

## Cambridge OMERO Workshop 06/12/2018

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

### Software versions used for this workshop:

- OMERO: 5.4.9
- OMERO.iviewer: 0.6.0
- OMERO.figure: 4.0.1
- OMERO.parade: 0.1.1
- OMERO.FPBioimage: 0.3.0
- OMERO training notebooks: 0.4.0
- Bio-Formats: 5.9.2
- CellProfiler: 3.1.3
- rOMERO-gateway: 0.4.3

### Content

- Introductory talk about OMERO
- OMERO core concepts
- Analysis using R - idr0021 example
- Analyze metadata using OMERO.parade
- OMERO.figure

## 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.




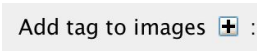
The **login names** of the 40 users are “user-x” where x goes from 1 to 40, We have given to each of the 40 users in OMERO a unique first name and surname which we picked from a list of 40 famous scientists e.g. Ada Lovelace or Francis Crick. This is the name (“your” name) which you will see in the top-right 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>

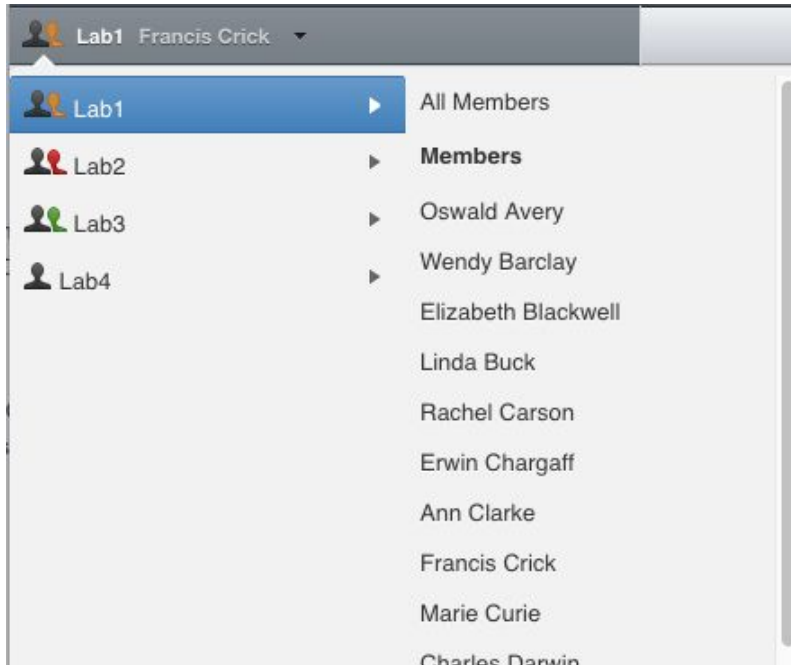
## Import images

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](https://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.

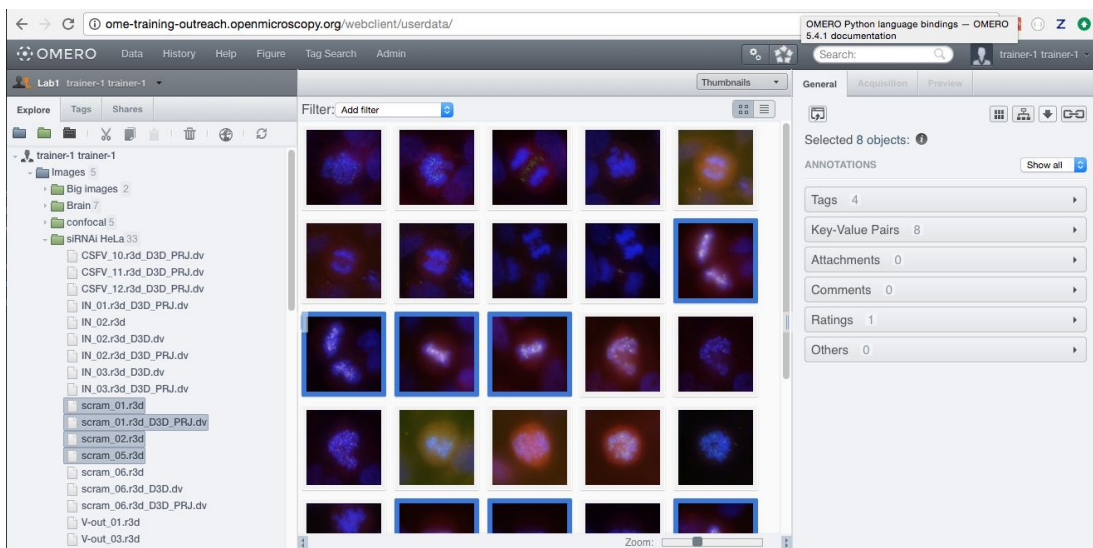
- b. Select the tag(s) on the left-hand side or create a new one.
  - c. Click  to move the tag(s) to the right-hand side.
  - d. Click *Save*.
9. Click on the *Import* button in the bottom-right corner of the Importer window. You should see two progress bars for every image imported, *Upload* and *Processing*.
  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.
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.

