### Integrated Cellular Imaging Core - MAJOR EQUIPMENT

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**Updated: April 2023**

**Major Equipment for Integrated Cellular Imaging Core (ICI) Users**

**INTEGRATED CELLULAR IMAGING CORE (ICI)**

The **Integrated Cellular Imaging Core (ICI)**, one of the **Emory Integrated Core Facilities (EICF)**, provides state-of-the-art light microscopy and image analysis platforms. ICI offers confocal, spinning disk confocal and live cell imaging, multi-photon animal and tissue imaging, widefield with deconvolution, super resolution, light sheet and image analysis. To effectively implement these technologies, ICI provides consultations, expert training, and support for all our systems. We assist our users from bench to publication, starting with designing experiments tailored to the specific microscope, through training and data acquisition, to data analysis and interpretation.

**Confocal Imaging:**

Leica SP8 (Winship): The Leica SP8 confocal laser scanning unit, attached to the latest Leica inverted DMi8 body, comprises an infinitely flexible prism based spectral detector system and multiple excitation laser lines (405, 488, 514, 561 and 633nm) for a vast variety of experimental and labeling options. Four channel detection, via two PMTs and two extremely sensitive Leica HyD detectors (GaAsP based), together with 8 kHz resonant scanning provides high speed imaging. A transmitted light detector allows for simultaneous laser scanned DIC or phase imaging. A stage and objective warmer facilitates numerous live cell imaging approaches such as FRET, FRAP, and photoconversion. High speed automated tiling/stitching and multipoint location acquisitions are also possible in combination with the above.

Olympus FV1000 (HSRB): This FV1000 confocal laser scanning microscope is mounted on an inverted Olympus IX81 microscope with a motorized x-y-z stage. It has a three channel PMT detection system with grating based spectral detectors on channels 1 and 2 (allowing for spectral unmixing). In addition, a transmitted light detector provides laser scanned DIC for label free imaging. These coupled with 6 excitation laser lines (405, 458, 488, 515, 559, and 635nm) facilitate highly flexible imaging combinations.

Olympus FV1000 (Whitehead): This FV1000 laser scanning microscope is identical to the HSRB FV above, except it’s filter based detection on all channels and a different combination of excitation laser lines (405, 488, 515, 543, 635). Having near identical systems in two locations provides easy secondary options without additional trainings during maintenance and repairs.

**Live cell confocal imaging:**

Nikon A1R HD25 (Whitehead): This live cell confocal laser scanning microscope is optimized for imaging cellular dynamics at high speed and a large view of view (25mm). A hybrid dual scanning confocal system with both galvanometric and resonant scanners, together with a piezo z controller, can acquire 1024x1024 images at 15 fps, 512x512 images at 30 fps, or with a reduced size up to 720 fps. A stage top environmental chamber at 37° C and 5% CO2 required for live cell imaging, coupled with a Perfect Focus system, provides extremely stable long-term imaging without drift. Flexible laser scanning control for photokinetics experiments can be conducted in multiple user-defined regions of interest. Six laser lines (405, 440, 488, 514, 561, & 640nm) and four channel PMT (2x GaAsP, 2x Multi-Alkali) detectors, in addition to a transmitted light channel for laser scanned DIC provide flexible live cell experimental options. A DUVB-2 GaAsP spectral detector unit provides arbitrary 10 – 320 nm wide spectral windows between 400 and 720 nm for complete spectral flexibility. NIS Elements packaged with analysis options coupled to JOBS tailorable data-based control system.

Nikon Ti2-E – Crest X-Light Spinning Disc Confocal (Winship): A Nikon controlled Eclipse Ti2-E with a CrestOptics X-Light V2 L-FOV ultrafast spinning disk confocal provides a 18mm FOV utilizing a sensitive high speed sCMOS camera (~100fps or more for cropped ROI). A stage top environmental chamber at 37° C and 5% CO2 required for live cell imaging, coupled with a Perfect Focus system, provides extremely stable long-term imaging without drift. A fast z-piezo and motorized xy stage provides high speed automated multi-location and tiling/stitching acquisitions. Seven laser lines (408, 445, 473, 518, 545, 635, & 750nm), DIC, and phase acquisitions are available. NIS-Elements packaged with analysis options coupled to JOBS enable tailorable data-driven acquisitions.

