

**Day 1: Tuesday, February 10<sup>th</sup>**  
**Emergence of Quantitative Imaging**

**9:00 – 12:00 Morning Symposium: Grace Auditorium**

**Keynote presentation:**  
**The Science and Technology of Cytometry**

**Presenter:** J. Paul Robinson, Ph.D., Purdue University Cytometry Laboratories, Purdue University, W. Lafayette, IN

*J. Paul Robinson is SVM Professor of Cytomics in the School of Veterinary Medicine and a professor in the Weldon School of Biomedical Engineering at Purdue University. He is the director of the Purdue University Cytometry Laboratories and Deputy Director for Cytomics and Imaging in the Bindley Biosciences Center there. He is Past President of the International Society for Advancement of Cytometry and Managing Editor of Current Protocols in Cytometry.*



*An active researcher with over 130 peer-reviewed publications and 20 book chapters, Dr. Robinson has edited 7 books, given over 100 international lectures and taught advanced courses in over a dozen countries. He was elected to the College of Fellows of the American Institute for Medical and Biological Engineering, was the winner of the 2004 Pfizer Award for Innovative Research, and the Gamma Sigma Delta Award of Merit Research in 2002.*

*Dr. Robinson's research has focused on reactive oxygen species primarily in neutrophils, but more recently in HL-60 cells and other cell lines. His lab is currently studying the biochemical pathways of apoptosis as related to reactive oxygen species in mitochondria. Over the past several years, his group has expanded their interest in bioengineering, with hardware and software groups developing innovative technologies such as the first high-speed multispectral cytometry, optical tools for quantitative fluorescence measurement and advanced classification approaches for clinical diagnostics and bacterial classification. An important focus of the laboratory is the "Cytometry for Life" program ([www.cytometryforlife.org](http://www.cytometryforlife.org)) which is targeted at developing low-cost, easy-to-use diagnostic tools for resource-limited nations.*

**Abstract**

For over 40 years cytometry has been defined, somewhat by default, as the measurement of cells, with a focus on flow cytometry. The implications of this definition are that flow cytometry has been the leading technology for cell analysis. However, this is far from the case. Simultaneous with developments in flow, other technologies for measurement of cells have opened up new opportunities. Imaging, laser scanning cytometry and flow cytometry have emerged, with well-defined advantages for each technology. It has become clear that no single tool is adequate, nor should it be, to solve difficult scientific questions.

Given that, we must ask the question, "Does science drive the advancement of technology or is the reverse true?" Clearly we can identify examples where both situations occur. While flow cytometry may identify a subpopulation of great interest, once it is identified and separated, other tools such as molecular analysis and advanced imaging may open up a field. There are many examples of this in the literature. Conversely when a new technology is invented, a diverse group of scientists studying different problems can engage that technology for discovery. The same situation occurs as existing technologies are advanced in their capabilities. This presentation will outline the role of technology in discovery and relate this to the impact on the field of cytometry.

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**Laser Scanning Cytometry: Where Does It “Fit In” with  
Modern Biomedical Imaging?**

**Presenter:** William G. Telford, Ph.D., National Cancer Institute, National Institutes of Health, Bethesda, MD

*William Telford obtained his Ph.D. in microbiology from the Michigan State University in 1994 and continued his postdoctoral training in immunology at The University of Michigan Medical School. He joined the National Cancer Institute of the National Institutes of Health (NIH) in 1999 and presently is Director of the flow cytometry core laboratory in the NCI Experimental Transplantation and Immunology Branch. Dr. Telford's research interests include laser scanning cytometry and other areas of instrument and assay development.*

**Abstract**

Image cytometry (also termed laser scanning cytometry) represents a unique hybrid technology between flow cytometry and traditional fluorescent microscopy. Imaging cytometers analyze the light scattering and fluorescent properties of biological specimens like a flow cytometer, but also collect correlated quantitative images of the cells simultaneously. This powerful technique is a dramatic expansion of traditional cytometric analysis, allowing the cell images and their morphological and structural information to become a cytometric parameter, like forward scatter or fluorescence.

In this introductory presentation, we will discuss the theory and practice of image cytometry, and provide a general overview of the technology available to biomedical scientists. We will also cover a wide variety of applications for this technology, from individual cells to intact tissues and whole organisms.

