

Freising Workshop

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

Software versions used for this workshop:

- OMERO: 5.5.1
- OMERO.insight: 5.5.6
- OMERO.insight-ij: 5.5.6
- OMERO.iviewer: 0.8.1
- OMERO.figure: 4.0.2
- OMERO.mapr 0.3.2
- OMERO.parade: 0.1.3
- OMERO.FPBioimage: 0.3.0
- OMERO training scripts: 0.6.1
- OMERO training notebooks: 0.5.1
- OMERO-downloader: 0.2.0
- omero-metadata: 0.4.1
- Bio-Formats: 5.9.2
- Fiji/ImageJ: 2.0.0-rc-68/1.52e
- CellProfiler: 3.1.8

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Import

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

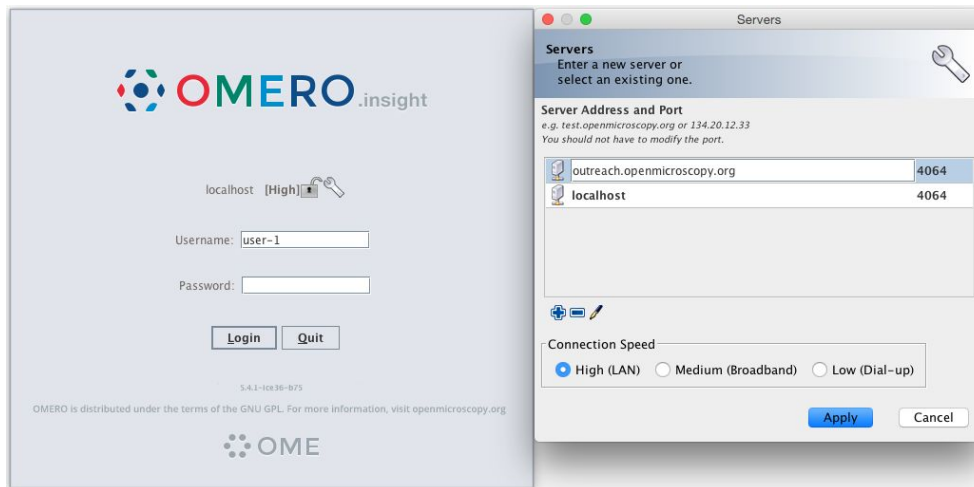
The import for yourself will be done by the OMERO user you have been allocated. The import for another user will be done only as demo since the user requires to have specific privileges. We will use the user *importer1* e.g. a facility manager.





Setup


- The Desktop client OMERO.insight has been installed on the local machine. The installation instructions can be found at
- <https://omero-guides.readthedocs.io/en/latest/upload/docs/import-desktop-client.html>
- 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 <https://docs.openmicroscopy.org/latest/omero/users/cli/installation.html>.

Desktop client Import

1. Open OMERO.insight and in the login dialog, click on the spanner icon . 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**



2. When done, click *Apply*.
3. Log in using the username and password provided.
4. In OMERO.insight, click on the Importer Icon  in the toolbar.
5. Browse your local hierarchy in the left-hand pane of the importer, select single images or whole folders and add these to the Queue by clicking on the *arrow*  icon.
6. In the *Import Location* window, select the target Project and Dataset (existing or create a new one) to import to.
7. Note: If no Dataset is selected or created, a new Dataset will be automatically created and named after the folder containing the images to be imported.
8. Optional: Go to the *Options* tab
 - a. Click on **Add tag to images**  to bring the Tag selection dialog.
 - b. Select the tag(s) on the left-hand side or create a new one.
 - c. Click  to move the tag(s) to the right-hand side.
 - d. Click *Save*.
9. Click on the *Import* button in the bottom-right corner of the Importer window. You should see two progress bars for every image imported, *Upload* and *Processing*.
10. Note: The import of the next image in the queue starts immediately after the *Upload* of the previous one is finished. The *Processing* phase of the import is done on the OMERO.server only, and can be finished while the next image(s) is/are being uploaded to the server.

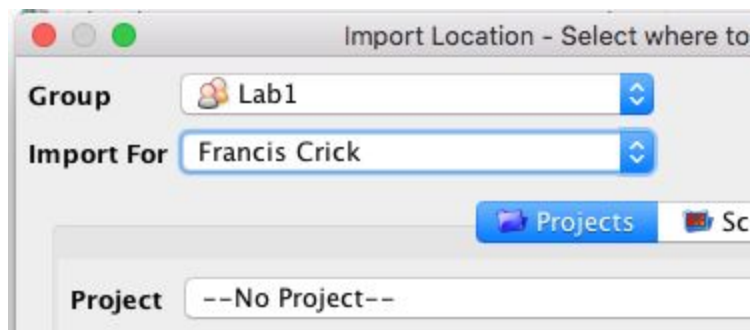
11. Once imports are finished, go back to the OMERO.insight main window and click the *Refresh* button  above the right-hand pane. This will display the imported images inside the Dataset and/or Project you specified previously in the *Import Location* window.
12. (demo only step) Now, the demonstrator will log out from OMERO.insight and 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.

