TECHNICAL PRESENTATIONS: SESSION II

3D imaging of HIV transmission and entry
Sriram Subramaniam, Laboratory of Cell Biology, NCI

This presentation will discuss new strategies to map viral landscapes at molecular resolution using novel 3D electron microscopic technologies.

Site-Specific Chemical Mapping of Individual Cells in Two- and Three Dimensions with Imaging Mass Spectrometry
Christopher Szakal, Surface and Microanalysis Science Division, Analytical Microscopy Group, NIST

Together with collaborators from the Laboratory for Cell Biology at NIH's National Cancer Institute, we have begun to explore the challenging prospect of chemically mapping molecules in single cancer cells in two and three dimensions. By utilizing the surface sensitivity and molecular imaging capabilities in time-of-flight secondary ion mass spectrometry (ToF-SIMS), along with newly developed sample preparation protocols, we have attained state-of-the-art 400-nm resolution chemical maps of immortal cell lines known as HeLa cells, including the distributions of lipid and salt signatures. This technology can provide the foundation for exploring the site-specific chemical changes responsible for disease progression and allow for the development of fast and robust imaging mass spectrometry technologies to be used in clinical settings.

Tools for Quantitative Imaging of Cells on Extracellular Matrix Mimics
John Elliott, Cell Systems Science Group, Biochemical Science Division, NIST

Quantitative fluorescence imaging and image analysis are powerful tools to measure the observable phenotypic characteristics of cells under experimental conditions. We have developed a robust two-color cell staining procedure that greatly facilitates image analysis procedures for determining cell morphology (structure, form and arrangement). We have also focused on the development of highly reproducible cell adhesion substrates coated with a fibrillar collagen type I extracellular matrix. The substrate preparation is compatible with many types of conventional cell culture plasticware and can be used in high-throughput imaging instrumentation. Our studies indicate that these materials mimic many properties of fibrillar collagen gels and provide excellent optical properties for cell imaging on an extracellular matrix substrate. These tools can be important components for observing phenotypic changes in cell behavior.

Live Cell Microscopy to Follow the Temporal Regulation of Gene Expression
Michael Halter, Cell Systems Science Group, Biochemical Science Division, NIST

Quantitative measurements of dynamic processes in single cells are challenging and require the identification, segmentation, and tracking of live cells. Collecting and storing live-cell image data has been greatly facilitated by automated microscopy, but determining quantitative metrics of cell behavior using image-analysis algorithms remains challenging. We illustrate the application of live-cell microscopy and automated image analysis tools developed at NIST to measure the dynamics of gene expression in single cells by monitoring levels of green fluorescence protein.

Processing for Cellular Metrology
Alden Dima, Computational Biology Project, Information Technology Laboratory, NIST

High-throughput technologies for measuring the characteristics of cells are generating large

amounts of complex data that are difficult to process and convert into knowledge. Under the auspices of the NIST Computational Biology Project, experimentalists and computational scientists are working together to address mutually defined image-based challenge problems (well-defined challenges embodying essential difficulties in a research area whose solutions have broad impact). One challenge has been to evaluate segmentation techniques (methods to locate objects and boundaries in images) and associated parameters to reliably determine the cell morphology (structure, form and arrangement) for the purpose of comparing cell lines as part of a new standard procedure under development. Another challenge involves the segmentation and tracking of live cells in an image sequence to quantify the total fluorescence intensity of individual cells over time, and thereby gain a better understanding of protein expression over the cell cycle.

Phantom Development to Support Quantitative MRI

Robert Usselman, Biomagnetics Program, Electromagnetics Division, NIST

NIST has recently initiated programs to support quantitative biomagnetic imaging. As part of the International Society for Magnetic Resonance in Medicine (ISMRM) Committee on Standards for Quantitative Magnetic Resonance, NIST is assisting in the design and fabrication of a new phantom, an object used to calibrate imaging systems. The system phantom is designed to measure geometric distortion, contrast properties, resolution, signal-to-noise ratio, and a variety of other parameters. This will be the first MRI phantom that has NIST traceability and will be calibrated for a range of temperatures and fields. The phantom will initially be used for quality control during image-based clinical trials, though widespread clinical implementation is envisioned. NIST is also working with the Quantitative Imaging Biomarkers Alliance (QIBA) to develop a dynamic, contrast-enhanced MRI phantom and will be developing susceptibility phantoms and flow/diffusion phantoms.

Color Contrast Agents for MRI Utilizing Magnetic Microstructures

Gary Zabow, Laboratory of Functional & Molecular Imaging (joint NINDS and NIST), Stephen Dodd, (NINDS), Alan Koretsky, (NINDS), John Moreland, (NIST)

A joint venture between NIST and NINDS/NIH has resulted in the development of microfabricated structures that can be used as MRI contrast agents with enhanced functionality or as micro-RFID (radio-frequency identification) tags. The microstructures can be engineered to appear as different effective colors when resolved using MRI as opposed to strictly grey-scale contrast of existing MRI agents. In this way they can be thought as radio-frequency analogs to quantum dots. A set of agents could be produced that would enable *in vivo* labeling and tracking of multiple different types of cells simultaneously. The agents can also act as radio-frequency probes of various physiological conditions. Potential applications for these structures include MRI, cardiovascular diseases imaging, drug development, drug candidate distribution tracking, diagnostics, and microfluidics.

Multicolored Fluorescent Cell Lines for High-Throughput Drug Discovery

Enrique Ubani Zudaire, Angiogenesis Core Facility, NCI

We have developed a series of immortalized cell lines that were selected to represent the different cell types found in angiogenesis *in vivo*, that constitutively express different fluorescent proteins. Based on these cell lines, the inventors have developed several *in vitro* angiogenesis assays and a software application that can be used to investigate the relationships between different cells involved in angiogenesis, to develop new combinatorial approaches to boost the efficiency of existing therapeutics, and to facilitate the discovery of new potential single or combination drugs. This technology could potentially be used to develop a high-throughput screening assay for

angiogenesis or anti-angiogenesis drugs, or to screen compounds for cytotoxicity. The inventors have already demonstrated proof of concept for this technology by developing a high-throughput screen for potential angiogenic drugs, and they have also recently developed a cytotoxicity assay.