Module 2: Histology and Imaging

Purpose of the module

To investigate normal and diseased visual systems, researchers evaluate ocular tissues at the cellular and subcellular level, which requires appropriate histologic methods followed by sophisticated microscopy and imaging techniques. This module meets those needs by offering guidance in (A) histology and (B) imaging services related to the eye. The eye is unique and preparing it for histologic processing demands specialized expertise. To obtain excellent ocular tissue sections, which are amenable to immunodetection of genes/proteins, analysis of cellular and subcellular structure, and rigorous morphometric analyses, requires well-maintained processors and microtomes. This module offers high quality sections of ocular tissues prepared by skilled technical experts. It also offers access to very expensive microscopes and other state-of-the art imaging technology and most importantly, advanced skill in the use of the technology.

Location and contact information:

Carl Sanders Research and Education building

Histology/EM suite: 1st floor, Room: CB1113
Imaging suite: 2nd floor, Room CB2309

Director: Dr. Xingjun Fan (XFAN@augusta.edu), phone 706-721-2019
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Staff members:

EM/histology: Donna Kumiski DKUMISKI@augusta.edu, 706-721-6278
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Imaging: Rachel Cui, PhD XCUI@augusta.edu, phone 706-721-1876

Hours of operations: 9:00 a.m. – 5:00 p.m.

Description of services:

HISTOLOGY

The histology services ensure timely execution of experimental procedures including:

- assistance with isolation/proper orientation of eyes for histologic processing
- guidance in fixation and embedding in paraffin, methacrylate, epoxy, or OCT (optimal cutting temperature) compound for frozen sections

- sectioning tissues for light and electron microscopy
- routine and specialized staining of tissue sections
- training laboratory personnel in immunohistochemical studies
- performing immunodetection experiments for light or electron microscopy including co-localization studies
- assistance with laser capture microdissection of ocular tissue

ELECTRON MICROSCOPY (EM)

ELECTRON MICROSCOPES:

(1) JEM 1400-Flash Transmission Electron Microscope (TEM) provides high contrast TEM imaging with a lateral resolution down to 0.2 nm. The special sample holder together enables dual-axis electron tomography to capture three-dimensional structures. JEM-1400Flash comes with a montage system capable of utilizing stage drive for the field shift. This new system allows for simple capture of a montage panorama image over a limitless wide area. Thus, an ultra-wide area, high pixel-resolution image is obtainable, which is comparable to the image taken by conventional photo film.

(2) JEOL JEM-1230: high performance, high contrast, 40-120kV TEM with very good imaging capabilities suitable for biological, polymer and materials science applications. Imaging modes include bright/dark field and electron diffraction. The electron gun is a standard tungsten filament. The instrument is capable of magnifications from 50x-600,000x; resolution at 120 kV is 0.2 nm, and features standard tilt/rotation parameters.

(3) JSM-IT500HR Field Emission Scanning Electron Microscope (FESEM) This state-of-the-art SEM, with its high-brightness electron gun system, provides amazing high-resolution imaging along with high sensitivity and high spatial resolution analysis at even faster speeds. It can achieve 1.5nm at 30kV and 4.0nm at 1kV.

IMAGING

State-of-the-art imaging instrumentation available to vision researchers:

