OMERO overview: View, Annotate, Measure, Create Figure

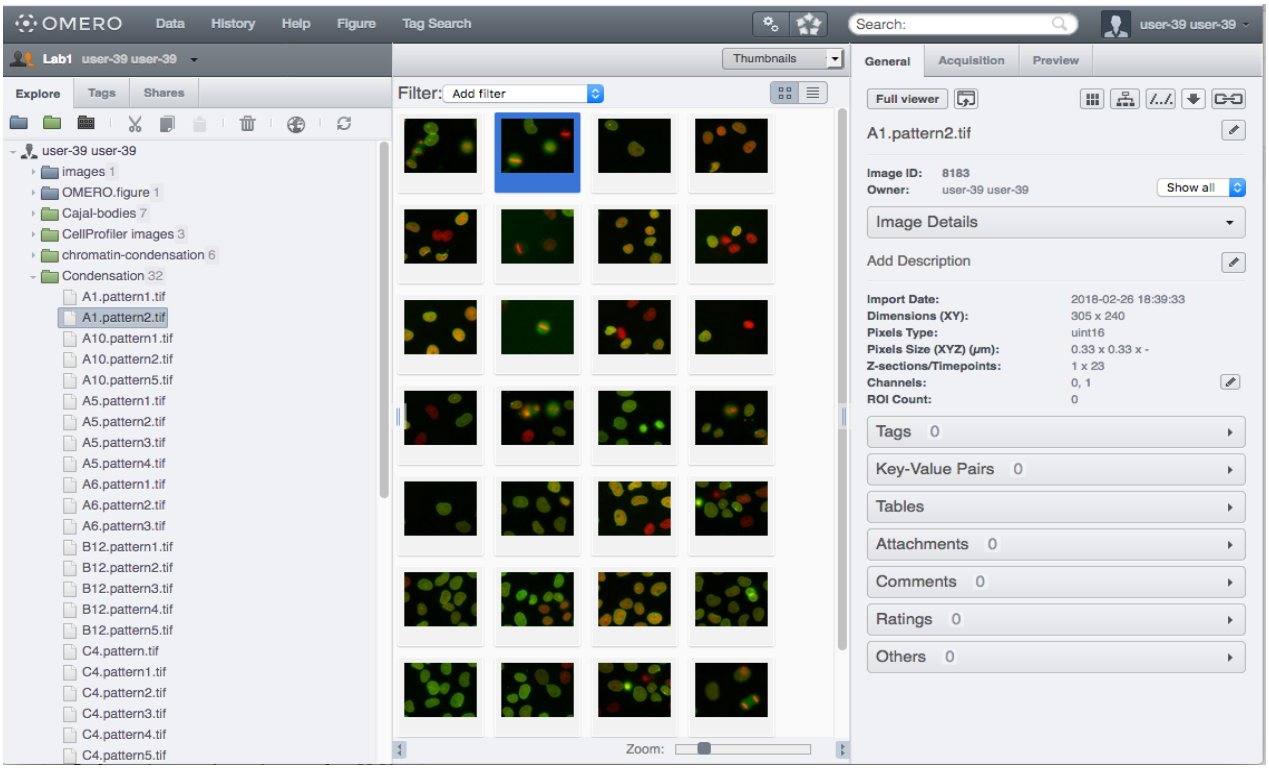
Description

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.

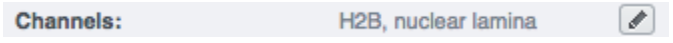
Browsing and Viewing Images

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 at http://outreach.openmicroscopy.org 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* named *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.

