University of Southern California OMERO Workshop 2019

The presentation and a PDF version of the workshop are available at
https://downloads.openmicroscopy.org/presentations/2019/LosAngeles

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

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 50 users on the OMERO.server. **Each** of these 50 users has **their own set of images.** These sets consist of i**mages 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 49 sets of images belonging to the 49 other users.

The **login names** of the 50 users are "user-x" where x goes from 1 to 50, We have given to each of the 50 users in OMERO a unique first name and surname which we picked from a list of 50 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.
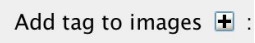

Import setup (not shown today)

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 (not shown today)

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 (no https)
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 ⸢Add tag to images ⊞⸣ : 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.
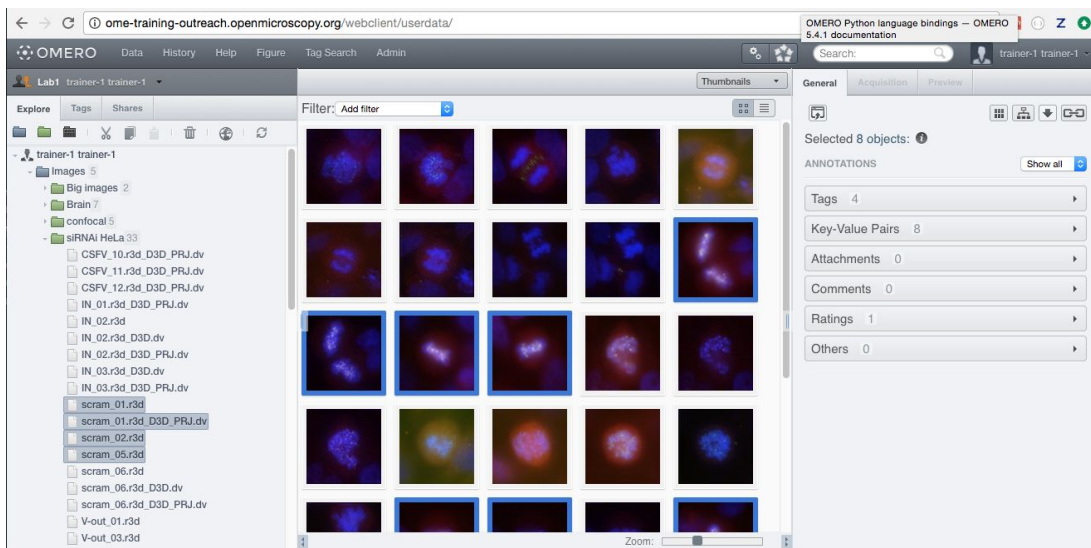
Browse Images

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.

4. 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.
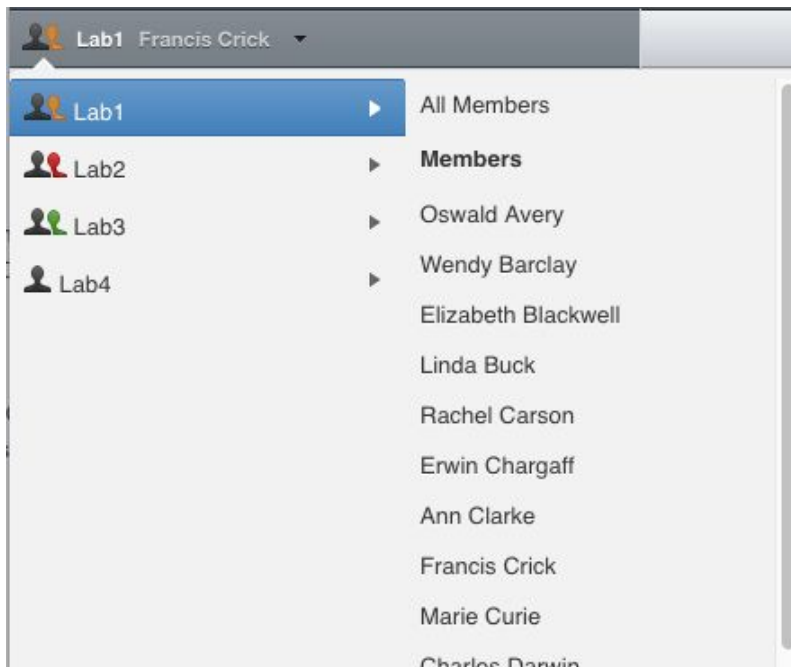

5. You can browse 'folders' in the left-hand pane: *Image* folders are called *Datasets* and they are within *Projects*.

6. When a Dataset is selected, Image thumbnails are shown in the centre panel.

7. These represent imported Images. The original Images are stored on the server and the generated thumbnails allow us to browse them.

8. 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.   .

User Settings:

trainer-1 trainer-1   user-25 user-25
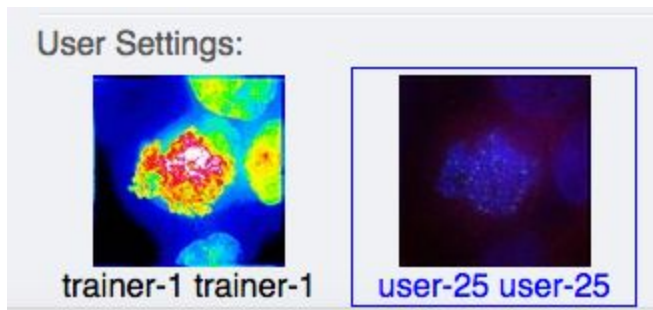
17. Your own settings are highlighted in blue.
18. You can revert to the original settings for an Image or Dataset. For example, using the context menu for a Dataset in the tree, select *Rendering Settings > Set Imported and Save*.

View Images

1. Double-click on an Image thumbnail, or click the *Full Viewer* [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.

