Manual Luxendo TruLive3D Imager

Knowledge of this manual is mandatory for the operation of the instrument. It is thus crucial to make yourself familiar with the contents of this manual. Please pay special attention to the hints concerning the safe operation of the instrument.

The specifications are subject to change; the hard copy of the manual, however, does not follow any update services.

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Introduction

The TruLive3D Imager including its original accessories and compatible accessories from other manufacturers, may only be used for the intended use, i.e. microscopy methods.

The TruLive3D Imager was original designed with the ideal geometry to image small, delicate biological samples, e.g. single cells, spheroids and organoids with high magnification and resolution. Luxendo TruLive3D Imager Light-Sheet technology is the most gentle way to observe live samples with high temporal resolution and / or longer time periods lasting several days. Luxendo Light-Sheet Microscopy systems enable high-resolution imaging including simultaneous dual channel acquisition of delicate samples with precise environmental control.

The inverted light-sheet geometry allows the TruLive3D Imager to image long-term 3D incubated samples at high speeds, while minimizing the effects of photobleaching and phototoxicity.

The TruLive3D Imager contains a set of internal X, Y, and Z stages that hold living samples in a sample carrier with proprietary design to ensure high mechanical stability, allowing for maximized acquisition speed and high precision of data acquisition.

TruLive3D Imager features two opposing light-sheets that are created by rapidly sweeping a Gaussian beam through the focal plane of the detection lens. The light-sheet forming optics allow the user to freely adjust the beam's length and diameter for an optimized illumination of individual samples. The laser light used to create the beam is supplied by the TruLive3D Imager's expandable, alignment-free light source and allows for a range of up to six lasers (405, 445, 488, 515, 532, 561, 594, 642, and 685).

Detection optics include one 25x NA 1.1 water immersion objective lens. In order to acquire two spectral channels simultaneously, fluorescence signal can be split up using a motorized dichroic switch (three positions) and is subsequently recorded using two separate detection paths, each featuring a ten position filter wheel and camera. Filter selection includes both long pass-, to maximize photon efficiently, and band pass-filters to minimize bleed through during multi-channel experiments. The detection paths also include a two-position magnification changer that can adjust the overall magnification of the 25x NA 1.1 objective from 31.3x to 62.5x with a click of a button. State of the art Hamamatsu sCMOS Orca Flash 4.0 V3 cameras provide high detection efficiency, quantification, and speed.

Light-sheet fluorescence microscopes (LSFM) are designed for fast and gentle acquisition of multi-dimensional, high-resolution images of fixed and live. The temperature-controlled sample chamber is filled with water based medium to the objective lenses used and the sample carrier is filled with sample medium. The sample is inserted into the small volume of the sample carrier inside the sample chamber. The sample carrier is connected to a holder that can be moved in three dimensions (x,y,z). The sample is illuminated by a light-sheet generated by laser

light. The light-sheet is positioned in the focal plane of a detection objective lens, whose optical axis is arranged perpendicularly to the plane of the light sheet. Hence, only light from the focal plane is registered by the system and used for image formation.

The manufacturer assumes no liability for any malfunction or damage caused by any non-intended use of the **TruLive3D Imager** system as a whole or any of its components or resulting from any repair or other maintenance services carried or tried out by any non-authorized personnel. Any such activity will void the warranty for the whole system including parts not directly affected. This includes any modification of the system computer (PC), for example equipment with new hardware or software components by the user. Every manipulation voids the warranty of the laser safety.

Furthermore, to guarantee your own safety you are strongly advised to read the manuals, in particular the safety instructions, of any modules and additional components of the **TruLive3D Imager** system as not all features might be listed here explicitly.

The lasers built into the **TruLive3D Imager** microscope are classified as laser safety class 3B. The safety interlocks ensure that the user is not exposed to dangerous laser radiation. The **TruLive3D Imager** itself is a laser safety class 1 product.

The operator of the system must comply to all applicable accident prevention regulations enforced by law.

The Luxendo **TruLive3D Imager** system complies with the following standards:

EN 61000-6-3:2007 + A1:2011

IEC 61000-6-3:2006 + A1:2010

EN61000-6-2:2005

IEC 61000-6-2:2005

DIN EN 60825-1:2008

DIN EN 50581: 2013

Dimensions

Component	Length (cm)	Width (cm)	Height (cm)	Weight (kg)
TruLive3D Imager main module	81	47	41	85
Power supply	30	30	14	12

Environment

The system must be installed in a closed room.

Operation	T = 22°C ± 5°C without interruption (24 h per day independent of system operation)
Storage	T = 15°C … 40°C
Temperature gradient	± 0.5°C/h
Relative humidity	< 40% at 30°C
Warm-up time	1-2 h
Maximum elevation	2000 m

Power supply configuration

The Luxendo **TruLive3D Imager** uses an external power supply module. This module is connected to the AC network using the provided country-specific power

supply cord. The PC and the camera(s) for detection are connected to the AC network using separate power supplies and cords.

Voltage Frequency	90 V AC ... 240 V AC 50 ... 60 Hz
Power consumption **TruLive3D Imager**	Max. 140 W
Power consumption per camera	Max. 70 W
Power consumption PC	Max. 1200 W

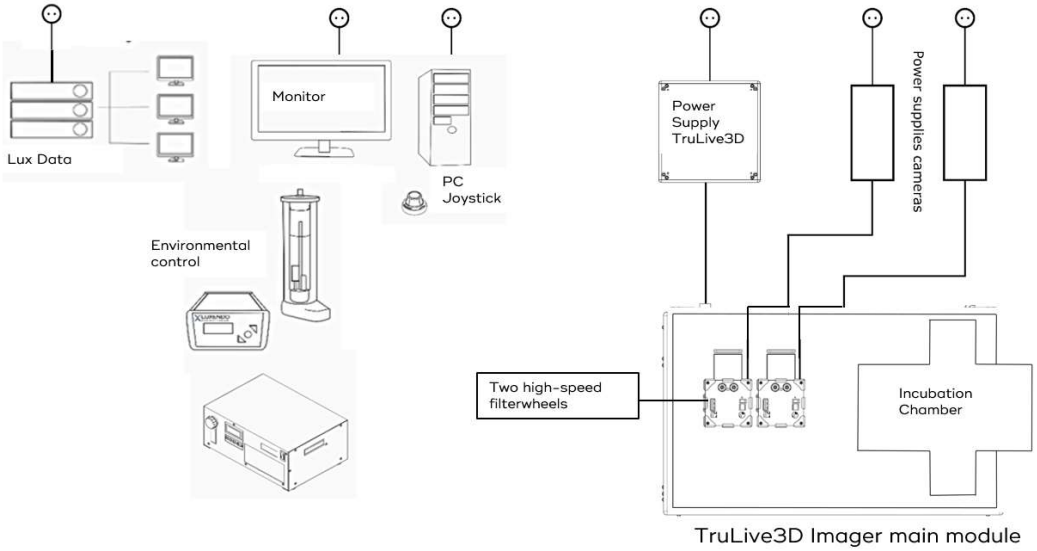

*Figure 1: Wiring scheme of **TruLive3D Imager** system*

Power on the microscope by using the switch on the plugged power supply module. Initialization period is approx. two minutes; the system is then ready for usage and can be controlled via PC.