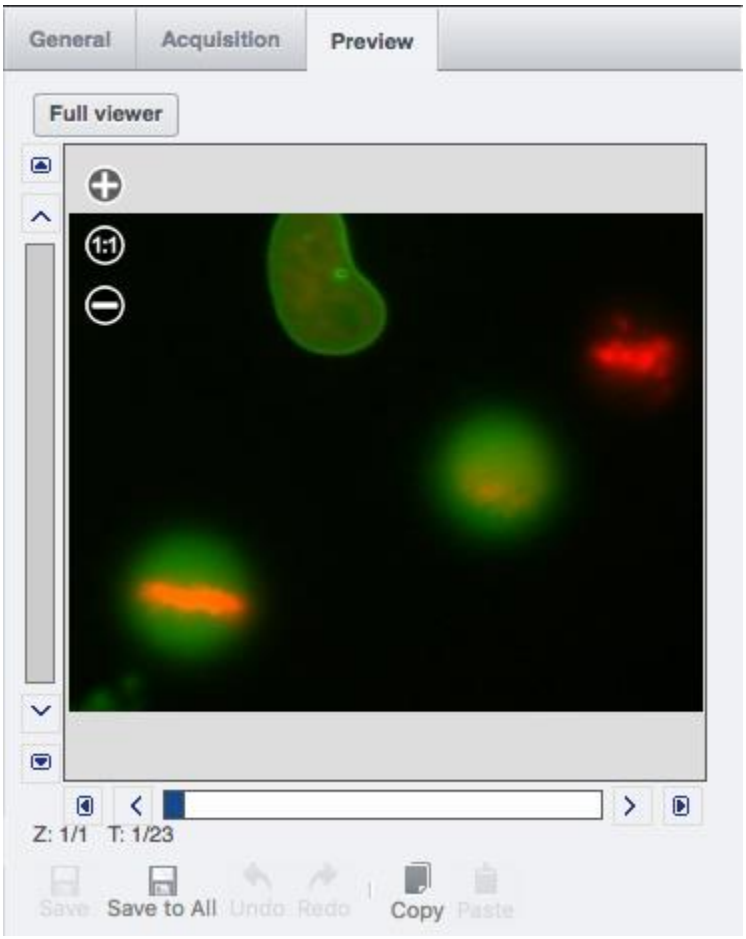


6. Channel names in the right-hand pane

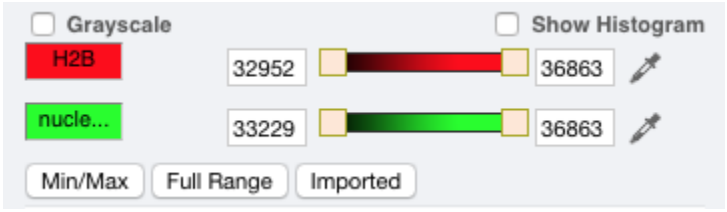


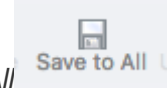
can be edited by clicking on the *pencil* icon and the new name can be saved for all images in the *Dataset.*

7. The *Preview* tab at the top-right of the page will show a small image viewer.



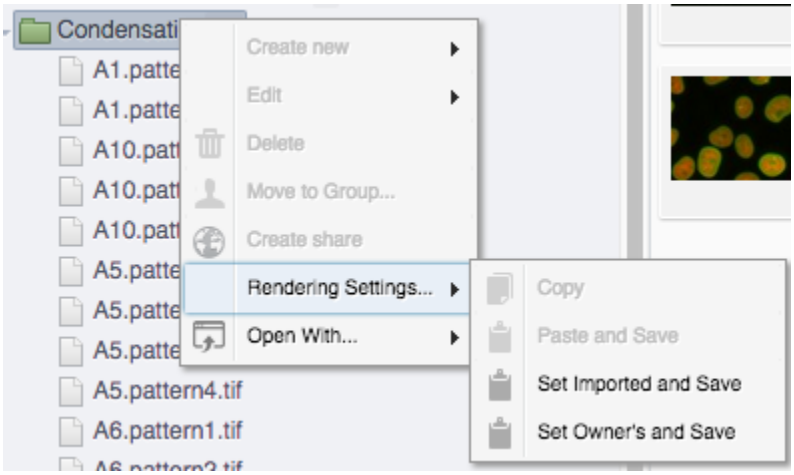
8. Play the video by clicking the *arrow in the box*  to the right-hand side of the *T* slidebar under the image viewer. In this way, you will be able to see whether or not there is a mitotic event in this particular image. You can select another image thumbnail in the central pane and continue playing the quick videos (eventually for the whole *Dataset*).
9. The image rendering settings can also be adjusted, such as turning channels on and off, adjusting levels and channel colors.

