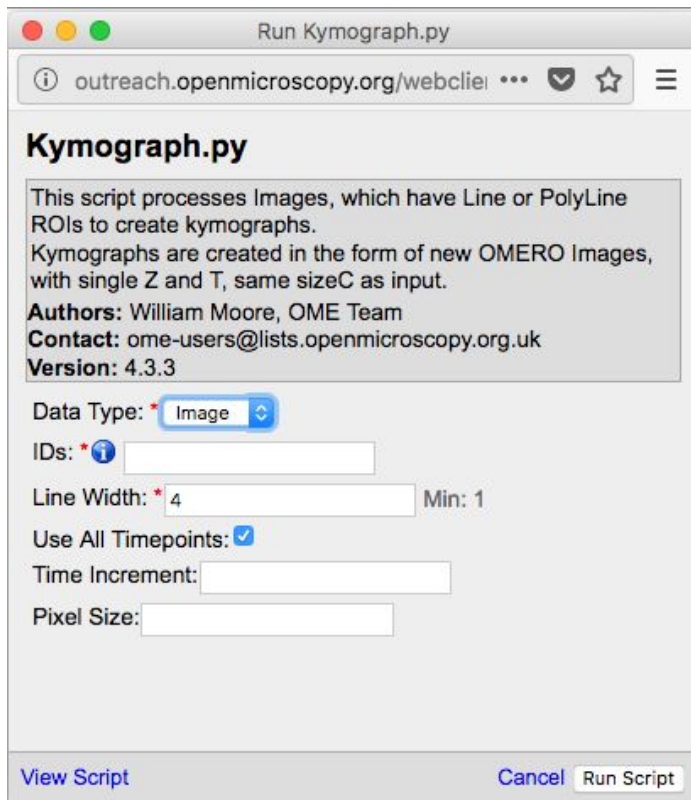
11. Run the script again and then check in the webclient for the Map Annotation on the Image.

Analysis workflow Server side

We will now use a server-side script for creating a Kymograph from an Image in OMERO. The Image we will use for the Kymograph demonstration has been published in a study investigating protein migration along microtubules <http://jcb.rupress.org/content/194/2/187>. (Bowen et. al. Journal of Cell Biology 194 (2): 187).

1. Go to the Dataset **Kymograph**.
2. Select the Image inside the Dataset.
3. Double-click to open the Image in OMERO.iviewer and draw one or more lines along microtubules which seems to have the most persistent trafficking of objects along them.

4. Save the line(s).
5. Go back to the webclient. Click the *Script* button in the top-right of the page .
6. Select *workshop_scripts* > Kymograph...



Kymograph.py

This script processes Images, which have Line or PolyLine ROIs to create kymographs. Kymographs are created in the form of new OMERO Images, with single Z and T, same sizeC as input.

Authors: William Moore, OME Team
Contact: ome-users@lists.openmicroscopy.org.uk
Version: 4.3.3

Data Type: * Image

IDs: *

Line Width: * 4 Min: 1

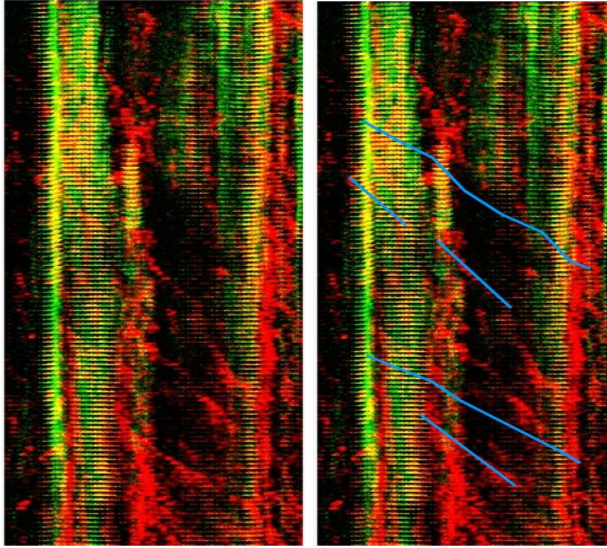
Use All Timepoints:

Time Increment:

Pixel Size:

[View Script](#) [Cancel](#) [Run Script](#)

7. The script will create a new image (=Kymograph) where the pixels under the line region you have drawn previously will be collated into this image timepoint by timepoint. The row of pixels from the first timepoint will be on the top of the new Kymograph Image.
8. Note: the direction in which you have drawn the line ROI on the original image matters with respect to the orientation of the stripes composing the Kymograph image. The start of the original line is on the left of the Kymograph Image, the end on the right.
9. Open the new Kymograph image in OMERO.iviewer.
10. Find some tracks (typically red stripes going under angles across the image, see screenshot below).



11. Draw some lines over these tracks and save them.
12. Go back to the webclient, select the Kymograph Image and select the script *analysis > Kymograph analysis...*
13. Run this script. The *Kymograph analysis* script will produce a CSV file attachment on the Kymograph Image.
14. Open the CSV in Excel for example and verify the speeds of the observed particles in the original image.

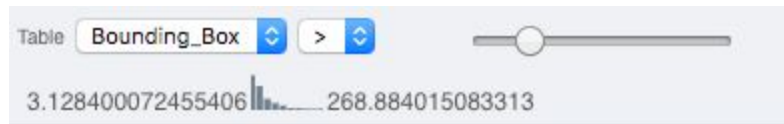
OMERO.parade and OMERO.figure

Analyze metadata using OMERO.parade

First let's look at the analytical results generated using the *Analyze particles* plugin in Fiji

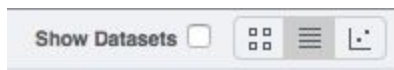
1. Select the Project **idr0021**.
2. Choose the *Parade* option in the centre panel dropdown menu.
3. Expand all Datasets by clicking on the *Open All* button.
 - a. All the Datasets will be expanded in the left-hand tree.
 - b. The Thumbnails will be loaded in the centre panel. This allows to browse a full Project.
 - c. Note that if you collapse a Dataset in the tree, the Thumbnails will be removed from the centre panel.
4. In the *Add filter...* selection box, select the *Key_Value* item.
 - a. When the Map Annotations are loaded, pick the Key *Gene Symbol* and enter the Value *CEP* to show all *CEP* genes and then *CEP120* to show only images with that gene.

- b. To remove this last filter, hover over the filter and click the X button that shows on hover.
5. In the *Add filter...* selection box, select the *ROI_Count* item.
 - a. Enter a Value > 20. When you hover over the area used to enter the value, the range is indicated in the tooltip.
 - b. Then enter < 3 or 4.
6. Remove all filters by clicking the X button showing on hover.
7. In the *Add filter...* selection box, select the *Table* item so we can find using the analytical results generated previously:
 - a. Choose the *Bounding_Box* item and drag the slider to filter the Images. Note that *PCNT* has the largest number of Images with large ROIs.



8. Switch to Table layout (middle button) .
9. In the selection box *Add table data...*, select
 - a. *Table_Bounding_Box*
 - b. *Table_Total_Area*
 - c. *Table_Image*

Note that it is currently not possible to remove a column.
10. Click on the name of a column to sort it.
11. Uncheck *Show Datasets* to sort all Images together e.g. by ROI count.

