1) Available machines (See AMCF website for analysis module details and training resources)

    a. <u>HALO workstation</u> ($7.50/h, unassisted) – ideal for 2D image analyses.

    b. <u>IMARIS workstation 1</u> ($7.50/h, unassisted) – ideal for 3D/4D image analyses.

    c. <u>IMARIS workstation 2</u> ($7.50/h, unassisted) – often used for super resolution image analyses (old version of IMARIS). Imaris workstation 1 may be used too.

    d. <u>Zeiss Workstation</u> (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 (advancedmicroscopy@unmc.edu). Core staff submit individual card access requests while setting up RSS scheduling permissions. Verify card access is working before scheduling data analysis.

3) ***All time in the data analysis workroom must be scheduled using RSS (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) *<u>Do not store data more than 3 months on these workstations, it will be removed without notice.</u>* 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) ***<u>HALO</u>*** *<u>can only access/re-analyze samples that are stored in exactly the same location</u>* 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 (current version below).

10) *<u>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.</u> Use the following, "**We acknowledge use of the University of Nebraska Medical Center - UNMC Advanced Microscopy Core Facility, RRID:SCR_022467, P30 CA036727 (NCI, Buffett Cancer Center), P20 GM103427 (NIGMS, NE-INBRE), P30 GM106397 (NIGMS, NCS), P20GM130447 (NIGMS, CoNDA), S10RR02730 (NIH), S10OD030486 (NIH), Nebraska Research Initiative, UNMC Vice Chancellor for Research Office**." As appropriate, please consider/including the following, " We additionally thank [INSERT APPLICABLE NAMES] of the UNMC AMCF for their assistance." James R. Talaska, B.S., Camille Hennerberg, B.S., Janice A. Taylor, B.S., Heather Jensen-Smith, Ph.D.*

Shared Research Resource Information for Data Management & Sharing Plan – Updated 07/2024	
Shared Research Resource Name:	*Advanced Microscopy Core Facility (AMCF)*

Shared Research Resource Director:	*Heather Jensen-Smith, PhD*	*heather.jensensmith@unmc.edu*
Shared Research Resource POC (if differs from Director):	*James Talaska, Camille Hennerberg*	*advancedmicroscopy@unmc.edu*
Shared Research Resource Website:	*Advanced Microscopy Shared Research Resource Facility	VCR	University of Nebraska Medical Center (unmc.edu)* *https://Shared Research Resourcemarketplace.org/RRID:SCR_022467*	

Data Management & Sharing Services Description	
Will Shared Research Resource assist with data upload in a repository?	*No for outside data repositories. Data stored on AMCF OMERO server will be eligible for public sharing from this location once established by UNMC RITO.*
If yes, estimated cost/data set for upload:	*No additional cost. Researchers may upload their own data to OMERO once access/permissions are established. AMCF will back up files to the OMERO server once a month. This is data analysis and sharing site, separate long-term, off-site data storage should be coordinated.* *To use the OMERO server, a lab permission tree must be in place. Fill out the form here: https://forms.office.com/r/6CirPy5BjH* *Available guide for assigning roles and permissions: https://docs.openmicroscopy.org/omero/5.6.1/sysadmins/server-permissions.html*
If using an outside repository, please email Peng Xiao at peng.xiao@unmc.edu. The bioinformatics Shared Research Resource may be able to assist with upload of different data types.	

Repository Information - **https://www.nlm.nih.gov/NIHbmic/nih_data_sharing_repositories.html**			
Types of Data Generated in Shared Research Resource	Metadata provided by Shared Research Resource (automatically in image files)	Suggested Repository	Tips
Biomedical/ Research Images *Zeiss Imaging Platform (710, 800, Elyra, Axioscan 7, Cell Discoverer 7)*	**.czi image files contain the following* *1) Image settings: size, pixel type, scale (x,y,z), annotations* *2) Microscope Settings: microscope, objective, reflector, contrast method, fluorescent dyes selected* *3) Camera Settings: camera, binning, exposure time, bit depth* *4) Document Info (optional, must be entered by researcher): title, subject, keywords, recording date, user information. ** these may facilitate file organization, sorting sharing*	*1) AMCF OMERO Server The public sharing of data function is under development with RITO. Contact the AMCF to verify availability is appropriate for your research needs.* *2) NIH domain-specific repository (discipline and/or funding agency). Note, researcher/funding source specific. Example: https://portal.imaging.datacommons.cancer.gov/* *3) Generalist repository if no appropriate domain-*	*See 'Other' section for details/tips.*