2. We can adjust the rendering settings and scroll through Z or T.
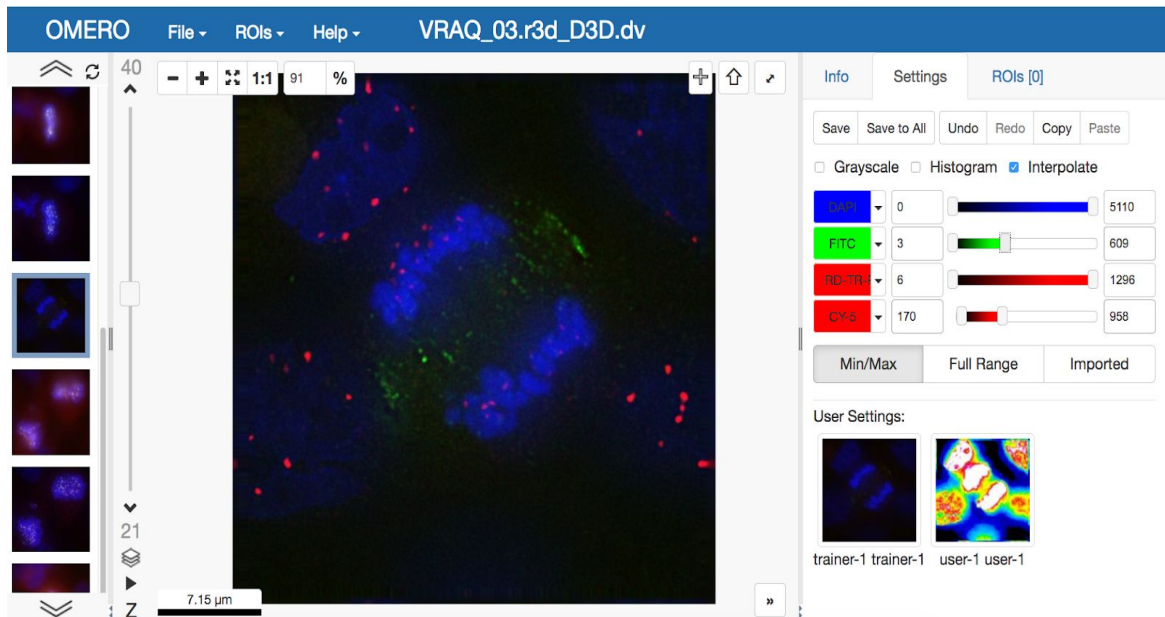
3. Click the *Crosshairs* icon ⊕ at the top-right of the viewer to enable the pixel intensity display for the mouse pointer. Then mouse over the Image to see the pixel intensities for the channels turned on.
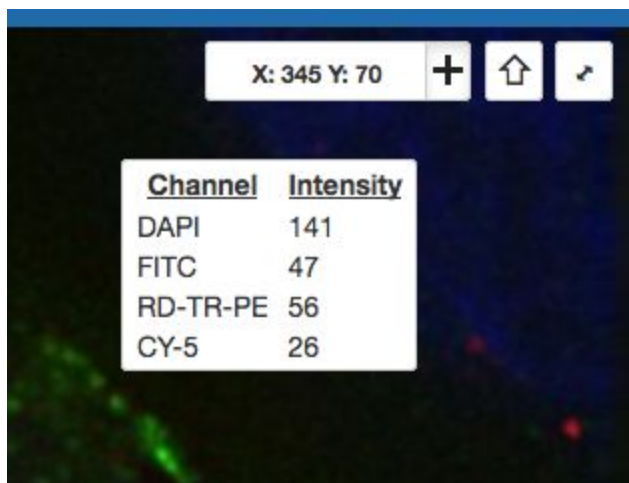

4. The ROIs tab ROIs [0] includes tools for viewing and drawing ROIs on the Image. These are saved back to the OMERO server. Select the *arrow tool* in the right-hand

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* Save 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).
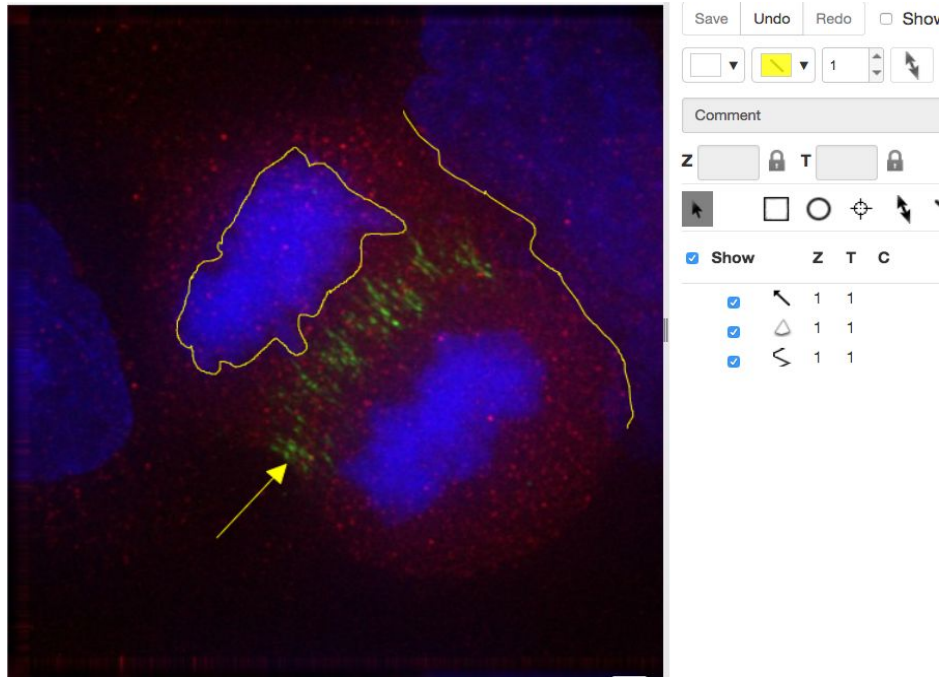

5. Clicking onto the ROIs in the right-hand pane table brings the viewport to the position where the selected ROI is in the middle of it and navigates the viewport to the timepoint the ROI is associated with.

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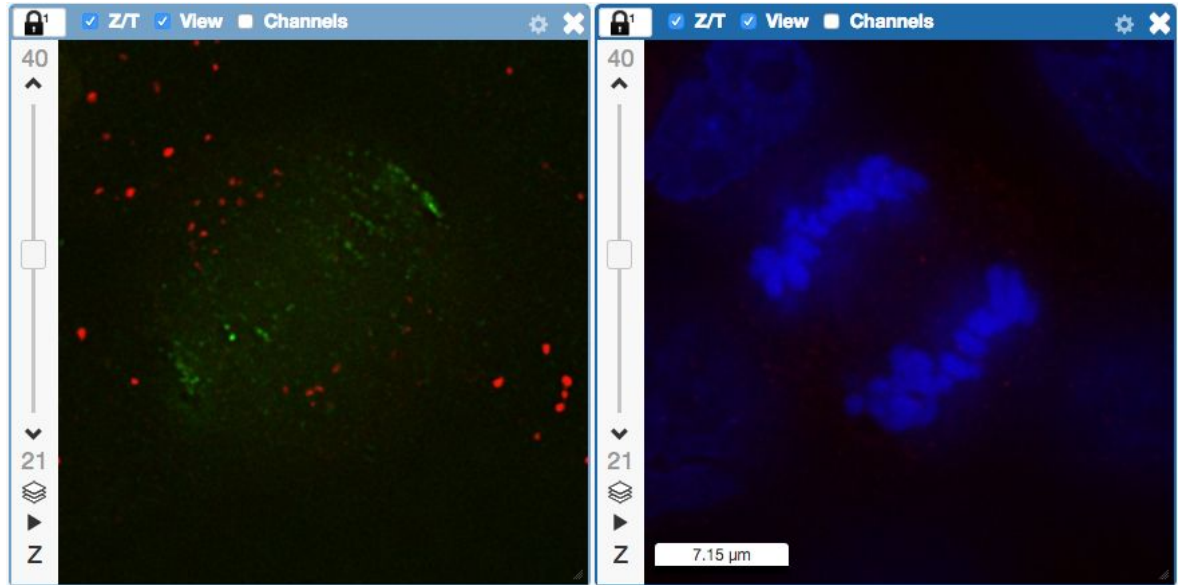
10. We can synchronize the viewers by adding them both to the same sync Group.

   a. Click the *sync icon* in the top-left corner and select the first option in the dropdown menu.
   b. Repeat for the other viewer.
   c. 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:*

12. Click on that link. This will bring you back to the webclient.
13. Adjust the channel names:

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

14. Open the mouse embryo image from the Dataset **mouse-14-days** with multiple Z-sections in a 3D viewer: OMERO.FPBioimage.