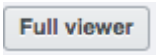


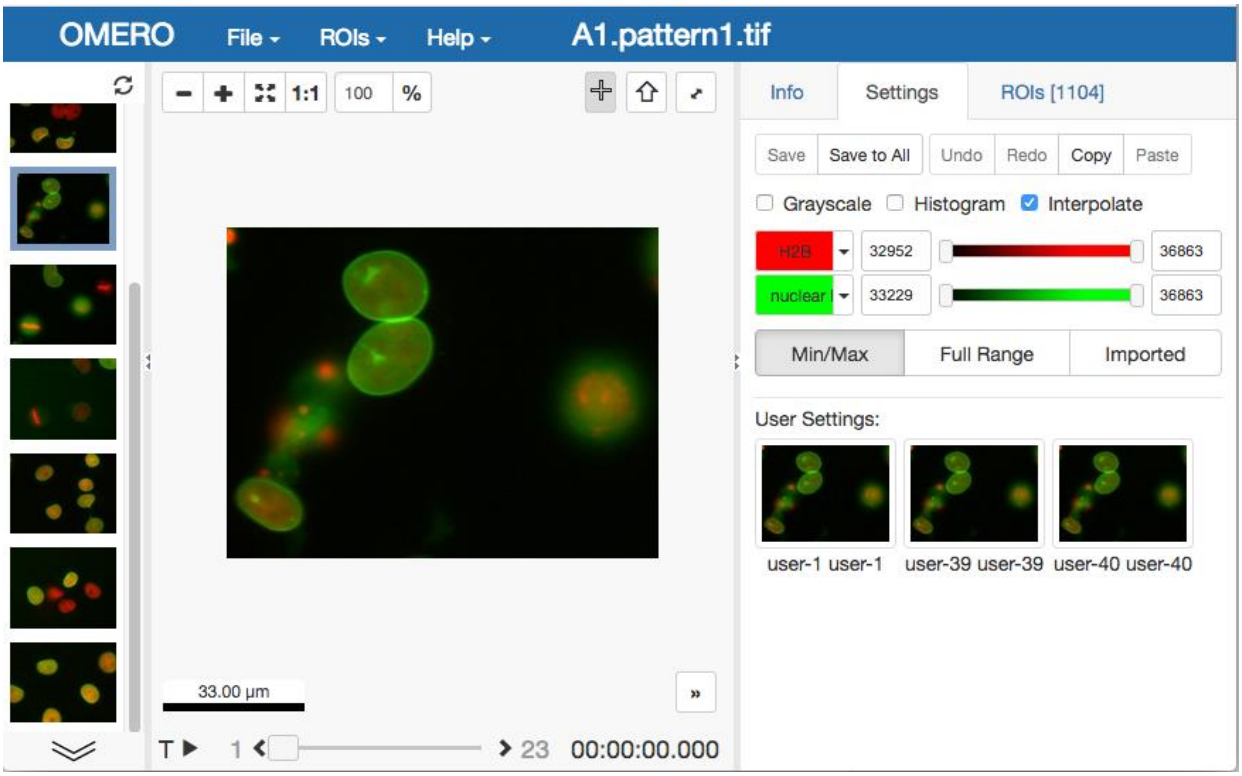
10. Saving the settings does not change the original pixel data for the image.
11. Click the *Save to All* 

button to apply the current settings to all other images in the Dataset.

12. In the left-hand pane tree, right-click on the Dataset *Condensation* and choose *Rendering Settings* > *Set Imported and Save* to revert to the original settings.

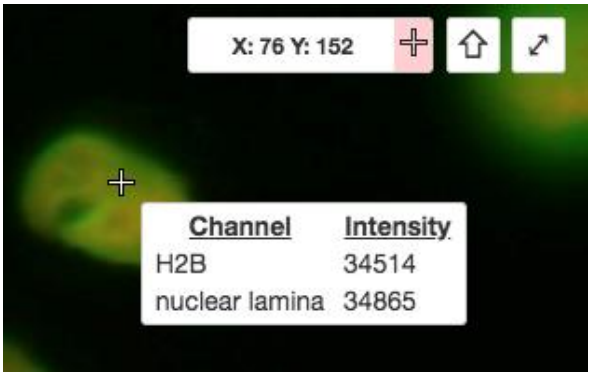


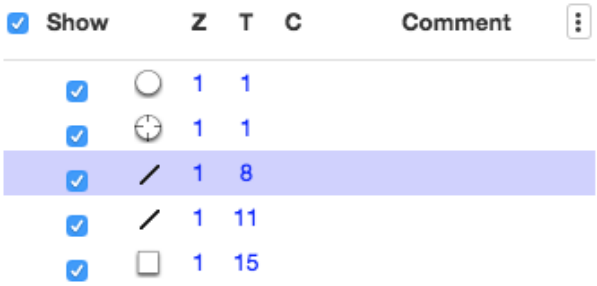
13. Double-click on an image thumbnail, or click the *Full Viewer*  button in the right-hand pane to open an image in a larger viewer (called OMERO.iviewer).