Import for another user (demo only)

In this example, we show how to import data for another user. A facility manager *importer1* with restricted admin privileges imports the data for *user-1*. The facility manager has been given the ability to import for others.

Desktop client

The steps are the same as before for the normal import, but as *importer1* has the permission to import for another user there are two drop down menus for selecting the user and group to import for:



- Select Group: 'Lab1'
- Select User: 'Francis Crick' (user-1)
- Continue import workflow as usual.

A restriction of Insight is that the user *importer1* needs to be a member of the groups he wants to import for. This restriction does not hold for the CLI import command.

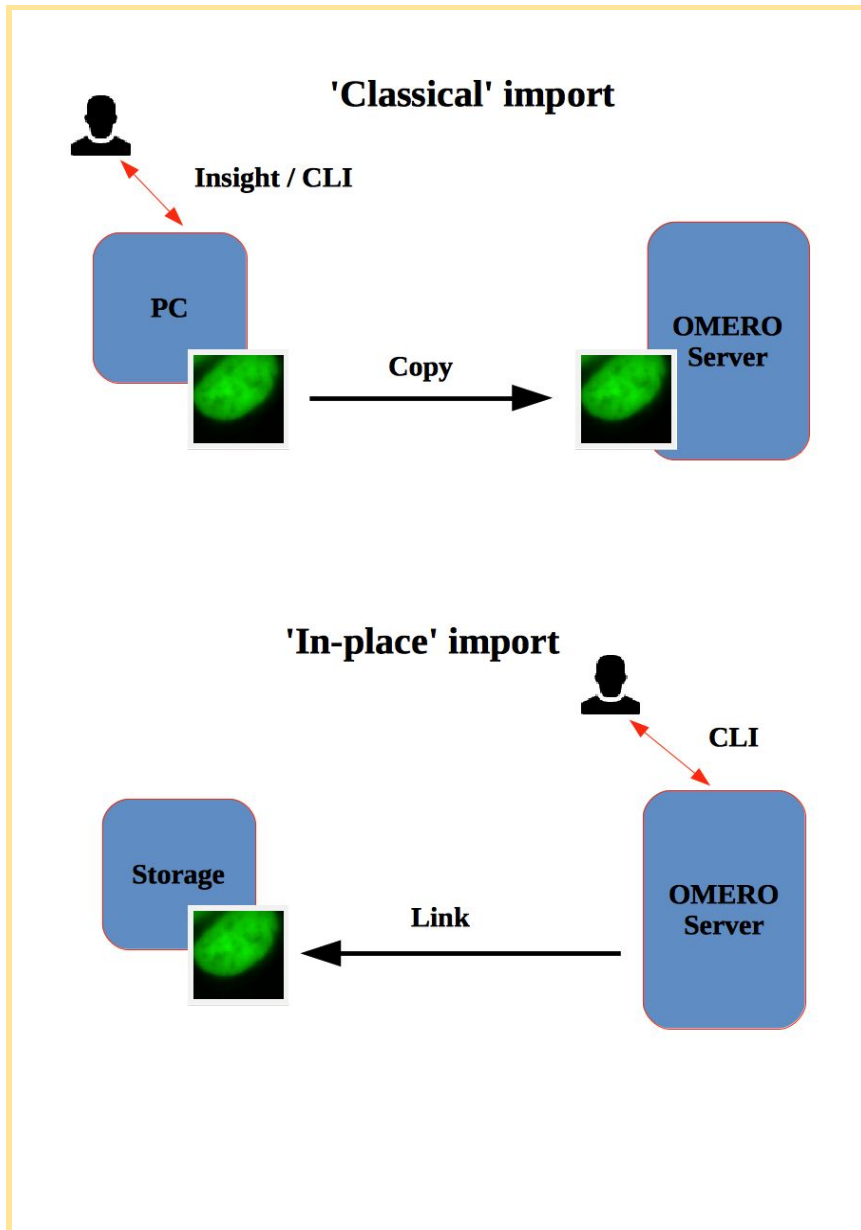
CLI (part of the OMERO.server package)

1. CD into `OMERO.server/bin` directory
2. Use `./omero --help` to get more information about the CLI and the various commands.
3. Login `./omero --sudo importer1 -u user-1 login` as *importer1* acting for *user-1*
4. Create a new dataset `./omero obj new Dataset name=import_one`
5. Import an image `./omero import -d Dataset:123 P-TRE_12_R3D_D3D.dv`

Check that the image is successfully imported.

