

The OHSU-FEI Living Laboratory and the Multiscale Microscopy Core (MMC):

The MMC is a 5500 sq. ft. University Resource Shared core facility that is housed in the CLSB that provides fee-for-service access to state-of-the-art imaging platforms including: a 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; a Teneo Volume Scope Serial Block Face SEM for automated 3D SEM image acquisition, a 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; a Talos Arctica 200kV Cryo-TEM, a 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 two Corrsight™ fluorescence microscopes, one equipped with cryo capabilities, dedicated for correlative light and electron (CLEM) microscopy projects. Thermo Fisher Scientific (formerly 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. 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 and MMC business operations by Dr. Heidi Feiler.

FEI Helios 660 Nanolab™ FIB/SEM. The Helios NanoLab™ 660 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. The outstanding imaging capabilities of the Helios NanoLab™ 660 begin with its Elstar™ FESEM, which utilizes an integrated monochromator and beam deceleration to deliver sub-nanometer resolution across the whole 1-30 kV range. The Elstar features other unique technologies such as constant power lenses for higher thermal stability and electrostatic scanning for higher deflection linearity and speed. It has Elstar 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 two novel detectors (MD and ICD): two multi-segment solid state detectors for low kV SE/BSE and S/TEM performance. For unsurpassed fast, precise and reliable milling, patterning and ion imaging, the Helios NanoLab™ 660 Dualbeam™ relies on the Tomahawk FIB. The Tomahawk's exceptional low-voltage performance is proven to produce the world's best quality thin samples for high resolution STEM or atom probe microscopy. It provides excellent ion image resolution, with integrated differential pumping and time of-flight correction. It also delivers a tighter beam and a more accurate scan profile for extremely precise ion milling. Creating the most complex structures at the nanoscale is equally possible with beam chemistries (gas injection, carbon and platinum), 16-bit pattern generator and integrated CAD, script or library-based patterning. 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™ 660 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.

FEI Teneo VolumeScope™ with ThruSight™ Multi-Energy Deconvolution: is a state-of-the-art serial block face imaging (SBFi) scanning electron microscope that combines physical and optical slicing technologies to produce up to 10nm isotropic 3D datasets of resin embedded biological samples. This field-leading 10nm isotropic resolution is possible through the use of FEI's innovative ThruSight™ multi-energy deconvolution technology, which allows optical sectioning to derive several virtual subsurface layers within each physical slice, thus dramatically improving resolution, particularly in the axial direction. The system tightly integrates FEI's latest high performance scanning electron microscopy (SEM) platform with an on-stage microtome for in-situ sectioning and ThruSight™ Multi-Energy Deconvolution to provide fully-automated, large-volume reconstructions with dramatically improved Z-resolution. The full integration of all SBFi capabilities ensures ultimate throughput, ease-of-use and utmost flexibility of the instrument for various samples and experiments. The compact ultra-microtome is modular and removable to allow low to high resolution 2-dimensional SEM imaging. The Teneo VSTM has detectors for highest collection efficiency of SE and in-column BSE, annularly segmented in-lens T1 and in-lens T2, and also an Everhart-Thornley SE detector (ETD). The novel T1 detector provides highest contrast BSE imaging, optimal for low kV imaging modes. The Teneo VSTM is provided with MAPSTM software integration for wide-field tissue mapping (Tiling and Stitching) and correlative light and electron microscopy (Correlative Workflow) studies. Furthermore, the SBFi data generated by the Teneo VSTM is fully compatible with feature extraction and analysis by Amira™ software.

RMC/Boeckeler ASH: is an Advanced Substrate Holder and Ultramicrotome for collecting serial sections upon a variety of rigid substrates for array tomography-like investigations (Spomer et al 2015, Wacker et al. 2015). The ASH is a novel substrate-clamping mechanism coupled to a normal ultramicrotome that offers a simple approach for the collection of tens to hundreds of sections at a time, enabling semi-high throughput EM. The ASH ultramicrotome is a standard unit and will accept any type of diamond knife.

