

Bankhead-Coley Cancer Research Program

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Shared Instrument Grant
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Project Title: Leica TCS SP5 AOBS Confocal Microscope with Tandem Scanner

Project Summary: University of Florida Shands Cancer Center (UFSCC) researchers are using a confocal microscope for the recording of high-resolution images that show the structural features of cancer cells, tumors, and the tumor microenvironment. Most importantly, researchers are observing high-speed dynamic processes within the living cancer cells, from which essential informative, quantitative data are extracted. Understanding the cellular and sub-cellular behavior associated with the onset of cancer will lead to the development of tools and procedures for identifying and treating cancer at the very onset of cellular damage, i.e., low grade dysplasia. Recent advances in optical microscopy have made it possible to image features that are thousands of times smaller than those using conventional microscopy approaches. Fluorescent tracers, which are designed to attach to specific cellular sites, offer the potential to gain information regarding cellular dynamics. Unlike routine light microscopy, in confocal microscopy, a scanned, focused laser beam and a pinhole spatial filter are used to provide high resolution both in the plane of the image and perpendicular to the image. Newer, more sophisticated, confocal microscopes also allow researchers to employ methods to directly study the molecular dynamics within living cancer cells. FRAP (Fluorescence Recovery After Photobleaching) is used to measure the dynamics of 2-D or 3-D molecular mobility, e.g., in diffusion, transport, or any other type of movement. FLIP (Fluorescence Loss in Photobleaching) is the decrease or disappearance of fluorescence in a defined region adjacent to a repetitively bleached region. FLIP is also used to measure the dynamics of molecular mobility in membranes or living cells. FRET (Fluorescence Resonance Energy Transfer) uses two fluorescent markers and a laser source to image interactions between cellular components (e.g., two different proteins) on the nanometer scale. After an exhaustive internal evaluation, the Leica TCS SP5 AOBS confocal microscope with tandem scanner was selected for research at UFSCC because it allows acquisition of both types of data (slow high-resolution imaging and fast dynamic quantitative measurements). The instrument has the following major options: 1) FRET/FRAP/FLIP capabilities, and 2) full environmental incubation systems for the study of live cell populations. This specific confocal microscope has been selected by a multidisciplinary team of UFSCC scientists because it brings functionality to the UFSCC that has been absent. The projects described in the grant are representative of the broad base of the UFSCC research that spans the College of Medicine, College of Liberal Arts and Science, Engineering, and IFAS. The projects cover areas ranging from the study of the transport of zinc within cancer cells (Cousins) to therapeutically targeting the blood vessels that feed tumors (Siemann). Three projects that highlight the utility of the Leica confocal microscope are described here. Drs. Cance and Lele use confocal microscopy to explore the role of focal adhesion kinase (FAK) in breast cancer progression. Dr. Ishov, an expert in microscopy, is investigating the exciting, newly-discovered relationship of the cancer-related protein Daxx to cell division. Finally, Dr. Tan continues to employ cutting edge

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nanotechnologies to explore important molecular events in the cancer cell. As fundamental discoveries identify targets for new cancer therapies, the need to identify their precise cellular location and study the dynamics of the molecular associations is of increasing importance.