To completely disconnect the system from the AC network, turn the power switch on the power supply to “OFF” and pull the plug out of the power socket. Access to this power socket must never be obstructed.

In case of an emergency, press the kill switch installed on the power supply’s front panel. This powers down the microscope immediately.

To reset the kill switch, turn it counter-clockwise.

Setting up the Luxendo **TruLive3D Imager**

Installation and initial startup of the **TruLive3D Imager** has to be carried out by authorized Luxendo service personnel. Before working with the system for the first time, a training by a Luxendo representative is inevitably required.

Do not set up the **TruLive3D Imager** close to a heat source, i.e. radiators or direct sunlight. An influence by air conditioning units is also possible and should be avoided.

Do not set up the system in an explosion-hazardous area.

Always use the provided power supply cord to connect the system to a properly installed safety socket. The protection by the protective ground contact must not be influenced by using an extension cord.

The system contains components carrying hazardous voltage. It may only be opened by authorized Luxendo service personnel. The power cord has to be disconnected prior to opening the system.

Maintenance and repairs, modification, exchange or removal of parts, the transport of the **TruLive3D Imager** module or any other encroachment on the system not described in this manual have to be executed by Luxendo or by personnel explicitly authorized by Luxendo.

This specifically applies to the microscope system, the built-in laser module, the lasers and cameras, the PC, power supplies, cable connections and other components of the system.

The system contains a safety interlock that shuts off the lasers as soon as the sample chamber's lid is opened. A notification will be displayed in the software user interface. In order to make the lasers operational again, close the sample chamber's lid and reset the lasers using the reset button at the lid.

After installation or modification, the **TruLive3D Imager** system has to be inspected thoroughly by Luxendo technical staff to ensure the system functions properly – particularly the mechanisms for protection from dangerous laser radiation.

Optional components such as the temperature control unit should not be put on the same table as the **TruLive3D Imager** system itself to avoid vibrations.

Warning labels

Please pay special attention to the warning labels on the **TruLive3D Imager** system. Check if all labels listed below are clearly visible on your system and contact Luxendo if any of those are missing. You will receive a free label substitute.

Meaning of labels:

Attention:
Possible exposure to hazardous laser radiation when working with the system.

Attention:
Malfunctions and dangers that can occur during operation and can possibly lead to damages of the system or injury of the user.

Attention:
Risk of electric shock. Possible exposure to hazardous voltage.

General information on handling laser components and illumination elements

The **TruLive3D Imager** is a laser safety class 1 product.
The lasers built into the system are encapsulated class 3B lasers.
Exposure to hazardous laser radiation is prohibited by full housing and safety interlocks on all customer-accessible parts.

Attention: By using control elements or performing adjustments or procedures not described in this manual, you risk exposure to hazardous laser radiation.

The following lasers are currently listed for use in the Luxendo **TruLive3D Imager**. Using any lasers other than those listed below is prohibited.

Laser	Class	max. output power
405 nm	3B	60 mW
445 nm	3B	60 mW
488 nm	3B	60 mW
515 nm	3B	60 mW
532 nm	3B	60 mW
561 nm	3B	60 mW
594 nm	3B	60 mW
642 nm	3B	60 mW
685 nm	3B	60 mW

When using the Luxendo **TruLive3D Imager** in compliance with the given instructions the user will not be exposed to hazardous laser radiation. Nevertheless, note the following warnings:

- Under no circumstances are you permitted to remove any cover panels nor any parts that are not explicitly described in this manual.
- Do not manipulate any safety interlock under any circumstances. Luxendo assumes no liability for damages caused by manipulations of the security mechanisms.

Proper precautionary measures are mandatory, if health-damaging gases, dust and vapors, secondary radiation or explosive substances are produced due to laser radiation.

Handling of computer and data media

By default, the Luxendo **TruLive3D Imager** comes with a high-end workstation computer and operating system WINDOWS 10. The PC handles both communication with the microscope and data storage and analyses.

Please note that the hard drives used and the RAID, respectively, work slower the more data they hold. Therefore, move the data not needed onto external storage devices. Backup your data on a regular basis.

Do not install any other software on the workstation computer unless authorized by a Luxendo employee.

To prevent possible loss of data power down the **TruLive3D Imager** and shut down the WINDOWS operating system before shutting down the PC.

The hardware and software configuration of the computer are optimized for the operation of the **TruLive3D Imager** microscope. It is not intended to be used as a common-purpose workstation. Therefore, do not install any additional hardware or software unless instructed otherwise by an authorized Luxendo employee. Also, refrain from regular updates and patches for the WINDOWS operating system unless instructed otherwise by an authorized Luxendo employee.

Components TruLive3D Imager

The sample chamber's lid is equipped with a safety interlock system to ensure laser safety. This safety system must not be manipulated.

Interfaces not described in this manual are reserved for service and only authorized Luxendo service personnel is permitted to use them. The following components however may be attached and detached by the user or are accessible to the user:

- sample holder

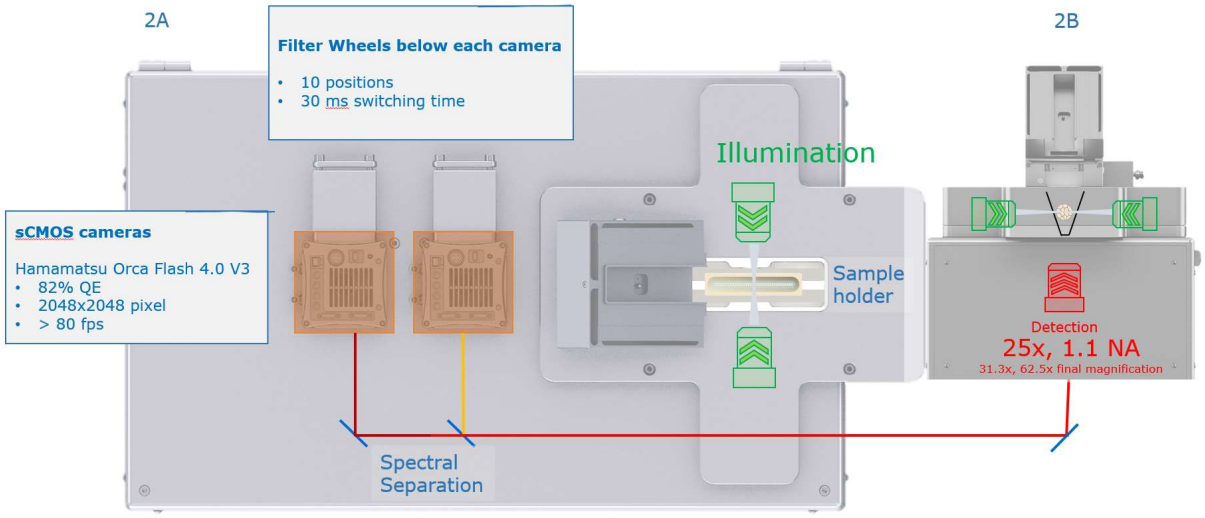

Figure 2: TruLive3D Imager: Components and Optical Concept 1A view from above, 1B front view

Sample chamber

The sample chamber has a water bath made of biologically inert PEEK. The illumination and detection objectives are embedded in the water bath from the side and below at a fixed position. A heated lid confines the sample chamber and guarantees temperature and atmosphere control. Within the confined sample chamber, you can find the sample holder device described below. The sample carrier dips into the water bath, connected to translational stages via the fixed mounting adapter. The customer must not disassemble the sample chamber nor any of its parts.

