Fluorescence Imaging Resources at the CCAM Microscopy User Facility

The Microscopy User Facility at CCAM (https://health.uconn.edu/cell-analysis-modeling/microscopy-facility/) provides access to instrumentation and technical support for light based microscopy for the research community.

Resources include:

- Zeiss Lightsheet Z1 with 4 laser lines (405, 488, 561 and 633 nm) with dual side illumination multiview optics. The optics are equipped for water-based or cleared specimens and dual sCMOS cooled cameras to provide high sensitivity, rapid, 3D imaging of live or fixed thick specimens.
- Zeiss LSM880 laser scanning confocal microscope with 6 laser lines (405, 488, 514, 561 and 633 nm) and multispectral GAsP detection (~2-fold increased sensitivity over conventional PMTs).
- Zeiss LSM780/NLO confocal microscope with 7 laser lines (405, 440, 458, 488, 514, 561,and 633 nm) and nonlinear excitation from a Coherent Chameleon Ti:Sapphire, and with multispectral GAsP detection. This microscope is also equipped with a Becker-Hinkl Fluorescence Lifetime Imaging Microscopy (FLIM) detector on a nondescanned detection port, particularly useful for detection of fluorescence resonance energy transfer (FRET) based probes.
- All confocal microscopes have environmental chambers to allow stable control of temperature and CO_2 for live explant or in vivo imaging situations.
- Dual channel (488 and 561 nm) TIRF (total internal reflection fluorescence) widefield system on a Zeiss Axio Observer inverted microscope with Photometrics Evolve EM-CCD camera and α-Plan-Fluar 100X/1.45 oil objective.
- Photo-Activated Localization Microscopy (PALM) super-resolution light microscopy is available using a home-built, fully automated PALM imaging system in a CCAM associated laboratory. This system is built on ASI's custom Rapid Automated Modular Mounting (RAMM) platform, uses 440, 515 and 594 nm (Coherent OBIS) laser lines, and is equipped with Hamamatsu sCMOS cameras for image acquisition.
- diSPIM (dual-view inverted Selective Plane Illumination Microscope) imaging system from ASI imaging Inc is available in the Wu laboratory (a CCAM associated laboratory) for lightsheet imaging on the cellular as opposed to tissue scale.
- Our microscopes are equipped with DIC optics, a cache of interchangeable objectives and options for different types of environmental control for in vivo or live cell imaging.

We utilize Metamorph image processing software (Universal Imaging) for quantitative image analysis, Imaris software from Bitplane Graphics for volume and surface rendering, and in house software for specialized applications. A full time staff microscopist is available to provide technical advice on any of our microscopes, and for training of students and other investigators. In addition, the faculty and staff of CCAM provide a wealth of expertise related to light microscopic imaging and analysis. CCAM is a highly interdisciplinary center with a research focus of developing quantitative, detailed molecular hypotheses of cellular physiology, using advanced optical tools coupled with rigorous mathematical modeling. Bringing together optical

engineers, chemists, cell biologist, physicists, and mathematicians in a strongly interactive environment, CCAM is home to an NIH-designated National Resource that has developed and hosts a web-based environment, the Virtual Cell, that provides the tools to create spatially realistic computational models of cellular processes. The broad expertise at CCAM is available to provide help and advice on methods for obtaining, analyzing and quantifying multi-dimensional data from live or fixed specimens whether obtained *in vitro*, *in situ* or *in vivo*.