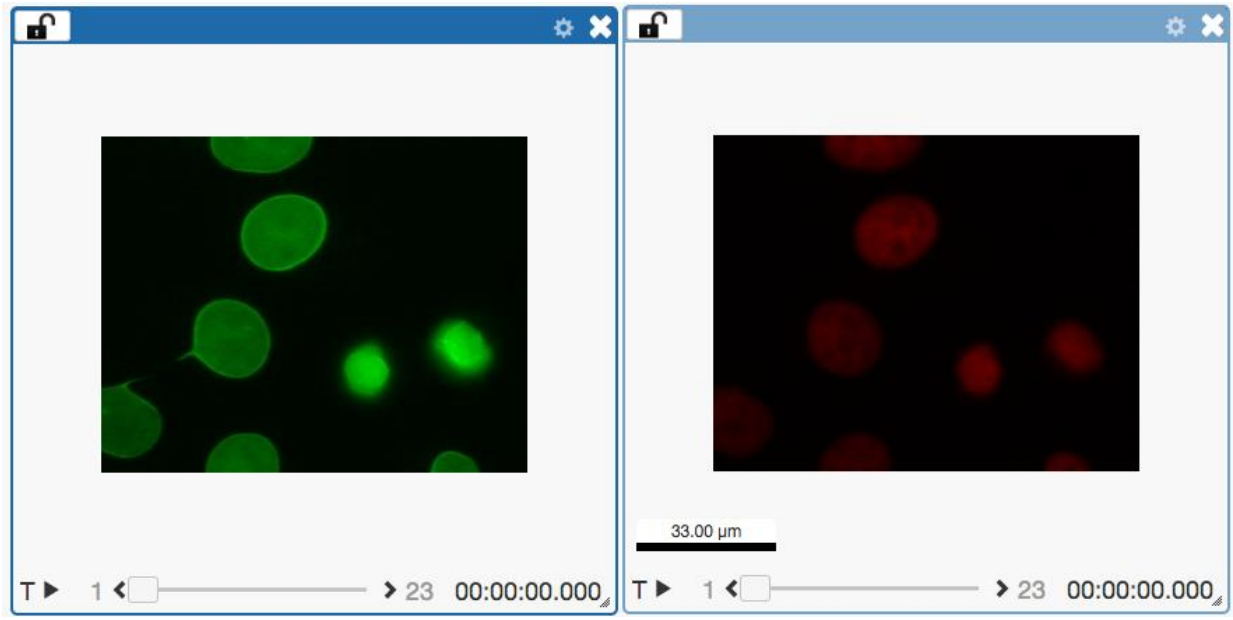
14. We can adjust the rendering settings and scroll through T and Z if it is a Z-stack image.

15. 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 pixel intensities for enabled channels.

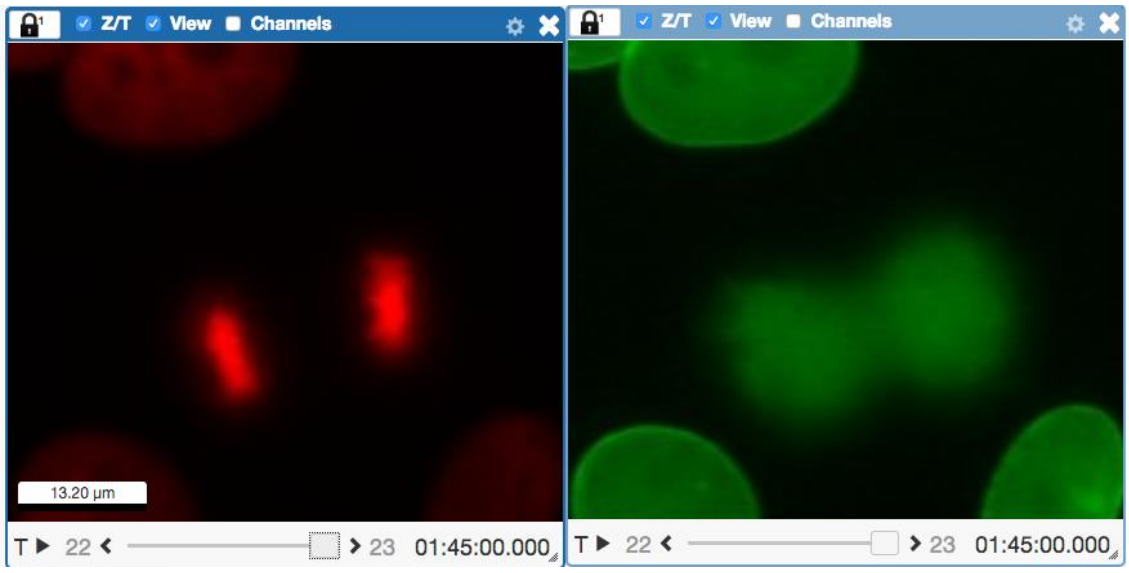
16. 

17. 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* Save located in the upper part of the right-hand pane. Draw also a couple of other ROIs on different T planes.
18. 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.
19. 

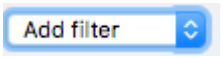
20. Save the viewport by selecting in the top-left corner *File* > *Save Viewport as PNG*
21. Compare the two channels of one of the images in multi-image view. Double-click on the thumbnail of the already opened image in the left-hand pane of the viewer. This will open the image again in a new window. Select one of the windows and in the right-hand pane switch the green channel off.