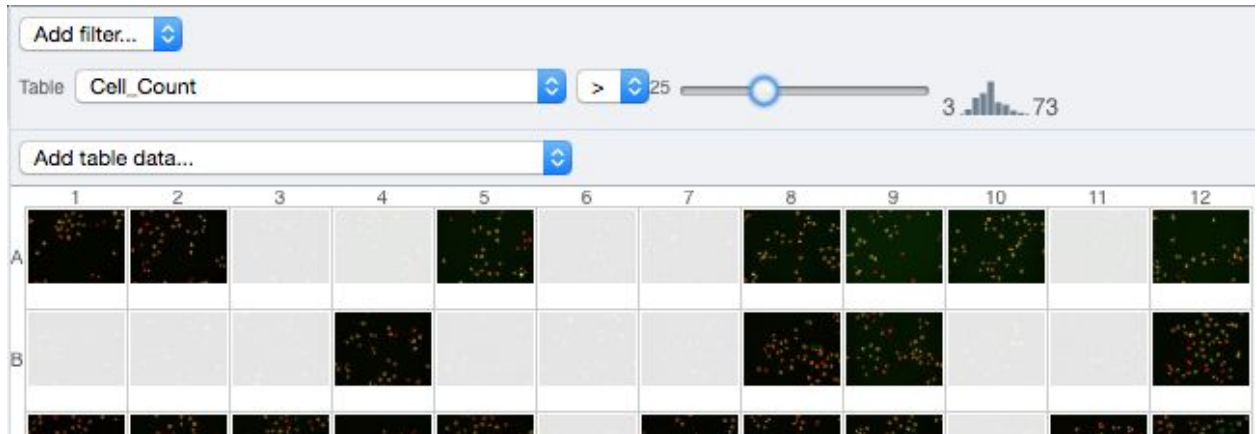


- 12.
13. Check the checkbox in each column to show the *Heatmap*. Note the corresponding pattern in the Heatmap.
14. Switch now to the Plot Layout (third button)
15. It takes the table data loaded and plot the values.
16. Filters can be added to plot the relevant results.
17. Try plotting by different Axis values.
18. Closing a Dataset in the left-hand tree removes the values from the plot.
19. Drag to select several outliers.
20. Note that you can use the selected images in right panel to annotate or *Open with...*
21. Choose *Open with Figure...*

Let's now look at the results generated by CellProfiler

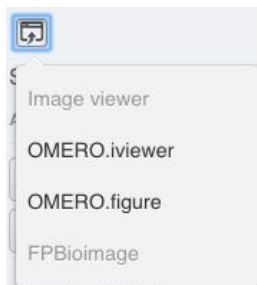
22. Return to the webclient and select the Plate named **plate1_1_013**.


23. Select a Well and open the *Tables* pane in the General tab in the right-hand panel. This will show all the CellProfiler values for this Well.
24. In the *Thumbnails* chooser at the top-right of the centre panel, select the *Parade* plugin.
25. At the top-left of the centre panel choose *Add filter...* -> *Table* to filter Wells by the data from CellProfiler.
26. Change the filter from *ImageNumber* to *Cell_Count* (at the bottom of the list).
27. Now you can use a slider to filter Wells by Cell Count.




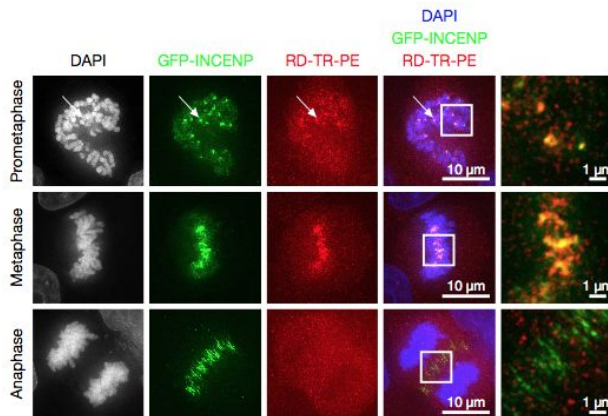
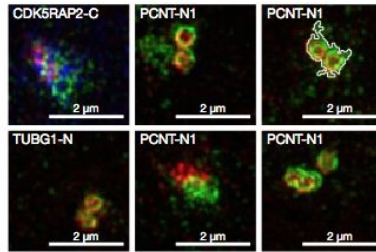
OMERO.figure: Use metadata in figure creation

1. In the webclient, select the **idr0021** Project, filter by e.g. Bounding Box and select several filtered images.
2. In the right-hand panel, click the *Open with...* button and choose *OMERO.figure*:



3. This will open these images in OMERO.figure in a new browser Tab.
4. Drag to arrange the Images into two rows, select all and click the *snap to grid* button  at the top of the page.
5. Select all Images and Zoom in around ~300%
6. Go to the *Labels* tab, select all Images and add a Scalebar of 1 μm and adjust the size of the Label to 12.
7. Add label from Dataset name: *color=white location=top-left*
8. Select one image. In the *Labels* tab, click the *Edit* button for ROIs.

