ELMI Workshop

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

Software versions used for this workshop:

- OMERO: 5.4.10
- OMERO.iviewer: 0.7.0
- OMERO.figure: 4.0.2
- OMERO.mapr 0.2.3
- OMERO.parade: 0.1.1
- OMERO.FPBioimage: 0.3.0
- OMERO training scripts: 0.6.0
- OMERO training notebooks: 0.4.1
- OMERO-downloader: 0.1.5
- Bio-Formats: 5.9.2
- Fiji/ImageJ: 2.0.0-rc-68/1.52e
- CellProfiler: 3.1.3

Content

- Introductory talk about OMERO
- OMERO core concepts
 - Data Management
 - Metadata
 - Search
- Visualization
 - Viewer
 - 3D Viewer
- Fiji as 3rd party tool for OMERO
- OMERO.parade
- OMERO.figure

Import via desktop client and CLI (for your information only)

Description

In this first part, we first show how to import data by yourself and for yourself into OMERO using various import strategies. This will be mainly done using the OMERO.insight desktop client.

Second part of this import section will show how to import data for another user, using OMERO.insight and Command Line Interface (CLI). The user importing the data needs to have some admin (or restricted-admin) privileges. More information about restricted privileges can be found at <u>https://docs.openmicroscopy.org/latest/omero/sysadmins/restricted-admins.html</u>

The import for yourself will be done by the OMERO user you have been allocated. The import for another user will be done only as demo since the user requires to have specific privileges. We will use the user *importer1* e.g. a facility manager.

Setup

- The Desktop client OMERO.insight has been installed on the local machine. The installation instructions can be found at http://help.openmicroscopy.org/getting-started-5.html#installing.
- Client libraries from the OMERO.server have to be installed on the client to import images using CLI. The installation instructions can be found at <u>https://docs.openmicroscopy.org/latest/omero/users/cli/installation.html</u>.

Desktop client Import

1. Open OMERO.insight and in the login dialog, click on the spanner icon S. This will open a list of servers to which you can connect. 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**

	Servers	
	Servers Enter a new server or select an existing one.	Z
localhost [High]	Server Address and Port e.g. test.openmicroscopy.org or 134.20.12.33 You should not have to modify the port.	
60	outreach.openmicroscopy.org	4064
localhost [High]	2 localhost	4064
Username: user-1		
Login Quit	 Connection Speed High (LAN) Medium (Broadband) 	C Low (Dial-up)
5.4.1-1ce36-b75		
OMERO is distributed under the terms of the GNU GPL. For more information, visit openmicroscopy.org		Apply Cancel
**• OME		_

- 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 $\textcircled{\blacksquare}$: 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 log in 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.

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 **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 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.

Browse Images

- 1. In your web browser, go to <u>https://outreach.openmicroscopy.org/</u>
- 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 use the menu to select the name of the user whose data you wish to see.

Lab1 Francis Cri	ick 🔻		
Lab1	•	All Members	
Lab2	×.	Members	
Lab3	•	Oswald Avery	
Lab4		Wendy Barclay	
		Elizabeth Blackwell	- 1
		Linda Buck	- 1
		Rachel Carson	
		Erwin Chargaff	
		Ann Clarke	
		Francis Crick	
		Marie Curie	
		Charles Darwin	

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, <u>https://www.openmicroscopy.org/bio-formats/</u>, 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.
- 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.

	X: 345 Y: 1	70 +	合。
Chan	nel Inten	sity	
DAPI	141		
FITC	47		
RD-TR	-PE 56		
CY-5	26		

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

and draw an arrow on the Image, using Click-Move-Click (not drag), pane

Save 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.



- 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.
- 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*:

Dataset:

siRNAi-HeLa . Click on that link. This will bring you back

🗹 Z/T 🗹 View 🔲 Channels

to the webclient.

- 12. 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 "GFP", "Aurora-B" and "CY-6" for the other channels.
 - c. Click the *Apply to All* button Apply to All and confirm by clicking *Continue*.
- 13. Go to data of trainer-1. Open an image from the Dataset **mouse-14-days** in a 3D viewer: OMERO.FPBioimage.
 - a. First select the Image.
 - b. Right-click menu on the image in the left panel, or use the Open with... icon on

top of the right-hand pane ^[52] to open the image with FPBioimage.

c. Click Start in the new viewer window.



