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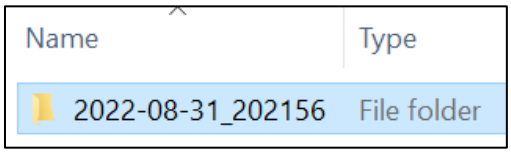
1. Overview of data structures

1.1 Raw data folder

Each time an experiment is initiated using the “Run” command, a new folder is created. The name of the folder is determined by the timestamp of the experiment. Here the data were collected at 8:21:56PM on 8/31/2022.



Within the experiment folder you may find the following folders and files:

1. raw – Image data and metadata written by the cameras during acquisition are stored here.

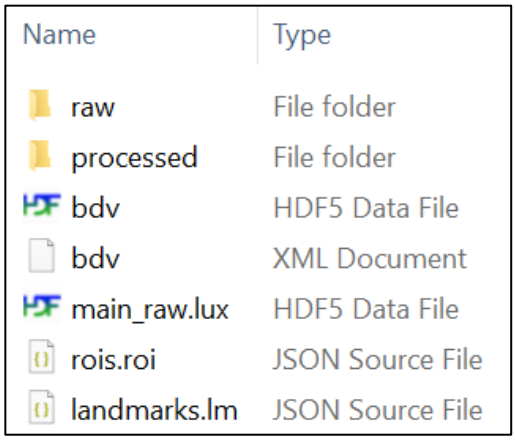
2. processed – Image data created with the Luxendo Image Processor are stored here. Metadata associated with the processing task are also stored here.

3. bdv.h5 – A .h5 file header for opening image data in ImageJ.

4. bdv.xml – A .xml file for opening image data using BigDataViewer

5. main_raw.lux.h5 – A .lux.h5 file header used by the Luxendo image processor.

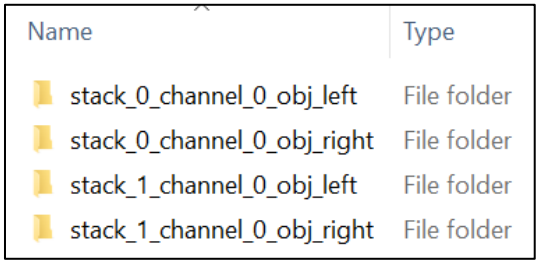
6. rois.roi.json – A .json text file created in the Luxendo Image Processor containing metadata specifying regions of interest.



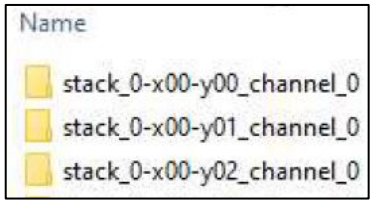
7. landmarks.lm.json – A .json text file created in the Luxendo Image Processor containing metadata specifying landmarks.

Image data and associated metadata are written into separate folders for each stack, channel, and detection objective.

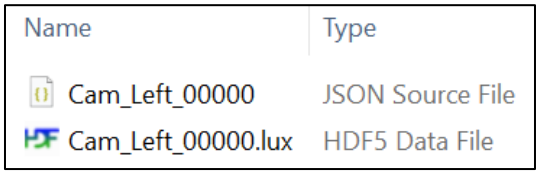
Here, data was acquired from two angles (stack_0 and stack_1), with a single channel (channel_0), and with two opposing detection objectives (obj_left and obj_right).



For tiled acquisitions, the XY positions of each tile are labeled with an index position.

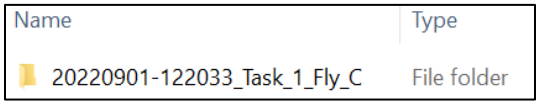


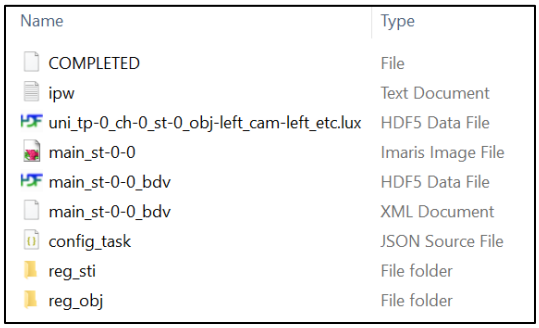
Within each folder above we can find two types of files. The .json file contains metadata of the imaging parameters and the .lux.h5 file contains image data. Each repetition or time point will be written into the same folder with a 5-digit index appended.



1.2 Processed Folder

Each task queued in the Image Processor generates a new folder containing a time stamp and description of the task specified during image processing. A suffix is appended if the task has been completed (_C), stopped (_S), or failed (_F).





Within the processed data folder, you may find the following folders and files:

1. COMPLETED – indicates the task has finished successfully.

2. ipw.txt – This is a log file generated by the Image Processor.

3. uni_tp-0_ch-0_st-0_obj-left_cam-left_etc.lux.h5 – This .h5 file contains the processed image data. The prefix indicates fusion settings (uni = union), tp refers to the time point, ch refers to the channel, st refers to the stack, obj refers to the objective, and cam refers to the camera. You may have several lux.h5 files if there are multiple time points or depending on the fusion parameters selected.