Sample holder for TruLive Dishes

The sample holder for TruLive Dishes connects the sample carrier with the translation stages. The sample holder can hold three TruLive Dishes with six compartments in total.

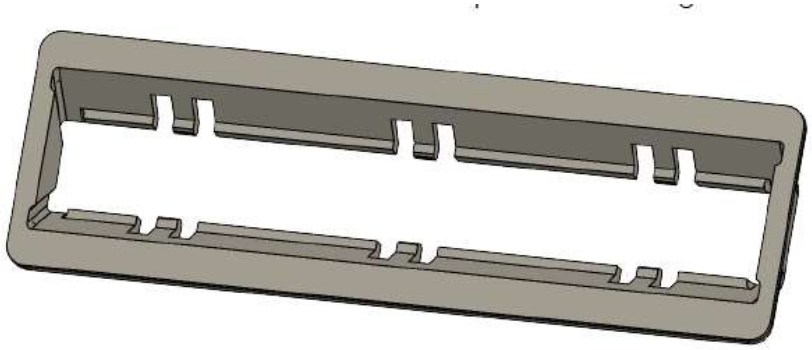

Figure 3: TruLive3D Sample holder

TruLive3D Dishes

The TruLive3D Dishes are ready-to-use sample holders for the Luxendo TruLive3D Imager light-sheet microscopes. The TruLive3D Dishes have been sterilized and are shipped in sealed sleeves. To retain sterile conditions, open the packaging sleeve only under a cell culture hood.

Similar to the classic sample holder, the FEP foil lining the holder serves the dual function of a “curved coverglass” and a physical barrier between the immersion medium and sample medium.

The new ready-to-use TruLive3D Dishes are fully compatible with the TruLive3D Imager. The possibility to enable the parallel imaging of samples grown under different media conditions.

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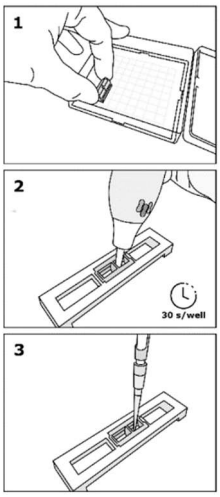
Figure 4: TruLive3D Sample holder with three TruLive Dishes

Sample preparation:



1. Remove the dish from packaging and place it in the specialized culture stand.

2. Carefully functionalize the TruLive3D Dish with cold plasma (PiezoBrush) for 30 seconds per well. For optimal ion transfer to the FEP foil, a metal working area of a culture hood below the dish is essential. It functions as a counter electrode.

3. Subsequently, apply your sample to the dish.

Figure 5: TruLive Dish

In order to clean/change the sample carrier, open the lid of the sample chamber. Remove the sample holder and clean with 70% Ethanol the complete chamber and let it dry out.

Correction collar

The medium embedding the sample can have a different refractive index than the immersion water. This changes the detection objective's effective working distance, which can lead to a degradation of image quality. To compensate for this effect the detection objective is equipped with a correction collar.

To control the correction collar, use the designated buttons in the subpanel "Stages" of the user interface. While manipulating the correction collar, one can see the change in image quality in the live images on the computer screen. Adjust the correction collar accordingly.

Cleaning, Maintenance and Service

The manufacturer cannot be held liable for damages caused by faulty operation, negligence or non-authorized procedures, particularly for the removal or exchange of components or use of improper equipment from other manufacturers. Any such action voids the warranty and laser safety is not guaranteed anymore.

Maintenance by manufacturer

To ensure a smooth operation of the Luxendo **TruLive3D Imager** over a long period, we recommend concluding a service contract with Luxendo GmbH.

Damaged devices or parts may only be repaired or replaced by the Luxendo service team. Modifications of system components have to be performed by the manufacturer or authorized and trained personnel.

We ask the customer to keep a safety distance during service and maintenance and to wear laser protection glasses if necessary. If the Luxendo personnel should give any further safety instructions, these are to be obliged accordingly and immediately.

During service, the room is classified as a laser safety class 3B area.

Exchange of fuses and electric connections

Only use original parts when changing fuses or replacing power cords.

In case of a malfunction of the power supply the customer may change the built-in fuses. The two fuses are located in the fuse holders right above the kettle plug as shown in Figure 2.

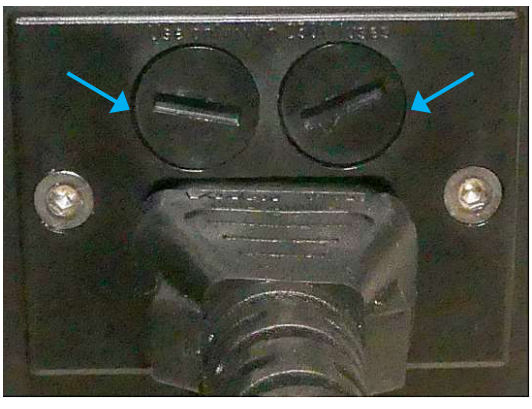


Figure 2: Position of fuses

The built-in fuses are type T 5 A/H, 250V, ø 5 mm x 20 mm cartridge fuses.

To change the fuse, proceed as follows:

- Unscrew protection cap (Figure 2, blue arrows)
- Pull out protection cap and replace the old fuse with a new one
- Reinsert and firmly screw in protection cap

The customer may not alter the wiring of the system. In case the power cord detached, reconnect it to the AC network as described above.

Maintenance by the customer

The maintenance by the customer is limited to cleaning the housing, cleaning and disinfection of the sample chamber, the illumination and detection optics in it, and the sample carrier.

a) To clean the housing proceed as follows:
   - Shut down the microscope and unplug the power cord.
   - Make sure liquids do not enter the system.
   - Wipe the housing using a clean cloth wetted with water and a bit of detergent. Dry off using a lint-free cloth.

   For sterilization wipe the surfaces with a cloth wetted with 70% ethanol or 70% isopropanol.

b) To clean the sample chamber, proceed as follows:
   - Shut down the microscope and unplug the power cord.
   - Make sure liquids do not enter the system.
   - Remove the sample holder as described above.
   - Remove remaining water from the water bath using the 50 ml syringe
   - Wipe the chamber using a lint-free cloth wetted with distilled water or 70% ethanol.
   - Pay special attention to avoid scratching the objective lenses during the cleaning process.

c) To clean the objectives, proceed as follows:
   - Remove the sample holder as described above.
   - Clean the front lenses as commonly recommended using optics detergents and lens cleaning tissue.
   - Do not wipe the front lens of the objective with dry lens cleaning tissue to prevent scratching.