		specific is available. Example: https://data.mendeley.com/	
Biomedical/ Research Images Miltenyi Biotech UltraMicroscope II (light sheet microscope)	*.ome tiff or *.ims (IMARIS file) contain the following:* *1) Light Sheet Microscope meta data is stored in the first image (only), do not delete this file.* *2) Image settings (geometry): date, time, size, scale (x,y,z)* *3) Parameters: channels, display settings, laser settings, filters, x,y,z acquisition settings* *4) The system currently registers the 515 nm laser as a 405 nm. This is incorrect and awaiting a software update from Miltenyi.*	*See repository options listed above.*	*See 'Other' section for details/tips*
Biomedical/ Research Images Analyzed using HALO or IMARIS	*IMARIS Software (*.ims)* *Images are converted to *.ims files for analyses. These new files contain the following meta data.* *1) Dataset: Contains the actual image data (by channel and timepoint, from the original image meta data).* *2) Dataset Info: Contains descriptive image parameters for IMARIS. A creation histogram reports display and analysis settings for IMARIS image file.* *3) Thumbnail: 2D thumbnail of the image.* *See detailed summary: Imaris File Format - Imaris - Oxford Instruments (oxinst.com)* *HALO Software* *Images are only read by HALO, there are no 'HALO' image files.* *1) HALO databases containing display and analysis settings are generated for each analyzed image.* *2) To access these settings, go to Studies tab → select images → advanced settings tab → select settings and analysis steps performed → export details (input and output info) as a *.csv file.*	****Only the raw image data is required to be uploaded at the end of funding according to funding source requirements.* ****At the time of peer-reviewed publication, data analysis parameters for those datasets should be reported/shared.* **** Any datasets that have be altered from their original parameters should report any changes that were made to the quantified/quantifiable dataset.*	*See 'Other' section for details/tips*

Data Storage Details

==IMPORTANT: All images and data collected/analyzed at the AMCF are not permitted to contain protected health information (PHI). It is the researcher's responsibility to ensure all samples, file names, and content do not contain PHI.==

Researchers are responsible for their data once it is collected (i.e. data should be immediately transferred to another location). Everything that happens to your data after collection (i.e., analyses) is the researcher's responsibility. The AMCF can help you transfer, document, and share your data using the OMERO server. The AMCF transfers (OMERO server) all properly stored data (acquisition computers) on a monthly basis. **Data should not be stored on acquisition computers for more than 3 months and is subject to automatic removal after 3 months.**

Research data is stored in the file types described above (*.czi, *.ome tiff, *.ims). Additional meta data details for all instrumentation are available on the AMCF website.

Temporary data storage on individual workstations is limited on AMCF instrumentation. ***Data may reside on acquisition workstations for 3 months. Data in temporary transfer sites (SharePoint) used prior to 01/01/2024 will be removed after 1 year. Data transferred via BOX may remain there but is the responsibility of the researcher to back up to an additional site. Starting 01/01/2024 all AMCF data transfers will be to the OMERO server.*** Contact the AMCF team to verify your OMERO storage settings/options. The following data storage and handling capabilities are available.