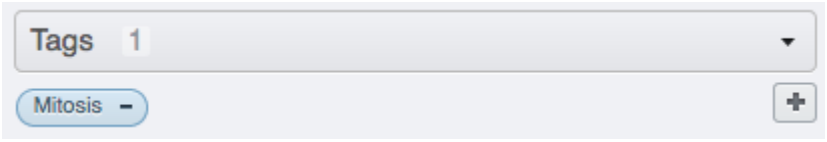
22. We can synchronize the viewers by adding them both to the same sync Group. Click the *sync icon*  in the top-left corner and select the first option in the dropdown menu. Repeat for the other viewer. Now both images have the *Z, T* and *View* (zoom and position of viewport) synchronized 

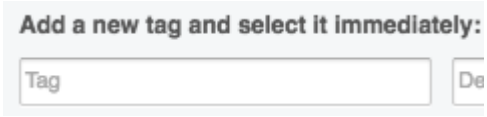
.
23. Select the red channel image (DNA staining) and play the timelapse by clicking onto the *arrow* at the bottom of the image window. After you have identified a mitotic cell, zoom in onto it by scrolling the mousewheel or expand the *cog* icon in the top bar of the window and click on *plus* sign. 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.
24. 

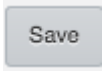
Use Tags to earmark images for analysis/publication

25. Go back to the webclient. We want to find the image *A1.pattern1.tif*. Select the *Dataset Condensation* again and in the *Add filter* menu 

above the centre pane select *Name* to filter by name. Then type into the box *A1.pattern1*


.This should filter out all the thumbnails in the centre pane except one. Note: the *A1.pattern1.tif* timelapse has a mitotic cell, which seems to undergo apoptosis as well.
26. Select the *A1.pattern1.tif* image and select the *General* tab. Expand the *Tags* harmonica in the right-hand pane. 

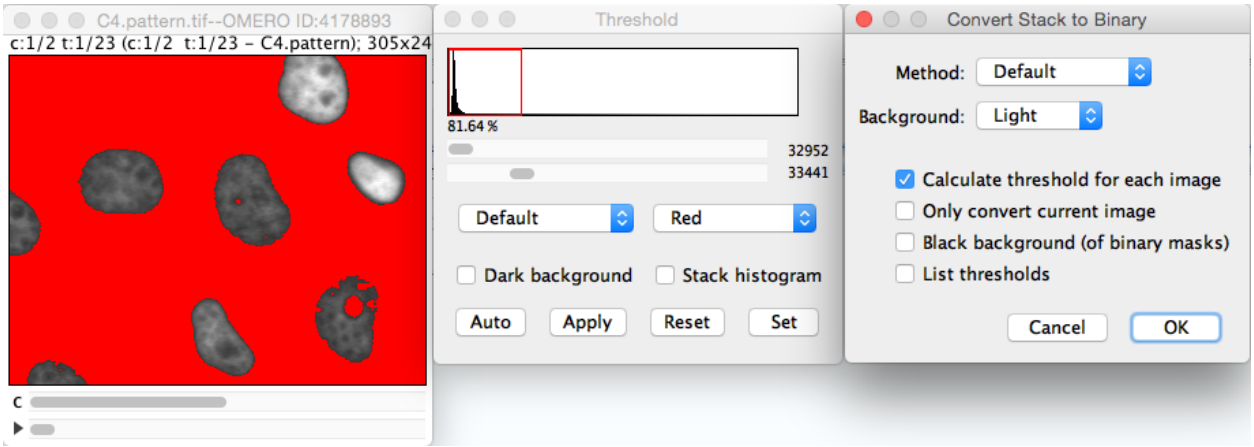
27. A tag named *Mitosis* was added prior to this workshop to 6 images. These 6 images, which include *A1.pattern1.tif*, will be used later in the **Analysis with Fiji: Scripted workflow** (see below).
28. Click on the plus icon. In the *Tag Selection* window select an existing tag *Apoptosis.* Click on the *arrow*  to move the tag into the *Selected Tags* area. If desired, new tags can be created using the *Add a new tag…* dialog 

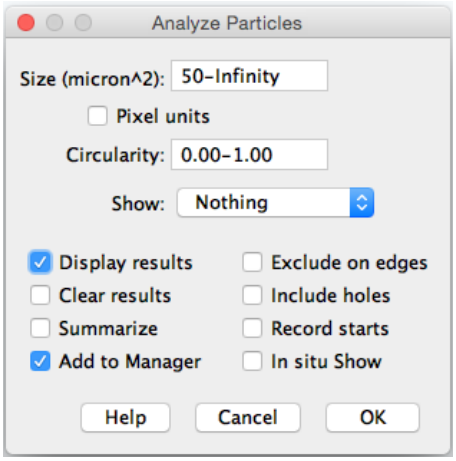
at the bottom of the *Tag Selection* window. Enter name of the tag to create and click *Add* button to the right.
29. Click *Save* button 

in the bottom-right corner.
30. Clear the text of the *Name* filter text to show all images.
31. Choose to Filter by *Tag* and select the *Mitosis* Tag to show images that we will analyse in the next step.