Advanced import options

'In-place' import



```
./omero import --transfer=ln_s /nfs/77917.svs
```

Advantages:

- All in-place import scenarios provide non-copying benefit. Data that is too large to exist in multiple places, or which is accessed too frequently in its original form to be renamed, remains where it was originally acquired.

Limitations:

- Only available on the OMERO server system itself.

Do not edit or move the files after an in-place import. OMERO may no longer be able to access them if you do.

Bulk import

If you are interested in importing large amount of files, please see Bulk import.

<https://omero-guides.readthedocs.io/en/latest/upload/docs/import-cli.html#bulk-import-cli>

OMERO core concepts

Here we go through the core concepts of OMERO, explaining cooperation between OMERO users, browsing, viewing and searching of data. The walkthroughs in this section are also presented in

<https://omero-guides.readthedocs.io/en/latest/introduction/docs/index.html>

<https://omero-guides.readthedocs.io/en/latest/iviewer/docs/index.html> and

<https://omero-guides.readthedocs.io/en/latest/fpbioimage/docs/index.html>

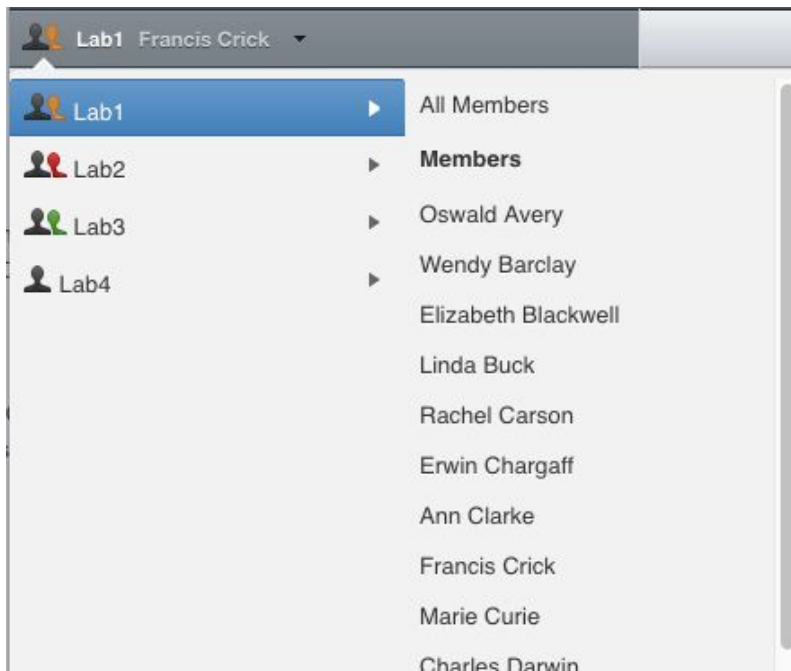
Data setup and ownership, usernames

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.

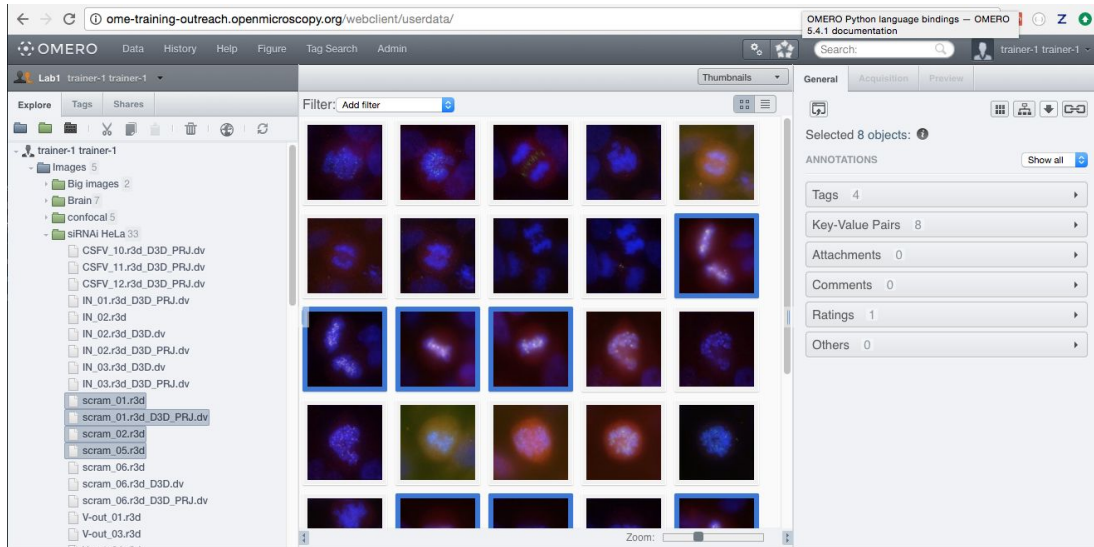
Browse Images

1. In your web browser, go to <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 use the menu to select the name of the user whose data you wish to see.



