# **Analytical Imaging Facility**

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**Introduction, Goals and Objectives**

The Analytical Imaging Facility (AIF) is a comprehensive microscopy shared resource that offers imaging technologies ranging from macro-imaging with a stereoscope, classical microscopy including brightfield, darkfield, phase contrast, Nomarski differential interference contrast and wide-field epifluorescence and confocal microscopy for cell culture and tissues, to high speed spinning disk confocal microscopy for live cells, intravital multiphoton microscopy for live animals, and super-resolution (SIM and STORM) microscopy, through standard transmission and scanning electron microscopy, to cryoEM of macromolecules and whole mount cells. The wide range of imaging modalities is especially important for studying a diverse range of biological models to support the diverse biomedical research within the Einstein community. For instance, cells labeled with fluorescent reporter genes may be imaged at the whole animal or whole organ level down to single cells or cell compartments. Having the technology and staff in one location provides continuity and adds value to Einstein researchers who use microscopy. The goal of the AIF is to make routine and complex imaging technologies available to the entire Einstein community and provide needed image analysis training or assistance to its users. This is accomplished by supporting routine microscopy applications, training users in microscopy and image analysis software, hosting regular workshops to be an educator on microscopy and image processing and introducing innovative imaging technologies developed at this institution as they become available to meet the needs of Einstein investigators.

In addition to offering microscopy services, the facility also offers customized full service sample preparation for electron microscopy ranging from chemical fixation, embedding in resin and ultrathin sectioning, negative staining, immunogold labeling, critical point drying and metal shadowing. The facility also offers a full range of low temperature techniques for electron microscopy including quick freezing in the millisecond range by plunge, metal mirror or high pressure freezing. Subsequently frozen samples can be freeze-substituted and embedded at low temperatures, deep etched and rotary shadowed, freeze fractured, ultrathin cryosectioned or viewed directly in the cryoTEM.

The facility has traditionally emphasized teaching new users, on an individual basis, the imaging techniques they require for successful execution of their experiments. The AIF has further expanded its service to include more quantitative, rigorous and advanced image data analysis to the community with newly purchased commercial software as well as home written codes to support diverse research needs in the Einstein community. The AIF user group varies from the novice to experienced microscopist, encompassing graduate students, postdoctoral fellows, technicians, and principal investigators. Appropriate staff effort is expended to address the needs of these groups assisting them in experimental design, data collection, quantitative analysis and presentation. Novice users are trained in the specific imaging technology appropriate to address the specific research objective. Experienced users may customize any imaging station, utilizing the large inventory of optics and accessories that are available. Each imaging station is returned to a “standard configuration” at the end of a session. In this manner a large user group can be efficiently accommodated.

The science of Einstein faculty drives the technology offered in the AIF. State of the art technologies are regularly introduced by the AIF Directors to address these needs. Intravital Imaging was specifically developed within the AIF to support the Tumor Microenvironment and Metastasis Program. CryoEM development is continuing as the AIF supports structural biology research. The AIF staff is currently developing specimen prep protocols for correlative fluorescence and scanning electron microscopy. The need for high resolution SEM was addressed by a successful SIG to purchase a FESEM with cryotransfer stage for imaging samples sensitive to dehydration artifacts, yeast capsule and bacterial biofilms for example, in a frozen hydrated state. This purchase included Atlas, for large area mapping, which will be used to reconstruct serial thin sections in 3D and Shuttle & Find for CLEM. Another successful SIG enabled the purchase of the 3DHISTECH P250 Panoramic Slide Scanner. This instrument will allow Einstein researchers to digitally scan and archive high quality images of large batches of slides in a very time efficient manner. Three most recent SIGs enabled the purchase of a Leica SP8 laser scanning confocal microscope for fixed and live cell imaging, a Nikon microscope equipped for Structural Illumination Microscopy (SIM) as well as localization based super-resolution (STORM/PALM) imaging and a JEOL 1400 Plus transmission electron microscope for ultrastructural studies in 2D and 3D.

**Services and Technologies Provided**

**Light Microscopy** - The light microscopy division of the AIF provides digital imaging from macro, on the order of cm, up to high resolution, on the order of 0.1 μm, or even up to angstroms when including information from Forster Resonance Energy Transfer (FRET) technology. Einstein researchers may utilize the resource by having AIF staff perform work for a fee although most researchers are trained in use of instruments and thereby have unlimited 24 hour access. The facility offers assistance with experimental design, image acquisition and post-acquisition data analysis.