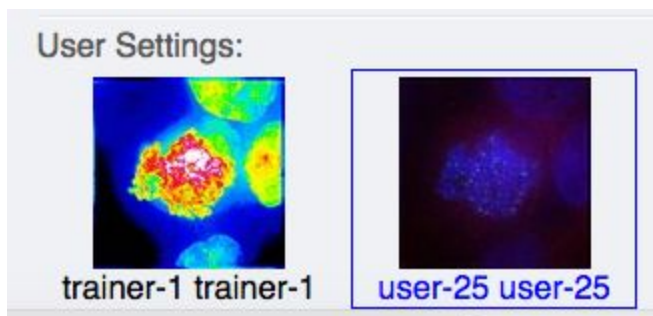


5. You can browse 'folders' in the left-hand pane: *Image* folders are called *Datasets* and they are within *Projects*.
6. When a Dataset is selected, Image thumbnails are shown in the centre panel.




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.

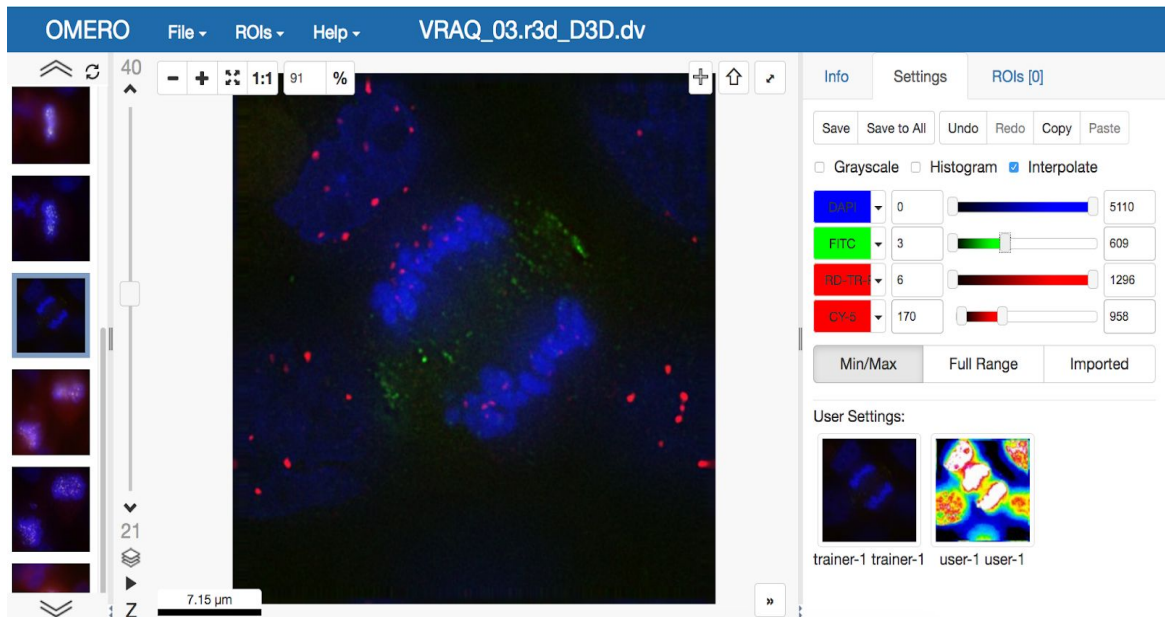



17. Your own settings are highlighted in blue.
18. You can revert to the original settings for an Image or Dataset. For example, using the context menu for a Dataset in the tree, select *Rendering Settings > Set Imported and Save*.

