The Multiscale Microscopy Core (MMC) is a state-of-the-art electron microscopy core facility that was established in 2013 through the collaborative efforts of the OHSU Center for Spatial Systems Biomedicine (OCSSB) and Hillsboro-based FEI Company. It offers comprehensive technical support for tissue, cellular, subcellular and molecular imaging, imaging services and training to academic and corporate users.

**Lab/physical space:**
The MMC is located in a 5,500 sq. ft. specially designed low-vibration microscopy suite in the new Collaborative Life Sciences Building (CLSB) on the OHSU South waterfront campus. The MMC, along with a satellite of the Advanced Light Microscopy Core (ALMC) co-located in the suite, work together to provide integrated services to analyze samples across the full microscopy resolution spectrum, including correlative light and electron microscopy capabilities.

**Instrumentation/Equipment:**
The MMC is a University Shared Resource core facility that is housed in the CLSB and provides fee-for-service access to state-of-the-art imaging platforms including:

- Helios NanoLab™ 660 DualBeam™ 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;
- FEI Teneo VolumeScope™ with ThruSight™ Multi-Energy Deconvolution technologies, which is a state-of-the-art serial block face imaging scanning electron microscope that combines physical and optical slicing technologies to produce up to 10nm isotropic 3D datasets of resin embedded biological samples;
- Titan™ Krios™ 300kV Cryo-Electron microscope (which is an automated transmission electron microscope (TEM) platform optimized for Single Particle Analysis and 3D cryo-TEM applications;
- Tecnai with iCorr™, an integrated 120kV TEM and light microscope that allows quick and easy navigation of the EM sample based on the fluorescence signal and a Corrsight™ spinning disk (SD) fluorescence microscope, an ideal platform for correlative light and electron (CLEM) microscopy projects.

*Specifications for the above can be viewed in a separate document on the core’s pure profile.
Ancillary Instrumentation and Wet Laboratory for EM sample preparation: 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 (including an EVOS inverted digital microscope). The wet lab is equipped with:

- ASH ultramicrotome (RMC Inc., Boeckeler Instruments) with a novel substrate clamping mechanism, for collecting tens to hundreds of serial sections upon a variety of rigid substrates for array tomography-like investigations or wide scale tissue/tumor mapping by SEM, enabling semi-high throughput EM and an easy to use solution for collecting ultra-thin or thick resin sections for CLEM and EM upon substrates that are ideal for imaging by light microscopy and SEM,
- UC7 Cryo-Ultramicrotome (Leica), UC7 Ultramicrotome (Leica), Vitrobot Mark IV (FEI) for cryo sample preparation, a single tilt holder tomography holder Gatan 626 Cryo Holder,
- high vacuum Sputter Coater and Carbon Evaporator (Leica ACE 600 Sputter with Platinum and Gold targets and Carbon Thread coater),
- Hummer-X low vacuum sputter coater with Platinum and Platinum/Palladium targets,
- 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 three Dell Precision T7610 Workstations for image analysis and processing, and dedicated image processing software for 3D image reconstruction and segmentation (FEI Amira 6). There is also 300 sq. ft. of MMC satellite lab at a second OHSU location that contains a FEI Tecnai Spirit 12 system interfaced to a 16 megapixels digital camera and associated software (Advanced Microscopy Techniques, Danvers, MA), High-pressure Freezer (Wohlwend) and Freeze Substitution Embedding System (Leica) for sample preparation.

Tissue Culture Facilities: Inverted tissue culture microscopes, biosafety cabinets, cell culture incubators, water baths, and accessories for maintaining cells in very close proximity to instrumentation for live-cell imaging and sample preparation equipment for EM analysis.

Imaging Analysis Stations: MMC users have access to three high-end workstations for data analysis of image data. Full-featured off-line licenses for vendor-specific software packages are available, paired with open-source Fiji/ImageJ/OMERO software, to facilitate simple data analysis task and image export tasks. Complex 3D and 4D data can be analyzed with advanced tools available in Amira 6 (FEI), Vision4D (Arivis), and Imaris (Bitplane). Core staff provides local training and support, with additional support available via web-based training by technology experts at the respective software companies.

Compute Infrastructure and Data Storage: OHSU’s recent capital investments included construction of a new off-site data center that houses OHSU’s ExaCloud advanced computing system, borne out of collaboration between locally headquartered Intel, Inc. and OHSU. The CLSB microscopy suite has high speed connectivity to a local server room and the data storage center. Due to extremely high data flows from light and EM microscopes, a pre-processing server cluster has been placed physically close to the
instruments. The microscopes are connected via 10 Gb/s copper and fiber-optic interconnects to the server cluster, where image data can be pre-processed in real-time to register and align images, extract targets, and be compressed in a data-dependent method. The pre-processed and compressed data is then transferred to a larger server cluster at the off-site data center for in-depth processing and analysis.