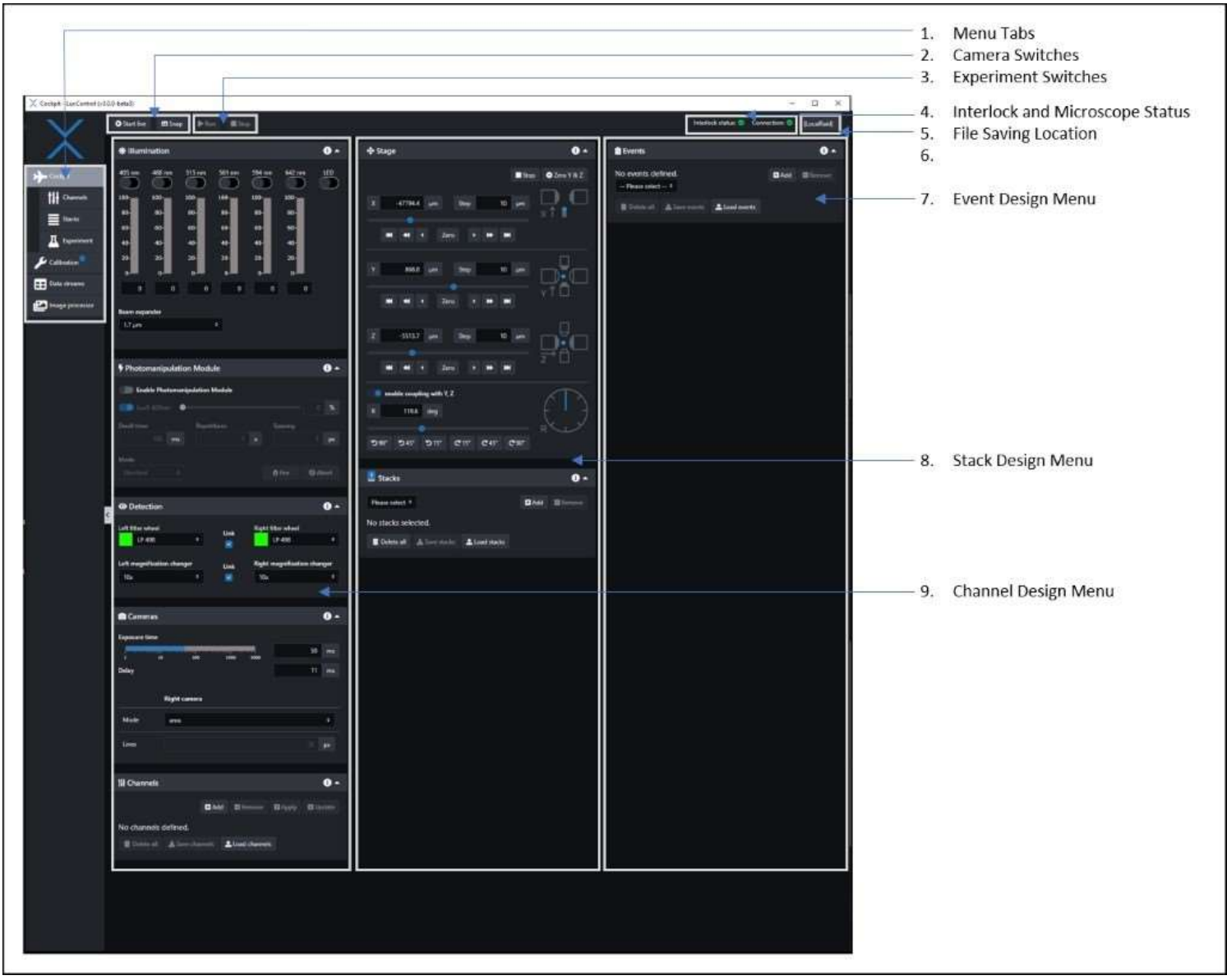
System Startup

Data Hub

1) Before starting the microscope, take care that the objective lenses and the sample chamber are clean and fill the chamber with sterile distilled water as immersion medium.
2) Turn on Cameras
3) Turn on Microscope
4) Turn on Computer, DataHub will start automatically
5) Check the taskbar for the DataHub
6) Reset the Interlock (push button on the sample chamber lid)
7) Start the LuxBundle application

Lux Control Overview

Application Layout

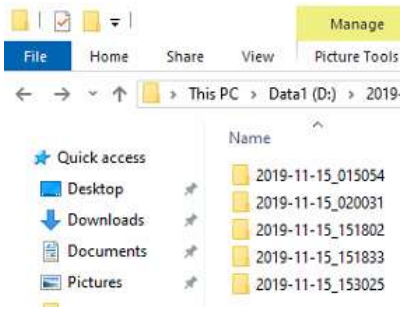


Data Review

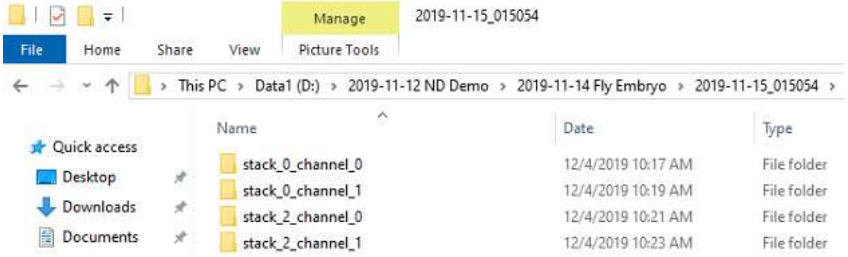
Luxendo TruLive3D Imager-SPIM File Naming Conventions

Folder Hierarchy

After an acquisition is initiated, a new experiment folder is created and named with the date and time the experiment was initiated; for example, an acquisition on November 15, 2019 at 01:50:54 am becomes **2019-11-15_015054**.



Within the main experiment folder, sub folders are created for every stack (ie. stage position) and channel combination. Each folder will be named, stack_(n)_channel_(n).



The sub-folders created for tiled **montage acquisitions** will have the same stack number and include the X and Y coordinates of each tile position. These sub-folders contain files related to the images and meta files generated by the short and long cameras.