Zeiss 710, 800, Elyra, CD7 acquisition workstations: Data may be temporarily (≤ 3 months) stored on the acquisition computer data storage drive (not desktop). Data drives will be transferred to the OMERO server once a month. ***No data left on these systems is guaranteed to be safe from loss, researchers are encouraged to backup data at time of acquisition and/or upload their data to the OMERO server as soon as it is generated.*** The AMCF will allow data to remain (only in the appropriate 'data drive' location) on individual acquisition workstations for up to 3 months. After this time (or improperly stored data) will be removed from the acquisition workstations without prior notification. Data not stored in the designated data drive location will not be backed up. This is not a guarantee of data safety nor security on these workstations.

Axioscan 7 Whole Slide Scanning, UltraMicroscope II Light Sheet Microscope acquisition workstations: These systems are currently connected to the internet. Data may be temporarily (≤ 3 months) stored on the acquisition computer data storage drive (not desktop). Data drives will be transferred to the OMERO server once a month. ***No data left on these systems is guaranteed to be safe from loss, researchers are encouraged to backup data at time of acquisition and/or upload their data to the OMERO server as soon it is generated.*** The AMCF will allow data to remain (only in the appropriate 'data drive' location) on individual acquisition workstations for up to 3 months. After this time (or improperly stored data) will be removed from the acquisition workstations without prior notification. Data not stored in the designated data drive location will not be backed up. This is not a guarantee of data safety nor security on these workstations.

Image Analysis Workstations (HALO, IMARIS): These systems are currently connected to the network. IMARIS rendered images (*.ims) are new images that may be stored on the OMERO server. Researchers may upload their own *.ims images to the OMERO server for transfer, storage, and sharing. HALO analyses do not modify initial images, instead a relational database (folders) store analysis parameters. HALO analyses are not compatible with the OMERO server architecture and should be stored elsewhere by the researcher. ***No data left on these systems is guaranteed to be safe from loss, researchers are encouraged to backup data at time of acquisition.*** *Data may reside on the acquisition workstation for 6 months. After this time (or data not stored on the designated 'data' drive) will be removed from the acquisition workstations without prior notification.* This is not a guarantee of data safety nor security on these workstations.

Long-Term Data Transfer Plans (starting 01/01/2024): The AMCF worked with RITO to establish long-term biomedical image transfer, management, and universal data sharing server using the OMERO platform, see below. ***OMERO is a critical organizational infrastructure for managing imaging data, is internationally accepted for publication-ready datasets, recognized by existing NIH data repositories, will allow us to eventually offer our own shareable data repository, directly reads/interfaces/integrates all raw imaging data collected on our imaging Shared Research Resource acquisition workstations, and can organize curated datasets with meta data meeting community standards for biomedical imagery.*** The OMERO server will read and organize meta data contained within biomedical images from hundreds of different systems (both inside and outside the imaging Shared Research Resource).
More information about OMERO can be found at https://www.openmicroscopy.org/omero/

Other

1) ***BEFORE IMAGING***, make sure you have an appropriate (funding specific) data sharing and management plan in place. ***DISCUSS*** your plan with AMCF staff and/or Director. Each repository has its own requirements with significant variability across data repositories. ***The AMCF anticipates the OMERO server will be appropriate for most, if not all, biomedical image data storage, sharing, and reporting.*** Researchers must let Shared Research Resource staff know prior to imaging if additional meta data is required.

2) Per NIH, "Regardless of the mechanism used to share data, each dataset will require documentation. Documentation provides information about the methodology and procedures used to

collect the data, details about codes, definitions of variables, variable field locations, frequencies, and the like. *The precise content of documentation will vary by scientific area, study design, the type of data collected, and characteristics of the dataset."* This is your meta data. When not otherwise stipulated by the funding agency, researchers are strongly encouraged to use community metadata standards (reference manuscripts below describe *evolving community standards*). Community standards generally exceed repository reporting requirements. Individual data repositories often provide guidance regarding appropriate metadata standards. ***The AMCF anticipates the OMERO server will be appropriate for most, if not all, biomedical image sharing and reporting.***

- Montero Llopis, P., et al. Best practices and tools for reporting reproducible fluorescence microscopy methods. Nat Methods 18, 1463–1476 (2021). https://rebecca-senft.shinyapps.io/MicCheck/
- Hammer, M., et al. Towards community-driven metadata standards for light microscopy: tiered specifications extending the OME model. Nat Methods 18, 1427–1440 (2021).