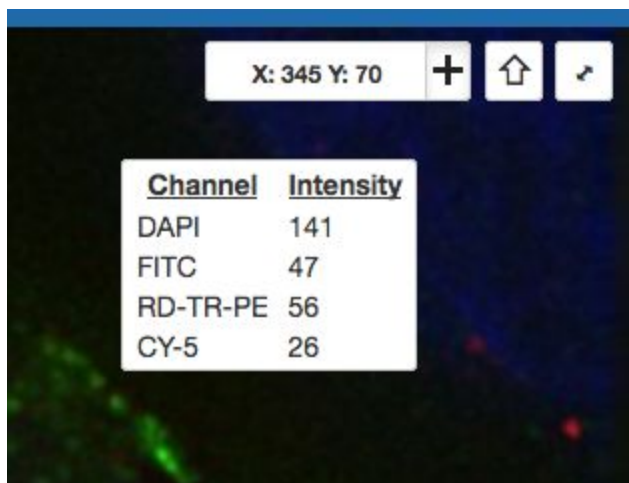
## View Images


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.

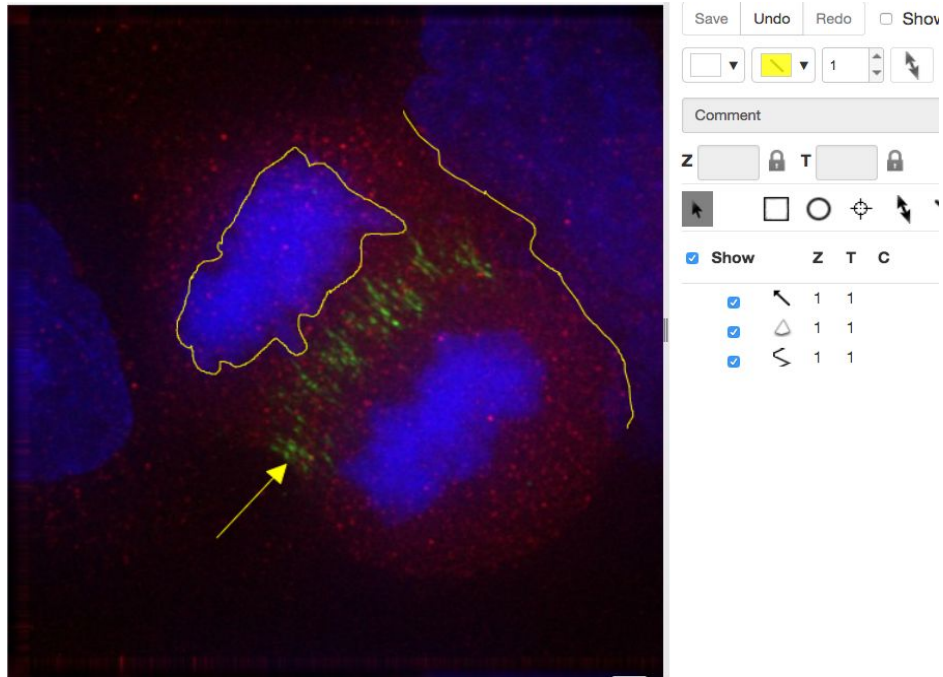


- The ROIs tab  includes tools for viewing and drawing ROIs on the Image. These are saved back to the OMERO server. Select the *arrow tool* in the right-hand

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).