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.
- 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 (to filter, type 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*.

Key-Value Pairs 6	•
Added by: user-40 user-40	+ 🗊 🖹 🗙
Кеу	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 webclient



- 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 at least 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. Select 2 images from two different datasets in the Results (you can judge by the thumbnails which images are from which dataset). Click onto the *Open with* icon in the right-hand pane and select OMERO.iviewer.
- 12. Go back to the Search results tab. Click on the *Browse* link ^{Browse} 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.
- 13. Images can also be filtered by Tag or Rating in the centre pane, using the *Add filter* chooser above the thumbnails.

- 14. For example, choose Tag and then select **Metaphase** from the list of Tags to show the images we tagged earlier.
- 15. 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



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

Analysis with Fiji

Description

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

We show how to analyze a batch of images stored in OMERO using the scripting facility in Fiji, the results of the analysis will be stored as a CSV and OMERO.table,

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

More information about restricted privileges can be found at

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

Setup

- Fiji has been installed on the local machine with the OMERO.insight-ij plugin. The installation instructions can be found at http://help.openmicroscopy.org/imagej.html#plugins.
- Installing the OMERO.plugin also adds the dependencies required to connect to OMERO using the Script Editor of Fiji.
- The OMERO.script <u>https://github.com/ome/training-scripts/blob/v0.6.0/practical/python/server/batch_roi_exp</u> <u>ort_to_table.py</u> has been uploaded to the server.

Fiji Manual simple workflow: Draw ROI

- 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.

	e o Servers	
	Servers Enter a new server or select an existing one.	Z
localhost [High]	Server Address and Port e.g. test.openmicroscopy.org or 134.20.12.33 You should not have to modify the port.	
A D	outreach.openmicroscopy.org	4064
localhost [High]	2 localhost	4064
Username: user-1		
Password:		
Login Quit	Connection Speed High (LAN) Medium (Broadband)	Low (Dial-up)
5.4.1-10x36=b75		
OMERO is distributed under the terms of the GNU GPL. For more information, visit openmicroscopy.or	A	pply Cancel
••• OME		_

- 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.

	Save ImageJ results	
Save results for Image from controls in the second	urrent window all image windows	
Save ROI Measurement	:s	
Measurements Fil	e Name:	
	Save Cancel	

- 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 see the ROIs and make sure that you can interact with them.

Fiji Manual simple workflow: Crop Image

- 1. This time we will crop an Image stored in OMERO and saved the cropped Image back to OMERO as OME-TIFF.
- 2. Select the **A-Fiji-dataset** Dataset.
- 3. Double-click on a thumbnail or on an Image in the left-hand tree to open an Image in ImageJ.
- 4. In the Bio-formats dialog, check that you have "Hyperstack" selected and click OK.

0 0		Bio-Format	s Import Options	
Stack viewing		Metadata viewing		Information
View stack with:	Hyperstack ᅌ	Display metadat	ta	Specify range for each series - Opens only the specified range of image planes from a dataset.
Stack order:	XYCZT 0	 Display OME-XML metadata Display ROIs 		After analyzing the dataset dimensional
				parameters, Bio-Formats will present an additional dialog box prompting for the desired
		ROIs Import Mode:	ROI manager ᅌ	range.
Dataset organiza	ation	Memory managem	ent	Example: You only want to open the range of focal planes in a z-series that actually contain
Group files w	ith similar names	Use virtual stack	k	structures of interest to conserve memory.
Open files inc	dividually	Specify range fo	r each series	
Swap dimensi	ions	Crop on import		
Open all serie	5			
Concatenate	series when compatible	Split into separate	windows	
Stitch tiles		Split channels		
Color options		Split focal plane	s	
Color mode:	Default ᅌ	Split timepoints		
Autoscale				

- 5. Note: You can also select **Split channels** checkbox in the Bio-formats dialog above, and do the following crop workflow on single channel images.
- 6. The image will open in Fiji.
- 7. Note: You can now use Image > Duplicate in Fiji and do the workflow on the duplicate.
- 8. Draw a Rectangle on the Image.
- 9. Select Image > Crop
- 10. A new Image will be displayed in a Fiji window
- 11. Go to Plugins > OMERO > Save Image(s) To OMERO
- 12. An Import dialog (specific to Fiji plugin) will pop up.