Light microscope imaging capabilities include epifluorescence, confocal and multi-photon imaging, as well as transmitted light options (brightfield, darkfield, phase contrast, Nomarski DIC). Available high quality optics represent current technologies of Olympus, Zeiss, Nikon, Leica, and 3DHISTECH. Dynamic experiments with live material can be performed on two widefield epifluorescence microscopes, three confocal microscopes, one multi-photon microscope, and a STORM/SIM super-resolution microscope, all fitted with environmental chambers and able to capture time series and z-series in multiple fluorescent channels. Two Eppendorf microinjection systems are available for use on any of the inverted microscopes and can be used for cell manipulations such as microinjection and chemotaxis assays. An Eppendorf cell collector allows researchers to collect individual unattached cells for further analysis. In addition, the AIF offers support for advanced microscopy techniques such as Foster Resonance Energy Transfer (FRET), Fluorescence Recovery After Photobleaching (FRAP), photoactivation and photoconversion assays, as well as Fluorescent Lifetime Imaging Microscopy (FLIM).

**Widefield Epifluorescence Microscopes:**

* **GE Delta Vision Core and Delta Vision Personal** stations are inverted microscopes using Olympus optics, with Applied Precision programmable, high precision stages and environmental chambers. Objectives range from 4-150x and fluorescent channels range from DAPI to Cy5. Photometrics cool-snap cameras are used for image acquisition. Due to the high precision stage, these instruments are ideally suited for acquisition of time-series and z-series in live samples. In addition, it is possible to mark coordinates, thus enabling point visiting and the acquisition of multiple time and z-series in each experiment. The software also allows for deconvolution of z-series post acquisition.
* **Digital Stations 1 and 2** are built around Olympus IX70 and IX81 inverted microscope stands and Cooke Sensicam CCD cameras operated with Scanalytics IP Lab software. Available objectives range from 4-60x. Each station can accommodate fluorescent probes ranging from DAPI to Cy5.
* **Digital Station 6** is built around a fully automated Olympus BX61 upright microscope stand with a Cooke Sensicam CCD camera operated with Scanalytics IP Lab software. Objectives range from 10-100x. It is equipped with filter cubes for 8 different fluorescent channels, ranging from DAPI to Cy7. It also has the Applied Spectral Imaging SKY SpectraCube and SKY camera for spectral karytyping of chromosomes. This station supports the microscopy requirements of the Molecular Cytogenetics Shared Resource.
* **Zeiss AxioObserver with Shuttle & Find** inverted microscope with objectives ranging from 5-100x. Brightfield imaging options include phase contrast and Nomarski/DIC. The microscope is equipped with two cameras, an Axiocam HRc for brightfield/histology color imaging and an Axiocam HRm for black and white fluorescence acquisition with Axiovision software and is equipped with a variety of fluorescent channels ranging from DAPI to Cy5. In addition, “shuttle & find” includes a Zeiss proprietary module in Axiovision to mark xy positions on samples in fluorescence and then find the same coordinates on the SEM microscope.
* **Zeiss Axioskop II upright microscope**. This microscope has objectives ranging from 1.25X through 100X. Imaging modes include brightfield, two types of darkfield, crossed polarization, and Nomarski DIC. Images are captured with a color Zeiss Axiocam using Axiovision software.
* **Zeiss Stemi 11 stereo microscope** with a Retiga Q-Image CCD Camera and a fluorescence module (DAPI, GFP and Rhodamine) for imaging entire yeast or bacterial plates and larger objects such as whole transgenic animals (for example C. elegans, zebrafish or mice).
* **Olympus Stereo microscope**. This microscope has a 1x lens with magnification boosters ranging from 0.7x to 11.5x. It is equipped with a cooled Olympus XM10 monochrome camera, four fluorescent filter cubes (CFP, GFP, RFP, CY5) and uses Cellsense Standard software.
* **3DHistech Pannoramic 250 digital slide scanner.** This instrument is a high capacity, high speed automated slide scanner. It can automatically scan up to 250 slides in one batch. It is equipped with a Lumencor LED light source, 20x and 40x air objectives and a scientific CMOS detector PCO edge 4.2. Imaging modes include brightfield as well as fluorescence (DAPI, FITC, TRITC, Cy5 and Cy7).

