

OMERO File Uploading Instructions for EMBRIO

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There are two ways to access the OMERO imaging database. For working with existing data, such as annotating images, creating ROIs, adding metadata, or organizing data sets, the OMERO web interface is used. For data uploading/downloading, the OMERO.insight application must be used. As of OMERO v5.6, image data cannot be transferred through the web interface.

The OMERO database is available to the public and is not behind a Purdue firewall. This also means that the username and password assigned to you for OMERO is not your Purdue LDAP (career account). It is a separate set of credentials created by the data management specialist.

If you need an OMERO account, contact the EMBRIO data management specialist.

1. Accessing your OMERO account through the web:

<https://omero.geddes.rcac.purdue.edu>

Log in with your credentials. This will verify that your credentials are working.

2. Download the OMERO.insight application for your operating system:

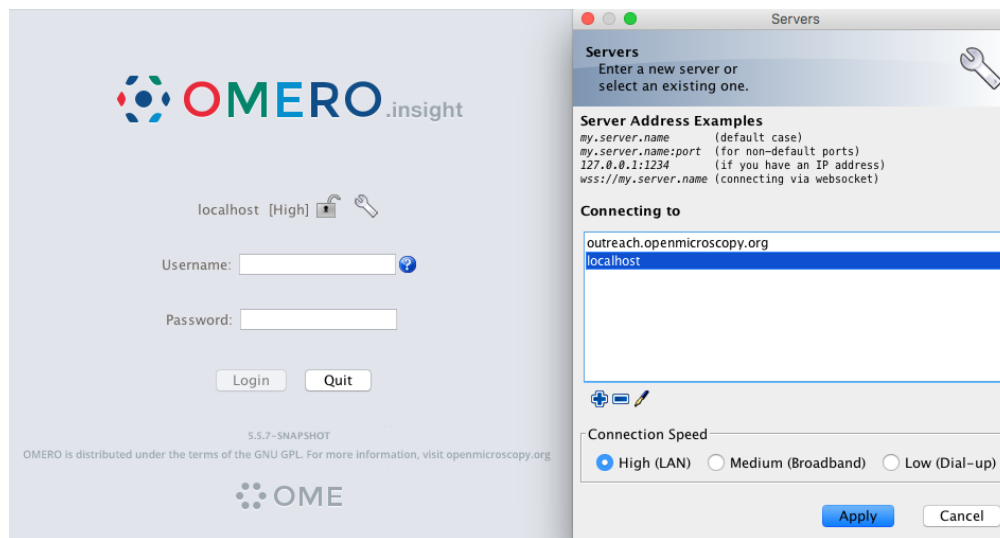
<https://omero-guides.readthedocs.io/en/latest/upload/docs/import-desktop-client.html>

3. Configure OMERO.insight

Launch the OMERO.insight application. The default login address is localhost (not used).

The login prompt for OMERO.insight must be set to the EMBRIO OMERO server. The steps are:

- Click on the wrench icon
- In the popup window, click on the + icon
- In the box, copy the following location:
wss://omeroserver.omero.geddes.rcac.purdue.edu:4066
- Click Apply



Now you will be able to log into the EMBRO OMERO server using the new address.

4. Use your OMERO web login credentials (from Step 1) to log into OMERO.insight

Once logged in, the interface should look familiar – it is very similar to the web interface.