- LEICA STELLARIS CONFOCAL MICROSCOPE provides an ideal combination of brightness, resolution and contrast. The next-generation White Light Lasers (allowing a perfect match to the fluorochrome) permits simultaneous use of up to 8 single excitation lines from 440 nm to 790 nm. Additionally, the LIGHTNING Super Resolution technology and the NAVIGATOR sample overview can achieve 120nm resolution.
- NIKON ECLIPSE TI RESEARCH INVERTED MICROSCOPE equipped for super resolution microscopy using STochastic Optical Reconstruction Microscopy (STORM) technology. The system is under the control of the universal NIS-Elements platform and features the SR Apochromat TIRF 100X 1.49 N.A. objective lens, which provides the highest quality point spread function for N-STORM super-resolution imaging.
- ZEISS 780 INVERTED CONFOCAL MICROSCOPE WITH AIRYSCAN is equipped with 6 lasers: 405, 458, 488, 514, 543, 633 nm and 5 lenses: 5x EC Plan-Neofluar(dry) - NA 0.16, 10x EC Plan-Neofluar(dry) - NA 0.30, 20x Plan-Apochromat(dry) - NA 0.8, 40x Plan-Apo(oil) - NA 1.4, 63x Alpha Plan-Apo(oil) - NA 1.46. It features atmosphere (CO_2/O_2) and temperature control ideal for imaging live cells. It has the Definite Focus feature, allowing focus drift accommodation. Modules associated with this instrument include: Physiology, FRET, FRAP, 3D, Tiling. This scope is equipped with the Zeiss Airyscan detector, which improves resolution and signal-to-noise ratio. It allows the scope to detect more light without sacrificing the S:N ratio.
- ZEISS 780 UPRIGHT CONFOCAL MICROSCOPE with GaAsp detectors (which enhance sensitivity). Its modules include: 3D, Deconvolution, HDR, Tiling, atmosphere (CO_2/O_2) and temperature control (for live cells). It permits confocal imaging and excellent resolution at the cellular level. It is equipped with 6 lasers: 405, 458, 488, 514, 543, 633 nm and 5 lenses: 5x EC Plan-Neofluar(dry) - 0.16 NA, 10x EC Plan-Neofluar(dry) - 0.30 NA, 20x Plan-Apochromat(dry) - 0.8 NA, 40x Plan-Apo(oil) - 1.4 NA, 63x Plan-Apochromat(oil) - 1.4 NA.
- ZEISS 780 MULTIPHOTON CONFOCAL MICROSCOPE is equipped with the same lasers as the inverted and upright scopes and the following lenses: 10x W N-Achroplan (water immersion) - NA 0.3, 20x W Plan-Apochromat (water immersion) - NA 1.0, 40x IR-Achroplan (water immersion) - NA 0.8, 63x LCI Plan-Neofluar (oil) - NA 1.3. It is particularly useful for visualizing thick samples. It features a fixed stage suitable for whole mouse studies. The lenses are “dipping” to permit

immersed imaging of saline-bathed living samples. The system is temperature controlled and includes Physiology, FRAP, RICS modules.

- Leica Microdissection Microscope LMD6 laser capture microdissection microscope enable users to isolate specific single cells or entire areas of tissue. Powered by a unique laser design and dynamic software, Leica LMD6 allow users to easily isolate Regions of Interest (ROI) from entire areas of tissue down to single cells or even subcellular structures such as chromosomes.
- Zeiss Lattice Light Sheet offers long-term volumetric imaging of living cells. This instrument makes light sheet fluorescence microscopy available for live cell imaging at subcellular resolution – while also allowing you to use standard sample carriers. With this automated, easy-to-use system, volumetric imaging of subcellular structures and dynamics over hours and days with best protection from photo damage becomes available to everyone.

Access, associated costs and responsibilities:

- The Module utilizes equipment within two existing cores on the Augusta University campus, both housed in the Department of Cellular Biology/Anatomy: (1) Histology/EM core (CB1113), and (2) Imaging core (CB2309).
- There is no charge to investigators for the use of Module 2. Costs of using the services and/or instrumentation of the two university cores (including training to use scopes in the imaging core) will be borne by the P30. However, investigators must provide reagents specific to their experiment (e.g. antibodies, etc.)
- PLEASE NOTE: For the imaging core, investigators are expected to honor their reservations for instruments and must adhere to guidelines for cancellations. **FAILURE TO ADHERE TO THE GUIDELINES WILL RESULT IN CHARGES TO THE INVESTIGATOR (NOT THE P30).**
- Investigators will access services via iLAB: https://augusta.corefacilities.org/homepage/

Citation

Please cite the NEI Center Core Grant for Vision Research - **P30EY031631** in your publications if you use the instrumentation and services offered in this module.