3) Researcher (funding source)-specific data management and sharing plans may require differing types of meta data/information. Basic system information (instrument type, date, time, excitation type/power, detector, and scan settings) are automatically contained in the image meta data and stored/reported when using the OMERO server to share raw image files (i.e. *.czi, *.ori, *.ims, *.ome tiff). Information regarding additional objective specifications (i.e., NA), filter parameters can be found on the AMCF website under equipment. The AMCF will assist researchers in obtaining additional information regarding global system configurations, as needed.

4) *.czi files (Zeiss) may be read using open-source software including ImageJ with bioformats plugin or FIJI, Zen Blue lite, QuPath. Individual repositories have specific requirements for acceptable file formats. If required by a repository, conversion of image files to opensource file formats such as OME tiffs (Open Microscopy Environment) can be used to preserve meta data in a universal file format. This option is available in most programs readily interfacing with *.czi files. *The OMERO server will readily open and report meta data for all image types collected in the AMCF.*

> https://imagej.net/formats/bio-formats
> https://imagej.net/software/fiji/downloads
> https://www.zeiss.com/microscopy/en/products/software/zeiss-zen-lite.html
> https://qupath.github.io/

5) Raw/unaltered image data is maintained in the *.czi file, if anything other than the initial raw data is analyzed for the final dataset, researchers should verify and record all downstream analysis parameters.

6) Volumetric mosaic images from the UltraMicroscope II/Light Sheet Microscope (TIBA affiliated instrumentation) can be stitched together using ImageJ/FIJI BigStitcher (OME Tiff) or using the IMARIS stitch program (requires initial storage as, or conversion to *.ims file type). IMS files can be viewed using free viewer software.

> https://imaris.oxinst.com/imaris-viewer
> https://imagej.net/plugins/bigstitcher/advanced-stitching

7) The AMCF OMERO server can accommodate over 160 different types of biomedical images in their parent/raw file format. Researchers are encouraged to use the OMERO server to transfer, access, and analyze all biomedical images, regardless of where they were acquired on campus.

8) When required, conversion/storage as *.ome tiff can significantly increase files sizes (2-3x). Please ensure adequate storage space and upload 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.

- 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.

detail?

Some helpful links:

[Data Management & Sharing Policy Overview | Data Sharing (nih.gov)](#)

ABRF [presentation](#) & [recording](#); NIH Webinar [Part 1](#) & [Part 2](#); [NIH Webpage.](#)
UNMC data sharing [website](#). Harvard Biomedical Data Management [website](#).

FAQs:

1. **Will shared research resources (Shared Research Resources) automatically share my data?** *No. shared research resources ONLY deliver data and associated shared research resource-generated metadata to the investigator. These data are the investigator's data.*

2. **Will shared research resources upload my data to a repository if I ask?** *It depends. Some shared research resources will assist with upload into a repository for an additional fee. Others do not have the personnel to support this effort. Please see the data management & sharing plan reference on the shared research resource's websites.*

3. **Does this mean shared research resources will store data for researchers the life of the grant and required storage when award ends?** *No, each UNMC shared research resource has its own data retention process and practices. Most UNMC shared research resources store data for 3- 6 months. The investigator is required to download data in that time frame.*

4. **What repository should I use?** *Investigators may choose to utilize a different repository to meet their needs or sponsor requirements. Of note, significant time and resources are often required to curate data for upload into these repositories. We strongly encourage investigators to use an electronic lab notebook to assist with downstream data management & sharing requirements.*

5. **What about metadata?** *Each repository has its own metadata requirements. Shared research resources will provide the investigator metadata elements that only the Shared Research Resource can provide (e.g., specific instrument model, vendor software version, etc.) accompanying the data you normally receive from the Shared Research Resource. Metadata elements for all instruments in the AMCF are available on the AMCF website.*