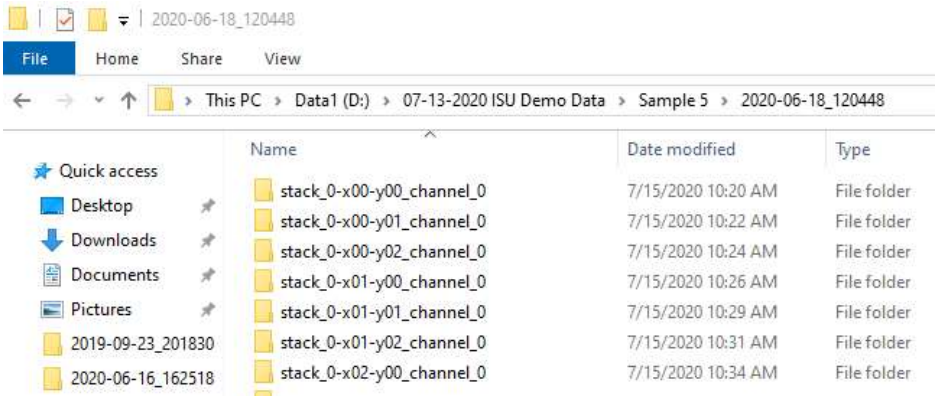
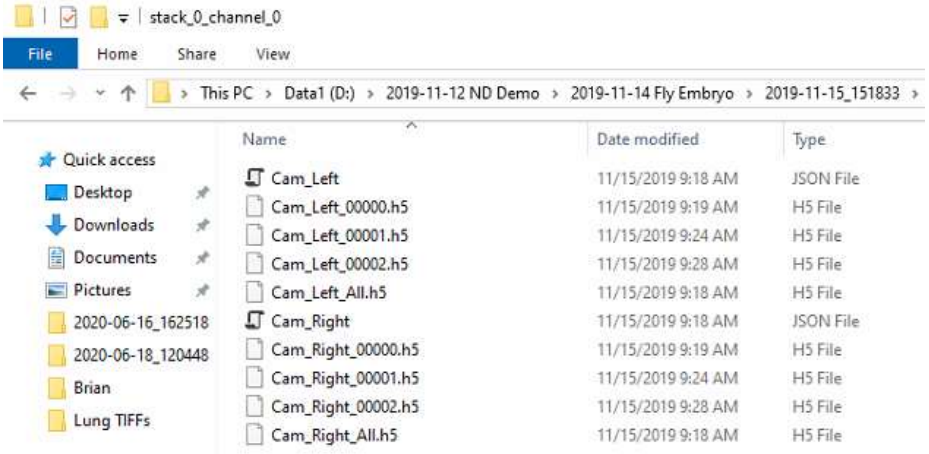


Image Data

- Image data for each stack and channel combination is stored in the **Cam_Long_OOOOO.h5** and **Cam_Short_OOOOO.h5** files
    - H5 files are an open source format that can be opened in FIJI
- Time series will have multiple .h5 files and be named according to time point (ie. **Cam_Long_(*****).h5** where (****) is the timepoint



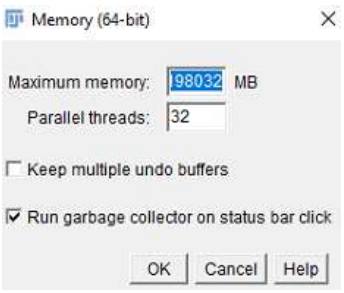
Meta Data

- The **Cam_Long.JSON** and **Cam_Short.JSON** files contain the metadata for each acquisition and can be opened in Notepad to view the microscope setting each for the experiment

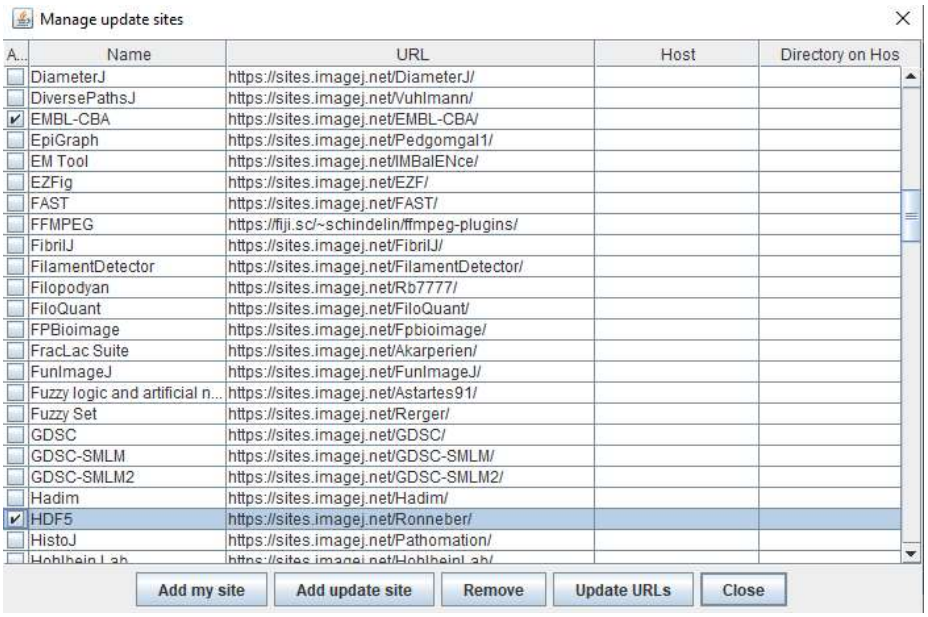
Viewing TruLive3D Imager-SPIM Data in FIJI

Downloading and Configuring FIJI

1. Download the latest 64-bit version of FIJI from https://fiji.sc/
2. Open FIJI > select Edit > Options > Memory and Threads
3. Set the Maximum Memory to match to the amount of RAM installed on the computer
    a. This setting will limit the size of the data sets that can be directly loaded in FIJI
    b. Maximum Memory can exceed the amount of RAM if Virtual Memory is enabled in Windows

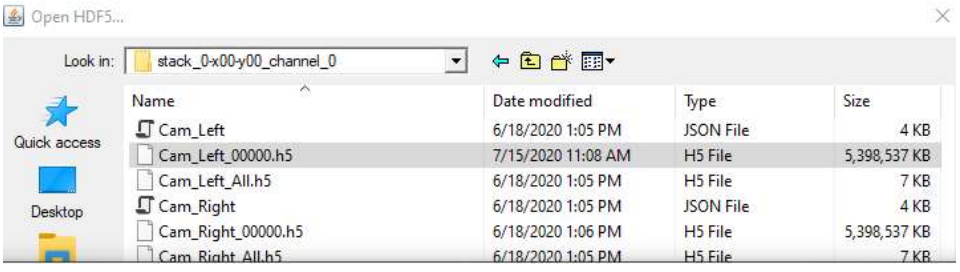


4. Select **Help** > **Update** > **Manage Update Sites** > check boxes for **EMBL-CBA** and **HDF5** > **Close** > **Apply Changes**



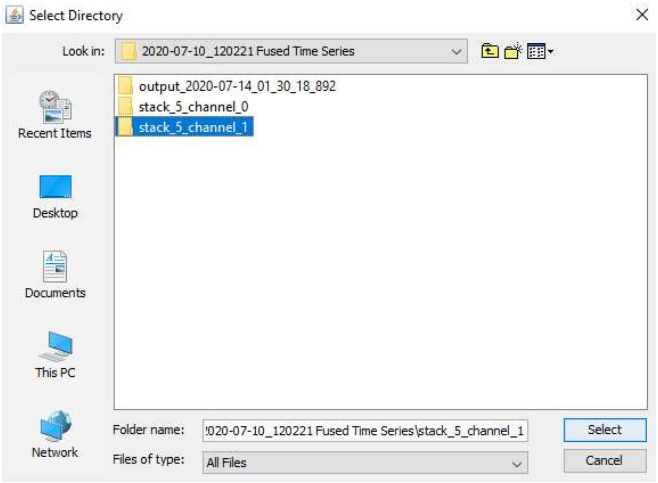
Opening Single Cam_(Long or Short)_00000.h5 Files