5. To start, create a new project to hold the associated data sets:

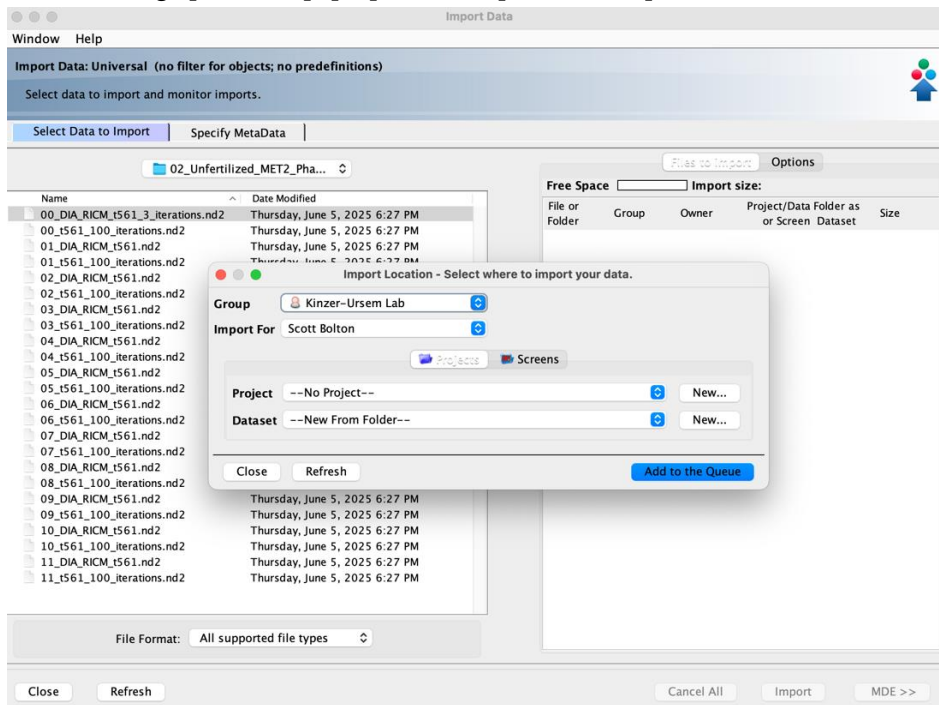
File -> New -> New Project

6. Add your project name and description, then click Create
7. Next, import your data set:

File -> Import

A pop-up window will display two panels, local source filesystem and upload queue. Navigate to the location of the local source data and select the desired data. Between the two panes are transfer arrows: click on the right

arrow to bring up another pop-up box that specifies the upload:



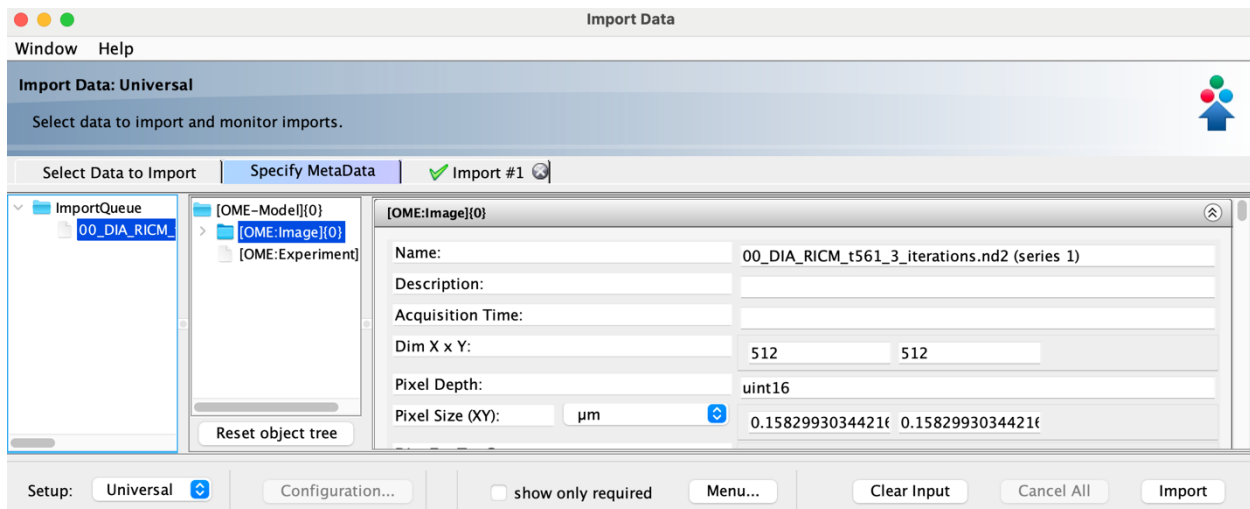
Choose the Group name and Import For person, then select the Project to assign the files. The Dataset field will automatically assume the source folder name when creating the new dataset, but this can be overridden by clicking on New... and adding the desired folder name and description.

Click on “Add to the Queue”.