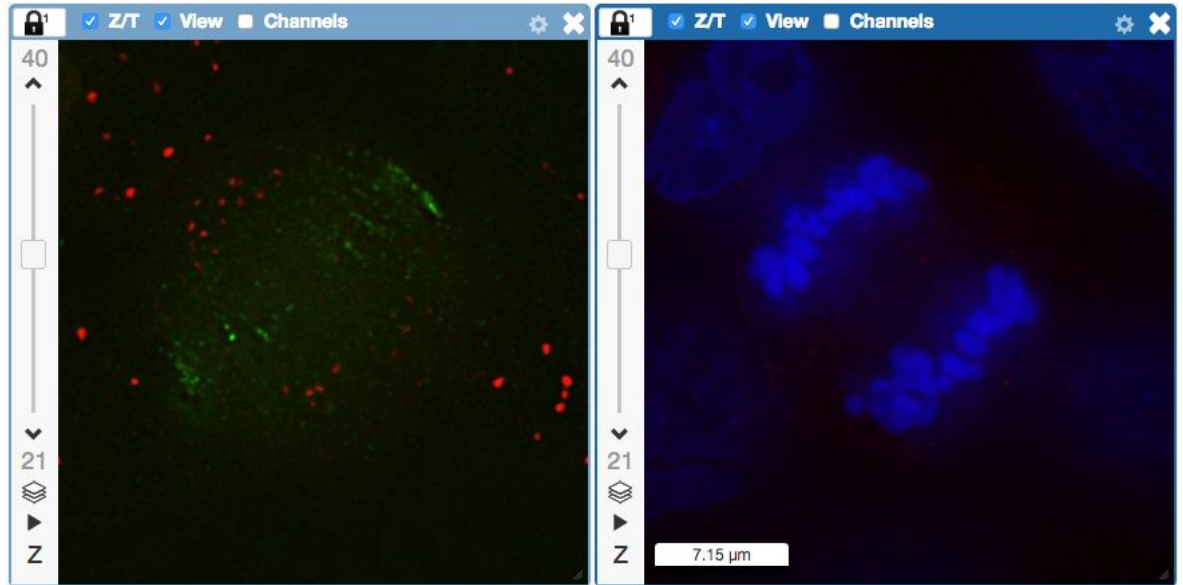


5. Clicking onto the ROIs in the right-hand pane table brings the viewport to the position where the selected ROI is in the middle of it and navigates the viewport to the timepoint the ROI is associated with.


<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	8	
<input checked="" type="checkbox"/>	1	11	11	
<input checked="" type="checkbox"/>	1	15	15	

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*.

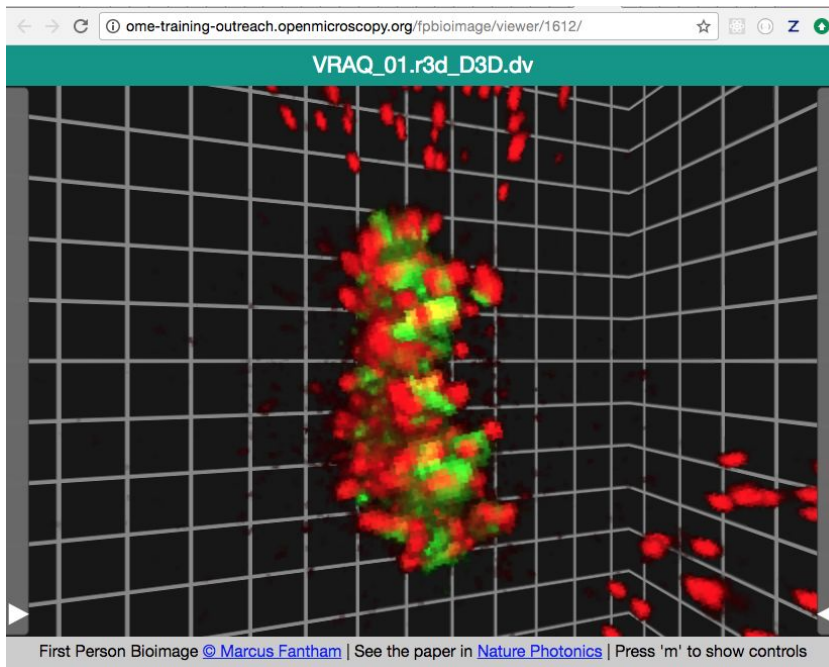
13. Open an image from the Dataset **siRNA-HeLa** with multiple Z-sections e.g.

**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 +   ×

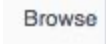
Key	Value
mitomycin-A	0mM
PBS	10mM
incubation	10min
temperature	37
Organism	Homo sapiens

- 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

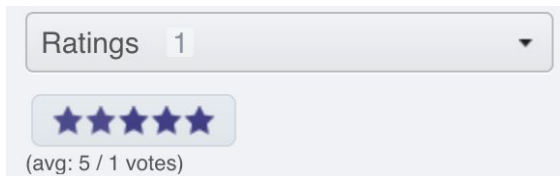


and press Enter.

- 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.

## Analyse data using R

For this section we use R as part of a Jupyter notebook. In order to be able to connect to OMERO from within R we need the `romero.gateway` and `rJava` packages. `rJava` can be difficult to set up, depending on the operating system you use. On the Jupyter server, however, everything has already been installed and we can simply access the notebook in the web browser. Everything we are going to do there can be done in Rstudio as well, if the `romero.gateway` package and its dependencies are available on your system. For installation instructions go to

[romero.gateway github page](#).

