Abstracts of posters displayed*

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Integrated -omics and Systems Microscopy

PARADIGM-SHIFT predicts the function of mutations in multiple cancers using pathway impact analysis

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Somatic cells acquire genomic alterations over the lifetime of an organism. Cancer cells accelerate this process due to an inability to sense and respond to such damage. While there exist well-described mutations in certain codons of key genes that drive oncogenesis, most somatic mutations in cancer are neutral with respect to overall cell fitness. Many approaches exist to predict the impact of a mutation. However, no existing method incorporates pathway-level information into the assessment of the consequences of a mutation. Investigating the impact of a mutated gene on its pathway neighborhood may provide complementary information to current approaches.

To predict the consequences of a given mutation (gain or loss of function), we adapted a method based on a pathway-guided model for integrative genomics interpretation called PARADIGM. The method makes use of gene activities inferred in the context of a set of pathway interactions to interpret gene expression and copy number data. The method can be used to predict whether a mutational event is a gain or loss-of-function, or neutral event.

We tested the method on several positive and negative controls for multiple cancers for which pathway information was available and demonstrate that it has both sensitivity and specificity. Application to the TCGA glioblastoma, ovarian, and lung squamous cancer datasets revealed several novel mutations with predicted high impact. The method shows promise particularly for those low-frequency mutational events that have a clear pathway signature but are difficult for current approaches to assess with statistical confidence.

* not inclusive of all posters

Signaling and immunophenotypic diversity in pediatric acute myeloid leukemia as defined by 31-parameter single-cell mass cytometry

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Acute myeloid leukemia (AML) encompasses a broad spectrum of cellular morphologies. This spectrum exists both within each patient in the form of a malignant developmental hierarchy, and across patients in the form of distinct disease classes with distinguishing mutational or pathological hallmarks. Flow cytometry has long been an essential tool for classifying AML surface markers, and has enabled improved diagnosis, minimal residual disease monitoring, and cell sorting for functional studies. Previous work by our laboratory and others (Irish et al., Cell 2004; Kornblau et al., Clin. Cancer Res. 2010) has shown that clinically relevant heterogeneity also exists at the level of single-cell intracellular signaling capabilities, particularly in response to *ex vivo* perturbations. Due to the constraints of fluorescent flow cytometry, the number of simultaneous parameters measured per cell in these studies was limited (e.g., 3 - 6). We hypothesized that there may exist additional layers of heterogeneity in both surface marker phenotypes and signaling responses which were invisible to the low-parameter systems used in these studies.

To address this challenge, we used a next-generation 31-parameter 'mass cytometry' platform to survey the diversity of signaling responses and their relationship to subsets defined by surface marker patterns. We measured the simultaneous co-expression of 16 surface markers and 15 dynamic intracellular signaling epitopes (e.g. phosphorylated kinase substrates) in a cohort of pediatric AML diagnosis bone marrow samples (n=18) and healthy adult bone marrow controls (n=3). Signaling dynamics were measured under 18 stimulation conditions, including a battery of cytokines, chemical stimuli, and small molecule kinase inhibitors. The resulting single-cell data was overlaid and compared using a new, unsupervised flow cytometry analysis and visualization program – SPADE (Spanning-tree Progression Analysis of Density-Normalized Events) – which links rare, transitional, and abundant cell types alike along a branching tree organized by phenotypic progression. Thus, we distilled the diversity of cellular phenotypes, their relative frequencies, and the corresponding signaling dynamics of each cell population onto a single graph structure representing the distinct and overlapping expression patterns in the 16-dimensional "immunophenotypic space" explored by the 21 individuals. Striking signaling and immunophenotypic diversity was observed between the AML samples, particularly in response to pervanadate stimulation. Pervanadate liberates intracellular kinase cascades by broadly inhibiting tyrosine phosphatases, and thus reveals the cell's potential for kinase activity and the interplay of signaling circuits in different cell populations. Interestingly, two transcription factors implicated in AML pathogenesis – CREB and c-Cbl – often exhibited mutually exclusive phosphorylation patterns in different subsets of primitive (CD34+) cells. Specifically, CREB was readily phosphorylated at Ser133 upon pervanadate exposure in the CD34+CD123- cells, while c-Cbl phosphorylation at Tyr700 was restricted to CD34+CD123+ cells. As CD123 (IL-3Ra) has been implicated as a marker of AML stem cells, the finding that these cells are poised for c-Cbl signaling may suggest a potential therapeutic avenue.

This work demonstrates the utility of 31-parameter mass cytometry for the simultaneous characterization of multiple cancer stem cell markers and comparison of their expression patterns to the normal tissue hierarchy. These results will inform future studies of AML signaling biology, prognostic biomarkers, and indicators of minimum residual disease.

EFS and SCB contributed equally to this work.