   a. First select the Image.
   b. Right-click on the image, or use the *Open with...* icon on top of the right-hand pane to open the image with FPBioimage.
   c. Inspect the embryo in 3D - zoom, rotate, pan.
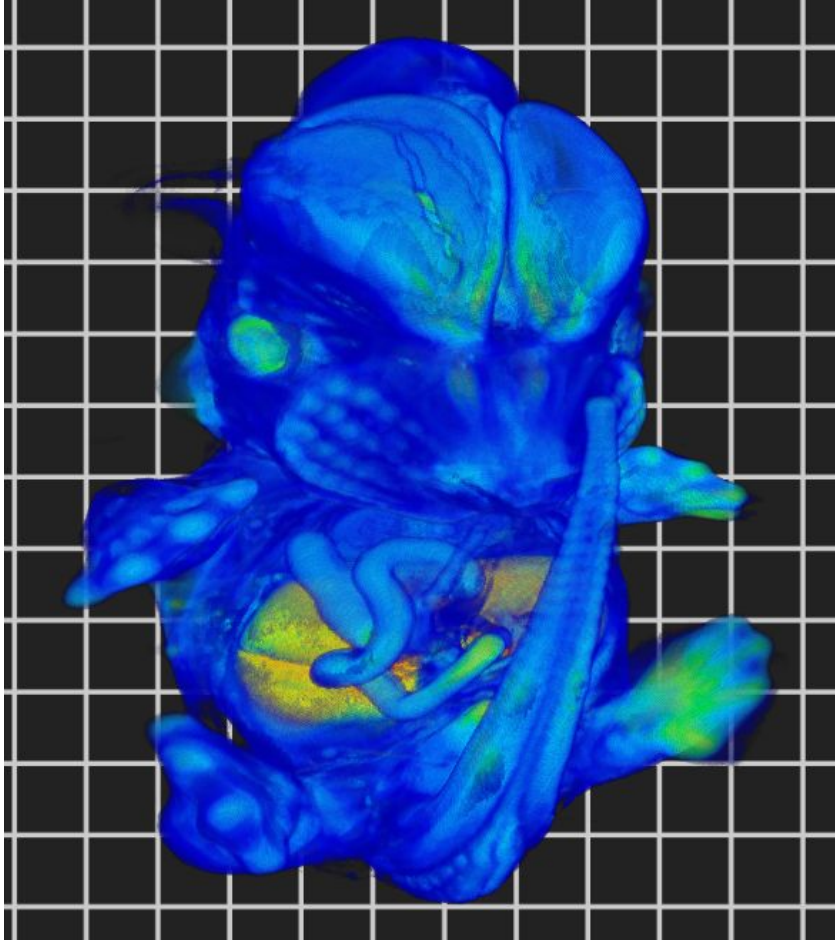
15.

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				

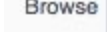7. The *Key-Value Pairs* allow you to add lab-book like additional metadata for the Image. These *Key-Value Pairs* are also specifically searchable.
8. It is also possible to add Comment, Attachment and Rating to selected Images.
9. Enter **mitomycin-A** into the search box in the top right corner of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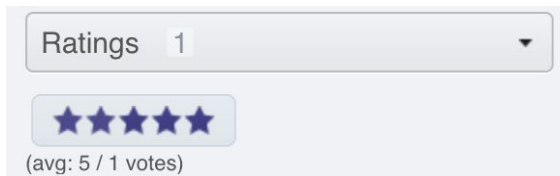
 and press Enter.

10. 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..
11. 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.

12. Images can also be filtered by Tag or Rating in the centre pane, using the *Add filter* chooser above the thumbnails.
13. For example, choose Tag and then select **Metaphase** from the list of Tags to show the images we tagged earlier.
14. 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

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

Analysis with Fiji (covered by slides only)

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 simple workflow

1. Launch Fiji.
2. Go to *Plugins > OMERO > Connect To OMERO.* This will show a login screen where you can enter the name of the server to connect to, the username and password. The OMERO plugin will allow you to browse your data in a similar manner to the webclient
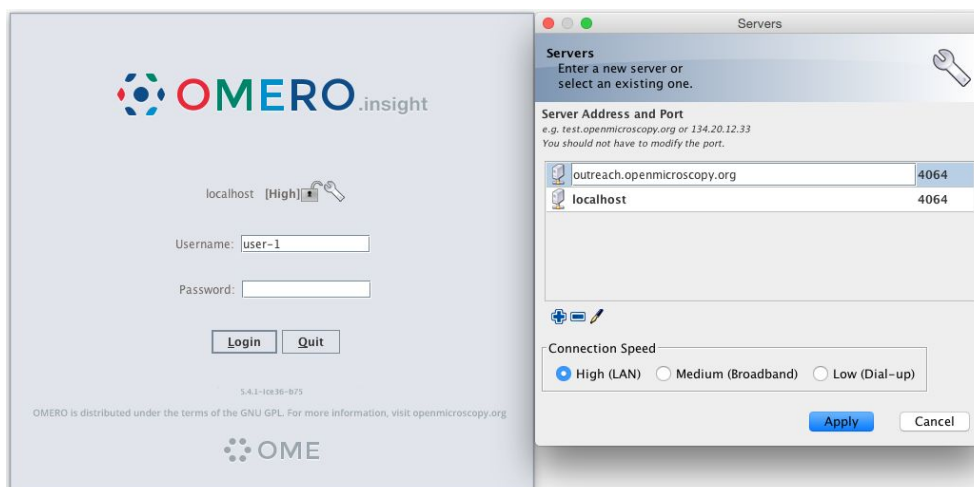
3. Select the **A-Fiji-dataset** Dataset.
4. Double-click on a thumbnail or on an Image in the left-hand tree to open an Image in ImageJ.
5. Go to *Analyze > Tools > ROI Manager…*
6. Draw a shape using for example the Freehand selection tool
7. In the ROI manager, click the button *Add [t]* to add the shape to the Manager.
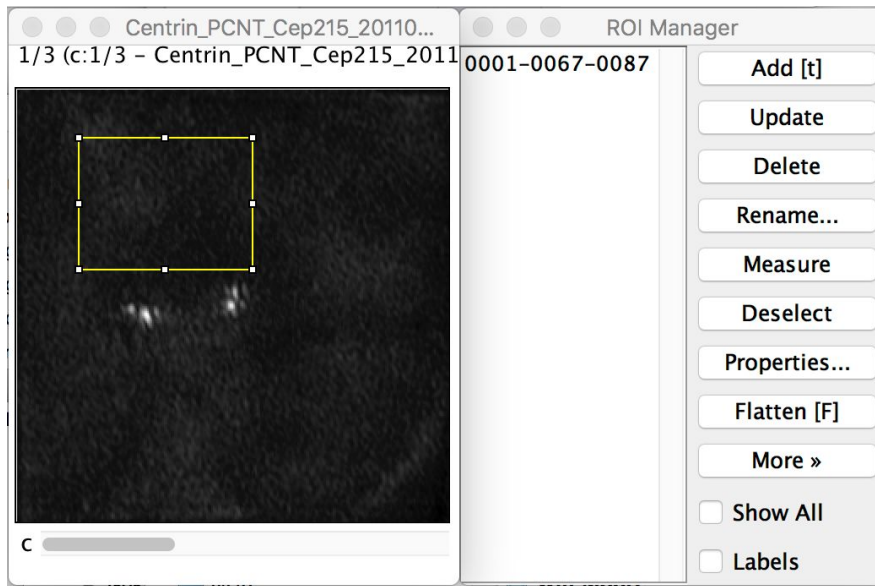
8. Move to another channel, using the *c* slider
9. Draw other shapes if desired. Add drawing a shape. Click *Add [t]* to add it to the Manager.
10. When done with the drawing, click the button *Measure* in the ROI Manager
11. A dialog with measurements for each shape pops up.
12. To save the ROI and the measurement back to OMERO, go to *Plugins > OMERO > Save ROIs To OMERO*
13. In the dialog popping up, under the *Save* section select *ROI* and *Measurements*.
14. The measurements are saved as a CSV file.
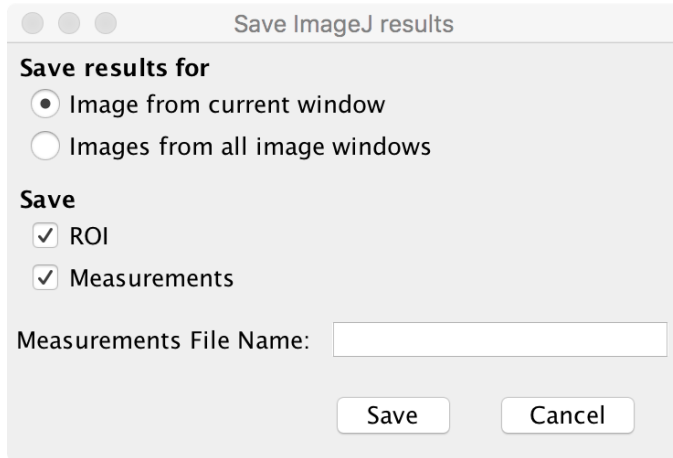
15. Go back to the webclient, in the right-hand panel, check that there is a new CSV file under the *Attachments* harmonica
16. Open the image in iviewer to the see the ROIs and make sure that you can interact with them.