In the notebook we are going to use the `idr0021` data. These are super resolution microscopy images showing certain proteins around the centrioles. With these images the authors of the article [Subdiffraction imaging of centrosomes reveals higher-order organizational features of pericentriolar material](#) showed that the area around the centrioles has a specific structure. By measuring the shape and diameter of these proteins and comparing them to each other they could show that each protein builds a specific part of this structure, see [Figure 1](#). Instead of manually measuring each centriole ring like the authors did, we are going to try to do this in an automated way in order to show that there is a significant difference between these proteins and create a plot similar to the one seen on [Figure 1](#).

In order to do that we need to...

1. Fetch the images from OMERO. (`romero.gateway`)
2. Perform image segmentation to identify the protein rings around the centrioles. (`EImage`)
3. Calculate the properties (features) of the identified objects (shape, diameter, etc.). (`EImage`)
4. Store these features as R dataframe. (in R)
5. The segmentation and feature calculation is a rather costly process. You don't want to do this all over again. Therefore:
  - a. Save the identified objects (ROIs, region of interests) back to OMERO. (`romero.gateway`)
  - b. Save the properties back to OMERO. (`romero.gateway`)
6. Perform statistical analysis on that data. (in R)

Note: We are going to do the image segmentation and feature calculation on a small subset. Everyone has a dataset called 'R-dataset' which contains four images of the `idr0021` data. Use this dataset for learning the segmentation tasks. The full analysis of the `idr0021` project has already been done and the result saved as 'Summary from R' table. We are going to load this table in order to do the statistical analysis at the end of the notebook.

- Go to <https://idr-analysis.openmicroscopy.org/training> .
- In the Files > notebooks > R folder, select the notebook `idr0021_Segmentation.ipynb`.
- Follow the steps in the notebook.

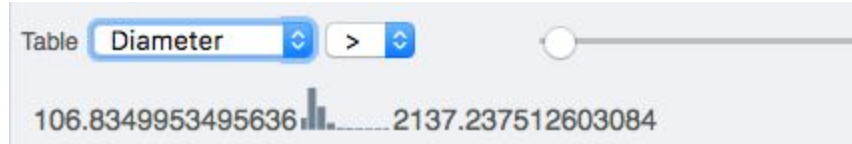
Further exercise: At the end of the notebook you will have created a plot which is similar to the one shown in [Figure 1](#) of the article. It won't be exactly the same. Can you explain why it is different? One dataset in particular has extreme outliers. Use `OMERO.parade` to find the images which cause the outliers. What went wrong in these cases? Note: `OMERO.parade` uses the 'Summary from R' table which is attached to the project, in order to provide enhanced filtering and plotting features.


## OMERO.parade and OMERO.figure

### Analyze metadata using OMERO.parade

First let's look at the analytical results generated using R. The way these results were obtained is described in the "Analyse data using R" chapter above.

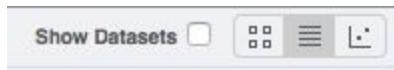
1. Select the Project **idr0021**.
2. Choose the *Parade* option in the centre panel dropdown menu.
3. Expand all Datasets by clicking on the *Open All* button.
  - a. All the Datasets will be expanded in the left-hand tree.
  - b. The Thumbnails will be loaded in the centre panel. This allows to browse 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 use the analytical results generated previously:
  - a. Choose the *Diameter* item and drag the slider to filter the Images. Note that *PCNT* has the largest number of Images with large ROIs.

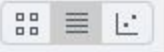


8. Switch to Table layout (middle button) . 
9. In the selection box *Add table data...*, select
  - a. *Table\_Diameter*
  - b. *Table\_Area*
  - c. *Table\_Perimeter*
  - d. *Table\_Image*

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.