Leica Stellaris 8 (HSRB II): A point scanning system, including a White Light Laser as excitation light source. An extended detection range up to 850 nm plus the expanded excitation range in the visible from 440 nm up to 790 nm allow the application and separation of an extended range of spectrally overlapping fluorophores, up to five simultaneously. It is equipped with a 405-diode laser as well.  The Stellaris has both Galvano and resonant scanners, 5 detectors, including 3 Power HyD-S and 2 Power HyD-X, a Tokai Hit stage top incubator for CO2 and temperature control, Navigator software for tiling, TauSense for FLIM, and LIGHTNING for optimal extraction of image details and maximum resolution.

**Super-resolution imaging:**

Nikon N-SIM (Whitehead): The Nikon Structured Illumination Microscope (SIM) doubles the resolution of widefield microscopy in all three dimensions (down to 120 nm x-y and 300 nm z resolution), and can image samples prepared with conventional techniques (same as confocal or widefield). A sensitive iXon EMCCD camera and three laser line system (488, 514, and 561nm) provides high speed multi-color acquisitions. A SIM-TIRF mode is also available. The system also includes a 37°C 5% CO2 and humidity-controlled stage insert, together with heated objectives for the option of live cell super resolution acquisitions.

Deltavision OMX BLAZE (HSRB I)*:*The DeltaVision OMX imaging platform is an advanced multi-mode, super-resolution microscope system. DeltaVision OMX provides 4 color super-resolution imaging using 3D structured illumination (3D-SIM) as well as ultra-fast widefield-deconvolution acquisition (3x sCMOS cameras for 300 fps imaging). The OMX Blaze also has Ring-TIRF capabilities to image biological processes at the cell surface-coverslip interface. The laser scanning optics of the TIRF beam path allow for fast switching of photoactivation and photokinetics in combination with live cell widefield-deconvolution, 3D-SIM imaging or TIRF. The system also includes 37°C 5% CO2 and humidity-controlled stage inserts, along with heated objectives for the option of live cell super resolution acquisitions.

3i Lattice Light Sheet (Winship)*:* The Intelligent Imaging Innovations (3i) lattice light sheet microscope (LLSM) is state-of-the-art for rapid high-resolution live cell imaging with low phototoxicity stemming from efficient lightsheet excitation/collection. Compared to a spinning disk confocal, the LLSM features 100x less phototoxicity/photobleaching in dithered mode. The collection objective, a Nikon 25X/1.1NA, is matched with a tube lens for an overall 62.5X magnification with better axial resolution than achievable through confocal microscopes. A one-channel 40x40x40um region can typically be imaged in 1 second. For super-resolution in x and z, a SIM mode is available at the expense of temporal resolution, and super-resolution may also be achieved through Super Resolution Radial Fluctuations (SRRF). The system has four lasers (405, 488, 561, and 642nm) for sequential acquisition with a quad-band bandpass filter. Samples are loaded onto 5mm glass coverslips and immersed in a 3- or 12-mL media bath between the inclined objective lenses.

Abberior Facility Line easy3D STED & Picoquant TCSPC (Whitehead): Abberior Instruments FACILITY LINE easy3D STED microscope with 4 excitation lines (405nm, 485nm, 561nm, 640nm), two pulsed STED lasers (595nm, 775nm). Includes adaptive illumination package (DyMIN, RESCue, and MINFIELD) for highest resolution and live cell super-resolution imaging at ultra-low light levels. Adaptive optics (OA) compensates for aberrations to improve STED in standard samples, and allow STED deep within tissues etc. Variable spectral detection and avalanche photodiodes (APDs) provide ultra-sensitive flexible detection. Picoquant time correlated single photon counting (TCSPC) provides additional time resolved techniques such as fluorescence lifetime imaging microscopy (FLIM) and fluorescence correlation spectroscopy (FCS). Includes steady focus during all STED modes to circumvent drift. Water, silicone, and oil lenses available to match sample refractive index for increased signal and resolution. Live cell chamber for time lapse images (utilizing adaptive illumination decreases photodamage and extends imaging speeds and durations).

Miltenyi UltraMicroscope Blaze Large fFeld of View Light Sheet (HSRB II): A fully automated light sheet microscope for imaging large or multiple cleared samples at subcellular resolution. It has a large sample chamber, holds 500mls of imaging solution, and allows for uni- and bi-directional illumination with 1-6 sheets. With 1.1X, 4x and 12x objectives one can achieve up 30x magnification and visualize large, cleared samples at subcellular resolution. This includes organoids, organs and tissue, as well as whole small animals.

