

Presentation is available at

<https://downloads.openmicroscopy.org/presentations/2018/ELMI-Dublin/OMERO-Workshop/index.html>

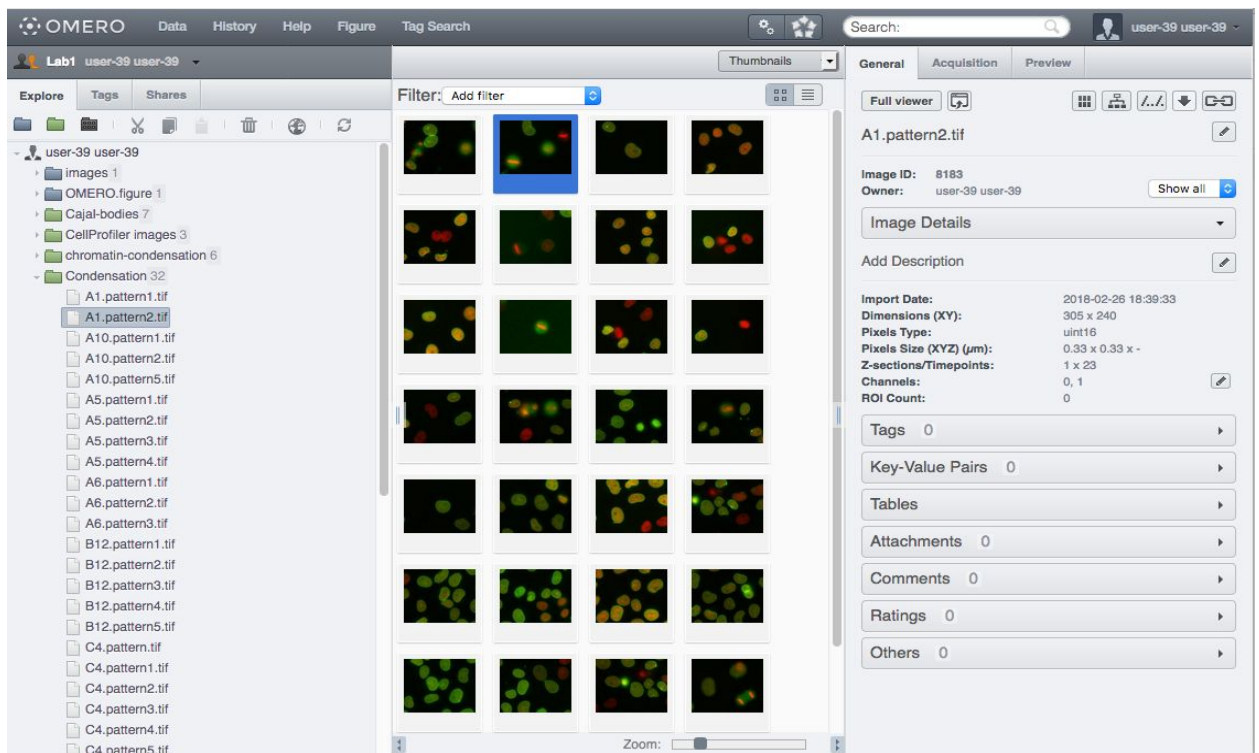
Description

We will demonstrate a number of features of the OMERO platform using an OMERO server based in Dundee, U.K. You can connect to the same server using login details provided to try the software for yourselves.

Browse Images

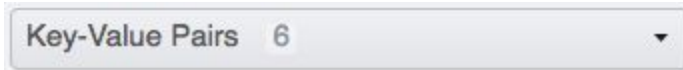
For this workshop, images have already been imported into OMERO. The import instructions can be found at <http://help.openmicroscopy.org/importing-data-5.html>

1. Log in to the webclient with the Username and Password provided.
2. You can browse the hierarchy of *Projects (blue)* > *Datasets (green)* > *Images* in the left-hand pane. Selecting a *Dataset* will show Images in the centre pane.
3. OMERO can handle images of many different file formats and sizes using Bio-Formats. In the *Dataset SVS* there are Images of size 96999 x 45667 pixels. We will use smaller timelapse Images for this workflow.
4. Open the *Dataset condensation*.
5. Select Images to view details in the right-hand pane. Image metadata such as width, height, number of Z sections or Timepoints, Channels and Pixel size is shown here.