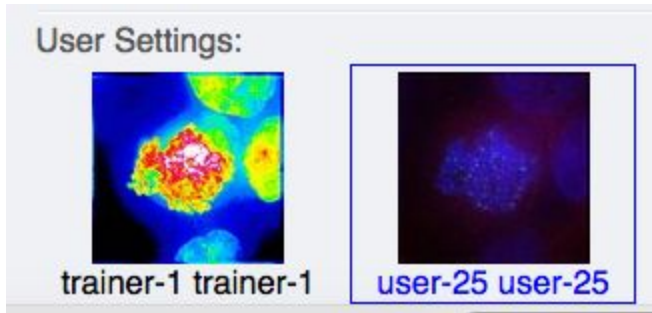
5. You can browse ‘folders’ in the left-hand pane: *Image* folders are called *Datasets* and they are within *Projects*.

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




- These represent imported Images. The original Images are stored on the server and the generated thumbnails allow us to browse them.
- 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.
- Select an Image. In the right-hand pane, metadata read by Bio-Formats and stored in a relational database is displayed:
 - core metadata in the *General* tab
 - additional metadata in the *Acquisition* tab. All the metadata read by Bio-Formats can be downloaded at any time.
- In the *Preview* tab in the right-hand panel, you can also view the Image.
- For multi-plane images, sliders allow you to move through Z or Time dimensions.
- 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.
- You can adjust the rendering settings for each channel e.g. turn on/off the channels, adjust color settings, look-up tables, etc..
- The rendering settings can be saved to the server. This **NEVER** changes the original Image data and can be reverted at any time.
- 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.
- 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

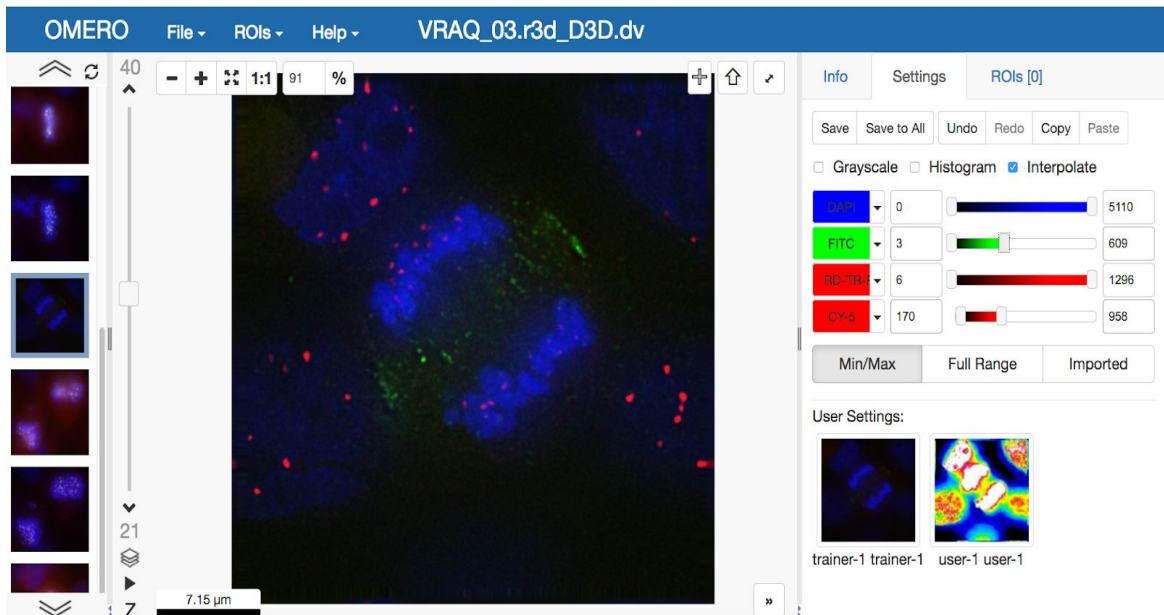
Preview pane.




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*.

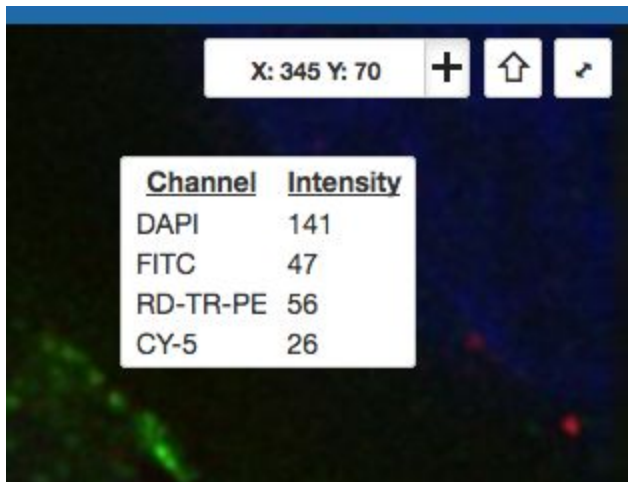
View Images

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.