Systems biology at the single cell level

Dr. Nolan's laboratory focuses on the analysis of biological events at the single cell level using novel genetic and FACS-based approaches at the intersection of immunology, autoimmunity, biochemistry, and cancer. The laboratory studies phospho-protein immune cell and cancer signaling, and other metabolic parameters by analysis of biochemical functions at the single cell level in primary cell populations. This includes interrogation of cancer (Cell, 2004) and immune signaling networks in complex cell populations (Science, 2005), drug screening approaches (Nature Methods, 2005, (cover article), Nature Chemistry and Biology, 2007a, Nature Chemistry and Biology, 2007b (cover article)) and using multiparameter data to stratify signaling maps from patient samples, (Cancer Cell, 2008, cover article). Other major interest areas of the laboratory include mapping of signaling networks within complex populations of immune cells, developing systems biology approaches to develop an atlas of immune cell differentiation (Nature Biotechnology, 2011), the development of mechanism-based diagnostics for use in clinical trial studies. This poster highlights recent work of the lab, including:

-Single cell analysis using mass cytometry
-Population analysis using the SPADE clustering algorithm
-Inspection of iPS progression using TimeSPADE
-Analysis of variable leukemic differentiation arrest in pre B-cell populations
-Inspection of hematopoietic system responses using mass cytometry
-Signal correlation distinctions between immune system components of infected and uninfected mice
- Automated differential population analysis

Automated identification of differential signatures in cellular populations

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Nuanced behavior of phenotypically distinct cellular populations plays a critical role in both immune response to and development of cellular diseases (i.e. cancer). Furthermore, recent work has highlighted the utility of subpopulation profiling in patient prognosis (see below). Accordingly, the ability to discern and identify condition-relevant populations can potentially play a critical role in disease diagnosis and treatment. To facilitate analysis of complex mixtures of cells, instrumentation technologies such as flow cytometry have emerged that enable high-throughput, simultaneous measurement of intra- and extra-cellular molecules within a single cell. However, the high-dimensionality of such data coupled with normal biological variation make comprehensive manual identification of phenotype-relevant subpopulations unfeasible.

Recent work on cell population-finding algorithms has enabled automated identification of clusters of cells in multidimensional space (1,2). We utilize these algorithms in conjunction with supervised learning models and present here a method for automated discovery of differential cell populations. Given multiple samples from patients belonging to two or more phenotypic classes, we automatically identify sub-populations of cells within each sample, extract meta-features describing each population, and train a supervised classifier for identification of a sample class. It follows that the stratifying features of a successful classifier correspond to class-differentiating populations. We demonstrate our method to by identifying differential populations in the blood of HIV patients challenged with two different antigens.

As technologies such as mass cytometry continue to increase the number of simultaneous measurements per cell, automated approaches such as the one described here will play a crucial role in the unbiased discovery and identification of populations involved in both disease mechanism and response.

Bulk and Spatial Elemental Analysis as Complementary Methods in Cancer biology

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High resolution fluorescent microscopy using cellular markers conjugated to fluorescent labels are common in cell biology to identify subcellular organelles and localize proteins within cells or organelles. Western blots or ELISA subsequently allow for determination of the relative abundances of tagged proteins but the spatial information is lost. Flow-cytometry uses single-cell suspensions in a multiparametric approach. As such modern flow cytometers can distinguish >18 different fluorescent tags with high throughput, however all spatial resolution is lost. Mass-cytometry, uses Ln3+-conjugated primary antibodies and can potentially distinguish between 40 – 60 different antibodies but is currently lacking the high throughput capabilities of flow-cytometry and, again, does not provide any information about spatial distribution of the targets. The mass cytometer consists of a TOF ICP-MS that was modified in its technology and sensitivity to measure Ln3+ concentration in single cell suspensions.

In our lab we are developing alternative uses for Ln3+ based antibodies. We are in the planning stages of using in Ln3+ based conjugates on complex sample mixtures, perform an online chromatography separation with subsequent ICP-MS detection of the label. This would in effect be equivalent to a Western blot with the exception that we don't depend on gel electrophoresis and blotting steps, thereby eliminating several unknowns of these processes. The second approach takes the Ln3+ based conjugates in the second (and third) dimension, we are conducting imaging experiments on labeled samples using hard X-rays as they are available at synchrotron facilities around the world. Synchrotron-based X-ray fluorescent imaging (SXRF) is a technique to quantitatively probe elemental distributions at high spatial resolution (<150nm soon to be <30nm), high sensitivity (attomolar, 10-18M), and very low background. It can complement mass-cytometry in the same fluorescent microscopy and Elisa complements flow-cytometry: it provides a second dimension and is fully quantitative! Additionally, it can provide chemical speciation such as the oxidation state of metals. Furthermore, the sample preparation allows for imaging of the native state (i.e. native elemental distribution), without artefacts from sample preparation need to be accounted for. We have used SXRF to determine changes in the intracellular distribution of copper during the progression of Wilson disease in a mouse model and found that the subcellular distribution changes dramatically during different stages. We furthermore observed impressive accumulations of copper in the periventricular area of mice brains, information that no other method could provide. We found that copper concentration close around the lateral ventricles in mice brains are >100 mM while the average copper concentration in brain tissue is about 60 µM! A major drawback of SXRF is that it is “blind” to cellular or organelle structures such as membranes unless they differ in their elemental content. For example, we can easily detect the nuclei by their elevated phosphorous content but we can’t “see” the trans-Golgi network unless it is enriched with some element. To expand the capabilities of SXRF we have begun to use Ln3+ conjugated antibodies of organelle markers to visualize organelles such as lysosomes or TGN. Specifically we used Pr3+ and Sm3+ in combination with anti-Lamp1. The l-absorption edges for both Pr and Sm are ideally located between the V and Fe k-absorption edges and should be easy to identify, fit, and quantify. This poster will outline the technique as well as results of our initial attempts to image Pr-antiLAMP1 cells and tissue. The experiments described here are the first towards our long term goal of developing SXRF as a complementary, quantitative, spatially resolved approach to mass-cytometry.