Add Annotations, Filtering and Searching

1. Let's now add annotations to Images in the *General* tab of the right-hand pane.
2. Select one or more Images from the Dataset **condensation** and click the harmonica *Key-Value Pairs* in the right-hand pane.



3. You should see a table or tables, depending on the number of selected Images:


Added by: user-40 user-40	
Key	Value
mitomycin-A	0mM
PBS	10mM
incubation	10min
temperature	37
Organism	Homo sapiens

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

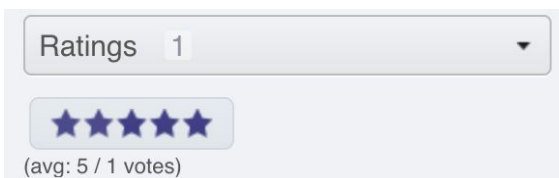


and press Enter.

7. The search results will show any objects e.g. Images or Datasets which have anywhere the string **mitomycin-A**. Several images should be found.
8. 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 10 images. Click on the *Browse* link  in one search result line, (in the right-hand part of the centre pane) to navigate back to the main webclient.


9. Images can also be filtered by Tag or Rating in the centre pane, using the *Add filter* chooser above the thumbnails,.
10. Choose the *Tag* option and then choose the **Mitosis** from the list of Tags. As mentioned already, the **Mitosis** tag was added to 6 Images before this workshop.
11. 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




- 12.
13. Repeat this to add a rating of 5 to the *Apoptosis*-tagged Image.

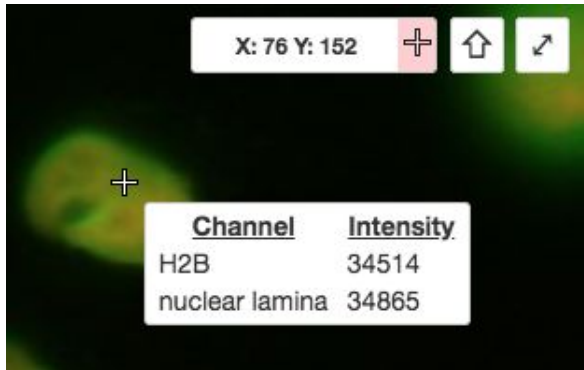
- Now we can remove the filtering by Tag and instead filter by *Rating of 5* to show only our favourite images.

View Images

- 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.



- We can adjust the rendering settings and scroll through T and Z if it is a Z-stack image.
- Click the *Crosshairs* icon  at the top right of the viewer to enable pixel intensity display for the mouse pointer. Then mouse over the Image to see the pixel intensities for the enabled channels.



5.

6. 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-Release-Drag, pointing to a feature in the Image, which will be later used by OMERO.figure and save it using the *Save button*



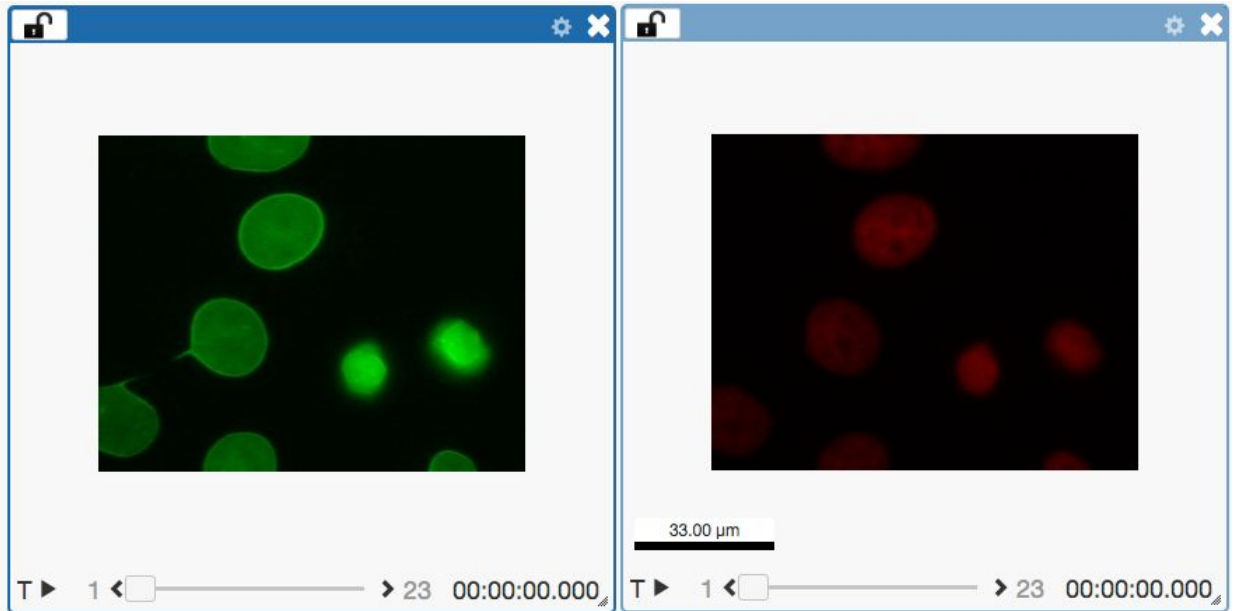
located in the upper part of the right-hand pane. Draw also a couple of other ROIs on different T planes.