8. Add additional files and add them to the queue as needed.

9. Review hardware metadata for correctness

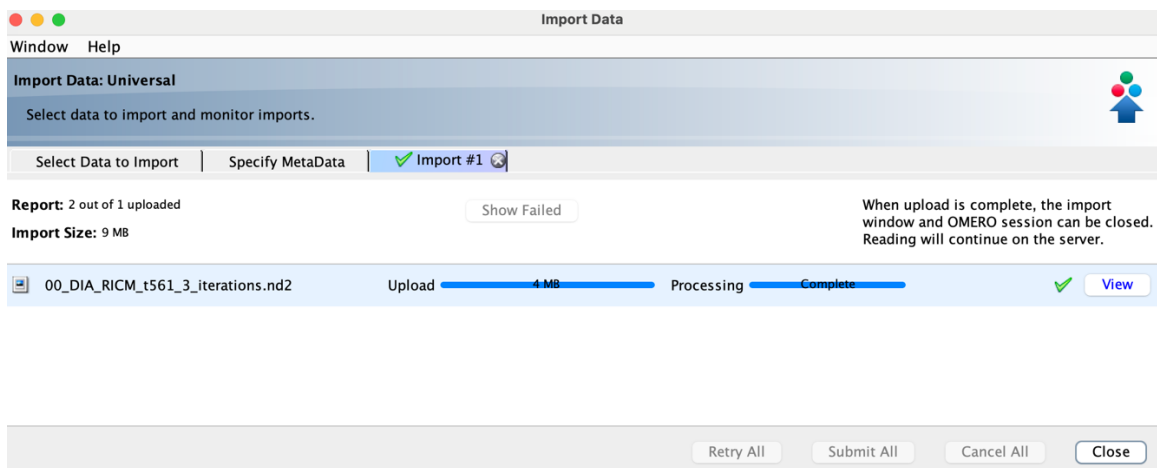
In the Import Data pop-up window, select the second tab labeled “Specify Metadata”, then select each file in the ImportQueue list on the left to evaluate its metadata embedded in the image file. Choose the [OME: Image] object to select the hardware metadata.



Review the information to ensure correctness and overwrite any erroneous values in the boxes.

NOTE: Do not populate the [OME: Experiment] object because higher-level metadata will be added in the OMERO web interface at the dataset and project levels.

10. When finished, click on the “Import” button.



After all files are uploaded, click “Close”.

NOTE: In the left browsing pane of the main window, click the reload icon (green bidirectional arrows) to refresh the filesystem. The data sets can now be browsed in the app as well as the web interface.

Data Organization

File architecture is flexible in OMERO and can be configured in a number of ways, depending on group needs. For EMBRIO, we have started with the following configuration:

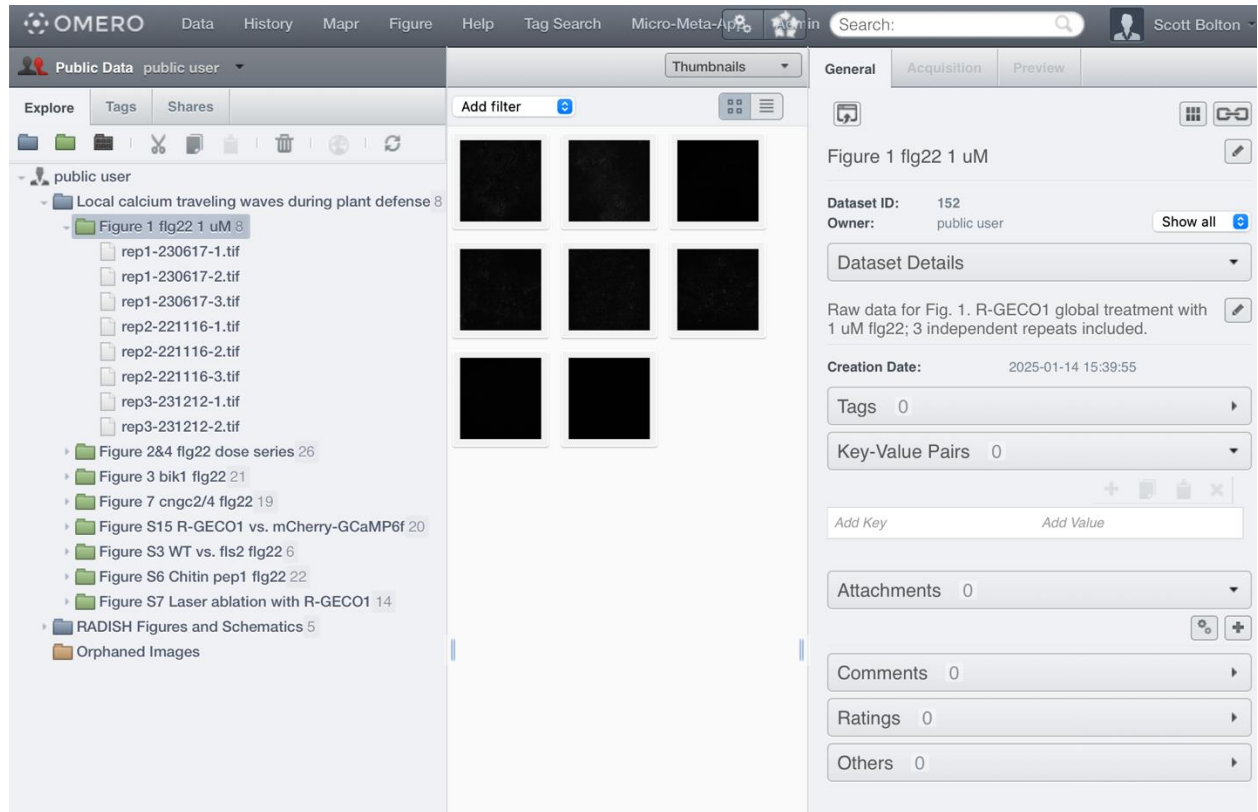
At the top level there are Projects. In the example below, the Project is delimited by a publication. High-level metadata is captured at the Project level in the Project Details section (seen in the right-hand side General tab).