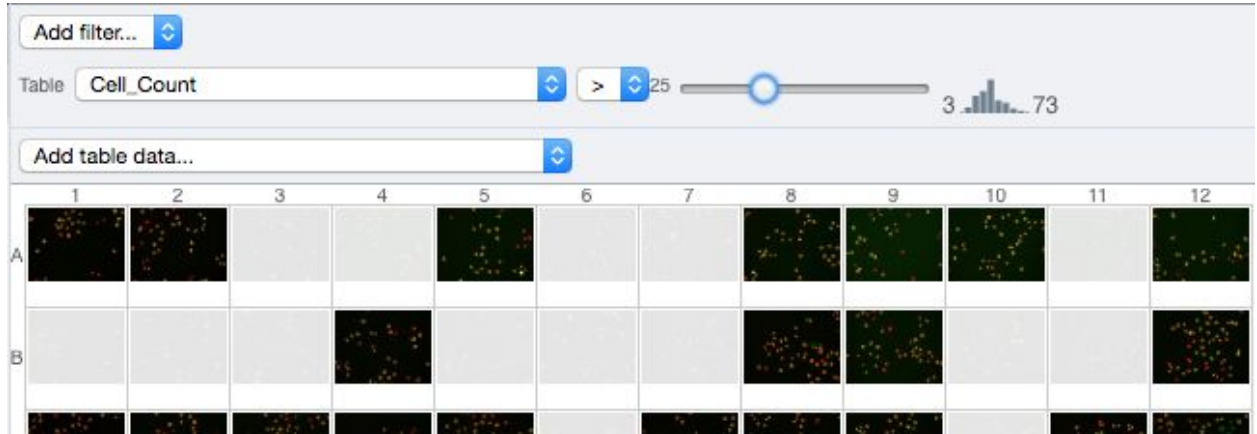


12. 
13. Check the checkbox in each column to show the *Heatmap*. Note the corresponding pattern in the Heatmap.
14. Switch now to the Plot Layout (third button)
15. It takes the table data loaded and plot the values.
16. Filters can be added to plot the relevant results.
17. Closing a Dataset in the left-hand tree removes the values from the plot.
18. Try plotting by different Axis values. For example, the *Table\_Diameter* is counted from the long and short axes of the object and thus independently of *Table\_Area* parameter, Thus, plotting those two parameters against each other gives a good idea about the shapes of the objects.
19. Drag to select several outliers. This is a way to perform a quality control of your segmentation.
20. Open the images underlying the outliers in OMERO.iviewer using the *Open with...* feature in the left-hand panel.
21. Inspect the ROIs in the OMERO.iviewer on those images. Are the ROIs correctly demarking the centrioles?
22. Note you can also work with the selected images in the right-hand panel further by annotating them or by opening them using *Open with....* In another application.
23. Choose *Open with Figure...*

Let's now look at the results generated by CellProfiler

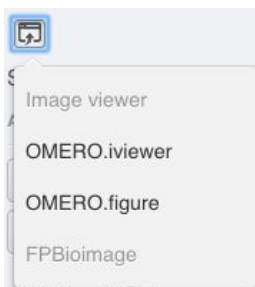
24. Return to the webclient and select the Plate named **plate1\_1\_013**.
25. Select a Well and open the *Tables* pane in the General tab in the right-hand panel. This will show all the CellProfiler values for this Well.


26. In the *Thumbnails* chooser at the top-right of the centre panel, select the *Parade* plugin.
27. At the top-left of the centre panel choose *Add filter...* -> *Table* to filter Wells by the data from CellProfiler.
28. Change the filter from *ImageNumber* to *Cell\_Count* (at the bottom of the list).
29. Now you can use a slider to filter Wells by Cell Count.




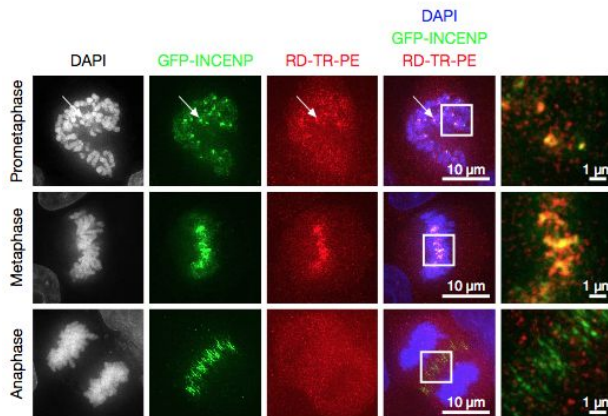
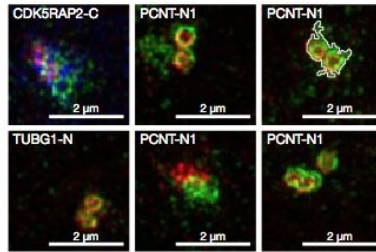
## OMERO.figure: Use metadata in figure creation

1. In the webclient, select the **idr0021** Project, Switch to OMERO.parade and filter by example *Table* and *Diameter* 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  $\mu\text{m}$  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.