7. 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			
<input checked="" type="checkbox"/>	⊕	1	1			
<input checked="" type="checkbox"/>	↗	1	8			
<input checked="" type="checkbox"/>	↘	1	11			
<input checked="" type="checkbox"/>	□	1	15			


8.

9. Save the viewport by selecting in the top-left corner *File > Save Viewport as PNG*
10. Compare the two channels of one of the Images in multi-image view. 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. Select one of the windows and in the right-hand pane switch the green channel off.



11.

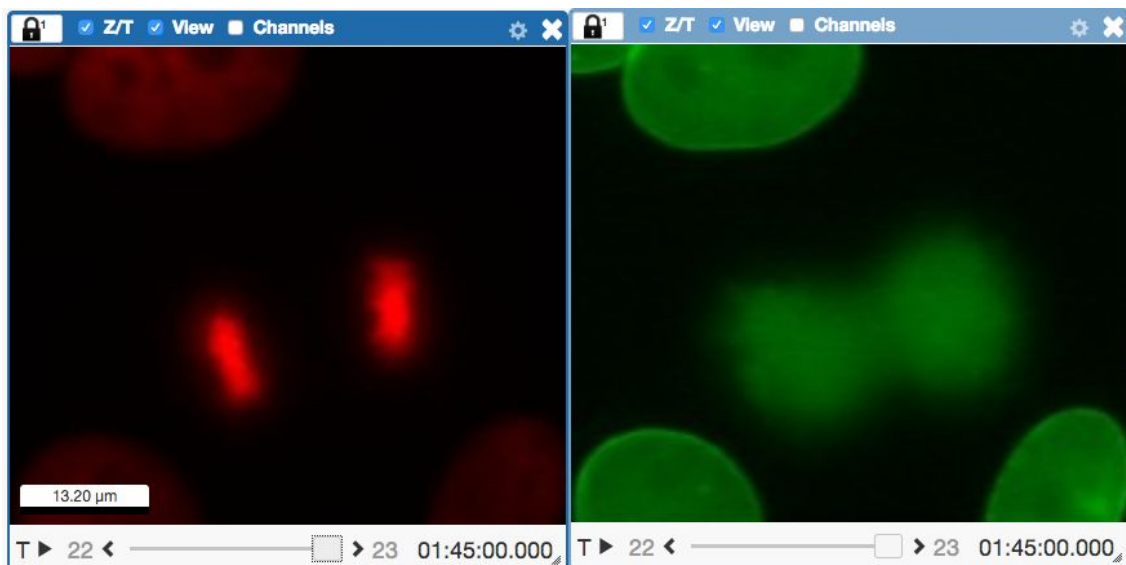
12. We can synchronize the viewers by adding them both to the same sync Group. Click the

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 .