5. For **single H5 files**, Select **File** > **Import** > **HDF5** > select **Cam_Long_OOOOO.h5** or **Cam_Short_OOOO.h5**
6. Repeat this process to open additional data from other stacks, channels or camera angles

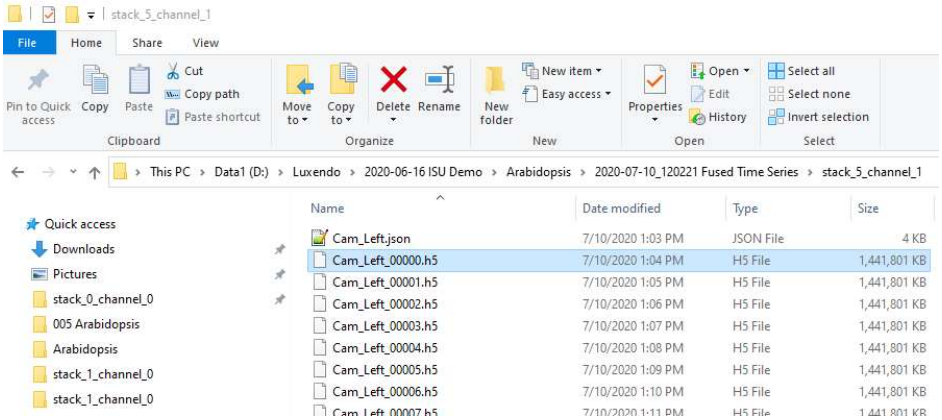


- Note: file sizes will be limited to the memory allocation configured in the FIJI Settings menu. Any files opened with this method will be stored in the computer's RAM.

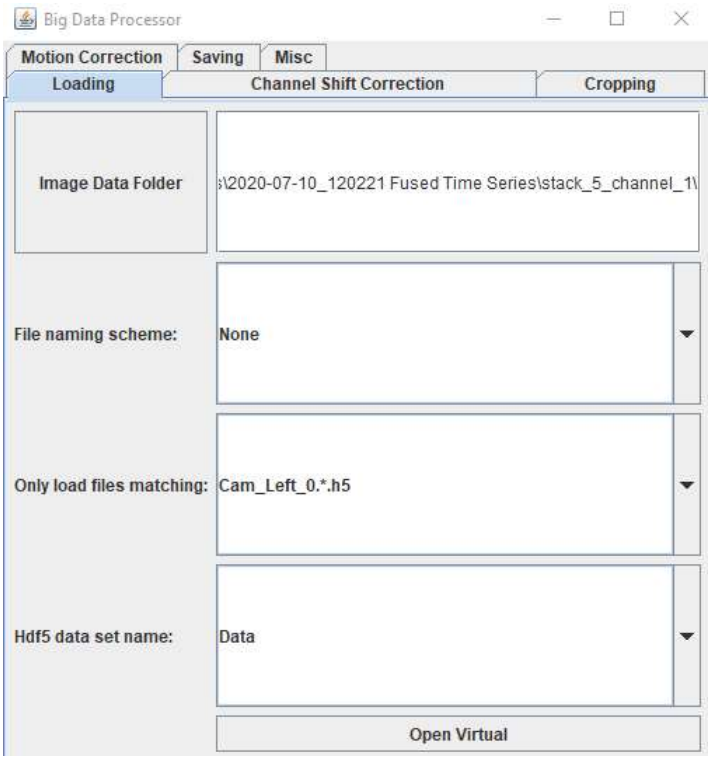
7. Stream **large data** sets, such as time series with the Big Data Processor in FIJI to bypass the memory limit of the computer and take advantage of the H5 file structure
    a. Select **Plugins** > **BigDataTools** > **BigDataProcessor** > select folder of interest
    b. Steaming data with the BigDataProcessor does not load the entire file to RAM



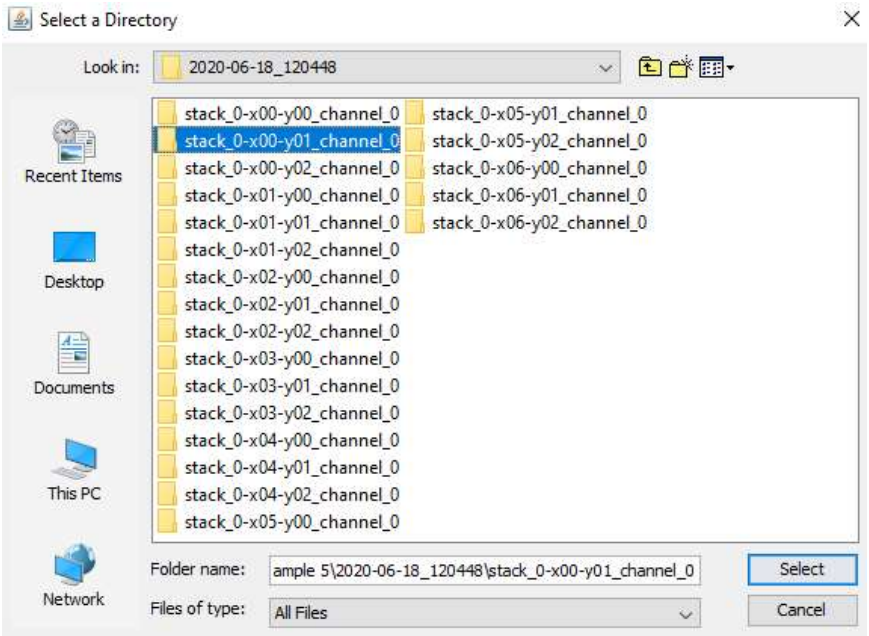
    c. Input camera specific information into the **Only load files matching:** field, ie. **Cam_Long_O.*h5**
        i. The file name information will be found in the data folder



        ii. Remove any numerical characters at the end of the file name and replace with “O.*h5”
            1. For example, **Cam_Long_OOOOO.h5** would be inputted in the **Only load files matching:** field as **Cam_Long_O.*h5**
    d. Select **Hdf5 data set name: Data** from the drop-down menu
    e. Click **Open Virtual**

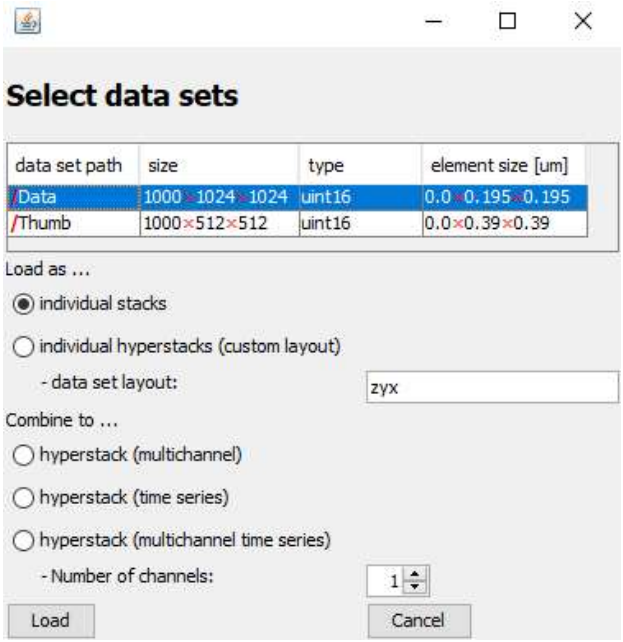


a. To open other camera angles, repeat process starting at Step 3a by inputting **Cam_Right_O.*h5** into the **Only load files matching:** field of the Big Data Processor window
b. To open other channels or stage positions, repeat process and select appropriate folder in **Image Data Folder** window of Step 3a