		Import Data	
Import Dat	a		
Select dat	a to import and monitor imports.		Ť
Select D	ata to Import 📔 🖌 Import #1 🞯		
Select wher	e to import the image(s).		Files to import Options
Group	🔏 Lab1 🗘		Free Space Import size:
Import For	trainer-1 trainer-1		File or Folder Group Owner Project/ Folder a Size or Sc Datase
	🍽 Projects 🛛 🝽 Screens		
	No Project No Dataset age from current window	New New	
	ages from all image windows		
Refresh		Add to the Queue	
Close			Cancel All Import

- 13. Check that the option *Add Image from current window* is selected
- 14. Select where to import the cropped Image e.g. A-Fiji-dataset Dataset.
- 15. Click Add to the Queue button
- 16. Then click Import. The import will start
- 17. When the import is done, go back to the Tree view in the Fiji plugin or Web client. Refresh. Check the new Image.

Fiji Manual workflow

- 1. Launch Fiji.
- 2. To open an image stored in OMERO, Go to *Plugins > OMERO > Connect To OMERO* if necessary.
- 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*.

	Servers	
	Servers Enter a new server or select an existing one.	Z
	Server Address and Port e.g. test.openmicroscopy.org or 134.20.12.33 You should not have to modify the port.	
	outreach.openmicroscopy.org	4064
localhost [High]	2 localhost	4064
Username: user-1		
Login		
Login Quit	Connection Speed	-
5.4.1-Ice36-b75	• High (LAN) O Medium (Broadband)	O Low (Dial-up)
OMERO is distributed under the terms of the GNU GPL. For more information, visit openmicroscopy.or	rg	Apply Cancel
*** OME		

- 4. Enter your Username and Password and click Login.
- 5. Browse to the 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*.

Auto Threshold	
Auto Threshold v1.16.4	
Method MaxEntropy	Centrin PCNT Cep215 201
Ignore black Ignore white	1/3 (c:1/3 - Centrin_PCNT_Cep215_201
White objects on black background	
SetThreshold instead of Threshold (single images)	
Show threshold values in log window	
🗹 Stack	
Use stack histogram	
The thresholded result of 8 & 16 bit images is shown in white [255] in 8 bits. For 16 bit images, results of 'Try all' and single slices of a stack are shown in white [65535] in 16 bits. Unsuccessfully thresholded images are left unchanged.	- - 1
Cancel OK	c

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?.

Size (micron^2):	10-Infinity	
🔽 Pixel ı	units	
Circularity:	0.00-1.00	
Show:	Nothing	0
🗹 Display resu	lts 🗌 Excl	ude on edges
🗹 Clear results	🗌 Inclu	ide holes
Summarize	Reco	rd starts
🗹 Add to Mana	aer 🗌 In si	tu Show

- 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. Let's go over the scripts to understand the logic and see how it matches the UI steps. This script explores the JAVA API using groovy. See

https://docs.openmicroscopy.org/latest/omero/developers/Java.html,

1. Go to <u>https://outreach.openmicroscopy.org/</u>.

- 2. Log in using the credentials provided
- 3. Make sure you are selecting your own data. Select the Dataset A-Fiji-dataset.
- 4. Launch Fiji. (relaunch if opened).
- 5. Go to File > New > Script....
- 6. A dialog pops up. In the Language menu select Groovy.
- Copy the content of the script <u>https://raw.githubusercontent.com/ome/training-scripts/v0.6.0/practical/groovy/analyse_d</u> <u>ataset_save_rois_and_summary_table.groovy</u> into the text script editor of Fiji.
- 8. Edit the following parameters: dataset_id, USERNAME, PASSWORD.



- 9. 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. Further, it will create a CSV and OMERO.table to be attached to that Dataset in OMERO. Both are not necessary when analysing data, this is mainly to show how it we
- 10. Click Run.
- 11. Return to the webclient and open an Image from this Dataset in OMERO.iviewer.
- 12. 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.