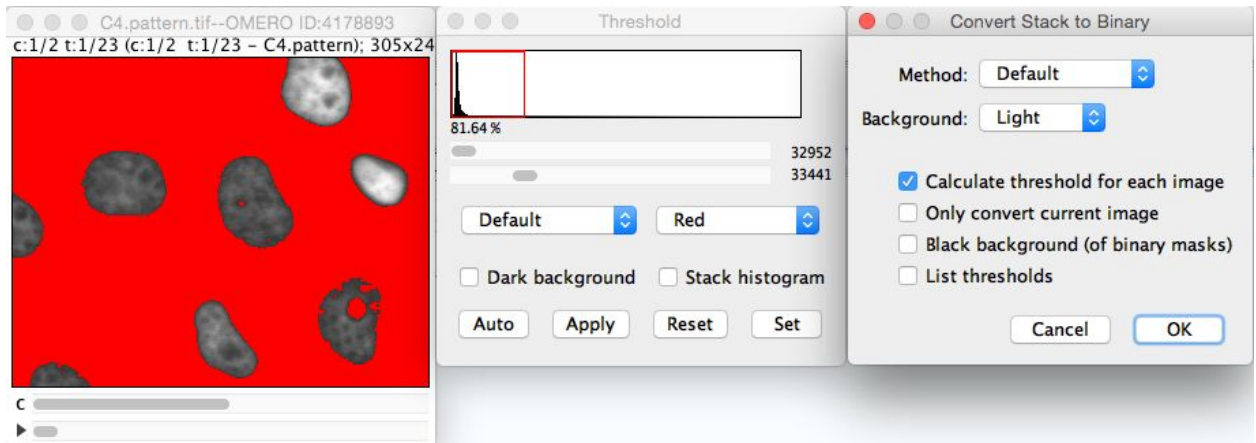
13. Select the Red channel Image (DNA staining) and play the timelapse by clicking onto the *arrow* at the bottom of the image window. After you have identified a mitotic cell, zoom in onto it by scrolling the mouse wheel or expand the *cog* icon in the top bar of the window and click on *plus* sign. Pan the viewport so that the mitotic cell is in the centre. The Green channel (nuclear envelope staining) window will mimic all the actions as well.



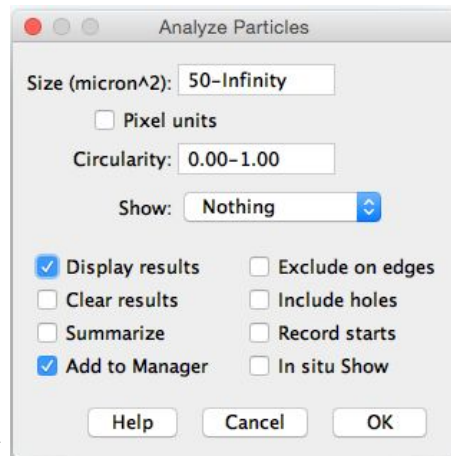
14.

Analyze with ImageJ/Fiji: Manual workflow (NOT PRESENTED, for information only)

1. We will **not** demonstrate this manual workflow, this chapter is for information only.
2. In this manual workflow section, we use ImageJ/Fiji and use the OMERO ImageJ plugin to analyze Images stored in OMERO. Instructions on how to install the OMERO plugin for ImageJ can be found at <http://help.openmicroscopy.org/imagej.html>.
3. 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 and open Images by double-clicking.
4. Select the Images in the **condensation** Dataset.
5. We will analyze cells by thresholding and segmenting the image. Go to *Image > Adjust > Threshold*.
6. The *Threshold* dialog will pop up, click *Apply*.
7. Another window *Convert Stack to Binary* will pop up.
8. Select the following parameters:
 - a. *Method: Default*
 - b. *Background: Light*
 - c. *Calculate threshold for each Image*
 - d. Then click *OK*.
 - e. Close the *Threshold* dialog if you want.



- 9.
10. Select *Analyze > Analyze Particles*
11. In the *Analyze Particles* dialog, select the following parameters
 - a. with *Size: 50-infinity*
 - b. Show: *Outlines*
 - c. Check: *Display results*, and *Add to Manager*.



12. Click *OK*.
13. A dialog pops up asking to Process all 46 images? Click *Yes*.
14. ROIs and Results table will be generated.
15. Go to *Plugins > OMERO > Save ROIs to OMERO* to save these ROIs and the measurements as CSV file to the Image in OMERO.