Analysis with ImageJ/Fiji: Manual workflow (not presented, for information only)

32. We will not demonstrate this manual workflow, this chapter is just for information, to show the same steps that will be performed by the scripted analysis. Please go to the section below **Analysis with Fiji: Scripted workflow (demo only)** if you want to follow the demonstration.
33. In this Manual workflow paragraph ImageJ/Fiji, connecting to OMERO, to analyse images. Instructions on how to install the OMERO plugin for ImageJ can be found at http://help.openmicroscopy.org/imagej.html but we are unlikely to have time to install this for all users during this workshop.
34. 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.
35. We will analyse cells by thresholding and segmenting the image. Go to *Image* > *Adjust* > *Threshold.*
36. The *Threshold* dialog will pop up, click *Apply.*

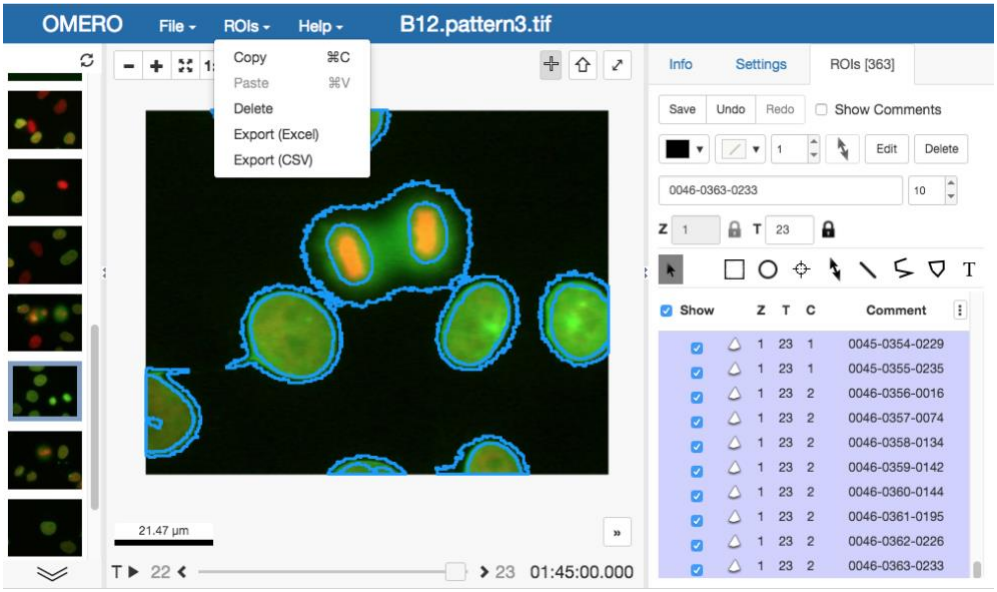
37. Another window *Convert Stack to Binary* will pop up.
38. Select the following parameters:
    a. *Method: Default*, *Background: Light* and *Calculate threshold for each Image.*
    b. Click *OK*.
    c. Close the *Threshold* dialog if you want.
39. 

40. Select *Analyze > Analyze Particles*
41. 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*.
42. Click *OK*.
43. 

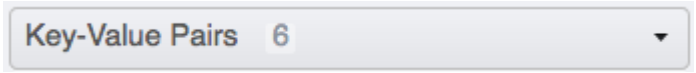
44. A dialog pops up asking to Process all 46 images? Click *Yes.*
45. ROIs and Results table will be generated.
46. Go to *Plugins > OMERO > Save ROIs to OMERO* to save these ROIs to the Image in OMERO.

Analysis with Fiji: Scripted workflow (demo only)

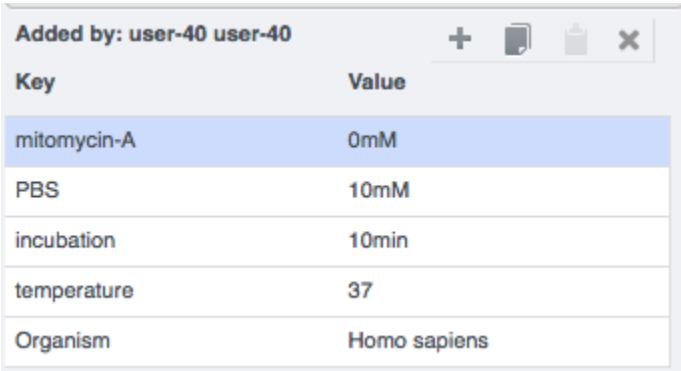
47. We will use a script in the Jython language to perform the same processing steps as above, including saving of ROIs to OMERO. This will analyse images within a Dataset that are tagged with a specified Tag. The tag *Mitosis* will be used which was added to 6 images in previous steps above.