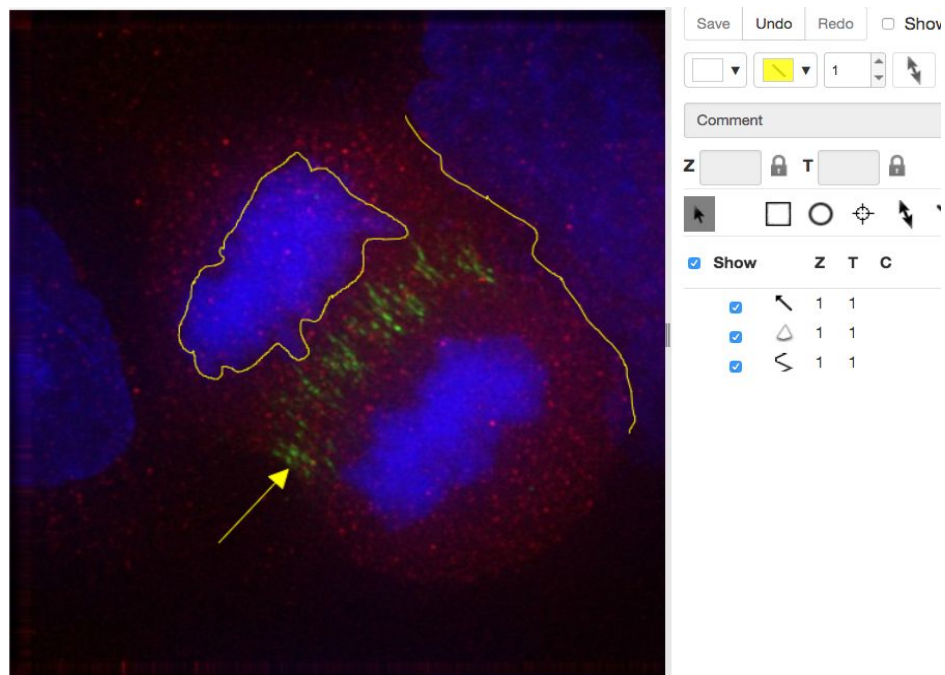


4. The ROIs tab ROIs [0] includes tools for viewing and drawing ROIs on the Image. These are saved back to the OMERO server. Select the *arrow tool* in the right-hand

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

pointing to a feature in the Image and save it using the *Save button* Save located in the upper part of the right-hand pane. Draw also a couple of other ROIs on different Z planes.

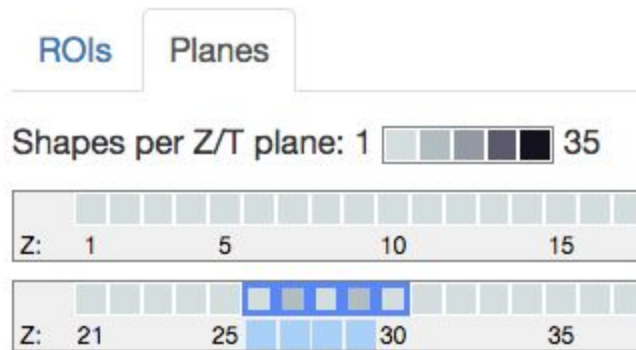
- Dragging on the Image is used for panning the image.
- Shift-drag* rotates the image (using selection tool) or draws “freehand” (polygon, polyline, rectangle and ellipse).



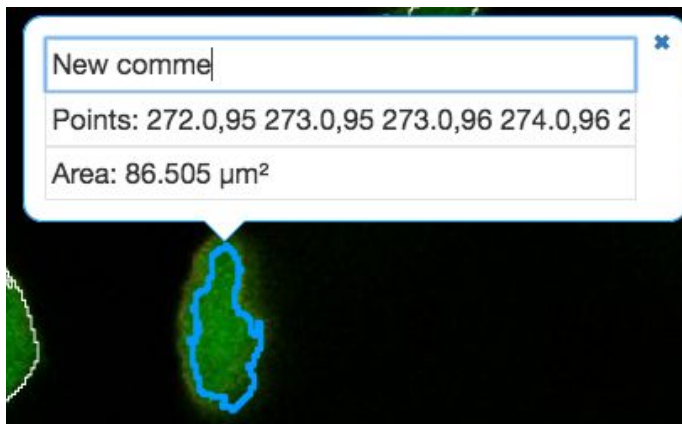
- Clicking onto the ROIs in the right-hand pane table brings the viewport to the position where the selected ROI is in the middle of it and navigates the viewport to the timepoint the ROI is associated with.