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*.
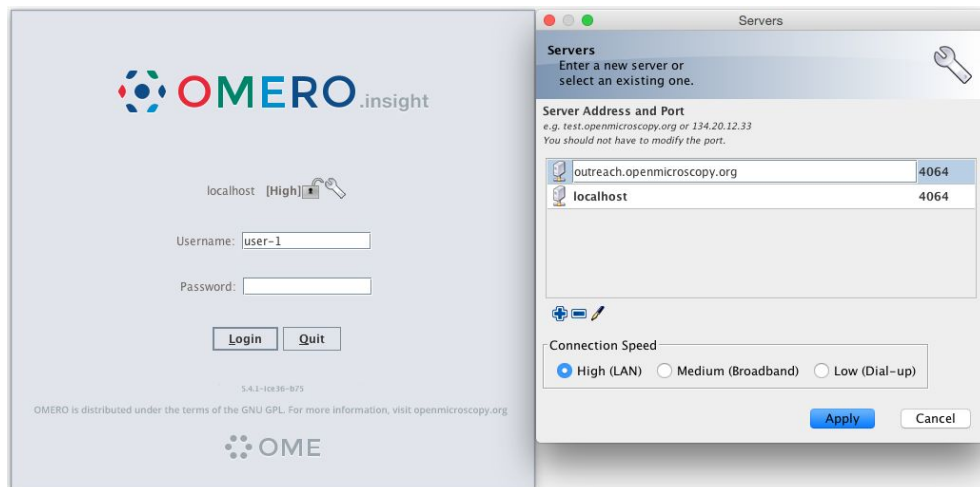
4. Enter your *Username* and *Password* and click *Login*.
5. Browse to Project **idr0021**, open any Dataset and double-click once on an Image to open it in Fiji. Bio-Formats is used to view the Image.
   - Make sure to select *View stack with: Hyperstack* in the *Bio-Formats Import Options* dialog.