4. main_st-0-0.ims – An Imaris file header for opening the experiment using Bitplane Imaris.

5. main_st-0-0_bdv.h5 – A .h5 header file for opening image data in ImageJ.

6. main_st-0-0_bdv.xml – A .xml file for opening image data using BigDataViewer

7. config_task.json – A .json text file containing metadata associated with image processing settings.

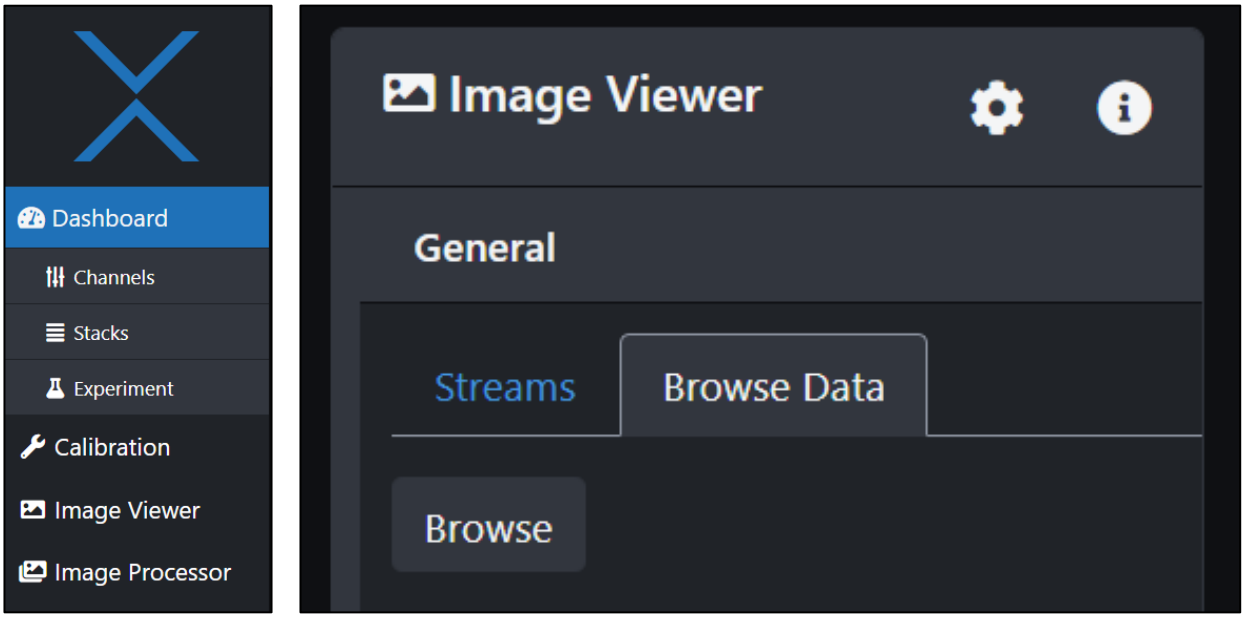
8. reg_sti – A folder containing transformations for tiles registration.

9. reg_obj – A folder containing transformations for objective registration.

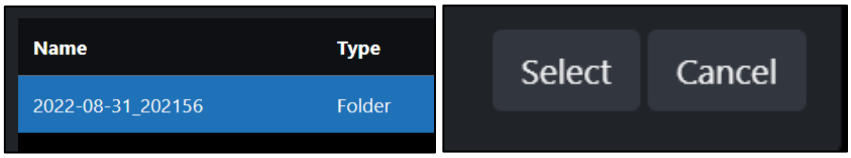
2. Inspecting Data

2.1 Opening Raw Data with Luxendo Image Viewer

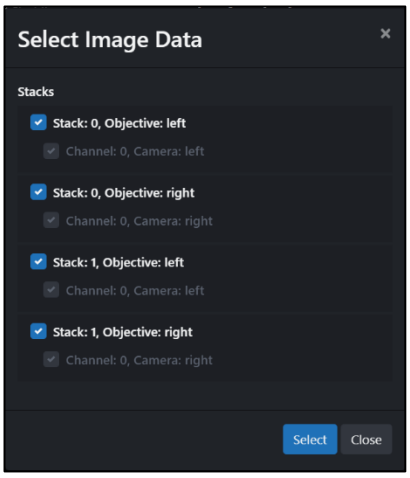
1. Open the Luxendo software package and select the *Image Viewer* from the control panel in the top left corner. In the Image Viewer window, select the *Browse Data* tab, then select *Browse*.



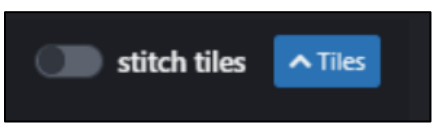
2. Navigate to the folder where the data is saved. Highlight the folder containing the experiment and then press *Select*.