**Laser Confocal Microscopes:**

* **Leica AOBS SP5 and SP8 confocal microscopes** are point scanning confocal microscopes well suited for multichannel imaging of fixed samples. Objectives range from 10-63x. In addition, the SP5 has an environmental chamber and the SP8 has a stage top incubation system for live cell imaging. They are true spectral imaging systems due to their AOBS technology. The SP5 has seven laser lines for excitation, ranging from 405 to 633 nm, and four photomultiplier tubes allowing users to set up four separate fluorescent channels, plus one additional detector for simultaneous Nomarski DIC. The SP8 is a state of the art confocal microscopy platform. It is equipped with a pulsed White Light Laser covering the entire excitation range from 470-670nm in addition to a 405 nm laser and five detectors, including two highly sensitive HyD SMD detectors for super-sensitive imaging and fluorescence lifetime imaging, one HyD detector and two PMT detectors. In addition, the SP8 is equipped with PicoQuant hardware to conduct fluorescence lifetime imaging (FLIM) experiments. The AOTF allows for small regions of interest to be illuminated at high intensity for photoactivation, FRAP,acceptor-bleaching FRET, spectral scanning and unmixing for challenging samples. The SP8 is equipped with an 8K Hz resonant scanner for low photo-toxicity live cell imaging.
* **Nikon CSU-W1 spinning disc confocal microscope** for live-cell spinning disk (Yokogawa) confocal imaging. The system has laser lines at 405, 488, 561 and 640 nm, a piezo for high-speed Z-axis control, a heated stage top incubator and objectives ranging from 10-100x. It utilizes Nikon Elements acquisition software and two ORCA-FLASH 4.0 sCMOS cameras for two-channel simultaneous imaging. Due to the nature of excitation via spinning disk, this microscope is also faster than point scanning confocal, therefore ideal for fast confocal imaging of live specimen. It is equipped with an Optimicroscan Device for Photoactivation and FRAP/ FLIP with either 405nm or 473nm lasers, for simultaneous imaging and bleaching/photoactivation experiments. The stage is fully programmable for multi-point visiting and large mosaic stitching. The system also includeds the Reflection-based Perfect Focus System.
* **Olympus Multiphoton PV1000-MPE** utilizes a Spectra Physics Tsunami pulsed laser to excite fluorophores and enable second harmonics generation by femtosecond pulses of highly concentrated long wavelength light. Currently, three fluorescent channels can be detected (CFP, GFP, and mCherry). An additional channel is set up for second harmonic detection. This instrument is the centerpiece of the Intravital Imaging Program and can accommodate a wide variety of samples ranging from anesthetized whole mice to whole organs, to sections of tissue. The deep penetration depth of multiphoton is ideal for intravital tissue imaging.
* **Nikon Super-resolution microscope** is a microscope with optical resolution beyond the traditional diffraction limit. It is equipped for TIRF imaging, Structural Illumination Microscopy (SIM) as well as localization based super-resolution (STORM/PALM) imaging. It has four laser lines at 405 nm, 488nm, 561 nm and 647 nm, a 100x (NA 1.49) oil objective, and an EMCCD (Andor iXon3) camera. It is fully motorized and equipped with environmental control in the form of a heated stage insert and objective heater.

**Electron Microscopy** - A wide range of sample preparation equipment and four electron microscopes permit AIF staff to offer full service sample preparation and imaging for standard and state of the art EM techniques. The AIF staff performs most of the specimen preparation protocols while some investigators with expertise in EM use the facility as an equipment resource. Investigators are trained as operators of the electron microscopes. The AIF offers all standard EM preparation protocols including embedding utilizing either epoxy or acrylic resins at ambient or low temperatures, thin sectioning, negative staining, immunogold labeling following pre or post embedding protocols. In addition, the AIF offers a full range of low temperature techniques for EM including cryofixation by plunge freezing, metal mirror freezing and high pressure freezing. Frozen samples can be further processed by freeze substitution and embedded at low temperatures. Specimens can be rotary shadowed for whole mount TEM, freeze fractured, and cryosectioned. Immunogold labeling can be performed using pre or post embedding protocols and by the Tokuyasu method for cryosections. Most traditionally prepared TEM samples are imaged with two JEOL TEMs (1400Plus and 1200EX) and recorded with Gatan digital cameras. Macromolecules and thin areas of whole mount cells frozen in vitreous ice can be imaged directly in the FEI Tecnai 20 cryo electron microscope under low dose conditions and recorded with a 4k x 4k TVIPS F415 CCD camera. 3D image analysis is performed with SPIDER or IMOD software. Surfaces of cells and tissues are imaged at high resolution at ambient or cryo temperature with a Zeiss Supra40 field emission scanning electron microscope (FESEM) with detectors for secondary backscatter and STEM signals.