FEI Tecnai with iCorr™ TEM. FEI-Tecnaï 12 system interfaced to a bottom mounted Eagle™ 2K TEM CCD multiscan camera and to a MegaViewG2 fast side-mounted TEM CCD Camera. The system is also equipped with cryo shields for cryo-TEM imaging. The most critical "tie point" between imaging modalities is between fluorescence microscopy (FM) providing nanometer to micrometer resolution information about functional molecular complexes and EM providing Angstrom to nanometer resolution information about molecular ultrastructure. Working at this interface has been difficult because cell preparation techniques differ substantially for FM and EM, transferring a sample between FM and EM instruments is time consuming, and computer algorithms to integrate and display the resulting multiscale images are lacking. The Tecnai with iCorr™ is a novel integrated light / TEM that was released in commercial form at OHSU in 2012. Scientists at FEI are working with scientists at OHSU to develop novel sample fixation and staining procedures for the Tecnai with iCorr™ that preserve both ultrastructural and molecular features for FM and TEM and reduce sample preparation times. Key applications include developing multi-parameter data management, visualization and modeling systems, and for correlative microscopy, the synthesis of labeled reporter capsids suited for FM/EM correlative

imaging, such as those that isolate the metal particles from the fluorescently labeled targeting groups on the surface. These methodologies combined with the Tecnai with iCorr™ instrumentation will allow us to overcome “tie point” limitations.

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.

CorrSight: Live cell imaging of dynamic events in intact cells or organisms using an integrated high-speed Andromeda spinning disk microscope offers fast optical sectioning and is conducted with environmental control. Direct chemical fixation and EM staining is conducted with automated microfluidics. 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.

Cryo Corrsight™: 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.

FEI Titan Krios™ Ultra-high resolution TEM. The Titan Krios™ is a state-of-the-art, dedicated imaging robot, optimized for both high-resolution 3D imaging of proteins and macromolecular assemblies, and high-resolution, dual-axis tomography. The Titan Krios™ is a 300 kV FEG Transmission Electron Microscope, which combines excellent performance with ease of operation in all modes of imaging such as TEM, STEM, TEM/STEM tomography, EFTEM. It is equipped with a unique, automated (cryo) sample loader/unloader system for sample handling at liquid nitrogen temperature. It masters the complete dynamic range from live observation of focused high intensity beams to low dose applications and diffraction. The Titan Krios™ is the most powerful and flexible high resolution electron microscope for 3D characterization of biological samples; tailored for use in protein and cellular imaging. Its revolutionary cryo-based technology and stability permits a full range of semi-automated applications, including: 2D electron crystallography, single particle analysis, cryo-electron microscopy, and dual-axis cellular tomography of frozen hydrated cell organelles and cells. Cryo-techniques preserve sample integrity by maintaining the sample in its natural condition and state. An enclosed platform combines excellent optical performance and thermal and mechanical stability with a high throughput sample loader. The automated dual-axis tomography uses a specially designed holder that allows the sample to rotate 90 degrees in plane while being present in the column, providing a reduction of the missing wedge information to a missing cone. The flexibility of operating the Titan Krios™ in the range of 80-300 kV allows for optimizing high tension to the requirements of the material under examination - ranging from vitrified suspensions to unstained cryo-sections. The Titan Krios™ has a Quantum energy filter

(Gatan), a FEI Falcon™ III 16 Megapixel Direct Electron Detector with Back-Thinned Sensor Technology and multi-frame readout capabilities and a high speed digital camera. The installed FEI Phase plate technology allow close-to-focus imaging of cryo-preserved biological material with significant phase contrast enhancement. For ease of use, a digital user interface allows for remote operation. A Gatan K2 Summit® electron counting direct detection camera is being installed and will provide electron counting modes to improve both contrast and resolution for large and small molecules alike. This camera provides the highest performance sensor for both resolution and contrast superior detective quantum efficiency (DQE) required for protein samples below 500 kDa at high resolution and also provides high quality cryo-tomography.

FEI Talos Arctica™: The Talos Arctica™ is a 200 kV FEG Transmission Electron Microscope equipped with a unique, automated (cryo) sample loader/unloader system for sample handling at liquid nitrogen temperature. An enclosed platform combines excellent optical performance and thermal and mechanical stability with a high throughput sample loader. The Talos Arctica™ is equipped with a Ceta FEI 4k × 4k CMOS based sensor to assure rapid access to high quality images. Its movie recording capabilities enables high quality 4k × 4k movies at 1 fps or 512 × 512 movies at 25fps. This electron microscope is also equipped with a Gatan K2 Summit® electron counting direct detection camera.

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), Vitrobot (FEI) for cryo sample preparation, a single tilt holder tomography holder Gatan 626 Cryo Holder, high vacuum Sputter Coater and Carbon Evaporator (ACE 600 Sputter with Platinum and Gold targets and Carbon Thread coater), High-pressure Freezer (Wohlwend) and Freeze Substitution Embedding System (Leica) for freeze-substitution sample preparation, 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 two Dell Precision T7610 Workstations for image analysis and processing, and dedicated image processing software.