3. We can select a subset of the data to display in the Select Image Data window. If the acquisition contains many tiles and/or views, we can reduce the number of displayed tiles to improve performance.



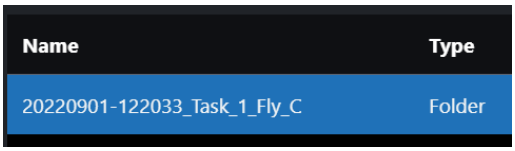
If the experiment contains multi-tiled data, there is a toggle switch to stitch the data before display.



2.2 Opening processed data with Luxendo Image Viewer

Opening processed data with the Image Viewer is similar to the process described above for raw data. One key difference is that the folder containing the individual processing task is selected, rather than the folder containing the entire experiment.

1. Open the Luxendo software package and select the *Image Viewer* from the control panel in the top left corner. In the Image Viewer window, select the *Browse Data* tab, then select *Browse*.



2. Navigate to the folder containing the processing task of interest. Highlight the folder containing the processing task and press *Select.*

2.3 Opening a single file with ImageJ/Fiji - HDF5 Plugin

In order to work with .h5 file formats, we recommend installing Fiji which contains plugins necessary to open our data. These plugins include HDF5, BigDataViewer, and BigDataProcessor. A fresh download of Fiji should include all of these plugins pre-installed.

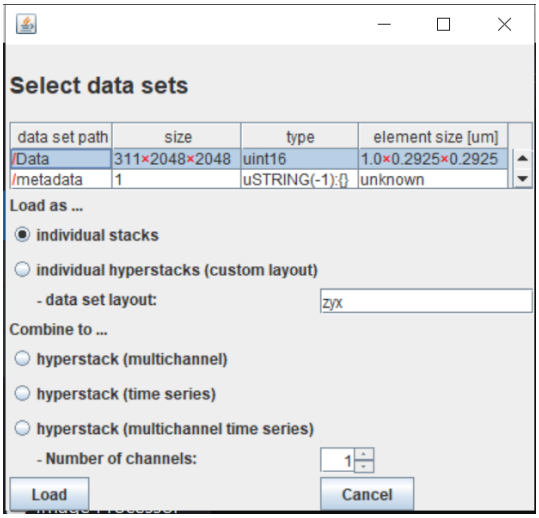
The native HDF5 plugin is a simple way to open a single .h5 file.

1. Within the ImageJ taskbar, navigate to File > Import > HDF5.

2. Select the lux.h5 file of interest and select *Open.*

3. In the Select data sets window, highlight the row containing /Data. Here the ZYX dimensions are shown in pixels and the pixel:micron conversion is shown under element size.

4. Select *Load.*

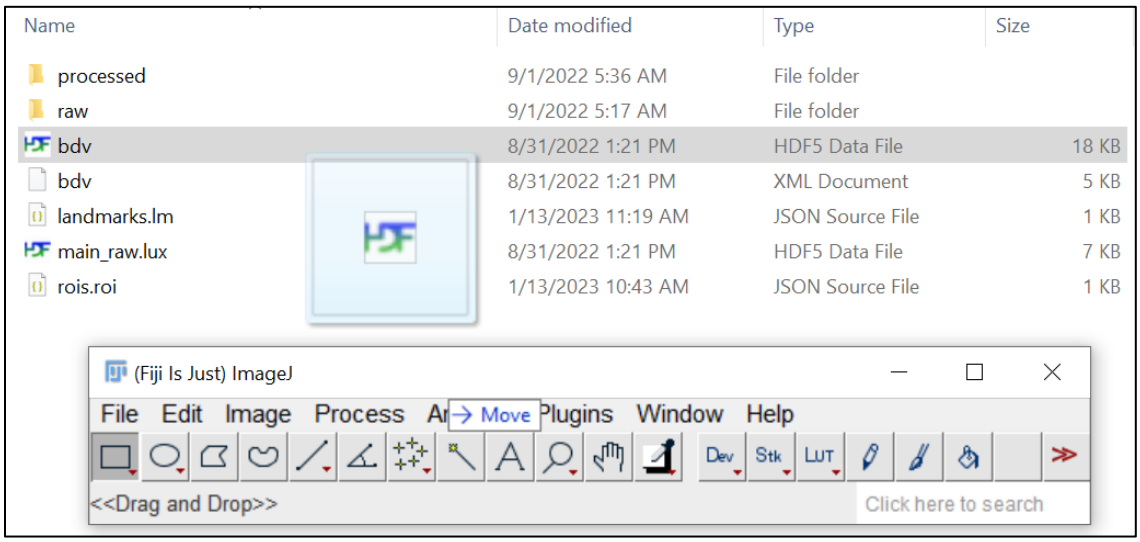


Note: This process with return a “length is too large” exception if the data exceeds ~4GB on disk.