- 13. In the *Settings* tab, turning channels on/off will also show/hide ROIs assigned to those channels.
- 14. 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.

- 15. This script was used previously to run on the whole **idr0021** Project and produced the resulting CSV file. We can now download this file from OMERO and open it in Microsoft Excel.
- 16. In Excel, select the Dataset column and the column bounding_box and then click on

Insert > X Y Scatter $\xrightarrow{\text{limit}}$ > Scatter . Depending on the version of Excel you are using, this option might not be available.



- 17. This will create a scatter plot. Right-click into the Chart now and select *Change chart type > Statistical > Box and Whisker.*
- 1. 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.
- 2. In the *Settings* tab, turning channels on/off will also show/hide ROIs assigned to those channels.
- 3. Open the image in OMERO.figure for a quick publication by going to *Info* tab in iviewer

Open With: OMERO.figure

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

OMERO.parade and OMERO.figure

Analyze metadata using OMERO.parade

- 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. Select several images in the dataset, and in the right-hand pane add a comment to them

saying "poor staining". Refresh, using the refresh button above the left-hand pane.

- 8. In the *Add filer...* selection box, select the *Comment* item.
 - a. Enter a text "poor staining"
- 9. Note that you can use the selected images in right panel to annotate or Open with....
- 10. Choose Open with Figure...

Let's look at the analytical results generated using the Analyze particles plugin in Fiji

- 1. Select the first dataset of Project idr0021, expand it and click on any image.
- 2. You can see the values stored in the OMERO.table produced by the Fiji script for that image in the right-hand pane under **Tables** harmonica. Note that these values are used by OMERO.parade to perform the filtering shown below.

Key-Value F	Pairs 6	۲
Tables		•
INFO		
Slice:	c:1/3 - Cep215_PCNT_NEDD1_20110505_Thu- 1504_0_SIR_PRJ.dv	
Count:	600	
Total Area:	29.795041680336	
Average Size:	4.96583990752697	
%Area:	28.99169921875	
Mean:	0	
Mode:	0	
Perim.:	90.70947766304016	
Feret:	33.201760053634644	
FeretX:	598.6200332641602	
FeretY:	648.7800121307373	
FeretAngle:	10633.987426757812	
MinFeret:	19.399362802505493	
Dataset:	CDK5RAP2-C	
Bounding_Box:	45.292896032333374	
Channel Index:	100	
Image:	9563	

3.

9.

- 4. Collapse all datasets inside the Project idr0021..
- 5. Select the Project idr0021.
- 6. Choose the *Parade* option in the centre panel dropdown menu.
- 7. Expand all Datasets by clicking on the Open All button.
- 8. In the Add filter... selection box, select the Table item so we can find using the analytical results generated previously:. 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.



- a. Table_Bounding_Box
- b. Table_Total_Area
- c. Table_Image
- d. Note that it is currently not possible to remove a column.
- 12. Click on the name of a column to sort it.
- 13. Uncheck Show Datasets to sort all Images together e.g. by ROI count.



14.

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

Let's now look at the results generated by CellProfiler

- 1. Return to the webclient and select the Plate named plate1_1_013_previously_analyzed.
- 2. 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.
- 3. In the *Thumbnails* chooser at the top-right of the centre panel, select the *Parade* plugin.
- 4. At the top-left of the centre panel choose *Add filter... -> Table* to filter Wells by the data from CellProfiler.
- 5. Change the filter from *ImageNumber* to *Cell_Count* (at the bottom of the list).
- 6. Now you can use slider to filter Wells by Cell Count.

Table Cell_Count					○ > ○ ²⁵						
Add table data					0						
1	2	3	4	5	6	7	8	9	10	11	12
							* * *		4		
			- (***) 15 - 21				1997 (12) 1997 (12)	and the second			1997 - 1997 -

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 position=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 ¹² 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 Labels tab.
- 29. Draw arrows or other shapes on the image, or load ROIs from OMERO. Click OK.
- 30. Click *Copy* ROIs in the *Labels* 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 pops 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 it 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.