Analyze with ImageJ/Fiji: Scripting workflow (NOT PRESENTED, for information only)

1. We will **not** demonstrate this workflow, this chapter is for information only.
2. We will now repeat the manual analysis on a Dataset using the scripting facility available in Fiji.
3. Log in to the webclient
4. Select the Project **idr0021** and first Dataset **CDK5RAP2-C**.
5. Note its ID on the right-hand panel, it will be used below.
6. Launch Fiji. (relaunch if opened).
7. Go to *File > New > Script...*
8. A dialog pops up. In the *Language* menu select *Python*.
9. Copy the content of the script https://github.com/ome/training-scripts/blob/v0.1.0/practical/jython/analyse_particles_for_another_user.jy into the text editor.
10. Edit the following parameters: `dataset_id`, `USERNAME`, `PASSWORD`.

```

62 # Setup
63 # =====
64
65 # OMERO Server details
66 HOST = "outreach.openmicroscopy.org"
67 PORT = 4064
68 group_id = "-1"
69 # parameters to edit
70 dataset_id = "1022"
71 USERNAME = "username"
72 PASSWORD = "passwd"
73
74 def open_image_plus(HOST, USERNAME, PASSWORD, PORT, group_id, image_id):
75     "Open the image using the Bio-Formats Importer"
76
77     options = ""
78     options += "location=[OMERO] open=[omero:server="
79     options += HOST
80     options += "\nuser="
81     options += USERNAME
82     options += "\nport="

```



Run Kill Show Errors Clear

```

Started New_.py at Thu Nov 30 11:28:33 GMT 2017
2732
P-TRE 10_R3D_D3D.dv.ome.tiff
uploading...
imported
2733
P-TRE 11_R3D_D3D.dv.ome.tiff
uploading...
imported
Done

```


a.

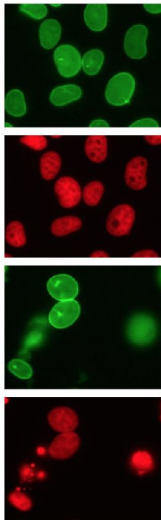
11. Click *Run*.
12. The script will process all Images in the specified Dataset, applying threshold, analyzing particles and saving ROIs back to the Images in OMERO
13. Go to the webclient, navigate to one of the images which were analyzed in the Dataset **CDK5RAP2-C**.
14. Double-click to open the image in OMERO.iviewer. Demonstrate that the Image has now the polygon ROIs exported from Fiji and these are channel-specific.
15. Select one ROI, from the *ROIs* menu in the top-left corner  select *Export CSV*
.
16. Download the output and open it in Excel to show data for ROI shapes.

Create a Figure

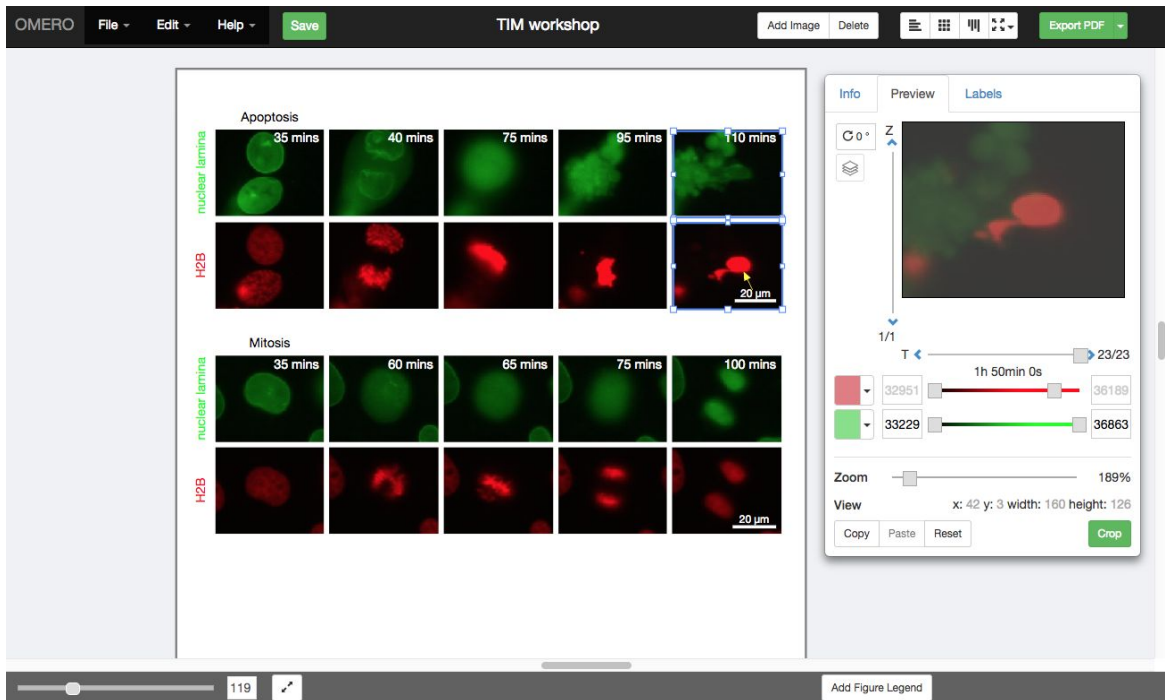
1. We now have both timelapse as well as non-time lapse images in the OMERO.figure. On all of the Images there are ROIs from the Fiji segmentation step above. The timelapse Images from the *Dataset condensation* were run through the Fiji script prior to this workshop.
2. Select the two panels of the Images from the *Dataset condensation* by clicking or dragging a select region. Arrange the panels by dragging and resizing them.
3. Duplicate both panels by selecting them, *Edit > Copy* and *Edit > Paste*, or use the keyboard shortcuts.
4. Use *Align > Width & Height* in the toolbar to ensure that the selected panels have all the same size.