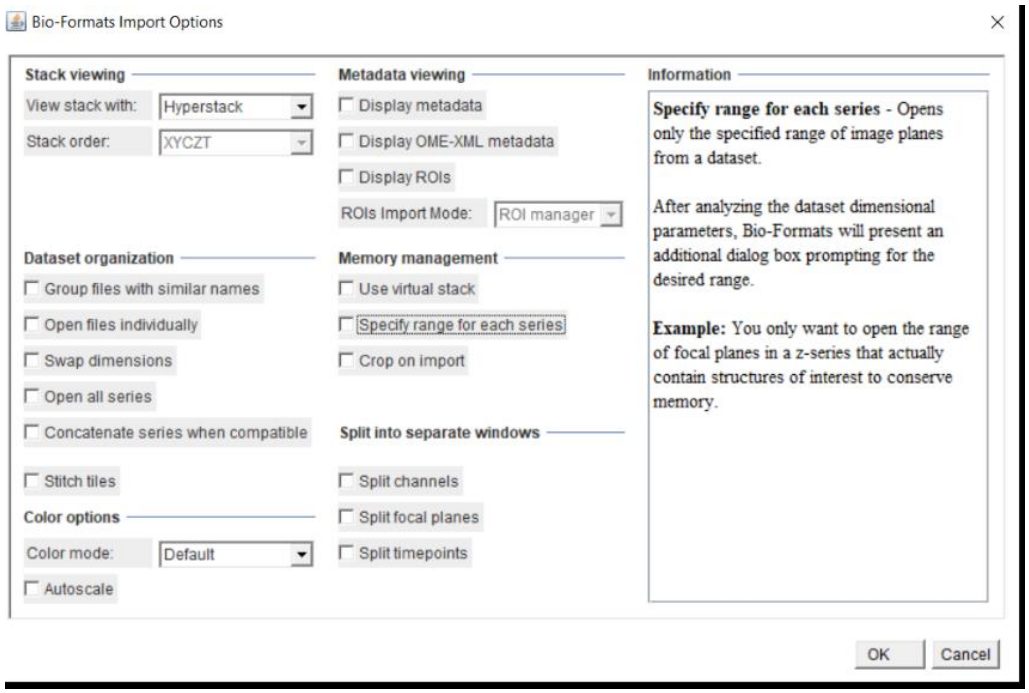
2.5 Opening raw data with BioFormats plugin

Opening raw data from a single large file, a time series, or a multi-tile acquisition can be done using Bioformats plugin and the bdv.h5 file header written into the ‘raw’ data folder. **We do not recommend BioFormats for large tiled or time-lapse acquisitions**, as reading the header file is very slow and can require a large amount of RAM. The advantage of BioFormats over BDV is that the entire dataset is loaded into memory and thus gives fast access to the data once loaded.

1. Drag and drop the bdv.h5 file from the ‘raw’ data folder into Fiji.

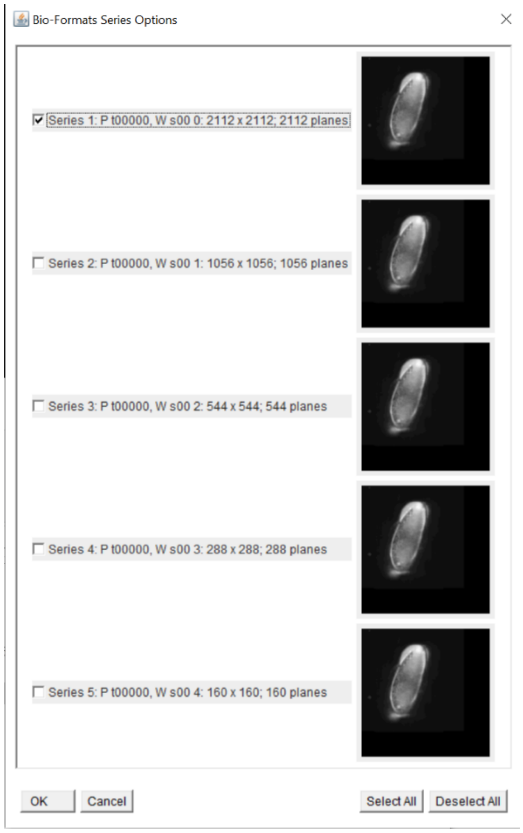


2. In the Bio-Formats Import Options, you may leave the default settings and select *OK.* You may want to use a virtual stack to reduce memory use (see section



3. Each .lux.h5 file can be selected or deselected in the Bio-Formats Series Options window. Select the data and press OK.

2.7 Opening processed data with BioFormats plugin



1. Drag and drop the main_st-0-0_bdv.h5 file from the 'processed' data folder into Fiji.

2. In the Bio-Formats Import Options, select *OK.*

3. Select the data series of interest and select *OK.*

By default, the Image Processor creates 5 data sets in a resolution pyramid, each down sampled by a factor of 2 in each dimension. These lower resolution data sets are useful for inspecting the data quickly or on less capable hardware (i.e., personal laptops).

