

**UltraMicroscope II: Image Handling Considerations**

AMCF UltraMicroscope II: DRC I Room 1063

Schedule through AMCF core staff

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The UltraMicroscope software (ImSpector) can only perform *basic visualization* of samples post-capture. The default file format set by Miltenyi is ome tiff, a universal image type (open microscopy exchange tiff). The default save/storage setting can alternatively be set to Imaris (\*.ims). This is dependent upon how individual researchers choose to work with their data, FIJI/ImageJ or Imaris. For researchers planning on using the data analysis workroom, set the file type to \*.ims (or plan on converting ome tiff to ims file, see below).

- a. Imaris file converter software has been placed on the LSFM acquisition computer and each of the Imaris workstations in the data analysis room (DRC I Rm 1036). Once converted from ome tiff to .ims, images can be viewed using the full Imaris software (data analysis room) or the free version of the Imaris software.
  - i. [Imaris Viewer - Imaris - Oxford Instruments \(oxinst.com\)](https://www.oxinst.com)
  - ii. ImSpector *does not* stitch files together.
  - iii. Mosaic/tiled images *must be stitched* into a composite image before viewing, see below.
  - iv. The Imaris stitcher program can only stitch ims files.
- b. Imaris stitcher software is part of the core facilities package and is therefore only available on the [IMARIS 1 workstation](#) in the data analysis workroom.
  - i. The Imaris stitcher program can only stitch ims files. Set the acquisition parameters to save as ims or convert individual images to ims files prior to stitching, see below.
- c. The Imaris file converter software can only convert individual tiles. Mosaic images must be converted as individual tiles then copied to the imaris stitcher program for fusing them together.
- d. There is a 'big stitcher' plugin for FIJI that can be used to fuse and visualize ome tiff files on any computer with sufficient memory to do so (<https://imagej.net/plugins/bigstitcher/>).

**LSFM/volumetric images are Large (10's to 100's of GBs)**

Please ensure adequate storage space and upload/transfer times. The AMCF has installed 10 Gb ethernet connections between the new instrumentation (Axioscan whole slide imager, Light Sheet Microscope) and the Data analysis room. Additional high-speed connections will be established as quickly as possible. Many locations on campus are 10 Gb 'ready,' not actively installed/configured. Researchers should verify individual transfer capabilities in their location/building and plan accordingly (table from UltraMicroscope II user guide).

- Data write *speeds* under good conditions are:
  - 10 Gb Ethernet: 500 Mb/s
  - 1 Gb Ethernet: 50-100 Mb/s
  - USB3: 50 Mb/s
- Data write *times* are shown in Table 11.1.

Amount of data	Transfer method		
	10 Gb Ethernet	1 Gb Ethernet	USB 3
1 Gb	2s	10-20s	20s
10 Gb	20s	1-3 min	3 min
100 Gb	3 min	15-30 min	30 min
1000 Gb	30 min	2.5-5 hours	5 hours

**Table 11.1.** Data write times for different amounts of data and transfer methods. Note that these are best-case values which are not always achieved in practice.

### **Image Analysis Workstation (IMARIS)**

These systems are currently connected to the network. Data may be securely transferred to individual researchers/research groups and/or temporarily stored ( $\leq 6$  months) using the AMCF SharePoint location ([contact James Talaska](#)) or BOX account ([contact Dr. Jensen-Smith](#)). Data may reside on the workstation (3 months) and/or SharePoint location for *up to 6 months*. After this time (or data not stored on the designated data drive) will be removed from the acquisition workstations *without prior notification*. The AMCF is working with RITO to establish local storage space for temporary transfer and storage of data ( $\leq 6$  months) from these workstations and/or between image acquisition and analysis workstations (estimated availability early spring 2023).

### **Data Analysis Workroom**

Located in the Data Analysis Room (DRC | RM 1036).

Contact [AMCF staff](#) to request permission to independently reserve/use workstations (RSS) and obtain card access for this area.



### **Terms of Use: AMCF Data Analysis Workroom**

Updated: 12.28.2022

- 1) Available machines (See AMCF website for analysis module details and training resources)
  - a. HALO workstation (\$5/h, unassisted) – ideal for 2D image analyses.
  - b. IMARIS workstation 1 (\$5/h, unassisted) – ideal for 3D image analyses.
  - c. IMARIS workstation 2 (\$5/h, unassisted) – used for super resolution image analyses. Imaris workstation 1 may be used too.
  - d. Zeiss Workstation (free) – Zeiss Zen software for exporting and/or very basic analyses of \*.czi images
- 2) AMCF core staff will provide you with scheduling permissions in RSS for unassisted use of these workstations. Assisted use must be coordinated with AMCF staff. Card access to the analysis workroom is not ‘controlled’ by the AMCF. Core staff submit individual card access requests while setting up RSS scheduling permissions. The complete process may take 1-3 days.
- 3) *All time in the data analysis workroom must be scheduled (free and fee-per-use).*
- 4) Users are expected to schedule times and machines appropriately. Computer usage will be checked regularly, anyone using systems without permissions and/or reservations will lose access.
- 5) *Do not store data more than 3 months on these workstations, it will be removed without notice.* Data should only be temporarily stored on the Data drive only (i.e. D drive). Files stored in other locations are subject to immediate removal without prior notice.
- 6) For temporary data storage during active analyses on the D drive, make a folder with your last, first name (Jensen-Smith, Heather).
- 7) *HALO can only access/re-analyze samples that are stored in exactly the same location* i.e. (D drive → Jensen-Smith, Heather (folder) → mitochondrial study 1 (folder). Label carefully and make sure you record where your image files are temporarily stored as HALO will not be able to re-read your prior analyses if you change the location of the reference image files.
- 8) HALO does not change image files, it only reads/references them from the temporary storage location. A meta data database is formed/contains the image analysis parameters.

- 9) IMARIS generates new image files in the IMARIS format (\*.ims) with meta data contained therein. See AMCF data management plan for more details (AMCF website).
- 10) *Researchers using the Data Analysis workroom to analyze AMCF or elsewhere acquired images MUST ACKNOWLEDGE use of, and funding sources for, this shared research resource. Use the following, "We acknowledge use of the University of Nebraska Medical Center - UNMC Advanced Microscopy Core Facility, RRID:SCR\_022467, P20 GM103427, P30 GM106397, P30 CA036727." As appropriate, please consider/including the following, "We additionally thank [INSERT APPLICABLE NAMES HERE] of the UNMC AMCF for their assistance." Janice A. Taylor, B.S., James R. Talaska, B.S., Heather Jensen-Smith, Ph.D.*