Quantitative imaging of individual mRNAs in situ and co-imaging of protein in breast cancer cells

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Measuring mRNA level provides key information of gene expression in biological systems. Northern blot, real-time PCR, and RNA-Seq have been used to quantify mRNAs, but those methods provide average gene expression information from bulk transcrpitome measurement. To get spatial and heterogeneous information in intact cells and tissues, we developed a fluorescence in situ hybridization (FISH) for imaging individual mRNA molecules in breast cancer cells using multiple probes labeled with single fluorophores, modified from Raj et al, 2008. We acquired images of 0.2 mm optical sections using automated widefield microscopy (60X objective, NA=1.42), performed "Deconvolution" to subtract blurred light or to reassign it back to a source, and generated three-dimensional image of whole cell. Our procedures greatly improved the resolution of mRNA particles' image, compared with the original work (Raj et al, 2008). Each transcript was clearly detected as a particle with a diameter of about 0.25 mm, counted by IMARIS software. We successfully performed simultaneous detection of three different mRNA species, Her2, Akt1, and Akt3, in single cells of breast sub-type cell lines, HCC1954, AU565, MDAMB231, and MCF7, using probes labeled with different fluorophores. Our results of quantifying each mRNA particle were closely correlated to the RNA-Seq data. To simultaneously visualize and quantify mRNAs and proteins, we combined immunocytochemistry and FISH, called as "immunoFISH". Using this technology, we counted Fra-1 mRNA molecules and detected an activated form of Akt protein (pAkt) in MCF7 cells, treated with insulin on different times. Membrane localization and intensity of pAkt were promoted with insulin treatment, and Fra-1 mRNA particles increased peaking with 2 hr treatment at 4.6-fold increase, suggesting that Fra-1 is an Akt-inducible gene in breast cancer cells. Our method could provide a spatiotemporal profile of both transcripts and proteins along with pathways activation in cells, tissues, and solid tumors in a quantitative manner.

Sunjong Kwon is the presenting author.

PIK3CA mutation status in HER2 positive breast cancer: influences on PI3K-AKT signaling and response to therapeutic regimens

James E. Korkola, Soulaiman Itani, Eric A. Collisson, Nora Bayani, Laura Heiser, Nicholas Wang, Heidi S. Feiler, Paul T. Spellman, Yi-ling Lu, Gordon Mills, Richard Wooster, Claire Tomlin, and Joe W. Gray

HER2+ breast cancer accounts for 20-30% of all new breast cancer cases, and has been shown to have a worse outcome than non-HER2+ cases. Targeted therapeutics such as trastuzumab and lapatinib have been developed that improve patient outcomes, but both suffer from both de novo and acquired resistance to therapy. In the case of trastuzumab, *PIK3CA* mutation has been implicated as a major effector of resistance. We previously observed an association between PIK3CA mutation status and response to combined treatment with lapatinib plus an AKT inhibitor (GSK690693). To investigate further, Reverse Phase Protein Array (RPPA) analysis was performed on a panel of HER2+ cell lines that had both mutant and wild-type PIK3CA. The RPPA data demonstrated that HER2+ samples with PIK3CA mutations had higher baseline activity of elements of the PI3K-AKT signaling network than their wild-type counterparts. Furthermore, this elevated level of activity was maintained following treatment with lapatinib, but was overcome with a combination treatment using lapatinib plus the AKT inhibitor. We also performed modeling experiments to interrogate the HER2 signaling axis. These models suggested that in wild-type cells, stimulation with heregulin prior to lapatinib treatment would result in sensitization of the cells to lapatinib, which was confirmed with experimental results. However, the modeling predicted that the two different PIK3CA mutations would affect response to lapatinib following stimulation in different ways. Modeling predicted H1047R mutants would be relatively resistant to such stimulation sensitization experiments, which was once again confirmed experimentally. Together, these experiments suggest that the therapeutic benefit of lapatinib can be enhanced by either pre-stimulating the cells prior to treatment in the case of wild-type PIK3CA cells, or by giving combinations of lapatinib with AKT inhibitors in the case of mutant PIK3CA cells. The fact that neither approach works in the opposite setting (i.e., lapatinib plus AKT inhibitor in wild type cells and stimulation prior to lapatinib in mutant cells) suggests that careful patient selection will be required if such treatment options are introduced into the clinic.