For reference, the file sizes for the following data are:

Series 1 (full resolution): 17.5GB

Series 2: 2.2GB

Series 3: 307MB

Series 4: 46MB

Series 5: 7.8MB

2.6 Opening raw data with BigDataViewer plugin

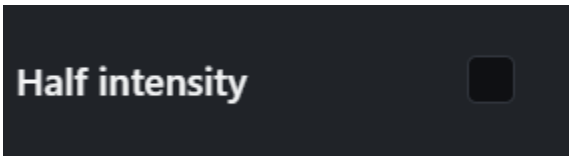
BigDataViewer and BigDataProcessor are useful plugins for quickly loading large tiled or time-lapse acquisitions.

The bdv.xml file header can be used to inspect raw data with the BigDataViewer plugin.

1. In Fiji, navigate to Plugins > BigDataViewer > Open XML/HDF5.

2. Select the bdv.xml file and press *Open.*

Note: You may need to select the "Half Intensity" option within the image viewer to open data in BDV. This is due to BDV using signed 32-bit integers, while we output unsigned 32-bit data.

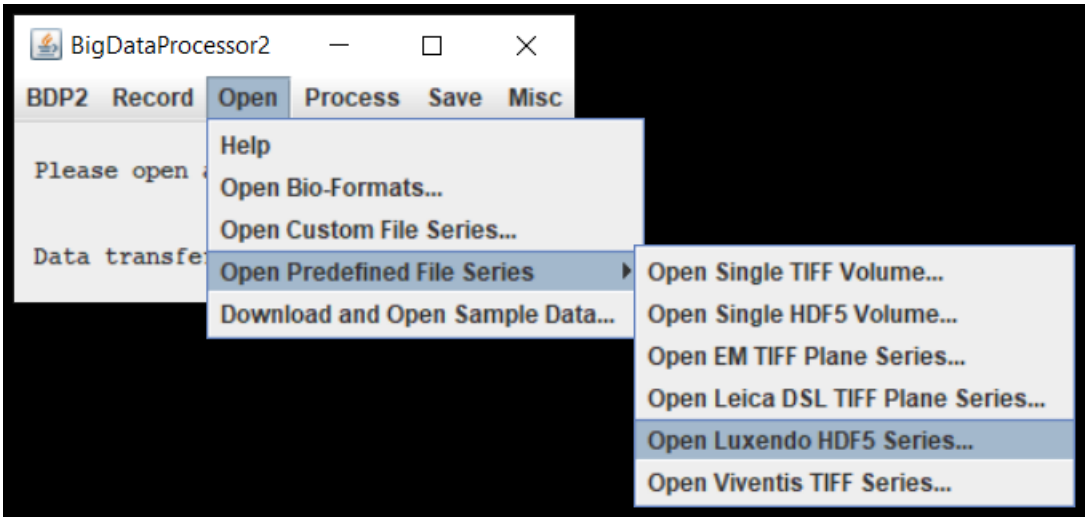


2.7 Opening raw data with BigDataProcessor2 plugin

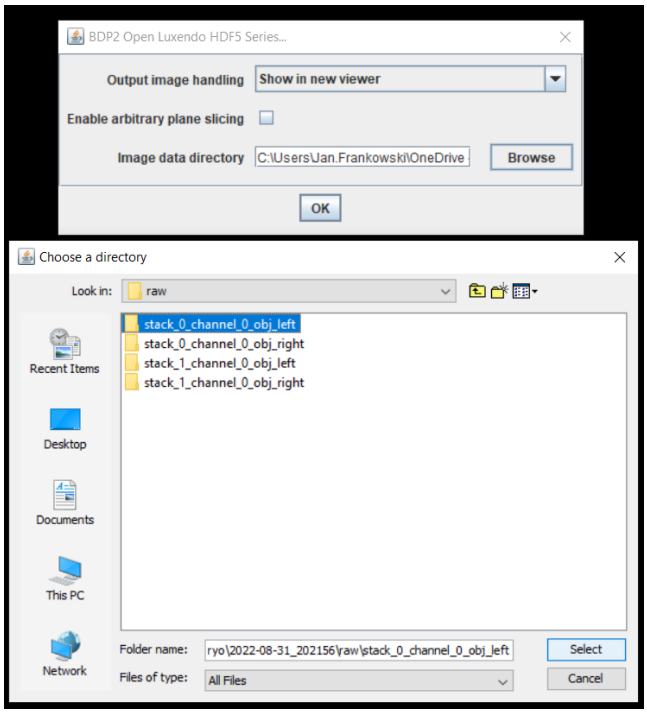
The BigDataProcessor2 plugin is an easy to use plugin to open raw (but not processed) data. It can be useful for opening a single stack of raw data or a subset of a time series experiment. Note: this does not work if you have added descriptions to the channel or stacks.