The screenshot displays the OMERO web interface. The top navigation bar includes links for Data, History, Mapr, Figure, Help, Tag Search, Micro-Meta-App, and a user profile for Scott Bolton. The main interface is divided into three panels. The left panel, titled 'Public Data' and 'public user', shows an 'Explore' view with a tree structure of datasets under the project 'Local calcium traveling waves during plant defense 8'. The datasets listed include: Figure 1 flg22 1 uM 8, Figure 2&4 flg22 dose series 26, Figure 3 bik1 flg22 21, Figure 7 cngc2/4 flg22 19, Figure S15 R-GECO1 vs. mCherry-GCaMP6f 20, Figure S3 WT vs. fls2 flg22 6, Figure S6 Chitin pep1 flg22 22, Figure S7 Laser ablation with R-GECO1 14, RADISH Figures and Schematics 5, and Orphaned Images. The right panel, titled 'General', shows the project details for 'Local calcium traveling waves during plant defense'. It includes the Project ID (101), Owner (public user), and a 'Show all' button. Below this, the 'Project Details' section provides a publication related to the dataset: Zhang, W., Kumar, N., Helwig, J.R., Hoerter, A., Iyer-Pascuzzi, A.S., Umulis, D.M., Pienaar, E. and Staiger, C.J., 2025. Local traveling waves of cytosolic calcium elicited by defense signals or wounding are propagated by distinct mechanisms in Arabidopsis. Sci. Signal. doi: 10.1126/scisignal.adw2270. The 'Experiment Description' section details the experimental setup: Epidermal pavement cells of seven-day-old Arabidopsis cotyledons expressing the cytosolic calcium reporter R-GECO1 were imaged with spinning disk confocal microscopy. Cotyledon epidermal cells were imaged at a single z focal plane with 5-s acquisition intervals for a total of 40 min. Samples were first imaged for 10 min without any treatment as a run-in period for any laser light-induced Ca2+ activities. MAMPs or elicitors were added after 120th frame. For more details, see the above publication. The 'Creation Date' is 2024-10-22 14:11:26. The 'Tags' section shows 0 tags. The 'Key-Value Pairs' section shows 0 key-value pairs. The 'Attachments' section shows 1 attachment: r-geco1_dynamics_with_flg22_treatment.json (3.49 KB). The 'Comments' section shows 0 comments. The 'Ratings' section shows 0 ratings. The 'Others' section shows 0 others.

On the left-hand side Explore panel, several Datasets can be seen for this project. In a finished project where data is publicly available, the Datasets may be named in support of the figures they comprise. For exploratory research, Datasets may be named after experiments, of which many may be attempted before final protocol and publication-worthy data is realized.

It is important to note that the “folder” structure is not a fixed structure. Images may be reorganized by tags or manually picking them and placing into “smart folders” much like the same songs can be organized into

multiple “smart playlists”. Rich tagging of images and Datasets enables flexible grouping of data.



At the Dataset level:

Tagging, and Key-Value Pairs

All data should have the “EMBRIO” tag.

Copy what Weiwei has done

There is a known issue with importing IMARIS IMS files directly into OMERO:

<https://forum.image.sc/t/problem-with-uploading-big-imaris-files-on-omero/50062>

The main problem is that the IMS file format (HDF-5) embeds multiple copies of each image in a pyramid of resolutions, and OMERO will import each resolution version as a separate image, leading to redundant images with different resolutions. It will also take substantially longer to import the file due to the extra images parsed and imported.

The workaround for the moment is to convert IMS files using IMARIS to another standard image format (such as TIF). One important detail to note is that the microscope metadata present in the IMARIS data should be configured to be exported in the TIF file.