**Multiphoton:**

Zeiss 710 NLO (Clinic B): This multiphoton allows for intravital (live animal) imaging with high magnification and resolution. This microscope is equipped with a Chameleon Vision S tunable multiphoton laser (680 nm to 1050 nm) that can penetrate farther into tissue than a traditional confocal laser. This system is mounted on an upright Zeiss Axioexaminer microscope, together with a flexible large area stage for imaging live mouse single cells, thick tissue sections, and cell lines. The confocal optics of the 710 house spectral detectors coupled with six lasers lines (405, 458, 488, 514, 543, and 633nm) for flexible acquisitions. Two non-descanned detectors (NDDs) provide ultrasensitive detection of multiphoton signals. Oil, air and water immersion objective lenses are available.

Leica SP8 (Whitehead): The Leica SP8 is an upright confocal and multiphoton microscope with a Coherent Chameleon Vision II laser tunable from 680 to 1080nm. Two highly sensitive non-descanned GaAsP based HyD detectors provide imaging of multiphoton processes. The confocal unit houses an infinitely flexible prism based spectral detector system and multiple excitation laser lines (458, 476, 488, 496, 514, 561, 594nm) for a vast variety of experimental and labeling options (coupled with LASX unmixing software for experiments with potentially 10 or more colors). Four channel detection, *via* two PMTs and two extremely sensitive Leica HyD detectors (GaAsP based), together with resonant scanning provides high speed imaging. A CCD camera allows– for brightfield and Dodt Gradient Contrast (DGC) for label free imaging and sample navigation. A stage and objective warmer facilitates numerous live cell imaging approaches such as FRET, FRAP, and photoconversion. A 12 kHz resonant scanner provides high speed scanning at up to 428 fps, and there is automated tiling and image stitching. Oil, air, and water immersion objective lenses are available.

**Widefield fluorescence:**

Zeiss Axioplan 2 (Winship): The motorized Zeiss Axioplan 2 is equipped for routine widefield epifluorescence and transmitted light applications (DIC, phase, and brightfield) using a range of high-end Zeiss Plan-Apo objectives. A Zeiss Axiocam color camera allows for histology imaging or fluorescence imaging via multiple filter sets.

Olympus IX71 (HSRB I): The inverted IX71 can perform epifluorescence and transmitted light microscopy. It contains long working distance objectives that allow for visualization through plastic culture dishes or glass. A sensitive color QImaging CCD camera and multiple filter cube sets provides fluorescence imaging in monochrome mode, and histology images in color mode.

Leica Thunder (HSRB II): The Leica Thunder wide field offers 3D live cell imaging and helps to maintain optimal physiological conditions by minimizing photobleaching, providing high performance imaging and will provide clear details in real time without out of focus blur utilizing Leica's Computational Clearing. It is a fully motorized DMi8 microscope with a highly sensitive K8 camera and a multi-line, high-intensity fluorescence LED light source andwill allow for optimal physiological conditions, keeping photobleaching to a minimum while providing high performance imaging. It has a Tokia Hit stage top environmental chamber at 37° C and 5% CO2 required for live cell imaging.

Olympus IX51 (Winship): The inverted IX51 can perform epifluorescence and transmitted light microscopy. It contains long working distance objectives that allow for visualization through plastic culture dishes or glass. An Infinity CCD monochrome camera allows for sensitive acquisitions from multiple filter set options.

BioTek Lionheart FX (Whitehead): The compact Lionheart FX widefield microscope provides flexible automated acquisitions in fluorescence, brightfield, and color imaging modes, together with multiple lenses and 37°C and 5% CO2 control allowing live cell multiple plate reading, to IHC, and widefield-deconvolution.

Keyence BX81 (Clinic B): The Keyence BTX is a bench top epifluorescence microscope with flexible stage adaptors allowing imaging of up to 3 slides and different types of multi-well plates in both fluorescence and color imaging modes. Multiple field of views can be acquired, with a flexibility to stitch these images.

**Image Analysis:**

Five image analysis workstations across the five ICI locations are available, ranging from Dell workstations to custom built towers designed to handle Terabite sized data sets. Users can analyze their data using a full suite of image analysis options. These include Bitplane Imaris, Volocity 6.2, Fiji Is Just ImageJ (FIJI), and CellProfiler. Deconvolution is available via Microvolution. Custom Fiji macros and java plugins can be tailored to individual needs and are [available to download](https://www.cores.emory.edu/ici/resources/plugins.html). Video analysis tutorials are also publicly available on YouTube via the channel [NoPhotonLeftBehind](https://www.youtube.com/channel/UCRVa5DSphB5gHMaFKPgyKSQ). MATLAB/Python code can be designed for stand-alone usage or for direct interface within Imaris.