1. In Fiji, navigate to Plugins > BigDataProcessor > BigDataProcessor2.

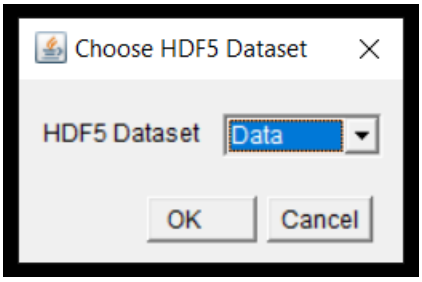
2. In the BigDataProcessor2 window, navigate to Open > Open Predefined File Series > Open Luxendo HDF5 Series



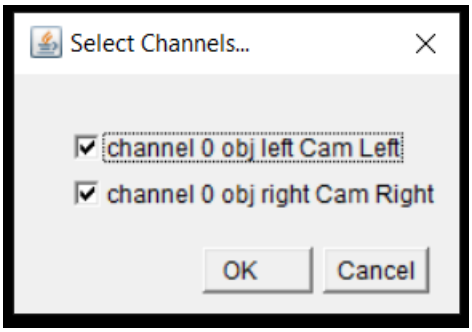
3. Press Browse, navigate to the folder containing data, and press Select. Then press Ok in the BDP2 Open Luxendo HDF5 Files Series window.



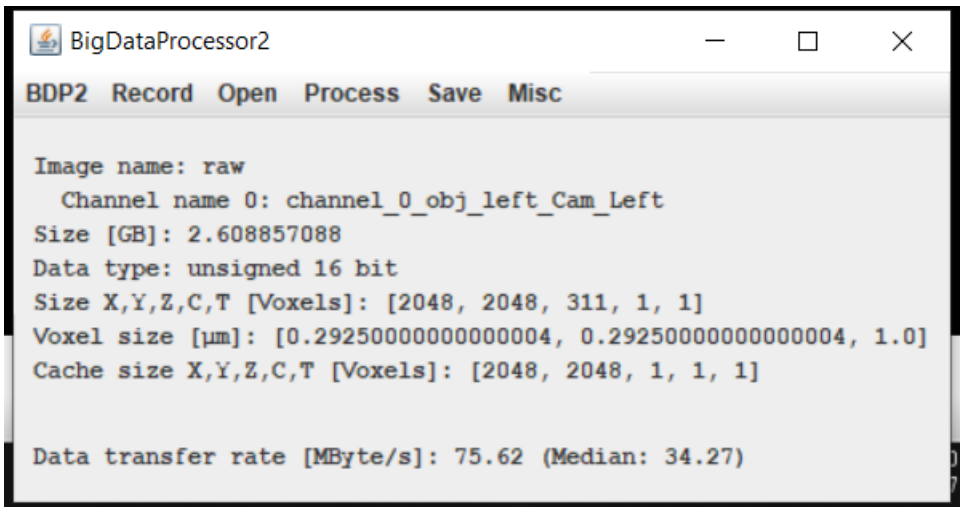
4. In the Choose HDF5 Dataset, Data is the default selection. Press Ok.



5. If the folder contains multiple data sets, you can select which ones to open using the check boxes.



6. The selected data will be opened in a new window. Go to Settings > Brightness & Color to adjust the display. Metadata is shown in the auxiliary window.



2.8 Opening raw data with BigDataProcessor1 plugin

1. In Fiji, navigate to Plugins > BigDataProcessor > BigDataProcessor1.

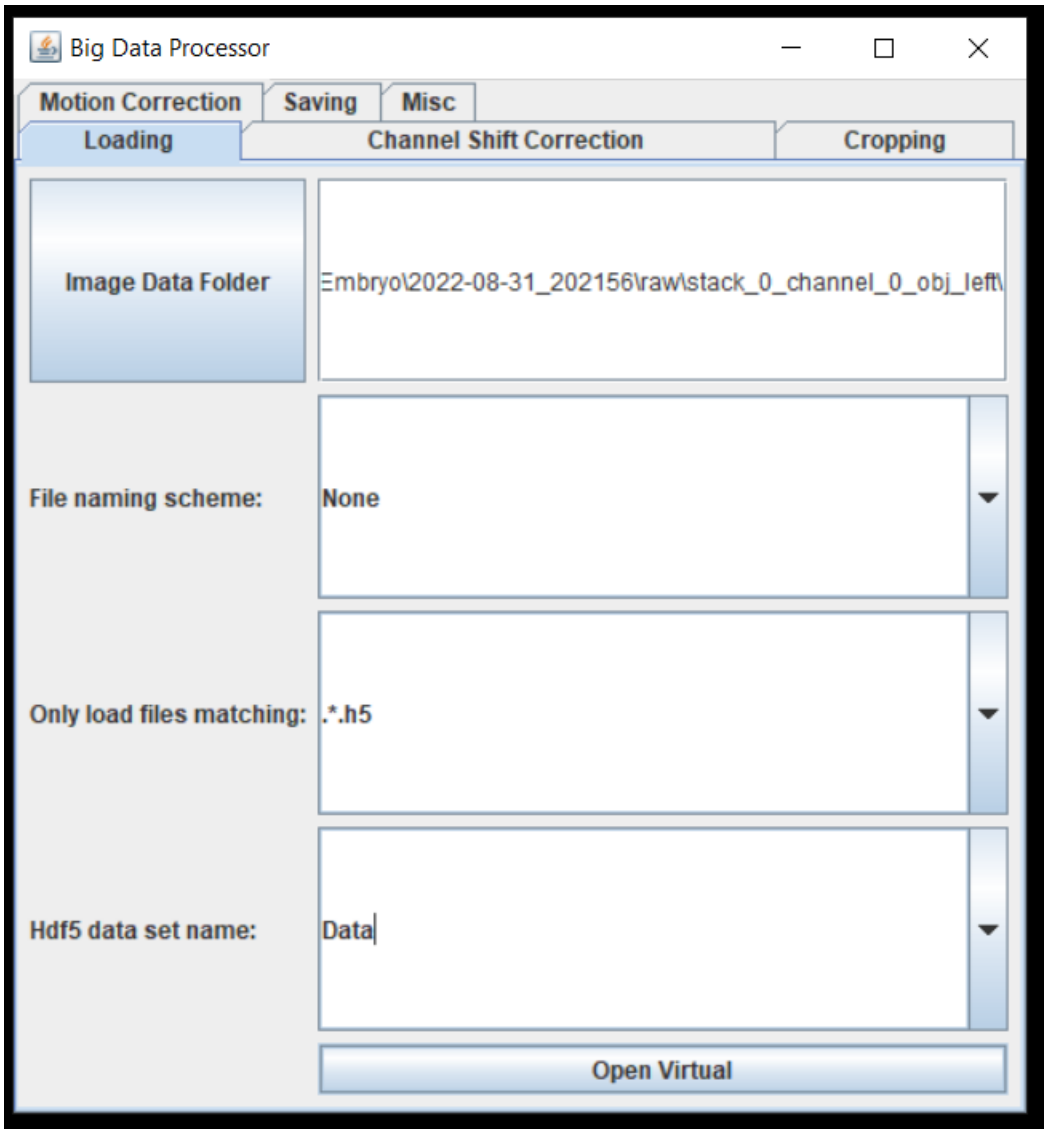
2. Press the Image Data Folder button and navigate to the folder containing data.

