Welcome to the third edition of the Cell and Molecular Imaging Core Newsletter. The Cell and Molecular Imaging Core is a shared resource of the Hollings Cancer Center and a component of the Center for Cell Death, Injury and Regeneration. The Imaging Core provides instrumentation and support for confocal/multiphoton microscopy and image analysis. We once again remind all users to please acknowledge use of the Cell and Molecular Imaging Core and the new Cancer Center Grant 1P30 CA138313-01 in their publications. This helps us greatly in providing you state-of-the-art services.

This month, we describe updates to the core and spotlight spinning disc confocal microscopy.

Special Happenings:

*Olympus Reference Site is here.* Olympus America has finished installing an Olympus FluoView1000 MPE intravital confocal/multiphoton microscope. This state-of-the-art microscope is configured especially for intravital imaging of tissues and organs in living animals. A multiphoton infrared laser and multiple visible wavelength lasers permit visualization of a wide range of fluorophores with optics especially designed for deep tissue imaging. The Cell and Molecular Imaging Core is proud to host this official Olympus Reference Site, and MUSC investigators are welcome to use the instrument for their research projects. Please email Dr. Venkat Ramshesh at ramshes@musc.edu for more information about this microscope.

**Spotlight on Spinning disc Confocal Microscopy:**

In confocal microscopy, a pinhole excludes fluorescent and reflected light originating from out-of-focus planes. In this way, confocal microscopes achieve narrow depths of field, allowing one to create thin optical sections through thick specimens and to image thick biological samples in 3-dimensions. The two commonly used types of confocal microscopy are the laser scanning confocal microscopy and spinning disk confocal microscopy. Here, we describe spinning disk confocal microscope and some applications well suited for the spinning disk confocal microscope.

In spinning disk confocal microscopy, multiple spots on the specimen are illuminated simultaneously by projecting an image of a spinning disk perforated with a spiral of pinholes (Nipkow disk). Thus as the disk rotates, spots of light rast across the specimen. Reflected and fluorescent light pass back through the objective and then the
pinholes. As in laser scanning confocal microscopy, the pinholes reject out of focus light to create thin optical sections. Unlike laser scanning confocal microscopy, spinning disk confocal microscopy creates images that can be viewed directly through the microscope oculars with the naked eye or captured recorded with digital camera. Because the disk rotates rapidly, full frame images can be collected at video rates (30 frames/sec) or even faster. The illumination light source can either be an arc lamp or laser light. A picture of the CARV II spinning disk confocal microscope is shown below (Figure 1). As with other forms of epi-illumination fluorescence microscope, a dichroic mirror reflects excitation light from a mercury halide lamp into the objective lens and transmits emission light to a detector, in this case a sensitive CCD camera.

One major application of spinning disk confocal microscopy is calcium imaging at video rates. Another application is of intravital (in vivo) imaging where the fast acquisition speeds help overcome the problem of motion artifact. Also, since spinning discs can use a panchromatic light source from ordinary non-laser light sources, allowing excitation of virtually any fluorophore using appropriate excitation and emission filters.

In summary, the principal advantages of spinning disk confocal microscopy are:

1. Image acquisition at video rates
2. Cheaper arc lamps panchromatic illumination makes systems cheaper compared to more expensive laser illumination.
3. Panchromatic light sources allowing excitation of a wide range of fluorophores
4. Comparable resolution to laser scanning confocal microscopy

The Cell and Molecular Imaging Core is home to a Becton Dickinson CARV II spinning disc confocal microscope housed in QF418 in the Quadrangle building. This spinning disc confocal microscope is equipped with various filters and a Photometrics Cascade 512B CCD camera. If your laboratory can benefit from spinning disc confocal microscopy, please contact Dr. Venkat Ramshesh at ramshes@musc.edu.
Fig. 1. Becton Dickinson CARV II spinning disc confocal microscope in the cell and molecular imaging core. Multiple points on the specimen are imaged simultaneously using the pinholes of the spinning disk enabling video rate confocal imaging. The microscope is housed in QF418.

To learn more about core services, equipment and charges, please go to:

www.musc.edu/ccdir

or

http://hcc.musc.edu/research/sharedresources/cellandmolecularimaging.htm

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