- 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:
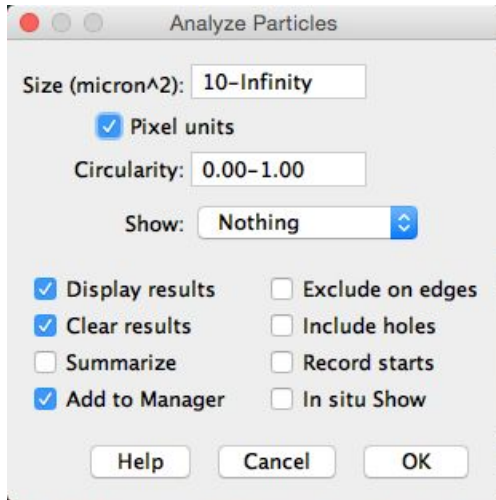   - Select *MaxEntropy* for the *Method* parameter.
   - Check the checkbox *Stack.*
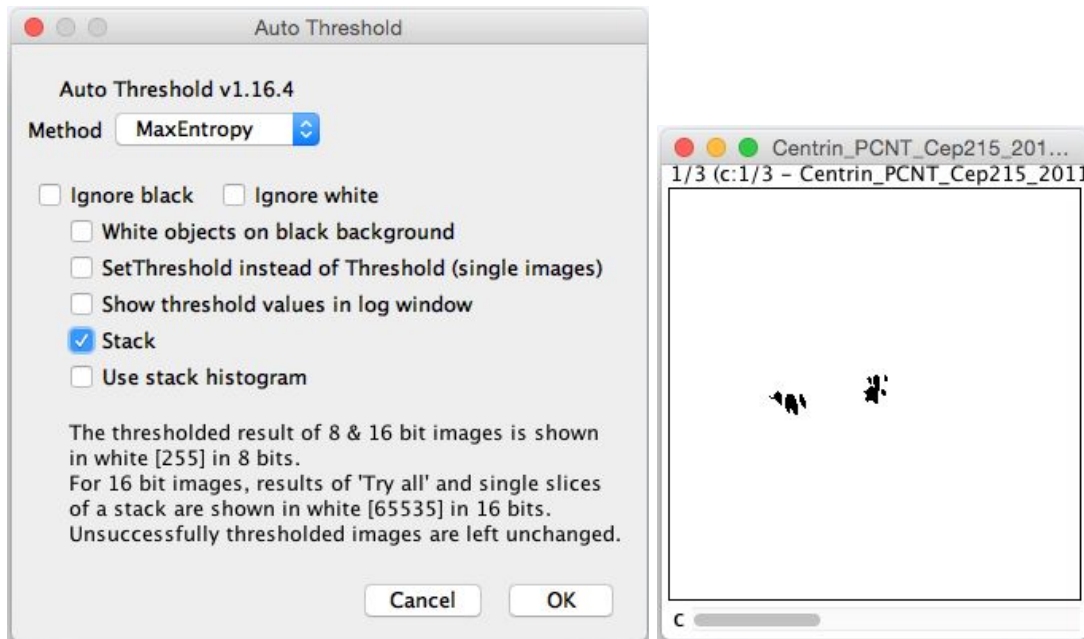
9. Click OK.
10. Then open *Analyze > Analyze Particles….*
11. In the dialog
    - Set *Size* to *10-Infinity* and check *Pixel units.*
    - Check the following checkboxes:
      i. *Display results*
      ii. *Clear results*
      iii. *Add to Manager*
    - Click *OK* then *Yes* in the popup dialog 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.
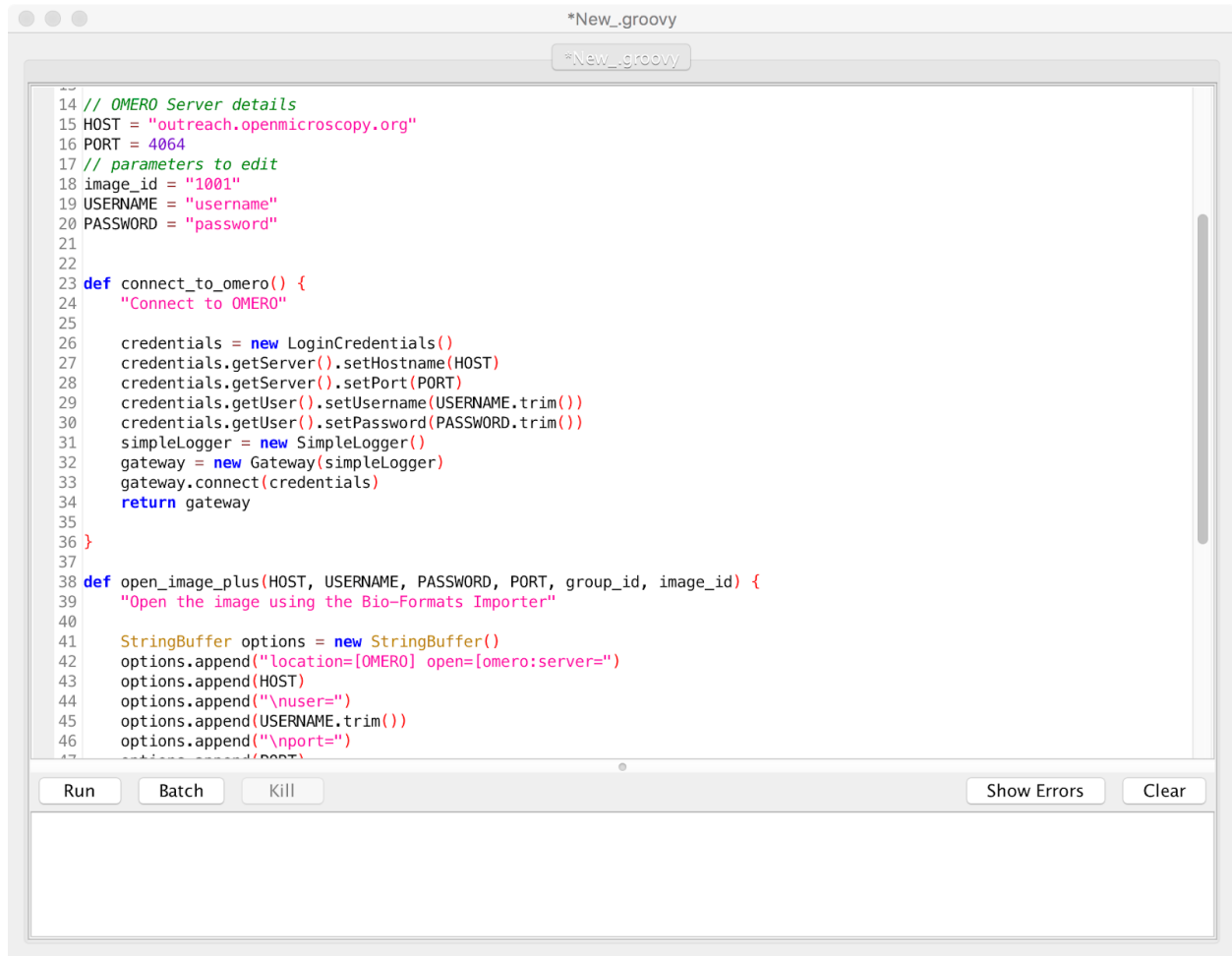
Fiji Scripting workflow

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.*

8. 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.
9. 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=")
```

Run | Batch | Kill | | Show Errors | Clear

10. Click *Run.*
11. 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.
1. Make sure you are selecting your own data. Select the Dataset **A-Fiji-dataset**. Note the Dataset ID from the right-hand pane.
2. Launch Fiji. (relaunch if opened).
3. Go to *File > New > Script….*
4. A dialog pops up. In the *Language* menu select *Groovy.*
5. 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.
6. 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. Open With: OMERO.figure |

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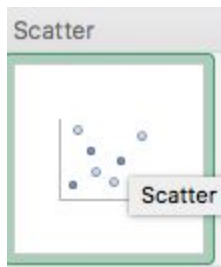
9. This will create a scatter plot. Right-click into the Chart now and select *Change chart type > Statistical > Box and Whisker.*

Analysis with CellProfiler

Description:
CellProfiler is a free open-source software for quantitative analysis of biological images. For more details, go to http://cellprofiler.org/.

Setup:
CellProfiler version 3.1.3 has been installed in a Jupyter notebook using docker as described at https://github.com/ome/training-notebooks.

1. We will use CellProfiler to analyse a Plate of Images in OMERO. We will run CellProfiler version 3.1.3 "headless" via a Jupyter Python notebook.
2. We will use the example pipeline from http://cellprofiler.org/examples/#PercentPositive to analyse RNAi screening data from IDR http://idr.openmicroscopy.org/webclient/?show=screen-102
3. First, open the webclient and find the Plate belonging to *trainer-1* named *plate1_1_013*.
4. Go to https://idr-analysis.openmicroscopy.org/training/hub/tmplogin and look under Notebooks > CellProfiler for *idr0002.ipynb.*
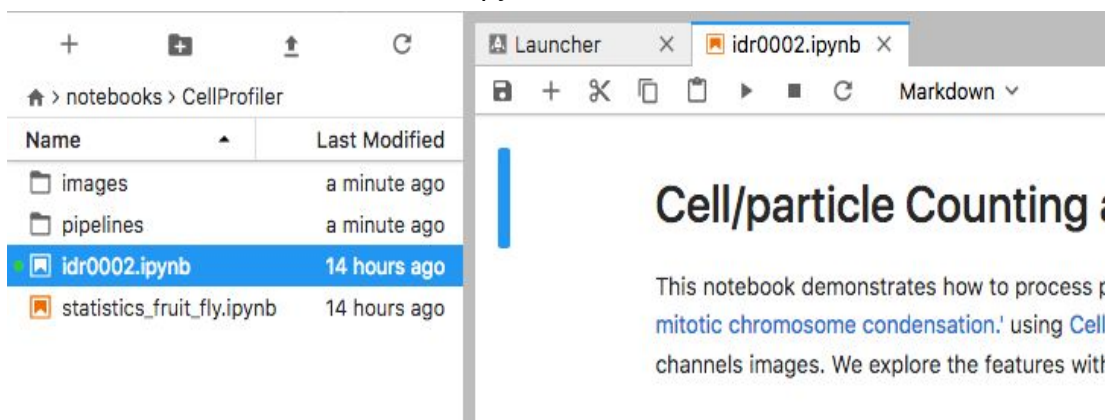
5. Select the first Step and click on the *Run* button to execute each step in turn.
6. For the connection to OMERO, you will need to enter your login details before running the OMERO credentials step.

7.

8. The following step loads the example pipeline and modifies it to remove the modules that are normally used for loading images from disk.
9. These modules are replaced by InjectImage modules, using numpy planes loaded from OMERO Images.
10. The pipeline is run on a 2-Channel image from each Well in the 96-well plate, generating a CSV file containing rows for different objects identified in the image and columns for various parameters measured.
11. N.B.: To save time in the workshop, we can run on a subset of all Wells in the plate:

```
# create list from generator
wells = list(plate.listChildren())
wells = wells[0:5]
well_count = len(wells)
```

12. CSV files are read into a Dataframe for each image. We add the Image ID and Well ID, as well as the total number of Objects, Cell_Count, to each Dataframe.
13. All the Dataframes are then concatenated into a single Dataframe.
14. We can visualise the data as histograms for each column with df.hist()
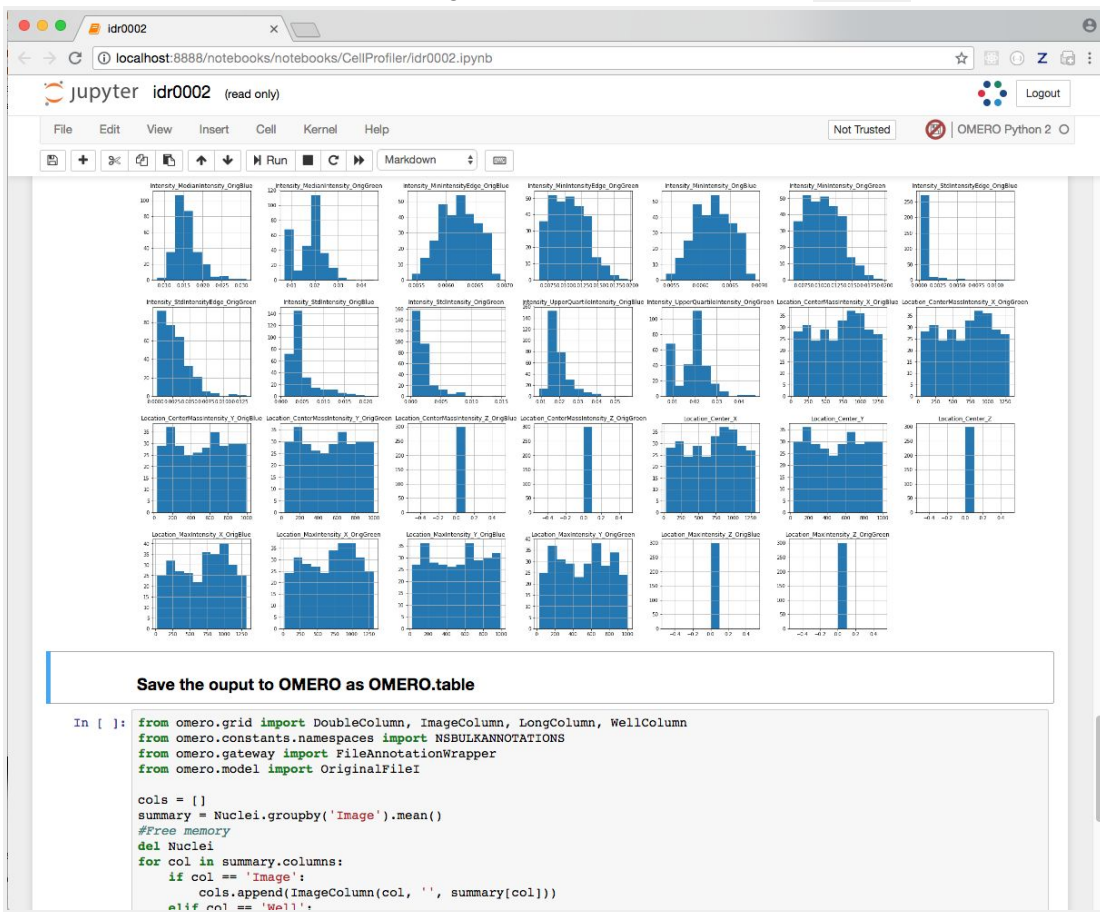
15. Finally, the Dataframe rows are grouped by Image to give an average value per Image of each parameter (column) in the table

16. This data is saved back to OMERO as an HDF5-based table attached to the Plate, which can be read by other clients.
17. Return to the webclient and select the Plate.
18. 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.
19. In the *Thumbnails* chooser at the top-right of the centre panel, select the *Parade* plugin.
20. At the top-left of the centre panel choose *Add filter… -> Table* to filter Wells by the data from CellProfiler.
21. Change the filter from *ImageNumber* to *Cell_Count* (at the bottom of the list).
22. Now you can use a slider to filter Wells by Cell Count.
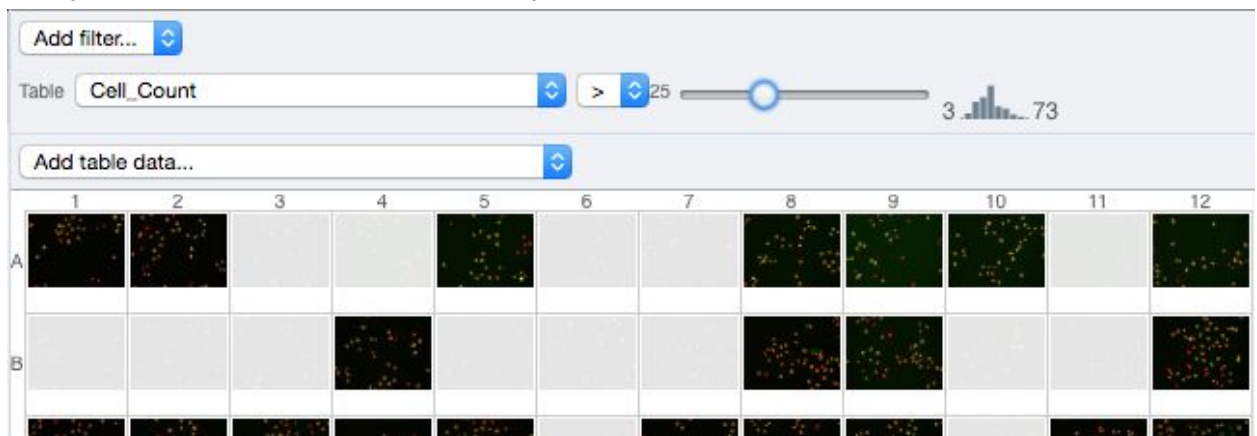
Orbit workflow: Manual training and segmentation (not covered today)

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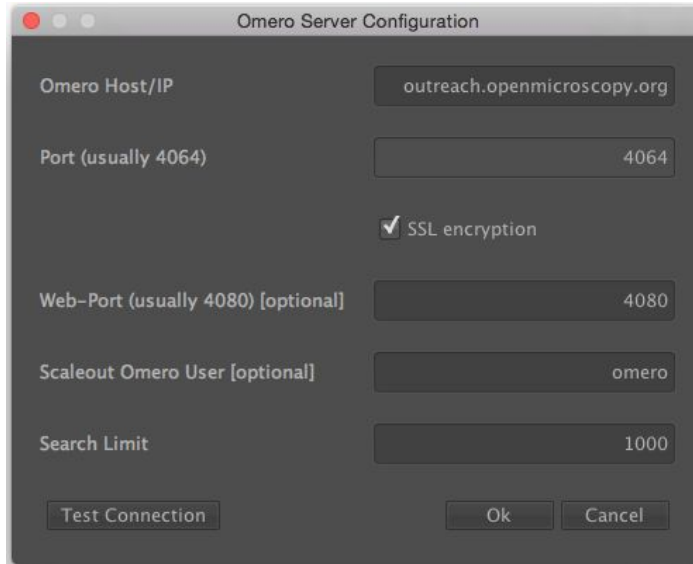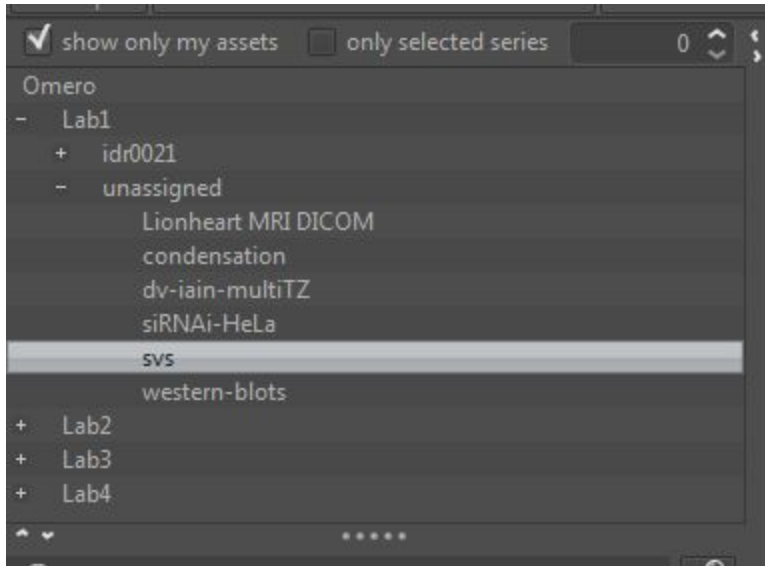
3. Then login to OMERO with the username and password provided:

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*.
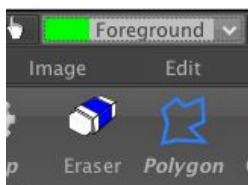
13. You should now have two classes named *Background* and *Foreground*:
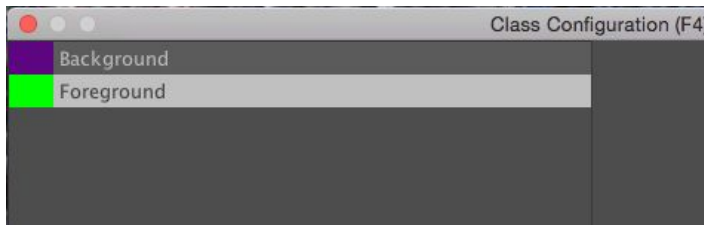
14. Click *OK* to close the dialog.
15. Now we will construct the model by defining regions of *Foreground* and *Background* on the Image.