3. In the “Only load files matching:” box, write a regular expression to select for the data series of interest. Examples are provided in the table below.

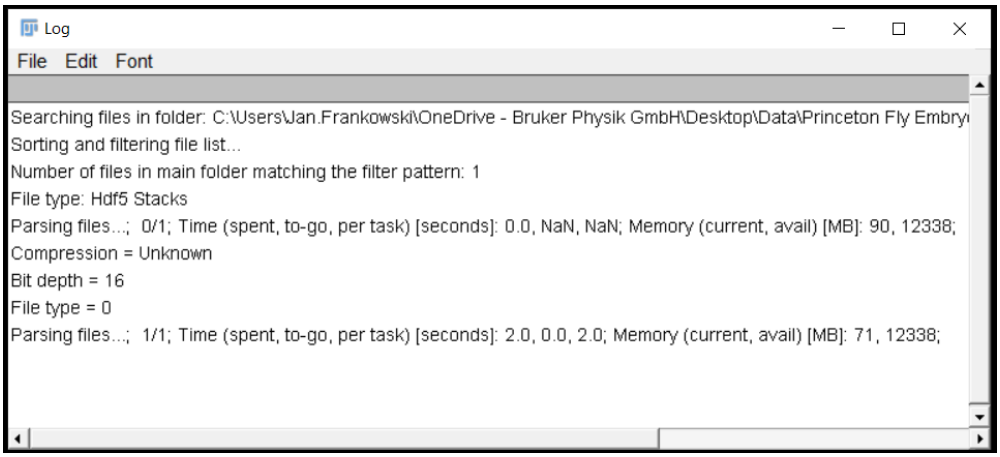
Regular Expression	Selection
.*.h5	All .h5 files
Cam_Left_0.*.h5	Only left camera files
Cam_Short_0.*.h5	Only short camera files