**Major equipment available for EM specimen preparation:**

* Leica ARTOS 3D ultramicrotome
* Leica EM UC7 ultramicrotome with ATUM automatic serial section collection system
* Leica EM UC6 ultramicrotome with EM FC6 cryo sectioning module
* Bal Tec HPM 010 High Pressure Freezer
* Thermo Scientific Vitrobot
* PIE Scientific Tergeo EM plasma cleaner
* Quorum GloQube
* Life Cell CF100 Slam Freezer
* Leica AFS2 and Bal Tec FSU-010 Freeze Substitution Units
* Cressington CFE-50 for freeze fracture and deep etch rotary shadowing
* Tousimis Samdri 795 critical point dryer
* EMS 150T ES combination sputter coater, carbon coater and glow discharge turbopump unit
* Pelco 3450 Laboratory Microwave System
* EMS Poly III infiltration unit
* Denton DV 502 vacuum evaporator.

**Electron Microscopes:**

* **JEOL 1200EX TEM**, accelerating voltage 20 KV to 120 KV, microprocessor control, equipped with side entry goniometer stage, side mounted Gatan 2k x 2k Orius CCD camera.
* **JEOL 1400Plus TEM** accelerating voltage 20 KV to 120 KV, LaB6 filament, bottom mount 11 megapixel Gatan Orius CCD Camera. SerialEM enables automated acquisition for electron tomography, serial sections and automated image montaging of large areas at high resolution.
* **FEI Tecnai 20 cryo TEM**, accelerating voltage from 60 KV to 200 KV, LaB6 filament, low dose software, Gatan cryo specimen holders, Gatan high tilt holder, Gatan dual axis cryo tomography holder, TVIPS TemCam-F415 CCD camera,. Electron cryomicroscopy of vitreous ice-embedded samples and electron tomography at ambient and cryo temperature have been developed utilizing this microscope.
* **Zeiss Supra 40 FESEM** with Gatan Alto 2500 cryotransfer stage, Oxford Inca EDS, Everhart–Thornley and in-lens secondary, backscatter and STEM detectors, Atlas large area mapping and Shuttle & Find. The relative ease of high resolution surface imaging, detection of 10nm gold by backscatter imaging for localization of surface receptors, elemental identification and mapping, serial reconstruction in 3D of large areas, observation of biofilms and other dehydration sensitive materials at low temperature and correlative light and electron microscopy (CLEM) are all capabilities of this microscope.

**Data Analysis** - The AIF provides three high-end computer workstations and an arsenal of state of the art commercial software for quantitative image analysis in a user friendly environment. The AIF routinely generates and customizes analysis protocols for users in the software modules listed below. The following commercial software is available:

* **Volocity** for Visualization, Restoration and Quantification is a high-performance 3D image analysis software for interactive, time-resolved volume visualization. It allows the user to identify, measure and track biological structures in 2D, 3D and 4D, and is able to deconvolve widefield fluorescence images to produce superior confocal quality images as well as quantify colocalization, ratio images, FRAP and FRET.
* **GE Applied Precision SoftWorx** for deconvolution and quantitative image analysis
* **Bitplane Imaris** is a powerful analysis software for data visualization, analysis, segmentation and interpretation of 3D and 4D microscopy datasets. It adds a set of high-performance tools to analyze multidimensional image data including interactive filtering, sorting, classifying, selecting and grouping objects based on statistical parameters. Its object tracking functionality is the best in the industry.
* **3DHISTECH QuantCenter** for analysis of whole slides that were scanned on the 3DHISTECH P250 slide scanner. QuantCenter has many modules that quantify immunohistochemistry stains and immunofluorescence of the whole scanned tissue. These include pattern-based tissue segmentation, classification of nuclear stain, and measurements of immunostain intensity.
* **IMOD** for 3D electron tomography and 3D reconstruction of serial sections.
* **Adobe Photoshop** for publication-quality figure preparation.