9. Load ROIs from OMERO and mouse-over the list to pick the largest ones.
10. Click to add it to the image and click *OK*.
11. Return to the webclient tab, select the **siRNAi-HeLa** Dataset and filter by Rating of 5 stars.
12. Select 3 images and in the right-hand panel click the link icon  then copy the link.
13. Return to the OMERO.figure tab, click *Add Image* button and paste the link into dialog. Click *OK*.
14. Arrange the 3 images into a vertical column, select all and click *snap to grid* button.
15. Copy the 3 images and paste (keyboard shortcut or *Edit > Copy/Paste*) 3 times to create 3 more columns.
16. Select the panels in the first column and adjust the rendering settings: Turn only the first channel on and set the color to white.
17. Repeat for the next 2 columns: 1 channel turned on for each column, adjusting the levels if desired, leaving the 4th column as 'merged' with multiple channels on.
18. Select all panels and zoom a little. Then select a row and drag the image in the *Preview* tab to pan.
19. Copy and paste the '**merged**' column again to create a 5th column. Zoom in to approx 500%.
20. Select the *Labels* tab, select the '**merged**' and **zoomed** columns and click *Show Scalebar* button.
21. Click the *Label* checkbox to add label to the scalebar. Select only the zoomed-in panels and change the scalebar to 1 micron.
22. Select the top-left panel and enter a label text in the *Add Labels* form.
23. Choose the label size, position (left vertical) and color and click *Add* to create label.
24. Now undo this (*Ctrl-Z* or *Edit > Undo*) and instead create labels from Tags:
25. Select the first column of panels and choose *Tags* from the label text-field drop-down options. Click *Add*.
26. Select the first row of panels and create the labels in the 'top' position using the *Channels* option.
27. Edit the created labels located at the bottom of the *Info* tab to rename the green labels to **GFP-INCENP**.
28. Select just the first '**merged**' image and click the ROIs *Edit* button in the *Info* tab.
29. Draw arrows or other shapes on the image, or load ROIs from OMERO. Click *OK*.
30. Click *Copy ROIs* in the *Info* tab, select the other panels in the same row and click *Paste* to add ROIs to these panels.
31. To create a Rectangle ROI indicating the region of the zoomed-in image, select the zoomed-in image on the first row and click *Copy* of the cropped region at the bottom of the *Preview* tab.
32. Now select the zoomed-out '**merged**' panel, and paste this region as an ROI by clicking *Paste* under ROIs section of the *Labels* tab.
33. Repeat for other rows of the figure.



34. Go to *File > Paper Setup...* and in the dialog that pop up choose *Pages: 2*. Click *OK*.
35. Finally return to the webclient, select 'Big' images from the **svs** Dataset, copy the link to them and paste into the *Add Image* dialog in OMERO.figure.
36. Move the big images to the 2nd page.
37. In the header, click on the *Save* button to save the Figure as "Figure 1".
38. To open other saved files, go *File > Open...*
39. We can view figures from our collaborators here and filter by name or Owner.
40. Open the **Aurora-B figure 2** from trainer-2.
41. Select a panel and click on the *Webclient* link in the *Info* tab to show the image in the webclient.
42. Back in OMERO.figure, go to *File > Open...* to choose the "Figure 1" file saved above.
43. Click on *Export PDF* to export it as PDF.
44. Download the PDF and open it. If opened in a suitable application e.g. Illustrator, the elements on the page will still be editable.