48. Setup: Just for your information, the script used here also needs the OMERO plugin for ImageJ/Fiji. Instructions on how to install the OMERO plugin for ImageJ/Fiji can be found at http://help.openmicroscopy.org/imagej.html but we are unlikely to have time to install this for all users during this workshop.
49. In Fiji, go to *File* > *New* > *Script*.
50. A dialog pops up. In the *Language* menu select *Python.*
51. Copy the content of the script https://github.com/jburel/omero-example-scripts/blob/cambridge-training/jython_scripts/analyse_particles_from_dataset.py into the text editor.
52. Edit the following parameters: dataset_id, tag_text, USERNAME, PASSWORD.
53. Click Run.
54. Back in the webclient, open one of the images tagged with *Mitosis* so that you can see the ROIs generated in Fiji saved to OMERO.
55. Each of the ROIs created by the script in Fiji are associated to a certain T and Channel, which can be seen in OMERO.iviewer.
56. Finally, we can export the pixel intensity for ROIs using iviewer. Select the ROIs to export using Shift-click to select a range of ROIs in the table. Then *ROIs* > *Export (CSV).*
57. 

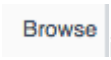
Adding Annotations, Filtering and Searching

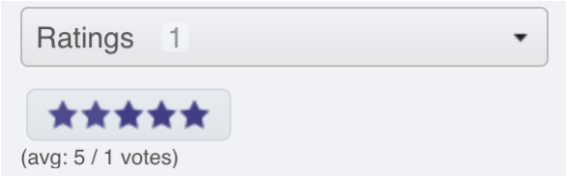
58. Return to the main webclient.
59. We can add annotations to images in the right-hand pane *General* tab.
60. Select one or more images from *Dataset Condensation* and click the harmonica *Key-Value Pairs* in the right-hand pane. 

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



62. The *Key-Value Pairs* allow you to add lab-book like additional metadata for the image. These *Key-Value Pairs* are also specifically searchable.
63. Type *mitomycin-A* into the search box in the top right of the weblicent 

and press Enter.
64. The search results will show any objects e.g. images or datasets which have anywhere the string *mitomycin-A.* 32 images should be found.
65. Refine the search now for only *Key-Value Pairs* which have the key *mitomycin-A* and value *0mM* by typing *mitomycin-A:0mM* into the search box and pressing enter. Now you should find only 10 images. Click on the *Browse* link 

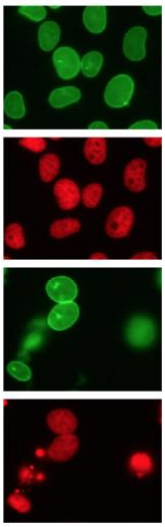
in one search result line, (in the right-hand part of the centre pane) to navigate back to the main webclient.
66. Images can be filtered by Tag or Rating in the centre pane, using the *Add filter* chooser above the thumbnails,.
67. Choose the *Tag* option and then choose the *Mitosis* from the list of tags. As mentioned already, the *Mitosis* tag was added to 6 images before this workshop.
68. 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
69. 

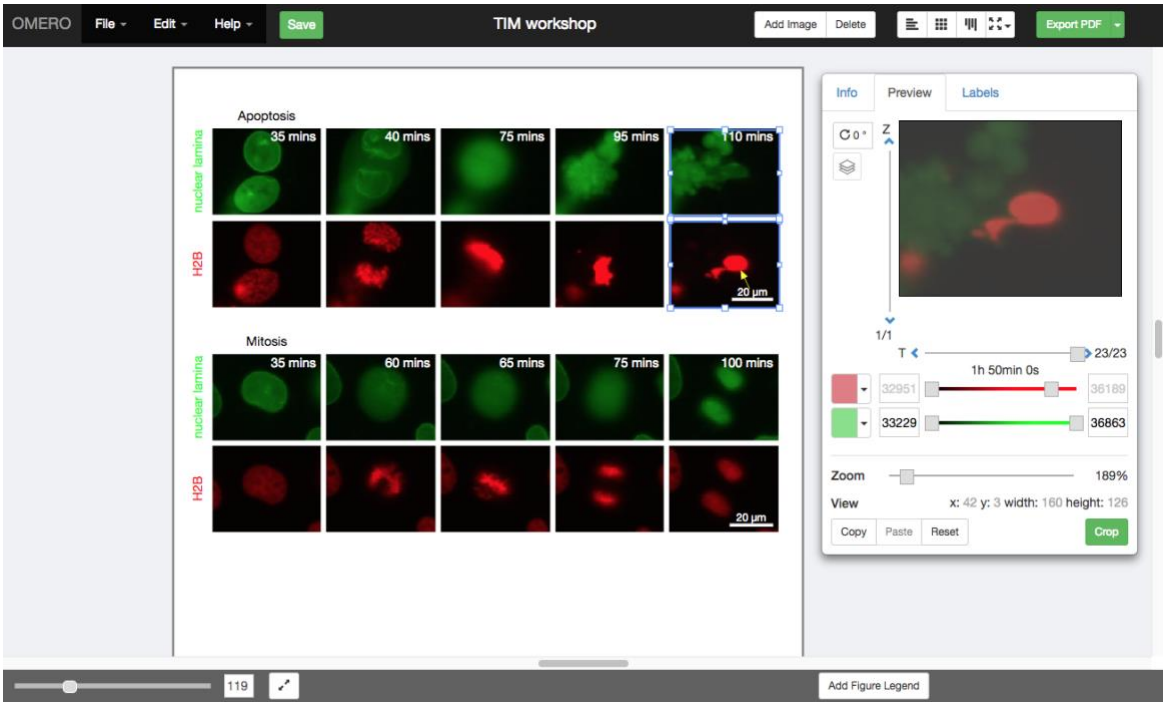
70. Repeat this to add a rating of 5 to the *Apoptosis*-tagged image.
71. Now we can remove the filtering by Tag and instead filter by *Rating of 5* to show only our favourite images.