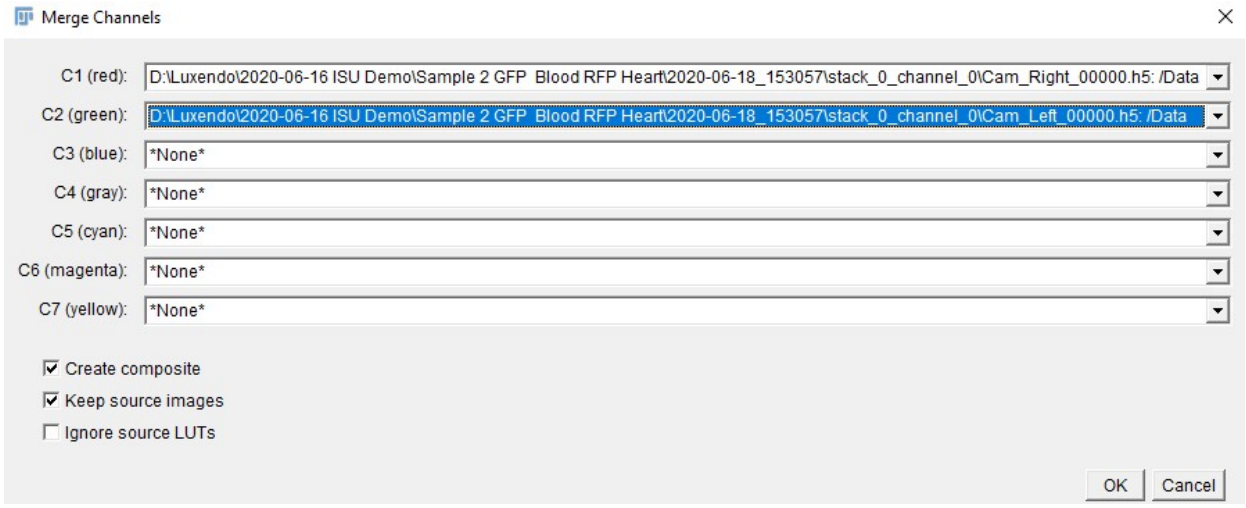


Opening Data Collected in Repetition Mode

8. For data acquired in **Repetition** mode, open data in FIJI using **File** > **Import** > **HDF5 file** > select Cam file of interest > **Open** > highlight **data set path "Data"** > click **Load**



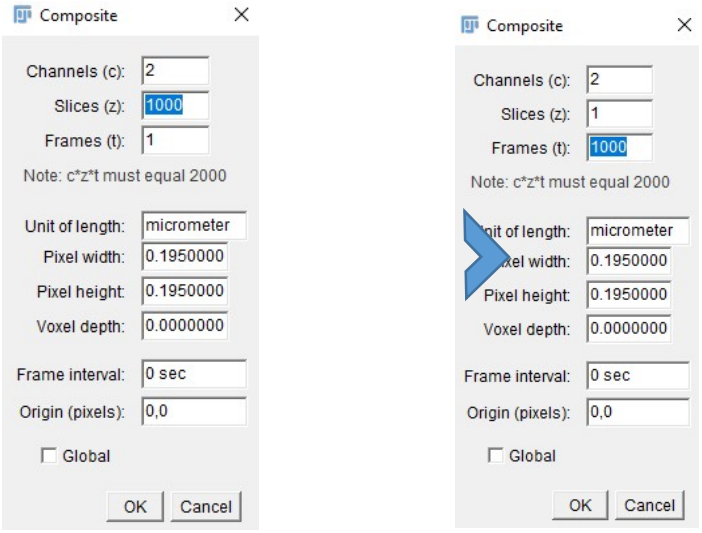
   a. If two channels were acquired, repeat this process to open the second Cam view
      i. For two color samples imaged using **dual channel simultaneous capture**, one data set may need to be flipped (depends on the model TruLive3D Imager-SPIM used for acquisition)
         1. Select **Image** > **Transform** > **Flip horizontally**
      ii. The two channels can be overlaid by selecting, **Image** > **Color** > **Merge Channels**



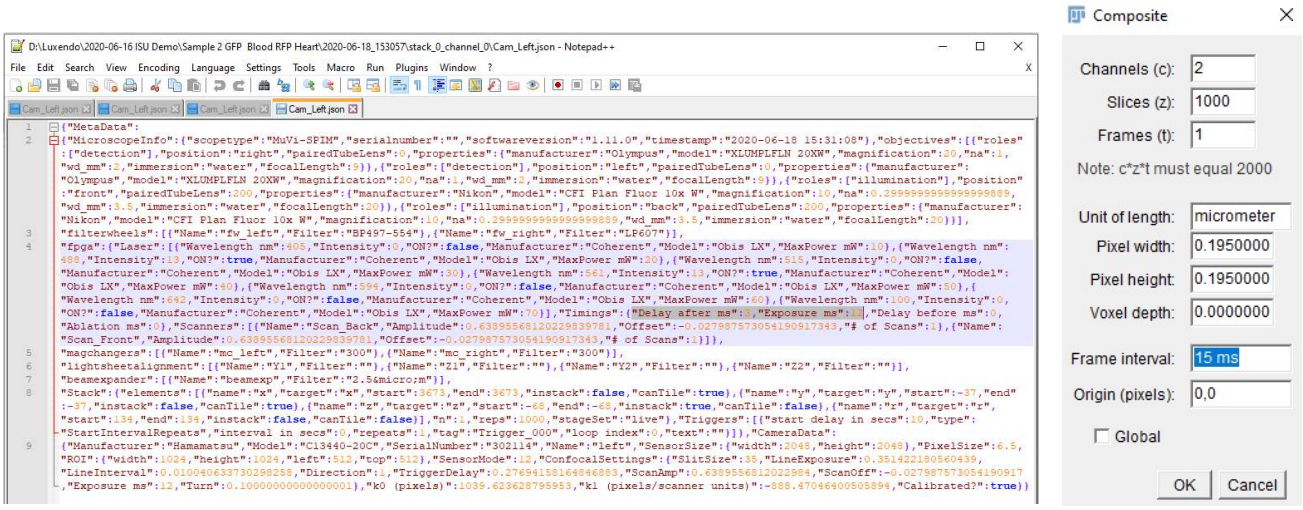
   a. During conventional capture time points are saved as distinct files, this takes a lot of computer overhead and makes the acquisition slower. When using Repetition mode the files are saved as one file – this format is typically reserved for Z. To correct this, select **Image** >