<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			

- The *Planes* tab gives you an overview of the ROIs distribution over the z or t sections. The boxes in the schema in the *Planes* tab represent single z or t planes and are selectable.

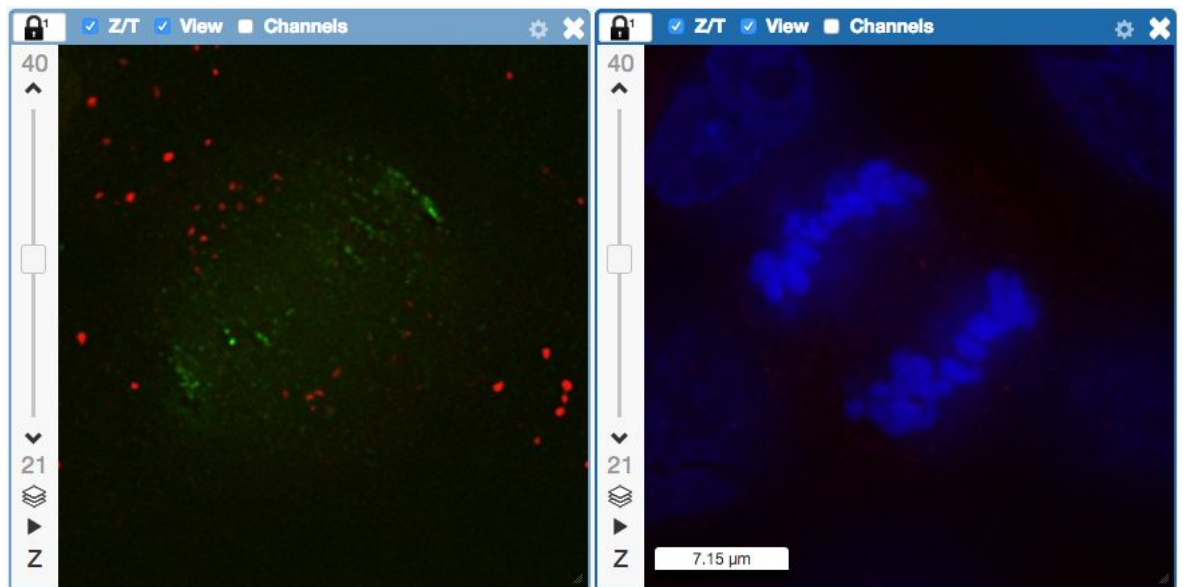


-
- You can select one box, this will navigate to the corresponding plane in the image viewer. Alternatively, select a range of boxes, which will display a projection of the range of the selected planes (in case of multi-z image) in the main viewer.
- Click onto a ROI in the image. A popup will appear. You can write a comment to this ROI directly into the popup




-

11. The popups can be disabled for all ROIs on the image by clicking onto the cross of one popup and closing it, and re-enabled by using a context menu accessed by right-clicking onto the image.
12. You can copy the values in the popup such as area (select and Ctrl + C) and paste them into your local documents.
13. 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.
14. 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.
15. Save the viewport by selecting in the top-left corner *File > Save Viewport as PNG*.
16. 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.



17. 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.



18. Click on *Info* tab in iviewer now, and find, in the right-hand pane, the link to *Dataset*:


Dataset:

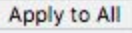
[siRNAi-HeLa](#)

. Click on that link. This will bring you back


to the webclient.

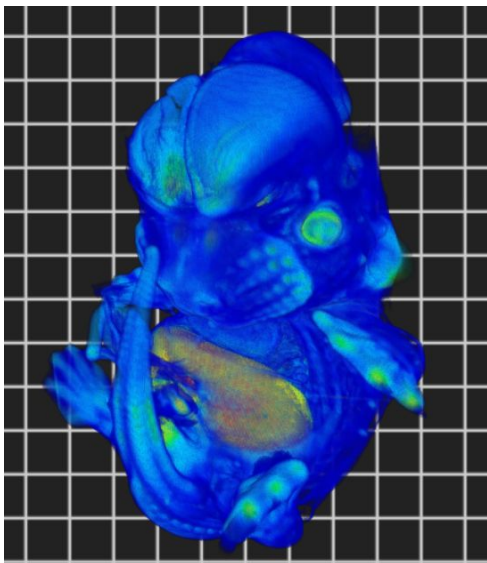
19. Adjust the channel names:

- 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 “**GFP**”, “**Aurora-B**” and “**CY-6**” for the other channels.

- Click the *Apply to All* button  and confirm by clicking *Continue*.

20. Go to data of trainer-1. Open an image from the Dataset **mouse-14-days** in a 3D viewer: OMERO.FPBioimage.

- First select the Image.
- Right-click menu on the image in the left panel, or use the *Open with...* icon on top of the right-hand pane  to open the image with FPBioimage.
- Click *Start* in the new viewer window.

