

Multiscale Microscopy Core (MMC):

The MMC is a 5500 sq. ft. University Resource Shared core facility that is housed in the RLSB that provides fee-for-service access to state-of-the-art imaging platforms including: a Helios NanoLab™ G3 DualBeam™ Focused Ion Beam (FIB) scanning electron microscope (SEM) which is used for automated high resolution 2D and 3D scanning electron microscope (SEM) image acquisition and Focused Ion Beam (FIB) sample preparation; a Volume Scope II Serial Block Face SEM for automated 3D SEM image acquisition, a Zeiss CrossBeam 550 outfitted with a Leica cryostage for Focused Ion Beam (FIB) sample preparation and 3D data collection; a Tecnai 120kV TEM for conventional TEM image acquisition and a Corrsight™ fluorescence microscope, equipped with cryo capabilities, dedicated for correlative light and electron (CLEM) microscopy projects. OHSU scientists in the MMC provide expert support with training, imaging and sample preparation services to all internal and external core users. The MMC is directed by Dr. Claudia López.

FEI Helios G3 Nanolab™ FIB/SEM. The Helios NanoLab™ G3 Dualbeam™ features FEI's most recent advances in field emission SEM (FESEM), FIB technologies and their combined use. This DualBeam™ platform is designed to access a new world of extreme high resolution 2D and 3D characterization, nanoprototyping, and higher quality sample preparation. This equipment is outfitted with detectors set for highest collection efficiency of SE (secondary electrons) and on-axis BSE (backscattered electrons), in-lens SE and BSE detector (TLD-SE/BSE), Elstar in-column SE detector (ICD), Elstar in-column BSE detector (MD) and also an Everhart-Thornley SE detector (ETD). The latest advanced detection suite includes high efficiency detectors for SE and BSE imaging and novel detectors (MD and ICD): two multi-segment solid state detectors for low kV SE/BSE and S/TEM performance. Robust, precise FIB slicing, combined with a high precision piezo stage and superb SEM performance support automated software for unattended sample preparation or 3D characterization and analysis. The Helios NanoLab™ G3 has the MAPS™ software for automatic acquisition of large images and correlative workflow, and AutoSlice and View™ software for automated sequential mill and view to collect series of slice images for 3D volume reconstruction.

Thermo Scientific™ Volumescape 2™ Serial Block Face-Scanning Electron Microscope (SBF-SEM). The Thermo Scientific VolumeScope 2 with Multi-Energy Deconvolution (MED) is a state-of-the-art serial block face-scanning electron microscope that combines physical and optical slicing technologies with 10 nm isotropic 3D datasets of resin embedded biological samples. This field-leading 10 nm isotropic resolution is possible through the use of MED-SEM technology, allowing optical sectioning to derive several virtual subsurface layers within each physical slice, thus dramatically improving resolution, particularly in the axial direction. Acquired volumes are typically larger than those collected with FIB-SEM technology. The instrument can also be utilized as a stand-alone SEM with low-vacuum capability for traditional imaging of non-conductive samples.

ZEISS Crossbeam 550 Microscope with Cryo-stage and Atlas 5 Software. The ZEISS Crossbeam 550 SEM combines high resolution, contrast, and signal-to-noise ratios of the GEMINI II column with the precise milling of the next-generation Ion-sculptor focused ion beam column. The Atlas 5 software can be used for automatic 3D image acquisition using FIB milling of bulk samples or array tomography of serial

sections on coverslips. The microscope is additionally equipped with a cryo-stage that allows for imaging and automated TEM lamella preparation of frozen, vitrified samples.

FEI Tecnai TEM. FEI-Tecnaï 12 system interfaced to a bottom mounted Eagle™ 2K TEM CCD multiscan camera and to a NanoSprint12S-B CMOS camera from Advanced Microscopy Techniques (AMT) fast side-mounted TEM CCD Camera. The system is also equipped with cryo shields for cryo-TEM imaging.

FEI Corrsight™. The Corrsight is an advanced light microscope that integrates multiple sample preparation protocols for correlative experiments, enables researchers to observe live cell dynamics using visible light microscopy and quickly fixes those cells for follow-on light and EM when a targeted event or structure is identified. The Corrsight system completes OHSU's correlative microscopy workflow and will be used by researchers to develop correlative light and electron microscopy (CLEM) assays for high-content drug screening applications. This advanced light microscope is a unique modular system that integrates multiple sample preparation protocols for CLEM experiments. Vitrified samples can be transferred to the cryo Corrsight module, where structures of interest are identified by fluorescent tags (such as GFP). The inverted design of FEI's Corrsight™ light microscope provides ample room for the cryo-cooling module required to keep the sample in its vitrified state during cryo-fluorescence imaging. Control of both light and EM platforms is accomplished using one single, easy-to-use software package called MAPS™. The Corrsight has various specimen holder to clamp conventional coverslips, small Petri dishes, IBIDI slides and TEM grids.

Thermo Scientific™ Glacios Cryo-Electron Microscope with Gatan K3 Camera (Summer 2020): The Glacios Cryo Transmission Electron Microscope (Cryo-TEM) from Thermo Fischer delivers a complete and affordable Cryo-EM solution to a broad range of scientists. It features 200 kV XFEG optics, the industry-leading Autoloader (cryogenic sample manipulation robot), and the same innovative automation for ease of use as on the Krios G4 Cryo-TEM. The Glacios Cryo-TEM bundles all this into a small footprint that simplifies installation. The Glacios microscope will be equipped with the Ceta 4k x 4k CMOS based sensor to assure rapid access to high quality images and a Gatan K3 camera, the new imaging performance benchmark for direct detection cameras. This next generation camera is optimized for the most demanding low-dose applications in both life science and materials science research. The K3 camera is the complete and latest expression of Gatan's deep experience in the delivery of real-time, single electron counting direct detection cameras.

The MMC also provides users with ancillary instruments for sample preparation and a modern wet laboratory with dedicated fume hoods for EM sample processing and a fully equipped tissue culture room. The wet lab is equipped with a UC7 Cryo-Ultramicrotome (Leica), 3D ARTOS ultramicrotome to produce multiple high-quality ribbons of plastic sections (Leica), High Vacuum Metal/Carbon Coater ACE 600 (Leica), cryo-Coater with e-Beam deposition ACE600 (Leica), VCM Vacuum Cryo Manipulation System (Leica), Vitrobot (FEI) for cryo sample preparation, a single tilt holder tomography holder Gatan 626 Cryo Holder, High-pressure Freezer (Wohlgend) and Freeze Substitution Embedding System (Leica) for freeze-substitution sample preparation, critical point dryer (CDP300 Leica), EasiGlow Discharge Unit

(Pelco) for TEM grid preparation, BioWave Microwave (Pelco) for sample processing, liquid nitrogen storage dewar for cryo-processed samples, -80C UltraLow Freezer, -20C Freezer, 4°C Refrigerator (Flammable-safe). The facility also provides the users with two Dell Precision T7610 Workstations for image analysis and processing, and dedicated image processing software.