16. Click the *Object Detection* tab, select the *Polygon* tool and choose the *Foreground* class from the chooser at the top-left of the screen.

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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:
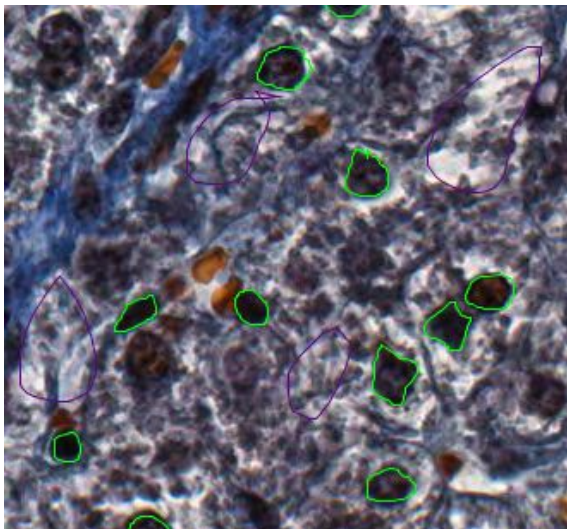

19. We can then train the model by clicking the *Train* button or press F7. You will see a progress bar in the right-hand panel.
20. To see how this model classifies objects within a region, click the *Define ROI* button and draw around a region of the image. Then click *Classify*. If no ROI is drawn, Orbit will
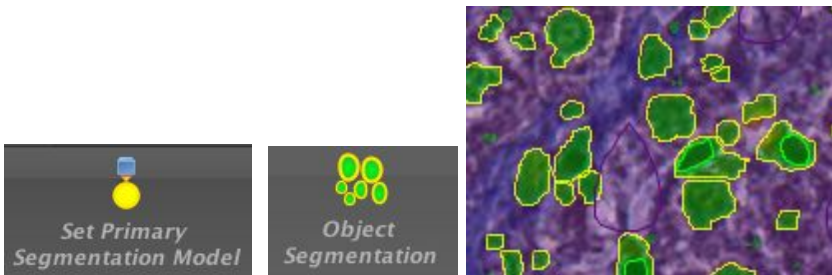
attempt to classify the whole Image which can be very time-consuming.

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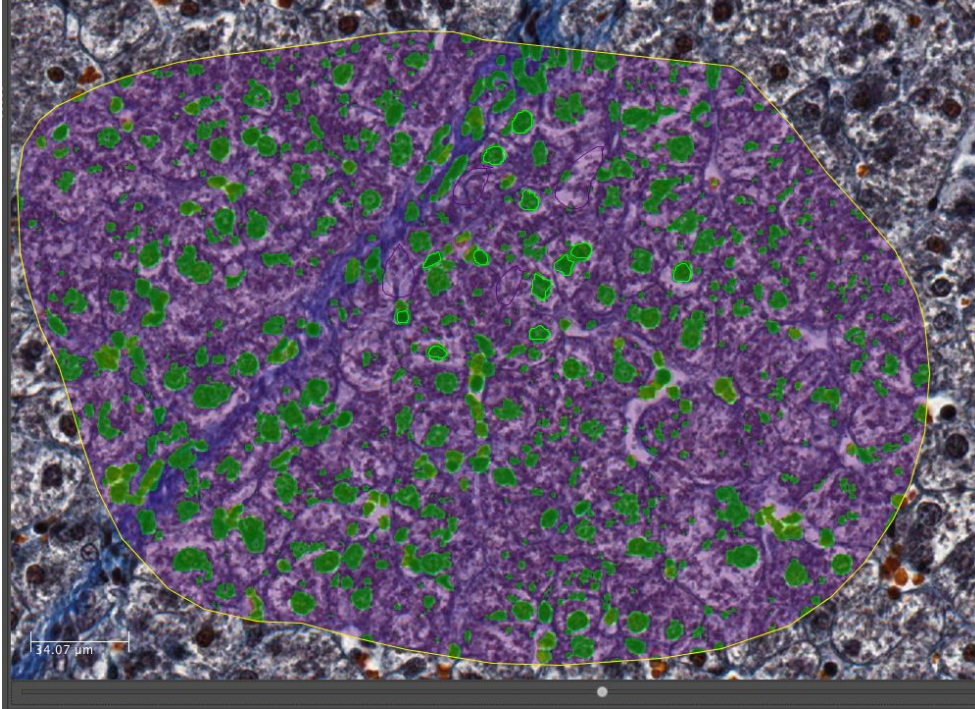
22. To segment the Image using this classification, click *Set Primary Segmentation Model* and then *Object Segmentation*.

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 (not shown today)

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 ic*on).
4. In the dialog, set the *Type* to *ROI.*
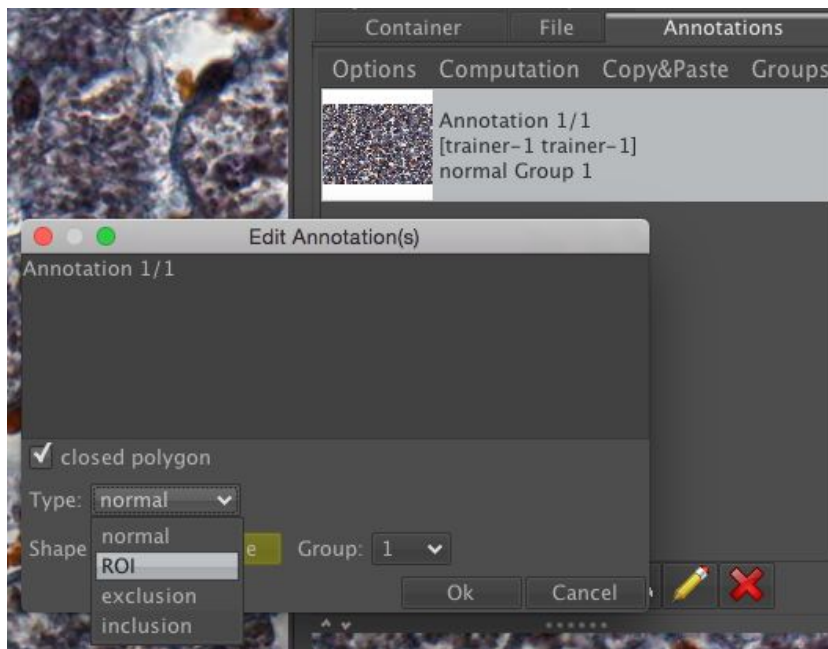
5. Click *Ok.* This will save the ROI as an annotation on this image in OMERO.
6. Click on *Tools > Script Editor* to open a scripting window.
7. Copy the script from training-scripts: https://raw.githubusercontent.com/ome/training-scripts/v0.3.0/practical/orbit/segmentation.groovy and replace the existing 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.

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.
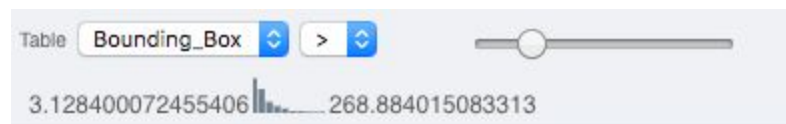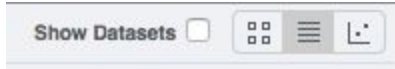

8. Switch to Table layout (middle button) .
9. In the selection box *Add table data*…, select
   a. *Table_Bounding_Box*
   b. *Table_Total_Area*

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