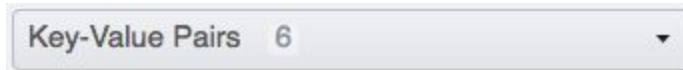


21.

Add Annotations, Filtering and Searching

- Let's now add annotations to Images in the webclient. We will add Tags to indicate Metaphase stages of these cells.
- Select one or more Images of cells which appear to be in metaphase.
- Choose the *Tag* option in the right-hand General tab and click [+] to launch the Tag dialog.

- Choose the existing **Metaphase** from the list of Tags (to filter, type above the list).
- Click > to move it to the right column, then click OK.
- Select an Image from the Dataset and in the right-hand *General* tab, click the harmonica *Key-Value Pairs*.



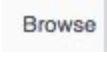
Added by: user-40 user-40 + [document icon] [trash icon] [close icon]

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

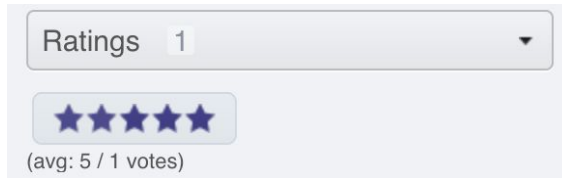
- The *Key-Value Pairs* allow you to add lab-book-like additional metadata for the Image. These *Key-Value Pairs* are also specifically searchable.
- It is also possible to add Comment, Attachment and Rating to selected Images.
- Enter **mitomycin-A** into the search box in the top right corner of the 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 at least 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. 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.
- Go back to the Search results tab. 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.


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.

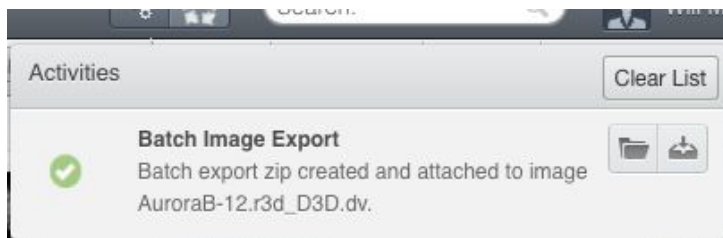
Image Export


Image export can be achieved using any of the three main clients for OMERO (OMERO.insight, OMERO.web or command line interface). Further, we have developed an “omero-downloader”, a command-line application dedicated specifically to the downloading and exporting of images and annotations from OMERO.

1. In OMERO.web, select one or more images you want to download. Then, find in the top of the right-hand pane the download icon  , click on it and select the desired action, for example *Download*, which downloads the data in original format.
2. Open the downloaded file(s) in the software of your choice.
3. To export images in a different format such as PNG, TIFF or OME-TIFF, we can use a script on the server to build a zip for download. Select images, click on the script icon



- and click on “*export scripts*” and then choose “*Batch Image Export...*”. You can choose to export individual channels and/or the “merged” image (current rendering settings). It is also possible to export multiple Z and T sections. The image Format can also be specified. If OME-TIFF is chosen, the Channels and Z/T sections are ignored and the entire image is exported as an OME-TIFF image.
4. Click *Run Script* to generate the zip on the server. When the script completes, the Activities panel will display the result. Click the *Download* button to download the zip.



5. Open one image in iviewer and draw several ROIs. Back in webclient, add several Tags onto the same image. Using the download icon in the right-hand pane,  export the image as OME-TIFF.

6. Reimport the OME-TIFF again into OMERO and verify the imported image still has the ROIs and Tags, same as the original image prior to export. Note: This workflow can be used to transfer the image with its metadata into another OMERO.server if available.

Image export using omero-downloader (demo only)

Setup:

- omero-downloader was installed by downloading and unzipping of the release artifact https://github.com/ome/omero-downloader/releases/download/v0.2.0/OMERO_downloader-0.2.0-release.zip
 - omero-downloader works on Mac, Windows and Linux
1. Open a CLI terminal on your machine and go into the directory where omero-downloader is installed.
 2. To download a single image, run the following command. It assumes that the target directory already exists e.g. `/tmp/repo`
 - a. On Mac or Linux
 - i. Note: Copy the following command from <https://raw.githubusercontent.com/ome/training-scripts/v0.6.0/practical/bash/downloader-commands>
 - ii. `$./download.sh -b /tmp/repo -s <server host> -u <user name> -w <password> -f binary Image:<image ID>`
 - b. On Windows
 - i. Note: Copy the following command from <https://raw.githubusercontent.com/ome/training-scripts/v0.6.0/practical/bash/downloader-commands>
 - ii. `$ download.bat -b /tmp/repo -s <server host> -u <user name> -w <password> -f binary Image:<image ID>`
 3. To download all images in a Dataset, run
 - a. Note: Copy the following command from <https://raw.githubusercontent.com/ome/training-scripts/v0.6.0/practical/bash/downloader-commands> into your Terminal.
 - b. `$./download.sh -b /tmp/repo -s <server host> -u <user name> -w <password> -f binary Dataset:<Dataset ID>`
 - c. Note that you will be able to deduce what tags and annotations the downloaded images were annotated with.
 - d. Go to OMERO.web and Tag several images from two datasets with a tag "your-name". Then, go back to the command line and run
 - i. Note: Copy the following command from <https://raw.githubusercontent.com/ome/training-scripts/v0.6.0/practical/bash/downloader-commands> into your Terminal.