Properties > switch values automatically generated in **Slices (z)** to **Frames (t)**

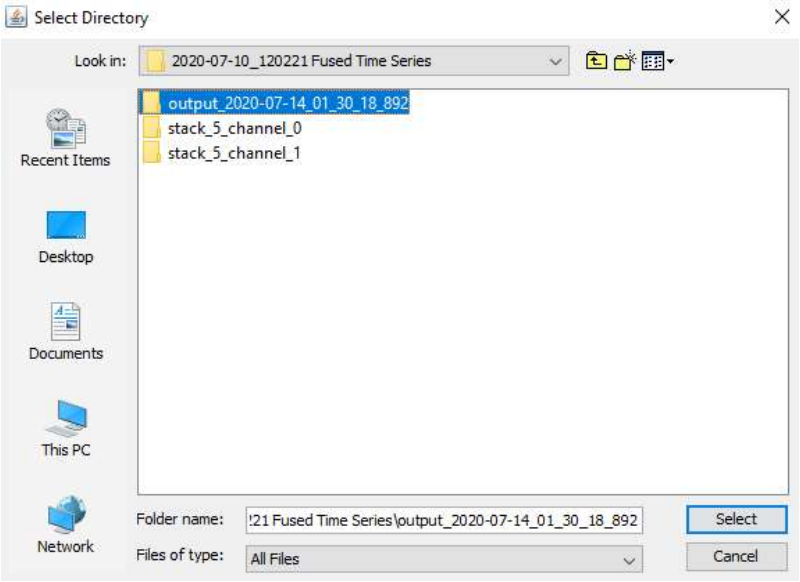
a. In the below example, Slices (z): 1000 becomes Slices (z): 1, and Frames (t): 1 becomes Frames (t): 1000



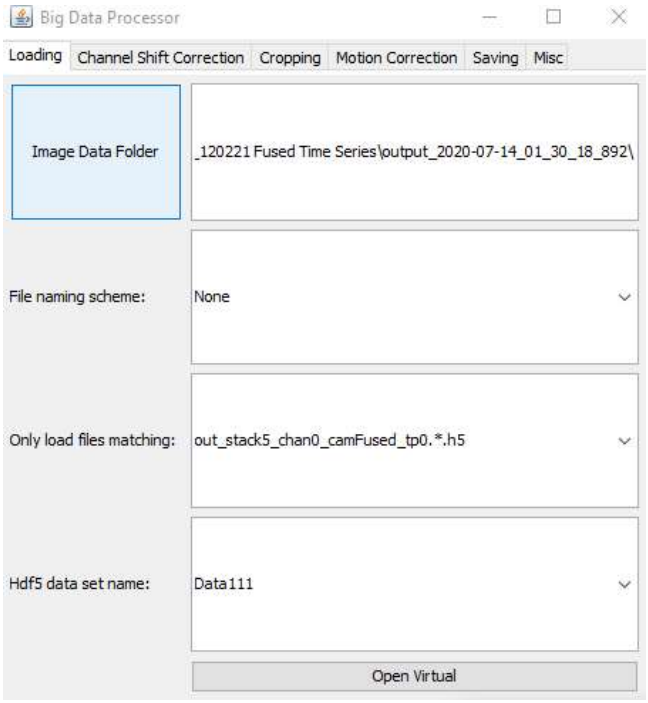
b. The **Frame interval** can be determined from the **JSON file** by adding the **"Delay after ms"** and **"Exposure ms"** values



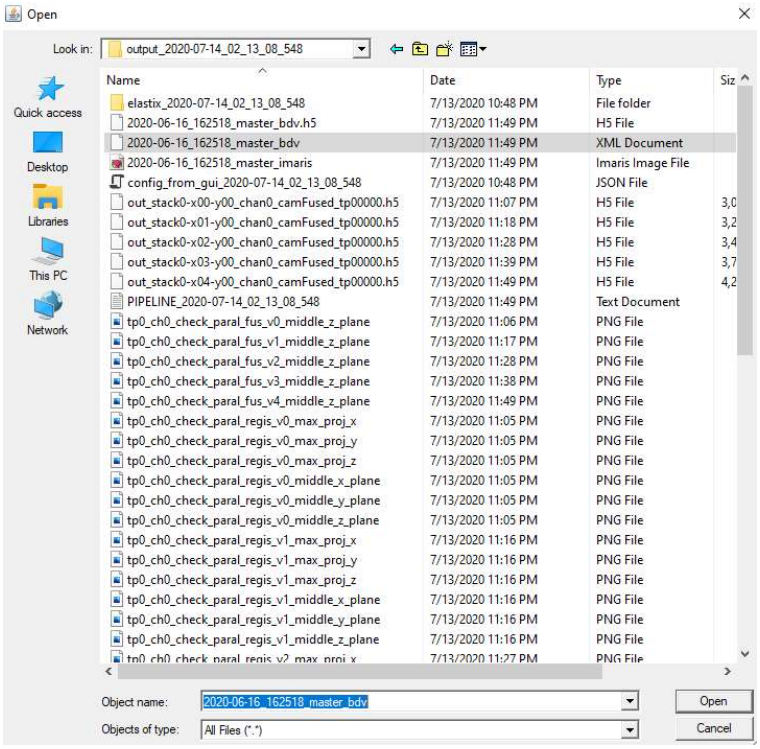
9. For raw data **fused** or **stitched** using the Luxendo Image Processing Pipeline, an "Output" subfolder will be present in the experiment's main folder. Depending on how the fusion was performed, this folder may contain compressed versions of the raw data and the fused data.



   a. These data can be opened within FIJI using **File** > **Import** > **HDF5** (Step 2) or with the **BigDataProcessor** plugin
      i. To use the **BigDataProcessor** plugin, follow the directions outlined in Step 3 with the following data-type specific changes:
         1. The **Open load files matching:** field must contain the H5 file name without the numerical characters + O*.h5
            a. For example "out_stackO-xOO-yOO_chanO_camFused_tpOOOOO.h5" becomes "out_stackO-xOO-yOO_chanO_camFused_tp).*h5
         2. Select **Data111** from the **Hdf5 data set name:** drop-down menu



10. To use **Big Data Viewer** plugin, select **Plugins** > **BigDataViewer** > **Open XML/HDF5** > select **"_master_bdv" XML file** of interest > **Open**



Opening TruLive3D Imager-SPIM Imaging Data in Arivis Vision4D and Imaris

Once the imaging data has been appropriately fused/stitched in the Luxendo Image Processing Pipeline, the channels and time points will open in a single

window in Arivis Vision 4D or Imaris (depending on the features of the software package being used).

- The "_master_bdv" XML file can also be dragged and dropped into **Arivis Vision4D** for rendering and analysis

- The "_master_imaris" Imaris Image file can be double clicked to open in **Imaris** or **Imaris Viewer**
    1. The free Imaris Viewer for Mac and PC can be download from: https://imaris.oxinst.com/imaris-viewer