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**Applications of Laser Scanning Cytometry for Cell and Tissue Analysis in Cancer Research**

**Presenter:** Heather Zecchini, Ph.D., Cancer Research UK, Cambridge Research Institute, Cambridge, UK



*Heather Zecchini graduated from Anglia University then received her PhD from Cambridge University in 2004 for studies of intestinal epithelial stem cells and progenitors using a novel in vivo clonal marking system. Her work included characterisation of the use of cre fusion genes as clonal markers and cell lineage analysis of specific cell types within the murine intestine, work that revolved around microscopy and sparked a keen interest in imaging technology. She is currently deputy head of the Imaging and Microscopy Laboratory at the Cancer Research UK Cambridge Research Institute, focusing on the development and application of qualitative and quantitative imaging to study the processes involved in controlling the normal biological state of cells and tissues, the perturbation of these mechanisms leading to cancer and to aid in the development of cancer drug treatments.*

**Abstract**

The iCys<sup>®</sup> Research Imaging Cytometer is an essential instrument for many studies undertaken within the Institute. Application examples are outlined, demonstrating the importance of laser scanning cytometry (LSC) in cancer research.

LSC analysis of protein subcellular localisation and correlation with parameters such as cell cycle is utilised in prostate cancer studies, for example in the characterisation of the role of  $\beta$ -arrestin. Recently it has been demonstrated that several membrane trafficking proteins can translocate to the nucleus and play a role in transcriptional activation. Using LSC in combination with confocal studies, the behavior of  $\beta$ -arrestin in prostate cancer cells is being elucidated, particularly its role in cell proliferation and its subcellular distribution throughout the cell cycle.

TIMP-3 over-expression causes apoptosis in tumor cells and smooth muscle cells in vascular disease models. We use LSC to further characterize the mechanism of TIMP-3 mediated apoptosis and to determine the death receptor involved in human smooth muscle cells. These large, irregular, slow-growing cells are difficult to analyse by FACS, but LSC protocols allow quantification of the efficiency of shRNA knockdown of receptors, determination of receptor expression within foci and the total number of foci per cell, and facilitate analysis of the effects of receptor reduction on these cells.

We also use LSC to assess compound distribution across large normal and tumor tissue samples, which can, for example, be applied to cancer drug delivery research. The vasculature within tumors and its relation to drug distribution can be analyzed efficiently and accurately.

Other applications include the measurement of ligand uptake in cancer cells and the assessment of DNA damage in human lymphocytes using  $\gamma$ H2AX staining.

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**Investigation of the Role of JNK-Stimulatory-Phosphatase 1 in Neurodegeneration in a Mouse Model of Parkinson's Disease**

**Presenter:** Li Li, Ph.D., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY



*Li Li received her PhD in Cell and Molecular Biology from St. John's University, NY, where her research focused on the structure and function of FLO11, a critically required adhesion molecule in several fundamental developmental processes in yeast. She identified the adhesion domain as well as its likely receptor on the cell surface and characterized the sequences required for subcellular localization of the protein. She is currently a Postdoctoral Fellow at Cold Spring Harbor Laboratory, where she has been investigating the physiological role of JSP1 (JNK-Stimulatory Phosphatase 1) in neurodegeneration in a mouse model of Parkinson's Disease.*

**Abstract**

JNK-stimulatory-phosphatase 1 (JSP1) was initially identified in our laboratory and shown to activate the JNK signaling pathway in cell transfection models, suggesting a new potential tier of control of JNK activation. In order to explore the function of JSP1 further, we generated a knockout mouse and tested for effects on JNK signaling. A key pathological feature of Parkinson's disease (PD) is a progressive loss of dopaminergic (DA) neurons in the substantia nigra (SNpc), with increasing evidence suggesting a role for JNK in neurodegeneration in animal models and patients with PD. For example, phosphorylation of c-JUN, a transcription factor and direct substrate of JNK, is considered a hallmark of DA neurons undergoing apoptosis in PD.

To investigate the function of JSP1, we used a mouse model of PD generated by treating animals with the neurotoxin MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine). Using various imaging technologies, including iCys<sup>®</sup>, to focus on JNK signaling in the SNpc region of the brain, we observed that MPTP-induced phosphorylation of c-JUN was delayed in *jsp1*<sup>-/-</sup> mice compared to their wild type counterparts. Furthermore, the phosphorylation of JNK itself and MKK4, the MAP Kinase Kinase that activates JNK directly in the signaling pathway, were both delayed in the absence of the phosphatase JSP1. These data indicate that JSP1 plays a *positive* role in promoting JNK signaling, at least in this model of PD. Currently, we are testing the hypothesis that JSP1 exerts its effects upstream of MKK4 by dephosphorylating an inhibitory site on a MAP Kinase Kinase Kinase, thereby promoting signaling. Furthermore, we are using microarrays to examine changes in MPTP-induced gene expression in wild type compared to *jsp1*<sup>-/-</sup> mice, to explore further the signaling function of the phosphatase. However, the delayed phosphorylation, and hence delayed activation, of the JNK signaling pathway components was not sufficient to affect the extent of MPTP-induced degeneration of DA neurons in *jsp1*<sup>-/-</sup> mice. Just as there is functional redundancy between individual *jnk* genes, it is possible that there is functional redundancy in the phosphatases. Future efforts will explore the role of the closely related phosphatase JSP2 in this system.