**EQUIPMENT**

*Washington University Center for Cellular Imaging (Fitzpatrick, WUSM)*:

The center occupies ~6,500 square feet and includes: (i) dedicated rooms for light microscopy (3 rooms, total of 16 stations, including 4 setups on large optical tables), (ii) dedicated vibrational isolated rooms to accommodate six electron/ion microscopes and large purpose-designed rooms for electron microscopy prep (including a large wet laboratory area for freezing and sample preparation and a sectioning room for ultramicrotomy), (iii) two separate tissue culture rooms, (iv) an animal holding space (for <24 hr use during imaging), (v) a 10-desk computer area for image analysis and processing, and (vi) a workshop for equipment maintenance.

*Light Microscopy*

**Confocal**

1. Nikon A1R Resonant Scanning Confocal Microscope (inverted, live cell capable)
   The Nikon A1Rsi is a scanning confocal microscope is based on a motorized inverted Nikon Ti-E microscope frame equipped with the PerfectFocus stability mechanism. The system is configured with six laser lines (405, 445, 488, 514, 561 and 640nm), 10 and 20X dry objectives and 40, 60 and 100X high NA oil immersion objectives as well as a motorized xy-stage and a nano-positioning piezo z-insert. The hybrid scan head, incorporates both a high-resolution galvo scanner and an ultra-high speed resonant scanner, enabling simultaneous photo-bleaching / photo-activation and image acquisition and is equipped with two GaAsP PMTs, and two high-sensitivity PMT detectors for four-color confocal imaging as well as a transmitted light PMT for simultaneous DIC imaging. The microscope is also equipped with a Tokai-hit stage-top incubation system to facilitate live cell imaging with control of $CO_2$, $O_2$, temperature and humidity.

2. Nikon Spinning Disk Confocal Microscope (inverted, live cell capable)
   The Nikon Spinning Disk (SD) confocal microscope is based on a motorized inverted Nikon Ti-E microscope frame equipped with the PerfectFocus stability mechanism. The system is configured with four laser lines (405, 488, 561 and 640nm), 10 and 20X dry objectives and 60 and 100X high NA oil immersion objectives as well as a motorized xy-stage and nano-positioning piezo z-insert. The system is fitted with a Yokagawa CSU-X1 variable speed Nipkow spinning disk scan head for the confocal illumination and imaging of an entire field of view (FOV) simultaneously on a camera. The scan head is equipped with an Andor TuCam two-camera adapter that enables simultaneous two-color confocal imaging at video-rate (30fps) on two separate Andor Zyla 4.2 Megapixel sCMOS cameras. The system is also equipped with an LED-based DMD system for ultrafast photo-stimulation and a Tokai-hit stage-top incubation system to facilitate imaging in living cells with precise control of $CO_2$, $O_2$, temperature and humidity.

3. Olympus FV1200 Confocal Microscope (upright, live cell capable)
   The Olympus FV1200 scanning confocal microscope is based on an upright BX61WI frame. The system is configured with six laser lines (405, 445, 488, 515, 559 and 635nm), 10X dry, 10, 20 and 40X water immersion objectives and 60 and 100X high NA oil immersion objectives. The FV1200 scan head is equipped with two GaAsP PMTs and three high-sensitivity PMT detectors (2 spectral and 1 filter-based) for five-color confocal imaging, and a transmitted light PMT for simultaneous DIC imaging. The spectral PMTs are capable of acquiring spectral image data at 2nm resolution. The system is also equipped with a motorized xy-stage and a separate high-speed SIM laser scanner enabling simultaneous photo-bleaching / photo-activation and multi-point image acquisition.

4. Zeiss LSM 880 Airyscan FAST Confocal Microscope (inverted, live cell capable)
   The Zeiss LSM 880 Airyscan FAST confocal microscope is based on a motorized inverted Zeiss Axio Observer Z1 microscope frame. The system is configured with six laser lines (405, 458, 488, 514, 561 and 633nm), 10 and 20X dry objectives and 40, 60 and 100X high NA oil immersion objectives as well as a motorized xy-stage and a nano-positioning piezo z-insert. The system is equipped with a unique scan head, incorporating a high-resolution galvo scanner along with two PMTs and a 32-element spectral detector as well as a transmitted light PMT for simultaneous DIC imaging. In addition, the Airyscan unit provides sub-diffraction limited imaging down to 140nm lateral resolution at 27fps with the FAST beam shaping module. The system is also equipped with a Pecon stage-top incubation system to facilitate imaging in living cells with precise control of $CO_2$, $O_2$, temperature and humidity.

