Welcome to 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 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 fluorescence resonance energy transfer (FRET) microscopy.

Special Happenings:
3rd Charleston Workshop on Light Microscopy for the Biosciences.
In June 6-11, the Cell and Molecular Imaging Core will hold the "Third Charleston Workshop on Light Microscopy for the Biosciences". The Workshop will provide theoretical and hands-on training in basic and advanced light microscopy techniques to postdoctoral fellows, graduate students and scientists. Limited scholarships are available for MUSC students from the Hollings Cancer Center. Major microscope vendors will bring their latest equipment for students to train on. Please email Dr. Venkat Ramshesh at ramshes@musc.edu for more information about this workshop.

Spotlight on fluorescence resonance energy transfer microscopy:
Fluorescence resonance energy transfer (FRET) microscopy is a distance-based technique involving the non-radiative transfer of energy from a donor to an acceptor molecule provided the two molecules are in close enough proximity (1 to 10 nanometers). FRET occurs when there is overlap between the donor emission spectrum and the acceptor absorbance spectrum. As FRET occurs the intensity and lifetime of the donor fluorescence decreases and that of the acceptor increases indicating the spatial proximity of the two molecules. These properties of FRET allows one to study molecular interactions, protein distributions, conformational changes and binding sequences with nanometer resolution in living cells and tissue. For example by using fluorescent proteins or by tagging proteins with fluorophore one can study its localization and proximity to other fluorescent protein molecules using FRET.

FRET microscopy can be performed using wide-field, confocal and multiphoton microscopy with each having its advantages and disadvantages. Wide-field FRET is the most commonly used FRET technique as it is the easiest to implement. However it suffers from out-of-focus light thereby limiting three-dimensional resolution. Confocal and multiphoton FRET eliminate out-of-focus fluorescence thereby enabling three
dimensional FRET imaging with depth resolution. Multiphoton FRET because of its penetrating ability is useful for thicker specimens. One important issue to consider with quantitative FRET is bleed-through of the donor and acceptor fluorescence. A number of algorithms are available in order to correct for these problems and extract the true FRET information.

Figure 1 shows an application of FRET in endothelial cells to distinguish depolarized and polarized mitochondria from the Lemasters laboratory (1). The left panels show Mitotracker green (MTG) fluorescence from the mitochondria. Excited with blue light, MTG fluoresces green. However, MTG does not absorb green light. Thus, green excitation results in essentially no red fluorescence. In the middle panels, tetramethyl rhodamine methyl ester (TMRM) was also added to the cells. As a result of FRET, energy of MTG excited by blue light is transferred to TMRM resulting in quenching of green fluorescence. In the right panels, potassium cyanide was added to depolarize the mitochondria, which releases TMRM but not covalently bound MTG. Consequently, green MTG fluorescence excited by blue light recovers as red fluorescence excited by green light decreases (lower right panel).

Fig. 1. FRET in cultured sinusoidal endothelial cells. Green MTG fluorescence excited with blue (488 nm) light was present in mitochondria (upper left panel). TMRM was subsequently added, which caused the appearance of bright red fluorescence after excitation with green (568 nm) light. After TMRM, green MTG fluorescence excited with blue light became quenched (upper middle panel). Mitochondrial de-energization with KCN caused red TMRM fluorescence (lower right panel) to disappear and green MTG fluorescence (upper right panel) to recover. (From (1))
In order to learn more about FRET microscopy and its potential applications please read references (2-4). The Cell and Molecular Imaging Core has confocal microscopes capable of performing FRET studies. Please contact ramshes@musc.edu if you are interested in using FRET for your research.

Reference List :


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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