Imaging Biological Function Across Scales: From Macromolecules to Cells to Tissues and Microbial communities

Manfred Auer, Life Science Division, Lawrence Berkeley National Laboratory

The Auer lab studies functional mechanisms underlying biological processes through multiscale, multimodal imaging, ranging in scope from the organization of macromolecular complexes to the large-scale organnization of cells in tissues and/or microbial communities. Our lab specializes in faithful sample preservation cryo-immobilization followed by freeze-substitution and resin-embedding, followed by TEM 2D and 3D imaging, as well as novel 3D SEM imaging approaches (FIB/SEM and SBF/SEM). Furthermore we are active in protein localization in cells and biofilms using SNAP-tag based labeling, high throughput epifluorescence, deconvolution optical microscopy as well as photooxidation and subsequent EM analysis. Among our biological systems of interests are the inner ear hair cells, underlying our senses of hearing and balance, mammary gland organoid undergoing branching morphogenesis and breast cancer cells cultured in 3D Matrigel, as well microbial communities relevant to toxic metal bioremediation and lignocellulose degradation. We found that integrating a variety of imaging techniques results in vastly improved insight into mechanistic function.

Biomateriomics: Multiscale Investigations in Cell and Tissue Engineering

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The emerging field of materiomics investigates the material properties of native and synthetic materials by examining fundamental links between formation processes, structures, and properties at multiple scales by applying experimental, theoretical, and computational methods. Our approach in studying the biomateriome includes multiscale simulation and experiments linked with mathematical theory. Our laboratory specifically examines the material properties of engineered cells and tissues while characterizing the multiscale phenomenon of mechanotransduction, with a particular focus on musculoskeletal diseases and health disparities. Our investigations have combined microfluidics and optical tweezers to create an optohydrodynamic trap for non-contact multiaxial loading of healthy and diseased single-cells. Original three-dimensional stress and strain formulations facilitate the full-field quantification of localized biomechanical properties as well as provide input for cytoskeletal structural models. More recently, we have fabricated a novel bioreactor to control the chemomechanical environment at the tissue-scale while exploring the interaction of millions of cells. This device is currently being used to investigate the biokinetics of vitamin D influence on bone and cartilage cells cultured in hydrogel scaffold designs while validating previous mathematical models of anabolic/catabolic dynamics. Future work will utilize these experimental/computational platforms to explore single-cell biomechanics and the phenotypic differentiation of adult mesenchymal stem cells.

Automated Correlation of Fluorescent and Electron Micrographs of Astrocyte Cells

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The accurate correlation of fluorescent and electron data is becoming increasingly popular among cell biologists but is often impeded by the lack of automation and certainty in their correlative approach. Our work focused on the automatic correlation of images taken with a fluorescent light microscope (FLM) and scanning transmission electron microscope (STEM). Astrocyte cells expressing enhanced green fluorescent protein (EGPF) collected from the hippocampus of transgenic mice were used for this experiment. The cells were also immunolabeled for EGFP with nano gold particles. Although EGFP expression was apparent in the light micrographs, images of the ultrastructure were also needed to confirm EGFP expression was confined to astrocyte cells. With the aid of newly designed software, images acquired from the FLM and an overview image from the STEM were accurately aligned. This subsequently allowed for automatic acquisition of higher resolution STEM images in selected EGFP expressing areas. Using this method it was possible to accurately locate the electron dense tags in the cellular ultrastructure and determine that the expression of EGFP was confined to only astrocyte cells.

Automated Image Cytometry in OTRADI: for basic biomedical research and technology development

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Cell-based plate reader assays typically report single or very few attributes from heterogeneous cell populations. While useful for high throughput applications, they have limited capability in elucidating complex cellular events.
The ongoing explosive advancement of our understanding of cellular processes highlights the needs for exploration of complex systems and the values of previously un-used cellular attributes for discovery. Automated image cytometry, also known as “high-content analysis” (HCA), is the amalgamation of fluorescence microscopy, instrument automation, image processing, and image analysis. In recent years it has been shown to be a valuable tool that can report intensity information of multiple probes as well as previously intractable attributes such as spatial re-distribution of markers, morphological changes, and rare events for use in assays. It has been used in drug screening and profiling. For biomedical research laboratories, HCA allows routine fluorescence microscopy and the subsequent analysis/quantitation to be scaled up to unprecedented levels.

OTRADI has established the capability of HCA with a fully automated multichannel laser confocal fluorescence microscope (Perkin Elmer Opera LX), robotics for sample handling, full complement of ability for image analysis algorithm development, and has built a complete environment for image and high dimensional numerical data exploration. This capability is perfect for quantitation of routinely performed fluorescence imaging tasks, assay development for basic biological research, and activities needed for various stages of drug discovery. This capability is to assist academic researchers as well as biotechnologists and will ultimately contribute to the technology innovation and transformation of the economy of our region.

Biostatistics Shared Resource, Knight Cancer Institute

Motomi Mori

The Biostatistics Shared Resource (BSR) plays a critical role in supporting cancer research conducted at the Knight Cancer Institute. The primary goal of the BSR is to provide biostatistics and research design support to the Knight members who are conducting cancer research. Support is provided at every stage of research, including grant and protocol development, study implementation and data monitoring, data collection and management, statistical analysis, and manuscript preparation and review. The BSR strives to foster sustainable, long-term collaborative relationships with the Knight research groups and to contribute to the science and discovery by serving as a member of the inter- and multi- disciplinary research teams. Current collaborative research projects include imaging studies involving breast, renal and prostate cancers, and early phase oncology clinical trials involving biomarkers and personalized medicine strategies.