**Super-resolution**

5. Nikon Structured Illumination Super-Resolution Microscope (inverted)
   The Nikon n-SIM microscope is based on a motorized inverted Nikon Ti-E microscope frame equipped with the PerfectFocus stability mechanism. The system is configured with four laser lines (405, 488, 561 and 640nm) and 60 and 100X high NA oil immersion objectives as well as a motorized xy-stage and a nano-positioning piezo z-insert. Fluorescence detection is provided by two Andor Zyla 4.2 Megapixel sCMOS cameras fitted to an Andor TuCam dual camera adapter, which facilitates simultaneous two-color and sequential four-color super-resolution imaging.

6. Nikon TIRF/STORM Super-Resolution Microscope (inverted, live-cell capable)
   The Nikon n-STORM TIRF Microscope is based on a motorized inverted Nikon Ti-E microscope frame equipped with the PerfectFocus stability mechanism. The system is configured with four laser lines (405, 488, 561 and 640nm), 10 and 20X dry objectives and 60 and 100X high NA oil immersion objectives as well as a motorized xy-stage and a nano-positioning piezo z-insert. Fluorescence detection is provided by either an Andor Zyla 4.2 Megapixel sCMOS camera when imaging in TIRF mode or an Andor iXon 897 EM-CCD when imaging in single-molecule or STORM (STochastic Optical Reconstruction Microscopy) super-resolution imaging mode. The system is also equipped with a Tokai-hit stage-top incubation system to facilitate imaging in living cells with precise control of $CO_2$, $O_2$, temperature and humidity.

**Two-photon**

7. Nikon A1RMP Confocal/Two-photon Microscope (upright, intravital capable)
   The Nikon A1RMP confocal microscope is based on an FN1 upright microscope frame. The system is configured both visible laser lines (405, 488, 561 and 640nm) and a Coherent Chameleon Ultra-II femtosecond infrared laser which spans 680-1080nm for two-photon excitation, 10 and 20X dry objectives, 16 and 25X water immersion objectives and a 10X glycerin-immersion objective as well as a motorized xy-stage and a Prior z-drive. The system is equipped with a unique scan head, incorporating high-resolution galvo and resonant scanners along with 4 PMTs (3 x GAsP and 1 x high-sensitivity PMTs for fluorescence detection. The system is also equipped with 4 non-descanned detectors (NDDs) (3 x GAsP and 1 x high-sensitivity) for two-photon imaging in thick tissue specimens, ex vivo preparations and in vivo imaging experiments.

8. Zeiss LSM 880 Airyscan Confocal/Two-photon Microscope (inverted, live cell capable)
   The Zeiss LSM 880 Airyscan confocal microscope is based on a motorized Zeiss Axio Observer inverted microscope frame. The system is configured both visible laser lines (405, 458, 488, 514, 561 and 633nm) and a Coherent Chameleon Discovery femtosecond infrared laser which spans 680-1300nm for two-photon excitation, 10 and 20X dry objectives, 40 and 60X water immersion objectives and a 100X high NA oil immersion objective as well as a motorized xy-stage and a nano-positioning piezo z-insert. The system is equipped with a unique scan head, incorporating a high-resolution galvo scanner along with two PMTs and a 32-element spectral detector as well as a transmitted light PMT for simultaneous DIC imaging. In addition, the Airyscan unit provides sub-diffraction limited imaging down to 140nm lateral resolution. The system is also equipped with two non-descanned detectors (NDDs) for two-photon imaging in thick tissue specimens and ex vivo preparations

**Fluorescence / Bright-field**

9. Zeiss Axio Imager.Z2 Fluorescence Microscope with ApoTome optical sectioning device (upright)
   The Zeiss Axio Imager is a motorized upright brightfield / fluorescence microscope equipped with an ApoTome 2 optical sectioning grid imager. The system is equipped with an LED illumination source and filtersets for DAPI, GFP, RFP and far-red imaging as well with the relevant prisms and sliders for Differential Interference Contrast Microscopy (DIC). The system is equipped with two cameras; a Hamamatsu Orca Flash sCMOS camera for fluorescence and a color CCD camera for histological imaging.

10. Zeiss Axio Zoom.V16 Fluorescence Macroscope (upright)
    The Zeiss Axio Zoom.V16 is a motorized zoom fluorescence macroscope equipped with LED illumination and filtersets for DAPI, GFP, RFP and far-red imaging as well with a white light source for transmitted light imaging. The system is equipped with two cameras, a Hamamatsu Orca Flash sCMOS camera for fluorescence and a color CCD camera for histological imaging.

11. Zeiss AxioScan combined Fluorescence and Bright-field Slide Scanner with 100-slide capacity
    The Zeiss Axio Scan Z1 is a fully automated brightfield / fluorescence slide scanner. The system is equipped with an LED illumination source and filtersets for DAPI, GFP, RFP and far-red imaging. The system is equipped with two cameras; a Hamamatsu Orca Flash sCMOS camera for fluorescence and a color CCD camera for histological imaging.