4. In the “Hdf5 data set name:” box, type Data to select for the full resolution data.

5. Press Open Virtual



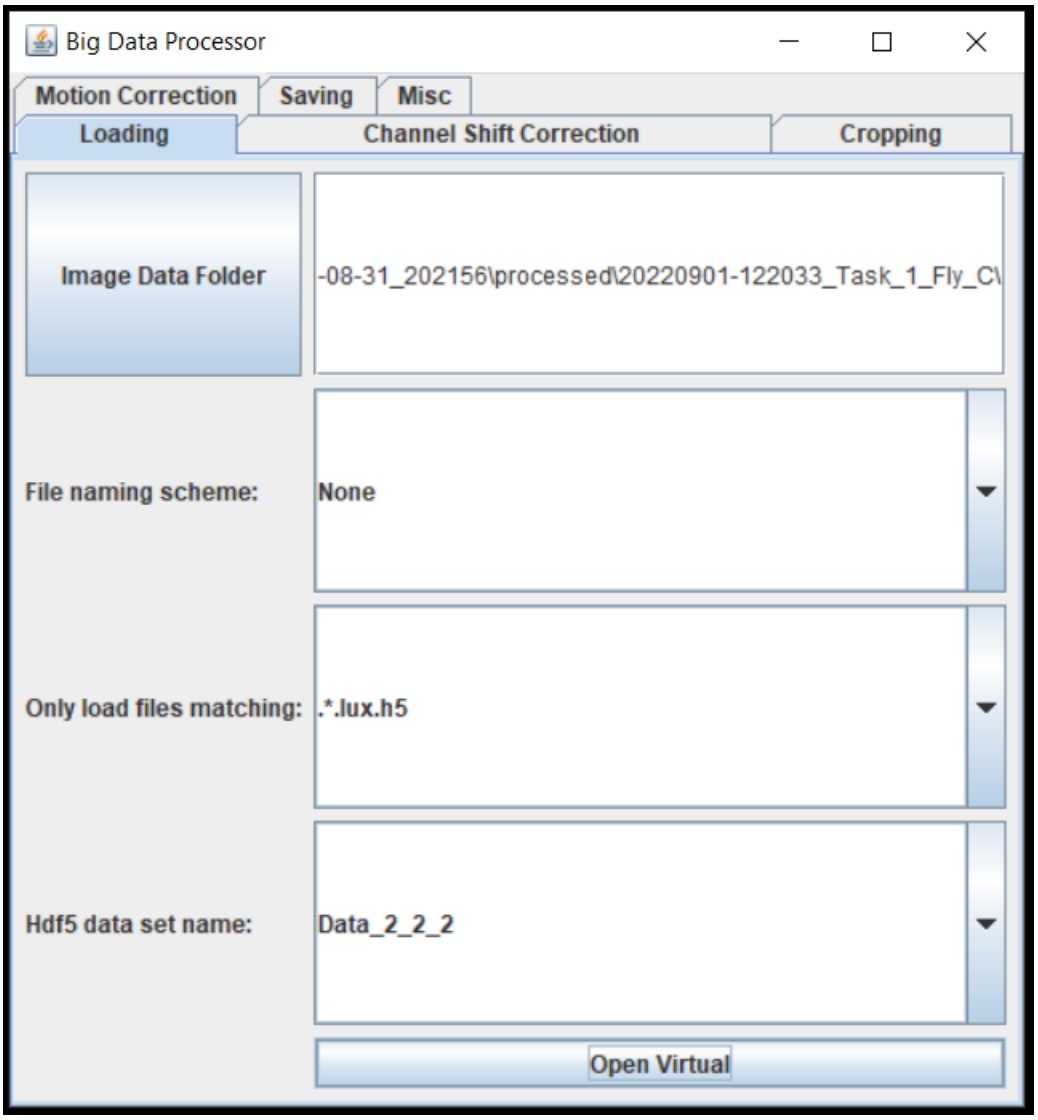
6. Information regarding the number of files matching the regular expression, memory use, and time to load the data are shown in the Log window.



2.9 Opening processed data with BigDataProcessor1 plugin

Opening processed data with BigDataProcessor1 is similar to the process described above for raw data. There are a few differences to note when dealing with processed data, described below.

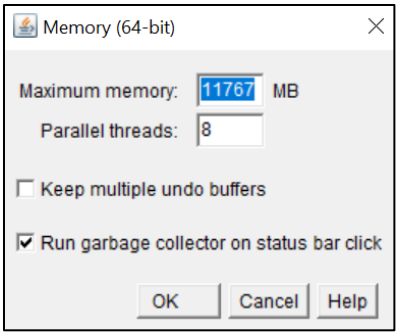
1. Using an expression .*.h5 will find one of the small .h5 header files written into the processed folder and try to open this as data. You must exclude this file using any prefix, or a suffix containing .*.lux.h5 to exclude this header file.



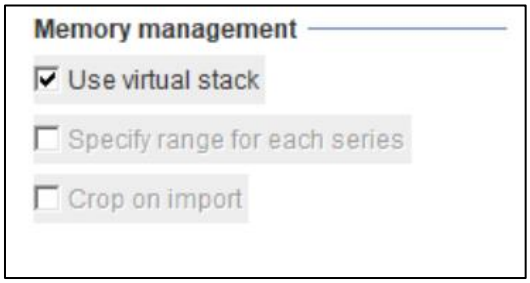
2. In the “Hdf5 data set name:” you may select a downsampled data set using one of the following inputs:

- Data_16_16_16
- Data_2_2_2
- Data_4_4_4
- Data_8_8_8

3.0 Considerations for memory management in ImageJ



Increase memory allocation – In ImageJ, navigate to Edit > Options > Memory & Threads. Here users can increase the amount of memory available to ImageJ.



Virtual stacks – ImageJ will load entire .h5 files into memory. Oftentimes this is not feasible as the image data may greatly exceed the amount of RAM available on the user's PC. Virtual stacks load data on-demand. This reduces the time to open a data set and overall memory allocation. However, while inspecting data the user will have to wait for individual planes to load.

Using the BioFormats plugin, select the "Use virtual stack" option under "Memory management" in the Bio-Formats Import Options. This window is available after opening a .lux.h5 file using BioFormats.