Phase II Trial Designs to Test Individualized Treatment Assignment

Motomi Mori, Yiyi Chen, Byung S. Park, Jeffrey W. Tyner, Marc M. Loriaux, Tibor Kovacsovics, Brian J. Druker

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We have recently developed a novel in vitro screening technique to evaluate sensitivity of primary cells from leukemia patients against a panel of small-molecule kinase inhibitors. To evaluate the clinical utility of drug selection based on in vitro drug sensitivity, we propose two different phase II trial designs, both involving multiple kinase inhibitors: 1) assay guided strategy design, in which patients are first randomized to an assay-guided vs. assay non-guided group, and 2) assay adaptive design, in which patients are randomized to one of the drug groups with a randomization ratio depending on the assay result. We will evaluate operating characteristics of two designs and discuss statistical issues and challenges in testing genome/biomarker-guided strategy or more generally “personalized medicine”.

Quantum Dots to study live, molecular dynamics of ligand-receptor complexes in neurons

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Growth factor ligands, such as brain-derived neurotrophic factor (BDNF), are highly expressed in the brain where they play key roles activating downstream signaling cascades that result in brain development, learning and memory. However, in spite of their clinical importance, the molecular mechanisms underlying their signaling and trafficking remain largely unknown since current technologies are population based, and do not offer visualization of individual receptor complexes to study their movements in time and space within cells. In order to better understand the spatiotemporal dynamics of ligand-receptor signaling, we use quantum dot technology to track individual ligand-receptor pairs inside live cells. Quantum dots (QDs) are bright, resistant to photobleaching nanocrystals that have been used as fluorescent probes for biomedical applications, making them ideal candidates for live animal targeting and imaging. We have recently developed *and characterized QD-BDNF probes (biotinylated BDNF protein conjugated to streptavidin-QD655) that will help us elucidate the complex spatiotemporal organization of cells during signaling. In the present study, w*e have validated the QD-BDNF probe’s use in live cells by testing the specificity of its binding *to their BDNF receptor TrkB, and QD-BDNF* bioactivity, using *in plate* assays and cultured neurons. In addition, we have demonstrated that, compared to Alexa Fluor-labeled BDNF which shows faint fluorescence at concentrations in the nM range, our QD-BDNF probes allow us to quantify individual ligand/receptor complexes at concentrations in the pM range. Some of the functional applications of our QD-BDNF probes so far include localizing the ligand/receptor complex within the cell at different time points, and tracking their translocations within the live neuron with high spatial and temporal resolutions.

Advances in serial block face Dual Beam electron microscopy for the exploration of cardiovascular and muscular tissues

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Traditionally serial section transmission electron microscopy has been the only method available in which images are acquired from sections that are cut and mounted on grids. These image representing a 3D volume can be used to reconstruct and analyze the structure of the elements within. This method is very labor intensive, requiring a greater deal of manual dexterity, and when small mistakes occur, a continuous dataset is lost.
Recent studies have explored an alternative method for serial image acquisition in which the block face of resinembedded neural tissue was imaged within a FEG SEM [1]. Sections were removed from the imaged face in the microscope using an ultra microtome and then immediately imaged by the SEM. This provides a series of aligned images of the tissue in the block face and has the clear advantage that image acquisition can be fully automated. However the quality of the sectioning is critically dependent on homogenous resin hardness which is difficult to maintain when the electron beam is being used at higher resolution and part of the block suffers from the differential heating effects.
We have continued [2] to explore an alternative approach that is seemingly unaffected by variations in resin quality that uses a focused ion beam directed perpendicular to the block face. This is used to mill the surface of the resin from which serial images can be acquired in either an arbitrary location or in a selected area of specific interest.
We have used automated repetitive cutting and imaging on the DualBeam (Slice and View) with consistent sectioning intervals to as low as 5 nm. I two separate experiments we used en EPON embedded vascular sample with an endothelial cell layer with an atherosclerotic plaque and block of heart muscle tissue to demonstrate the specific site 3D analysis (plaque region) as well as a bulk 3D analysis for the muscular tissue.
Results show that this focused ion beam technique is capable of reliably sectioning tissue, and using the back scattered electrons we were able to visualize the detailed ultrastructure. Organelles like ER, mitochondria, myosin are clearly visible. In a fully automated mode we were able to collect serial images that allowed us to make detailed morphological analyses of the cellular architecture in 3D.