**Light-sheet**

12. Zeiss Lightsheet Z.1 (live-cell capable)
    The Zeiss Lightsheet Z.1 planar illumination microscope is suitable for imaging dynamic processes in developing organisms, live organoid cultures as well as the anatomical content of cleared tissues. The system is configured with dual lightsheet illumination arms, visible laser lines (405, 488, 561 and 638nm), 5X dry and 10X, 20X, 40X and 63X water immersion and 20X (1.38ri - CLARITY) and 20X (1.45ri - SCALE) (Note +/- 0.03 on RI) objectives and a motorized xyz stage. The system is also equipped with a unique pivot scanning mechanism to avoid striping artifacts. In addition, the system is equipped with a live-cell incubation system enabling the physiological imaging of living specimens over long timescales.

*Electron Microscopy*

**Transmission EM (TEM)**

1. JEOL JEM 1400 PLUS TEM
    The JEOL JEM-1400Plus is a 120 kV Transmission Electron Microscope (TEM) that boasts high resolution and high contrast imaging on an easy to use platform. The system features 0.38 nm point resolution and is configured for imaging at either 80 kV or 120 kV. It has a 5-axis high-precision piezo motorized stage suitable for large-area montages and can accommodate tilt angles up to + 70˚ for anaglyph or automated tomographic analysis.

**Scanning EM (SEM)**

2. Thermo Helios 5 UX FIB-SEM
    The Thermo Helios 5 UX is a Focused Ion Beam Scanning Electron Microscope (FIB-SEM) is capable for both the analytical imaging of whole samples and 3D nanotomography of embedded samples. Equipped with an Extreme High-Resolution (XHR) Elstar column, this SEM has superb low kV performance. The voltage can be adjusted from 0.35-30 kV (0.8 pA to 100 nA probe current) with 1.0 nm resolution at 0.5 kV, 0.7 nm resolution at 1 kV, and 0.6 nm resolution at 15kV. In order to accommodate a large array of sample types, the system is equipped with Everhart-Thornley SE, In lens SE and BSD, Solid-state BSD and Solid-state STEM detectors. The system is also equipped with a Phoenix ion column that is capable of 2.5 nm milling resolution at 30 kV. When coupled with the XHR Elstar column, imaging can be performed using the SEM column, the FIB column, or simultaneous SEM imaging/FIB imaging or milling for easy monitoring of routines. For masking and charge compensation purposes, the instrument is equipped with two gas injection systems with both Pt and C precursor gasses.

3. Zeiss Merlin SEM
    The Zeiss Merlin FE-SEM is a high-resolution Scanning Electron Microscope (SEM) workhorse that is available for the analytical imaging of whole mount or thin section samples. Equipped with a GEMINI II column, this SEM has superb low kV performance. The voltage can be adjusted from 0.02-30 kV (10 pA to 40 nA probe current) with 3.0 nm resolution at 0.2 kV, 1.8 nm resolution at 1 kV, and 0.9nm resolution at 15kV. In order to accommodate a large array of sample types, the system is equipped with Everhart-Thornley SE, In lens SE and BSD, Solid-state BSD and Solid-state STEM detectors

**Cryo-EM**

4. Thermo Titan Krios 300kV Cryo-TEM system
    The Thermo Titan Krios G3 300kV Cryo-Transmission Electron Microscope is the premier cryo-TEM that is available for both single-particle averaging and 3D tomography of vitrified samples. Equipped with a high-brightness field emission gun (X-FEG) and a high-stability 300kV high-tension (HT) generator, the system easily facilitates the high-resolution, sub-nanometer scale imaging of frozen biological specimens. The platform is also equipped with an image corrector that removes the increased spherical aberration present due to the larger pole-piece gap of the C-TWIN objective lens, which is required to accommodate large angle

specimen tilts in tomographic data acquisition. In addition, the system is equipped with a unique, one-of-a-kind cryogenically cooled sample handling and auto-loading system. The Titan Krios G3 platform has been configured with two detection cameras, a scintillated CMOS camera (Thermo Ceta), which will be used for regular TEM/STEM imaging and diffraction applications and an energy-filtered Direct Electron Detection camera (Gatan K2 Summit on a GIF energy filter) for the low dose imaging of frozen biological specimens. In addition, the platform is equipped with two other capabilities, phase contrast imaging and STEM imaging. The phase contrast system almost entirely removes the necessity to acquire images of low contrast objects under beam defocus conditions. Finally, the STEM imaging functionality is essential in high-resolution studies of cellular macromolecules in situ.