The Intel-OHSU ExaCloud is a high performance computing system developed to deploy large-scale, computational and data intensive workflows. Through collaboration with OHSU, Intel engineers develop computational hardware and software to enable and accelerate the computational workflows and make their solutions available first at OHSU and later world-wide (all software solutions are open source). The primary cluster includes 10,000+ Xeon cores with 40+ TB of memory distributed across 300+ compute nodes. A subset of higher-capacity interconnected compute nodes are primarily used for complex image transformation tasks, such as performing single particle reconstructions derived from data acquired with the cryo-TEM microscope. Recently, the Intel-OHSU collaboration was expanded to include the development of an ambitious Collaborative Cancer Cloud system, combining Intel's strengths with OHSU's clinical workflows and innovative four-dimensional approach to imaging and analyzing the molecular-level drivers of cancer. The server architecture includes more than a petabyte of fault tolerant data storage, available to Investigators on payment basis. Off-site tape backup, with tape rotation to a secure location, keeps mission-critical data safe while maintaining compliance with OHSU data retention policies.

Quality Control: Acquisition of high quality image data is critically dependent on proper instrument functionality. MMC staff regularly assesses performance of individual instruments and completes routine maintenance tasks to keep instruments in good working condition. All major instruments in the MMC are covered under service contracts from the respective manufacturers that include yearly preventative maintenance visits and ensure rapid response time when equipment fails. In addition to routinely checking instrument function, MMC staff also offer users assistance in evaluating proper sample preparation.

Scientific Environment:
The MMC receives support from the Knight Cancer Institute, the OHSU Center for Spatial Systems Biomedicine (OCSSB), the OHSU School of Medicine, the University Shared Resources, the Murdock Foundation and FEI. FEI is a leading diversified scientific instruments company developing electron and ion-beam microscopes and other instruments for nanoscale applications across many industries. As part of the OHSU-FEI Living Laboratory FEI scientists work onsite with MMC staff and OCSSB faculty to develop new instrument capabilities and optimized protocols for user applications. For example, Danielle Jorgens, Ph.D. (Research Assistant Professor, BME), works with Dr. López toward the development of novel, integrated CLEM and 3D-EM workflows that incorporate microwave-assisted and cryo-based sample preparation techniques (high pressure freezing and freeze substitution, plunge freezing), as well as back-scattered EM techniques. Craig Yoshioka, PhD (Research Assistant Professor, BME), provides support for collaborators with optimizing collection of TEM data, processing that data efficiently and effectively, and assisting with interpretation. This assistance also incorporates many aspects of system administration and software development, and ties into longer-term efforts to improve the quality of TEM tooling. This includes working with FEI and the MMC in the development of near-real-time data processing software, and testing prototype hardware from Intel with the goal of making TEM data processing cheaper and more efficient.
Expertise:

Claudia López, PhD, Research Assistant Professor in the Biomedical Engineering Department, MMC Manager. Dr. López manages MMC technical operations and promotes the use of EM in basic and translational research at OHSU. Her formal training is in biochemistry, biophysics and molecular biology. Dr. López has 8 years of experience with EM research including a variety of TEM and SEM applications for analysis of both biological (cells and tissues) and non-biological materials (hard materials and fabrics). She has extensive expertise in conventional and cryo-EM sample preparation and drives protocol development of CLEM sample preparation for the OHSU-FEI Living Lab. Dr. López manages day-to-day EM operations and her responsibilities include instrument maintenance and standardized testing; meeting with investigators to advise, plan and schedule new experiments; providing sample preparation and imaging services; and distributing data and explaining results. For Investigators who request training of their own staff, Dr. López performs all training to ensure that users are fully prepared for independent use of the EM equipment. Since 2012 she has partnered with FEI to develop and present a series of technology-based workshops.

Heidi Feiler, PhD, Research Associate Professor in the Biomedical Engineering Department and Deputy Director of the OHSU Center for Spatial Systems Biomedicine. Dr. Feiler has focused her research in the human genomics and cancer fields. As a senior scientist at the Gallo Institute/UC San Francisco (UCSF) she directed a GWAS laboratory and at Lawrence Berkeley National Laboratory (LBNL) she directed a high-throughput microarray core facility. As a scientist and project manager she has coordinated multi-site grants awarded by NIH, NCI, DOD and also Stand up to Cancer at UCSF, LBNL, and now at OHSU, for over 15 years. She is currently the Project Manager of Joe Gray’s NCI/Integrative Cancer Biology Center for Cancer Systems Biology, and a NIH LINCS Center aimed at understanding the influence of microenvironmental perturbations on cell signaling networks. As the Business Director of the MMC, her responsibilities include: optimizing the business model; coordinating the generation of proposals for the acquisition of new core instrumentation; managing the activities of the EM user groups, and garnering input from OHSU faculty and MMC users to expand EM services. Dr. Feiler also assists Dr. Gray with the management of the OHSU-FEI Living Laboratory, including negotiation of FEI sponsored instrument upgrades and the development of a Nanoport showcase of jointly developed CLEM workflows for cellular and subcellular visualization of targeted proteins in cancer cells.

Melissa Williams, MS, Senior Research Associate, has over 18 years of experience as an EM technician, in part helping to establish an EM facility at Max-Planck-Institut für Verhaltens-physiologie. Ms. Williams worked as an EM assistant for both a research lab and the Department of Pathology at OHSU before taking on the dual role of support for EM research conducted in the Joe Gray lab and also providing technical support to MMC users. She has a strong background in standard and microwave assisted sample preparation techniques and immune-labeling. Ms. Williams assists Dr. López in guiding EM users on all parts of EM workflow, from specimen preparation to ultramicrotomy to imaging.