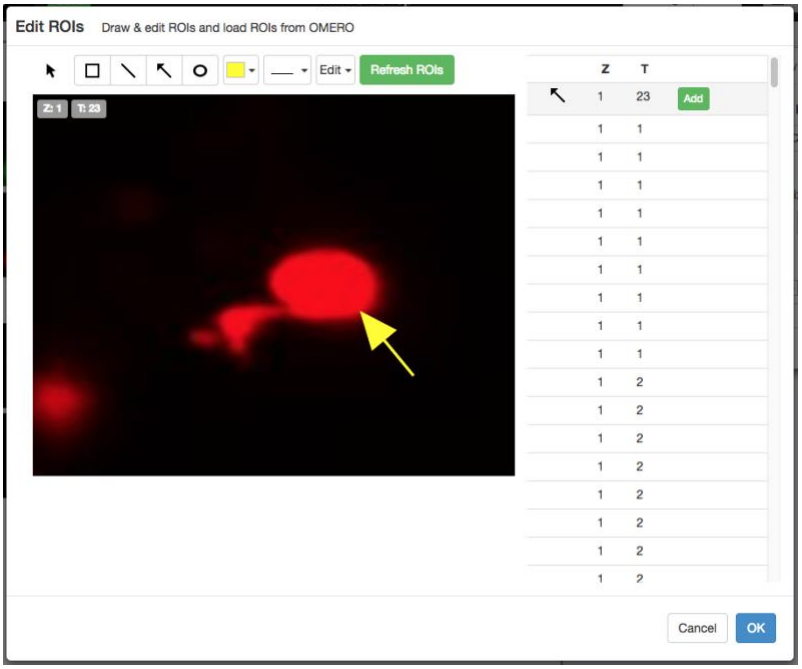
Creating a Figure

72. Select these 2 images and use the context menu on the images in the left-hand side tree to *Open With* > *OMERO.figure.* This will open the OMERO.figure app in a new tab.
73. Select panels by clicking or dragging a select region. Arrange panels by dragging and resizing them.
74. Duplicate both panels by selecting them, *Edit* > *Copy* and *Paste,* or use keyboard shortcuts.
75. Use the *Align* > *Width & Height* toolbar to ensure selected panels are all the same size.

76. 

77. Arrange the 4 panels in a single column and snap selected panels to a grid with the  button.
78. In the *Preview* panel you can adjust the Time position and the rendering settings of selected panels.
79. Toggle channels on and off to show a ‘split-view’ of each image.
80. 
81. Select all 4 panels, set the T-position to the start of Mitosis, e.g. position 8 (~35 minutes). *Copy* and *Paste* to create a new column.
82. With these new panels still selected, increment T by clicking the > button one or more times.
83. Copy, Paste and increment T-index two or three more times to create a timelapse figure.
84. 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.
85. With ALL panels selected, use the Zoom slider to zoom in to approximately 200%.
86. 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.

87. 

88. Click on the *Labels* tab to add a scalebar and labels to selected images.
89. Select one of the *Apoptosis* images that we previously edited in OMERO.iviewer and click the *Edit* button under the ROIs section.
90. 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)
91. 

92. A list of ROIs will be displayed. N.B.: Polygons and Polylines are not yet supported in OMERO.figure, but will be supported in the next release.
93. Clicking on each row of the list will show the image plane that the shape is saved on. Clicking the *Add* button will add the shape to the image. Click *OK* to close.

94. 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.
95. Change the scalebar length to 20 µm and check the *Label* checkbox to show a label.
96. 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.
97. Alternatively you can manually enter label text.
98. 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*.
99. 

100. 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*.
101. 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
102. 

103. 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.
104. 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.