5. Thermo Aquilos 30kV Cryo-FIB System
   The Thermo Aquilos 30kV Cryo-Focused Ion Beam Microscope is the most advanced platform for the preparation of thin lamella of vitrified cells and tissues for cryo-Electron Tomography. The Aquilos is equipped with a pre-aligned electron optical column containing a Schottky field emission gun optimized for high brightness/high current facilitating the low-noise imaging of frozen biological samples at a resolution of 6 nm at 2kV. The system is also equipped with a Sidewinder column that generates a focused beam of gallium ions that has a precision milling resolution of 7 nm. The Aquilos is equipped with multiple detection options including a secondary electron detector (Everhart-Thornley SED), optimized for use across the available keV and current range and a Trinity detection system, which is comprised of a segmented, lower in-lens detector (T1), an upper in-lens detector (T2) and an in-column detector (T3). An integrated infrared-CCD camera is present for in-chamber viewing of the sample and a “Nav-Cam” color optical camera is also present for taking top-down images of samples for navigation purposes. In addition, the platform is configured with an “ICE” detector for secondary ion and electron detection. In addition, the system is equipped with a unique, one-of-a-kind cryogenically cooled sample handling and loading system and fully rotatable stage which is specifically designed to keep plunge frozen non-conductive biological samples at vitreous temperature (-170$^{o}$C). The Aquilos is also equipped with a unique decontamination system to prevent contamination from the outgassing of non-conductive samples as well as the MAPS 3 correlative workflow for spatially targeted lamella production based on imported fluorescence microscopy data.

6. Thermo Glacios 200kV Cryo-TEM system
   The Thermo Glacios 200kV Cryo-Transmission Electron Microscope is a mid-range cryo-TEM that is available for both single-particle averaging and 3D tomography of vitrified samples. Equipped with a high-brightness field emission gun (X-FEG) and a high-stability 200kV high-tension (HT) generator, the system easily facilitates the high-resolution, sub-nanometer scale imaging of frozen biological specimens. In addition, the system is equipped with a unique, one-of-a-kind cryogenically cooled sample handling and auto-loading system. The Glacios platform has been configured with three detection cameras, a scintillated CMOS camera (Thermo Ceta-D), which will be used for regular TEM/STEM imaging and diffraction applications such as Micro-ED, a Thermo Falcon 4 Direct Electron Detection camera and a Gatan K3 Direct Electron Detector for the low dose imaging of frozen biological specimens. In addition, the platform is equipped with two other capabilities, phase contrast imaging and STEM imaging. The phase contrast system almost entirely removes the necessity to acquire images of low contrast objects under beam defocus conditions. Finally, the STEM imaging functionality is essential in high-resolution studies of cellular macromolecules in situ.

*X-Ray Microscopy*

1. Zeiss Xradia Versa 520 X-Ray Microscope
   The Zeiss Versa 520 is a highly versatile platform allowing sub-micron imaging of large sample volumes. The Versa is unique in that it offers Resolution at a Distance (RaaD) in addition to being a completely non-destructive imaging modality. The systems offers 700nn resolution over centimeters of sample volume.

*Tissue Culture*

2 x 4 foot Biological Safety Cabinets
2 x HeraCell incubators
2 x HeraCell incubators with hypoxic O2 control

*EM Sample Preparation*

Leica UC7 ultramicrotome

Leica UC7 ultramicrotome including FC7 cryo-sectioning attachment
Leica CPD300 Critical Point Drier
Leica ACE600 Sputter Coater / Carbon Evaporator
Leica ACE900 Freeze Fracture Machine
Leica ICE High Pressure Freezer including light stimulation unit
Leica AFS2 Free Substitution Unit including FSP robot for automated sample management
Leica KMR Glass Knife-maker
Home-built Deep Etch-EM freezing machine
Balzer BAF400 Freeze Fracture Machine
Thermo Vitrobot Vitrification System (x2)

*Data Analysis*
4 x HP Z840 High-End Data Workstations including Multi-Processor, Multi-TB RAID, GPU and 256GB RAM
2 x Dell Precision High-End Data Workstations including Multi-Processor, Multi-TB RAID and 384GB RAM
2 x High-End Linux Data Workstations including 3TB SSD, 256GB RAM and 4 x GPU for cryo-EM processing
1 x High-End Linux Data Server with 512GB RAM and 8 GPU for motion correction and cryo-EM processing
1 x Apple Max Pro High-End 3D Graphics Workstation including 1TB SSD, 2 x GPU and 64GB of RAM
5 x Imaris 3D Visualization Licenses
4 x Amira 3D Segmentation and Tracing Licenses
6 x MATLAB Licenses
2 x Visiopharm Oncotopix Licenses

*Misc. Equipment*
Refrigerators (-4C) and freezers (-20C and -80C), centrifuges, milliQ water supply and precision balances