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THE 4.7Å CRYO-ELECTRON MICROSCOPY STRUCTURE OF AN ENGINEERED ADENO-ASSOCIATED VIRUS GENE THERAPY VECTOR

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Adeno-associated virus (AAV) is a leading candidate vector for gene therapy. Therapeutic gene delivery using AAV serotype vectors is far from ideal due to broad tissue tropism, low transduction efficiency, and a prevalent immunity to the natural virus. Recently, a capsid library, created by random shuffling of several AAV serotypes, was put under selective pressure in hepatocytes using pooled human anti-AAV polyclonal sera. A promising chimeric vector, AAV-DJ, emerged from this screen and has greatly enhanced liver specificity, while avoiding recognition by neutralizing antibodies. Our previous high-resolution crystal structures of AAV serotype capsids have provided insights into the functions of AAV capsids, and serve as templates for the rational design of vectors with altered specificity. Crystallographic approaches, however, require large quantities of AAV and significant efforts to optimize crystal quality for highresolution studies. In the current study, we overcome these limitations and establish new methods to determine the structure of the AAV-DJ capsid, in an effort to understand its unique phenotype. State of the art high-resolution *cryoelectron* microscopy and image reconstruction were used to determine the structure to 4.7Å resolution, revealing insights into the altered functions of AAVDJ.

A MULTIFUNCTIONAL SHEAR FORCE MICROSCOPE AND THE POTENTIAL BIOLOGICAL APPLICATIONS

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A scanning ion conductance microscope (SICM), with expanded capabilities, is being developed for use in the detection of gentamicin to assist in the determination of the source and mode of transfer of gentamicin through a confluent layer of barrier cells. Gentamicin is an aminoglycoside antibiotic used to treat many types of bacterial infections. About 10% of patients receiving aminoglycosides experience acute nephrotoxicity and/or permanent ototoxicity as a side effect. To address this question, a microscope is being developed to allow the simultaneous imaging of topography, nonlinear fluorescence, and chemical activity of cellular cultures and drug transport. The project has yielded promising results in the form of sub 20 nm topographic imaging resolution, and has shown to reliably image a wide variety of samples including large topographical features and biological samples. Two photon fluorescence imaging has also been incorporated into the system and has achieved diffraction limited resolution using continuous wave (CW) excitation. In addition, steps have been taken to produce functionalized chemical probes intended to detect a known redox reaction in drug-resistant varieties of bacteria between gentamicin and N-acetyltransferase involving the exchange of an acetyl group from acetyl coenzyme for a proton on a gentamicin amino group. Initial characterization of low level current measurements has also been performed in preparation for incorporation into the system.

Membrane Rafts and Resonance Energy Transfer Study

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Resonance Energy Transfer (RET) is a widely used technique to study membrane structure. RET is a non-radiative dipole-dipole interaction that may occur when the emission spectrum of the donor fluorophore overlaps with the absorption spectrum of the acceptor molecule. In the ternary lipid system DOPC (1,2-dioleoyl-sn-glycero-3-phosphocholine) / DSPC (1,2-distearoyl-sn-glycero-3-phosphocholine / Cholesterol, there are coexisting lipid-ordered (lo) and lipid-disordered (ld) phases. In this project we mark the lipid mixture using di-16:0-NBD-PE as the donor and di-18:1-Rhod-PE as the acceptor. Previous work has shown that NBD-PE prefers the ld phase, while Rhod-PE prefers the lo phase, thus the RET efficiency of these donor-acceptor pairs provide us with information about the size of these different domains, which are related to lipid rafts. As we change the composition of the ternary mixture, different efficiencies are obtained, which shows us that the lipid raft sizes depend on the membrane composition.

Electronic sensors for early detection of ovarian cancer

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Ovarian cancer is the fifth-leading cause of cancer death in women. Due to its nonspecific symptoms and lack of an adequate physical exam, over 75 percent of cases are diagnosed in the late stages when the disease has metastasized beyond the primary site, leading to a five year survival rate of less than 30 percent. By comparison, patients diagnosed in the early stages have a 90 percent survival rate over the same period. Our objective is to employ the advances in microelectronics to produce a portable, integrated sensor for early detection of ovarian cancer. These devices form the basis of new analyzers consuming sub-microliters of reagents and patient sample, and because of their small size and light weight, provide low cost and portability.

Currently, CA-125 and CEA are the two most commonly used biomarkers to detect and determine the effectiveness of post-surgical treatment for ovarian cancer and colon cancer, respectively, and to monitor for disease recurrence. Another promising biomarker is He4, which was discovered in 2003 and has been linked to the occurrence of ovarian cancer. It is currently gaining ground for ovarian cancer detection since it is found to be elevated in most patients at the early stage of the disease, hence can potentially be used for early detection. We will present our results to date on detection of He4 with our electronic biosensor and compare these results to simultaneous detection of CEA and CA-125 on multiplexed sensors.

Alumina nanoparticles help induce the immune system to destroy tumors

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Therapeutic cancer vaccination is an attractive strategy because it induces T cells of the immune system to recognize and kill tumor cells in cancer patients. However, it remains difficult to generate large numbers of T cells that can recognize the antigens on cancer cells using conventional vaccine carrier systems. Here we show that alumina nanoparticles can act as an antigen carrier to reduce the amount of antigen required to activate T cells *in vitro* and *in vivo*. We found that alumina nanoparticles delivered antigens to autophagosomes in dendritic cells, which then presented the antigens to T cells through autophagy. Immunization of mice with alumina nanoparticles that are conjugated to either a model tumor antigen or antigens derived from tumor cells resulted in tumor regression. These results suggest that alumina nanoparticles may be a promising adjuvant in the development of therapeutic cancer vaccines.