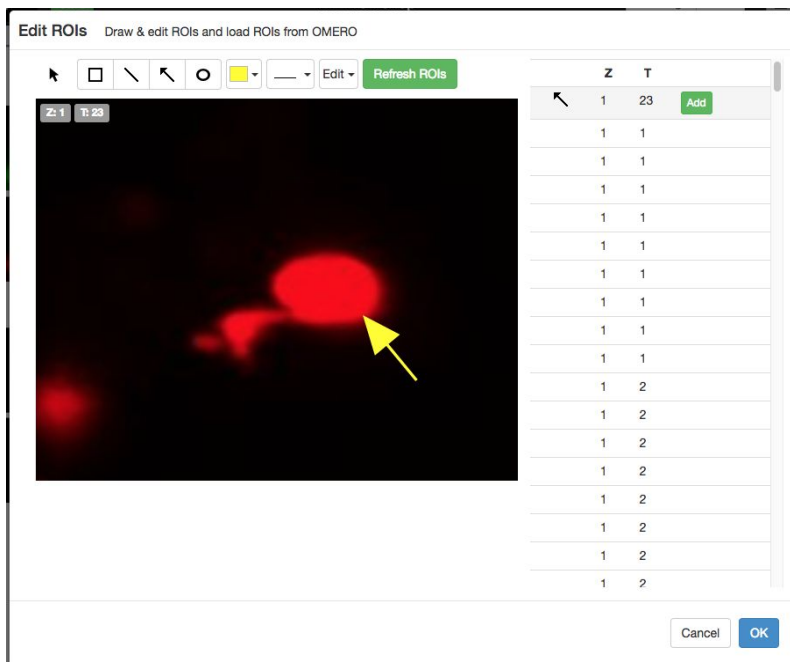
- 5.
6. Arrange the four panels in a single column and snap the selected panels to a grid with the  button.
7. In the *Preview* panel you can adjust the Time position and the rendering settings of the selected panels.
8. Toggle channels on and off to show a 'split-view' of each image.




- 9.
10. Select all four panels, set the T-position to the start of Mitosis, e.g. position 8 (~35 minutes). *Copy* and *Paste* to create a new column.
11. With these new panels still selected, increment T by clicking the > button one or more times.
12. *Copy*, *Paste* and increment T-index two or three more times to create a timelapse figure.
13. Select the lower panels for the second Image and use the *Down Arrow* key to nudge them down to create a space between them and the upper panels.
14. With ALL panels selected, use the Zoom slider to zoom in to approximately 200%.
15. Then select all the upper panels and in the *Preview* panel pan the Image to show the cell of interest. Repeat for all the lower panels.



16.  119 
17. Click on the *Labels* tab to add scalebar and labels to the selected Images.
18. Select one of the *Apoptosis* Images that we previously edited in OMERO.iviewer and click the *Edit* button under the ROIs section.
19. In the *Edit ROIs* dialog you can draw shapes on the Image using the toolbar buttons. Instead, we will click *Load ROIs* to retrieve ROIs from OMERO (if we have saved some on this image).