- ii. `$./download.sh -b /tmp/repo -s outreach.openmicroscopy.org -u <user name> -w <password> -f ome-xml Dataset:$ID2,$ID1`
- iii. This will download the xml metadata for images in those two datasets (Note: we could download all the images as well, but we can do it at any later point, as downloader “remembers” what is downloaded already and does not re-download it twice.)
- e. Note: Copy the `grep` command from <https://raw.githubusercontent.com/ome/training-scripts/v0.6.0/practical/bash/downloader-commands> into your Terminal.
- f. This will find the IDs of all the images from the two datasets tagged with “your-name” tag.


OMERO parade (part 1)

For more information, see also

https://omero-guides.readthedocs.io/en/latest/parade/docs/omero_parade.html

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 filter...* 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...*

OMERO figure

OMERO.figure is a popular application for OMERO.web which allows creation of publication figures from images stored in OMERO in a very efficient and speedy manner.

See https://omero-guides.readthedocs.io/en/latest/figure/docs/omero_figure.html for the walkthrough.

For advanced scripting using OMERO.figure, see

https://omero-guides.readthedocs.io/en/latest/figure/docs/omero_figure_scripting.html

Image data resource

1. Open a new tab in your browser and go to <https://idr.openmicroscopy.org>.
2. Next to the “Search by”, click on the dropdown menu and select “Gene”
3. In the next dialog, start typing “CDK...” and then select from the suggestions a CDK... gene
4. In the results, click onto the right-most column on “more...” - this will navigate you to the OMERO.web where you can view the images you found

Day 2

Analysis with 3rd party tools

Analysis with Fiji

For setup of the Fiji plugin see

<https://omero-guides.readthedocs.io/en/latest/fiji/docs/installation.html>

For the walkthrough in this workshop, see the three Fiji chapters

<https://omero-guides.readthedocs.io/en/latest/fiji/docs/installation.html>

https://omero-guides.readthedocs.io/en/latest/fiji/docs/threshold_manual.html and

https://omero-guides.readthedocs.io/en/latest/fiji/docs/threshold_scripting.html

Orbit (machine learning tool)

See https://omero-guides.readthedocs.io/en/latest/orbit/docs/orbit_manual.html

Analysis in OMERO using Cell Profiler

See <https://omero-guides.readthedocs.io/en/latest/cellprofiler/docs/cellprofiler.html>

Analysis in OMERO using Matlab

See <https://omero-guides.readthedocs.io/en/latest/matlab/docs/matlab.html>

Server side analysis

How to write script

We are going to use a Jupyter Python notebook to develop a simple script for FRAP analysis:

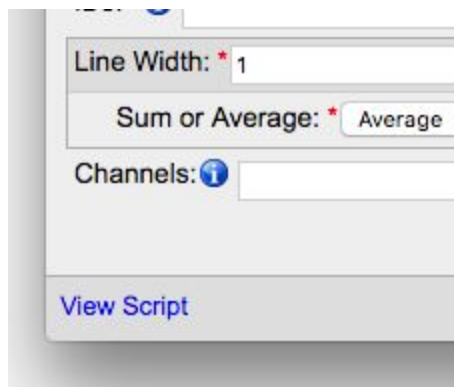
- Go to <https://idr-analysis.openmicroscopy.org/training> .
- In the Files > notebooks > Python folder, select the notebook simpleFRAP.ipynb.
- Follow the steps in the notebook

To create a script which is executable on the OMERO.server and can be interpreted by OMERO.web the code has to be transferred into a regular Python script:

See https://github.com/ome/training-scripts/blob/v0.6.1/practical/python/server/simple_frap.py

Getting started with your own script:

Get a script from <https://github.com/ome/training-scripts> and use it as a template or find a script which already does something similar in OMERO.web and copy its source code. You can always see the code by clicking on “View Script” at the bottom left of the script dialog:



How to manage script (demo only)

1. CD into `OMERO.server/bin` directory
2. Upload the script `./omero script upload --official simple_frap.py`
3. See which scripts are available and to get their ids: `./omero script list`
4. Modify a script and re-upload it with: `./omero script replace 123 simple_frap.py`

Data mining using OMERO.parade

See https://omero-guides.readthedocs.io/en/latest/parade/docs/omero_parade.html