The Dimeric Nature of Ras/Raf Signaling Revealed by Single-Molecule Super-Resolution Microscopy

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The Ras/Raf/MAPK signaling module regulates cell proliferation and other essential cellular functions. Deregulated activation of Ras/Raf is directly linked to human cancers. Despite extensive research, an accurate molecular model of Ras/Raf interaction is still missing. In this study, we used photoactivated localization microscopy (PALM), a new imaging technique that can precisely count and localize individual proteins with nanometer accuracy, to study Ras and Raf interactions in mammalian cells. We demonstrated that PALM provides sufficient resolution to directly resolve individual proteins and protein complexes such as dimers in cells. With PALM, we confirmed that Raf forms dimers under activating conditions. When over-expressed, Ras organizes into nanoclusters (2-6 molecules per cluster) on the cell membrane. By varying the expression level of a mutant Ras (GTP loaded), we discovered that formation of Ras dimers coincides with Raf/MAPK activation. Furthermore, artificial dimerization of lowly expressed, monomeric Ras-GTP induces a strong activation of Raf/MAPK. These findings strongly suggest that Ras-GTP dimers mediate activation of Raf, perhaps in part by facilitating Raf dimers. Based on these findings, we propose a new, 'dimeric' model of Ras/Raf signaling that contrasts the textbook, 'monomeric' model. Along with previous results on EGFR, Raf, and Erk, our work suggests that protein dimerization may be a common regulatory mechanism of cellular processes.

RNA-SEQ BRAIN GENE COEXPRESSION NETWORKS: SPATIAL COLOCALIZATION PROPERTIES AND RELATIONSHIP TO BEHAVIOR

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We have constructed gene coexpression networks using gene expression data from the mouse striatum, across genetic backgrounds and experimental conditions. First, we compared the coexpression structure across three increasingly complex mouse crosses; this analysis determined that the coexpression structure was largely preserved, groups of coexpression transcripts (modules) had distinct biological annotation and, importantly, displayed spatial colocalization within the striatum (1). In the first example of using RNA-SEQ data for de-novo coexpression network analysis, we compared the coexpression structure across microarray and RNA-SEQ platforms, revealing important advantages and high suitability of the sequencing data in regards to network inference (2). Using a population of heterogeneous stock mice, we performed short term selective breeding for haloperidol induced catalepsy; after three generations we detected large phenotype differences and genome-wide genetic differences. Using a novel network comparison algorithm, we detect topological changes between networks constructed independently from the two selected mouse populations. We conclude that selected breeding results in genetic, behavioral and gene coexpression changes; gene connectivity changes assist in the prioritization of potential gene targets.

1. Iancu OD*, et al.* (2010) Genetic diversity and striatal gene networks: focus on the heterogeneous stock-collaborative cross (HS-CC) mouse. *BMC Genomics* 11:585.
2. Iancu OD*, et al.* (2012) Utilizing RNA-Seq Data for De-Novo Coexpression Network Inference. *Bioinformatics, accepted*.

A “Rare Disease” Approach to Cancer Therapeutics

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Current approaches in cancer treatment are typically not selective, affecting both cancer cells and normal cells. However, inactivation of DNA repair pathways, an event that occurs frequently during tumor development (Rai, 2007), can make cancer cells over-dependent on a reduced set of DNA repair pathways for survival. Targeting the remaining functional pathways may be useful for single-agent and combination therapies in such tumors. The most successful example of this approach so far is specific targeting of BRCA-deficient tumors with PARP (Poly (ADP-Ribose) Polymerase) inhibitors (Fong, 2009). This targeted chemotherapeutic approach could improve cancer therapy with more specificity for tumor cells and with less toxicity for normal cells (Iglehart, 2009). The challenge in this approach is that patients must be stratified for specific treatments through biomarkers and surrogate indicators to find the (sometimes rare) subpopulation that is likely to respond.

Treating BRCA-deficient tumors with PARP inhibitors appears to block repair of single strand breaks. Unrepaired single strand breaks can lead to DNA double strand breaks (DSBs) that must be repaired for the cell to survive. In normal cells, DSBs can be repaired by homologous recombination (HR), a process that is defective in certain tumors. The ability of a cell to perform HR could be evaluated indirectly by identifying mutations in BRCA genes or by determining if cells are capable of forming RAD51 foci, a surrogate marker of HR function. The ability of a cell to form RAD51 foci formation may indicate “BRCA-ness,” and sensitivity to PARP inhibitors (Lord & Ashworth, 2012).

PARP inhibitors may also have broader indications. A deficiency in the tumor suppressor gene PTEN results in HR defects and reduced RAD51 foci formation (Mendes-Pereira, 2009). Reduced RAD51 foci formation due to certain PTEN mutations could indicate defective HR, and sensitivity to PARP inhibitors. However, only a subset of PTEN mutations are likely to result in reduced RAD51 foci and HR deficiency.

We are interested in:

1. Developing a screening assay for RAD51 foci as a predictor of PARP inhibitor sensitivity.
2. PTEN sequencing and expression analysis to identify PTEN mutations associated with reduced RAD51 foci and PARP inhibitor sensitivity
3. Analysis of primary tumor samples with a PTEN mutations for potential HR defect by screening for PTEN expression, RAD51 foci formation (as biomarker for HR) and PARP inhibitor sensitivity.