20.  
21. A list of ROIs will be displayed. Add the polygon ROIs to the Image.

22. Clicking on each row of the list will show the Image's plane that the shape is saved on. Clicking the *Add* button will add the shape to the Image. Click *OK* to close.
23. Use Shift-click to select just two panels (the last red panel for each Image). Under the *Scalebar* section, click on *Show* to display a scalebar created from the pixel-size metadata for each Image.
24. Change the scalebar length to 20 μm and check the *Label* checkbox to show a label.
25. In the *Add Labels* form, the drop-down list of the text input field can be used to create labels from existing image metadata such as timestamps, channel names or tags.
26. Alternatively you can manually enter label text.
27. Select 4 Images in the first column of the figure. From the *Add Labels* drop-down list, choose *Channels*, select *Left Vertical* from the label position chooser and click *Add*.

Add Labels M↓

[channels] 12 ↕ [↺] [■] Add

- 28.
29. Select all the Image panels showing the Green channel, using Shift-drag selection. Choose *[time-mins]* in the *Add Labels* input, *Top right* position and color: *white*. Click *Add*.
30. Finally, select the two Green Images in the first column and add labels from *Tags* metadata to the *Top* position of each panel. This will create labels for *Mitosis* and *Apoptosis*. N.B.: if unwanted labels are created from tags, they can be removed or edited in the *Edit Labels* section

Edit Labels

Apoptosis	12 ↕	↺	□	×
nuclear lamina	12 ↕	↺	■	×
Mitosis	12 ↕	↺	■	×
[time-mins]	12 ↕	↺	□	×

- 31.
32. Save the figure and check that the URL updates to a new URL for this figure which can be shared with colleagues. *File > Open* can be used to open your own figures or those of colleagues.
33. Click *Export PDF* and when complete, click the *Download* button. Open the downloaded pdf in any suitable program and find the second page of the pdf. Click on the link to the image and see that you are navigated back to OMERO in your browser and the appropriate image, which was used in the Figure, is highlighted.

Analyze metadata using OMERO.parade (PRESENTED depending on time)

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.

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.
5. Repeat the previous step but this time:
 - a. Pick the Key *siRNAi*.
 - b. Enter the Value *INCENP* or *Aurora-B* or just *B*.
 - c. To remove this last filter, hover over the filter and click the X button that shows on hover.
6. 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 .
7. Remove all filters by clicking the X button showing on hover.
8. In the *Add filter...* selection box, select the *Table* item so we can find Images using the analytical results generated previously:
 - a. Choose the *max_points* item and drag the slider to filter the Images. Note that *PCNT* has the largest number of Images with large ROIs.



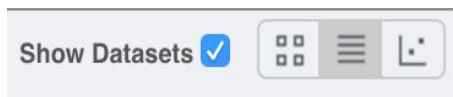
- b.
 - c. Adjust the controls to select < 50 .
 - d. Note that in that case *CPAP* and *CENT2* have the most of the Images as expected.

9. Switch to Table layout (middle button) 

10. In the selection box *Add table data...*, select
 - a. *Table_max_points*
 - b. *Table_mean_points*
 - c. *ROI_count*

Note that it is currently not possible to remove a column

11. Click on the name of a column to sort it.
12. Uncheck *Show Datasets* to sort all Images together e.g. by ROI count.



- 13.
14. Check the checkbox in each column to show the *Heatmap*. Note the corresponding pattern in the Heatmap.
15. Switch now to the Plot Layout (third button)
16. It takes the table data loaded and plot the values.

17. Filters can be added to plot the relevant results.
18. Try plotting by different Axis values.
19. Closing a Dataset in the left-hand tree removes the values from the plot.
20. Drag to select several outliers.
21. Note that you can use the selected Images in right-hand panel to annotate or *Open with...*
22. Add a new Tag to the selected Images then find the Images with the tag.
23. Preview Images and add Rating 5 to 1 or 2 Image(s) (1 per Dataset).
24. We can now want to find Images in a Plate, open the Plate named **INMAC384-DAPU-CM-eGFP_59223_1**
25. In the *Add filter...* selection box, select the *Table* item
26. Then choose *ChOMax* and drag the slider to filter Wells. Note that the plate layout does not change as Wells are found.
27. Return to the **idr0021** Project.
28. Filter by Rating - select all the Images then *Open with Figure...*