Let's now look at the results generated by CellProfiler

21. Return to the webclient and select the Plate named **plate1_1_013**.
22. 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.
23. In the *Thumbnails* chooser at the top-right of the centre panel, select the *Parade* plugin.
24. At the top-left of the centre panel choose *Add filter… -> Table* to filter Wells by the data
   from CellProfiler.
25. Change the filter from *ImageNumber* to *Cell_Count* (at the bottom of the list).
26. Now you can use a slider to filter Wells by Cell Count.
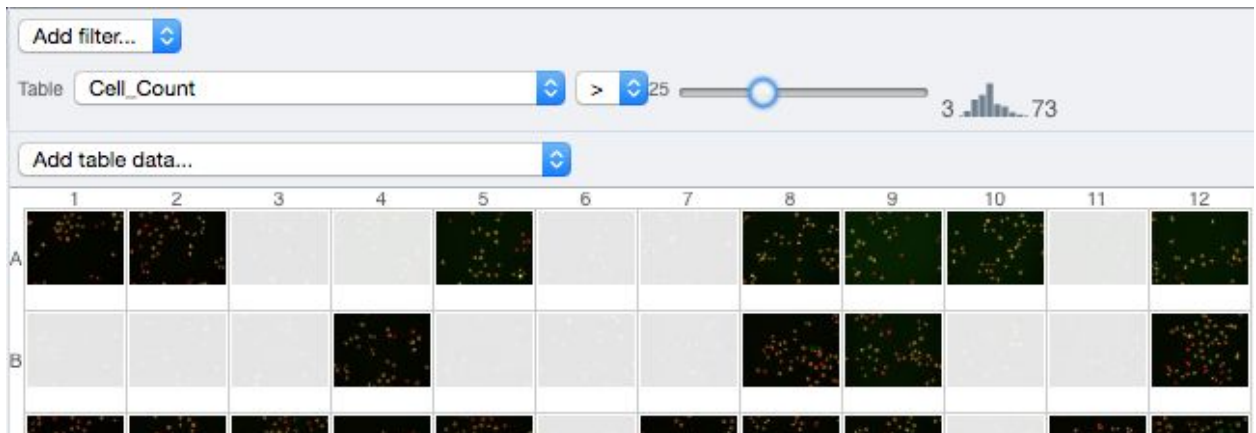
OMERO.figure: Use metadata in figure creation

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*:
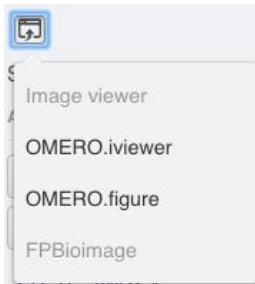
3. This will open these images in OMERO.figure in a new browser Tab.
4. Drag to arrange the Images into two rows, select all and click the *snap to grid* button ⠿ at the top of the page.
5. Select all Images and Zoom in around ~300%
6. Go to the *Labels* tab, select all Images and add a Scalebar of 1 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 three 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 three images into a vertical column, select all and click *snap to grid* button.
15. Copy the three images and paste (keyboard shortcut or *Edit > Copy/Paste*) three times to create three 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 two 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.
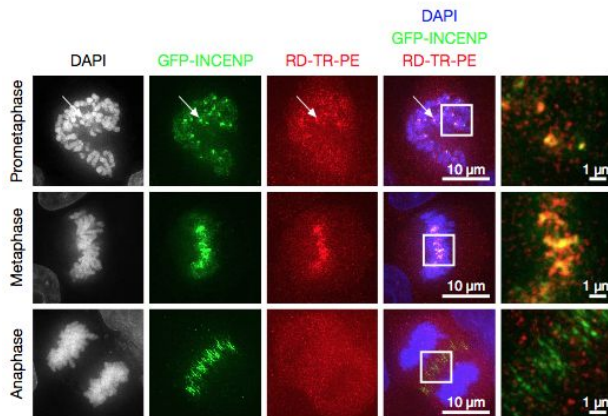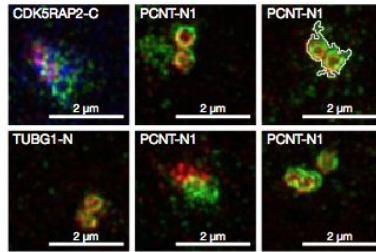
34. Go to *File > Paper Setup…* and in the dialog that pop up choose *Pages: 2.* Click *OK*.
35. Finally return to the webclient, select 'Big' images from the **svs** Dataset, copy the link to them and paste into the *Add Image* dialog in OMERO.figure.
36. Move the big images to the 2nd page.
37. In 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.