Can low-dose radiation modulate the early stages of cancer development?

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Considerable experimental and modeling work has addressed the question of the effects of low-dose ionizing radiation on the early stages of cancer development. Given the high rate of cancer incidence and the ubiquitous nature of low-dose radiation exposure, this issue has become a concern of the Department of Energy’s Low Dose Radiation Research Program (http://lowdose.energy.gov). I am interested in the use of computer simulations to investigate this question. My work has two parts: (1) simulation of electron-beam irradiation of skin tissue and (2) simulation of cancer development in skin. Both parts are facilitated by collaboration with members of the research group led by Bill Morgan at the Pacific Northwest National Laboratory. Confocal microscopy from the laboratory of Marianne Sowa has enabled Monte Carlo track-structure simulations to estimate the penetration of electron beams into the MatTek skin model EpiDerm™ full thickness. Tom Weber is developing engineered skin tissue models that will contain transformed cells related to basal cell carcinomas. I expect to model results from Tom’s lab using hybrid models of tumor growth like those reviewed by Rejniak and Anderson (*WIREs Syst Biol Med* 2011 3 115–125 DOI: 10.1002/wsbm.102).

Identification of Hyaluronan Binding Level that Predicts the Invasive Behavior of Breast Cancer Cell Subsets

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Abstract:
Breast cancer (BCA) is a heterogeneous disease at both the cellular and clinical levels. Increasing evidence suggests the presence of rare “stem-like” cell subpopulations in primary BCA tumors that may contribute to cancer metastasis and therapy resistance. These subpopulations are characterized in part by elevated expression of CD44. CD44 is a receptor for hyaluronan (HA) –a function it shares with RHAMM/HMMR. Since the increased accumulation of HA in BCA cells and their microenvironments correlates with poor prognosis and chemo-resistance, we explored if HA probes could be utilized to identify aggressive BCA subpopulations. We synthesized fluorescent HA probes and developed methods for multiplexed analysis of HA probe binding to CD44 and RHAMM receptor positive cells by fluorescent activated cell sorting (FACS), comparative profiling of HA probe binding to basal and luminal cell line surrogates, and analyses of low vs. high HA binding (HA-/low vs. HAhigh) subpopulations. The binding profiles of HA probes were evaluated in a panel of human BCA lines differing in their molecular subtype, cancer “stem-like” surface marker (e.g. CD44+/CD24-/low), and invasiveness. HA probes bound to most BCA lines, and unlike CD44 and RHAMM surface expression, HA binding profile was heterogeneous. The highest average binding was observed in basal subtypes and invasive cell lines such as MDA-MB-231. HA-/low and HAhigh subpopulations of this BCA line showed distinct surface markers, proliferation rates, and tumor phenotypes. The HAhigh subpopulation displayed a surface phenotype of CD44+/CD24-/low/RHAMM+, exhibited the highest degree of morphological heterogeneity, and contained slow-growing cells which were highly invasive. These findings suggest that HA probes can survey BCA cell heterogeneity and allow separation of slowly-proliferating and highly invasive cells from rapidly-proliferating and poorly invasive BCA subpopulations. These probes appear to identify subpopulations that contribute to BCA progression and treatment resistance.

Keywords: Hyaluronan, CD44, RHAMM/HMMR, breast cancer, invasiveness, tumor cell heterogeneity

Spatiotemporal dynamics of Akt signaling in breast cancer

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Activated protein kinase (pAkt) plays a critical role in cellular processes in normal and cancer cells. It is often up-regulated, promoting breast cancer progression. In this study, we explore the dynamic of pAkt in breast cancer cell lines using multi-spectral imaging technology. The study is difficult to perform, using conventional biochemical methods, such as western blotting, because the spatiotemporal features of the pAkt signaling cannot be maintained during preparation of the sample. In the study, pAkt was found to be up-regulated in the plasma membranes of basal-like cell types, which makes Akt an attractive drug target for breast cancer. However, basal-like cell types were found not to respond to Akt inhibitors in another study. To better understand the mechanism of the regulation of pAkt signaling, we treated MCF7 and MDA468 cells, classified in luminal subtype and basal-like subtype respectively, with insulin or epidermal growth factor (EGF) and stained pAkt and total Akt at multiple time points up to 180 minutes. We found pAkt was remarkably up-regulated in MCF7 within 5 minutes and the second peak of pAkt expression at 60 minutes after the treatments, however, up-regulation and changes of pAkt expression in MDAMB468 cell after insulin stimulation were moderate. Regarding how up-regulated pAkt activates downstream targets, we need further studies on, 1) proteins interacting with pAkt may regulate pAkt signaling by functioning as molecular scaffolds, 2) activation status of downstream targets in pAkt signaling: p70 S6kinase and 4E-BP1, and 3) transcriptional status of target genes of pAkt such as Fra-1. This study will provide a better understanding of the molecular mechanism of the up-regulated pAkt signaling complex, to inform future research investigating prognostic predictors and therapeutic targets for breast cancers.