National Cancer Institute

PHYSICAL SCIENCES in ONCOLOGY

PERSPECTIVES

News from the Collaborative Network of Physical Sciences-Oncology Centers | Summer 2010

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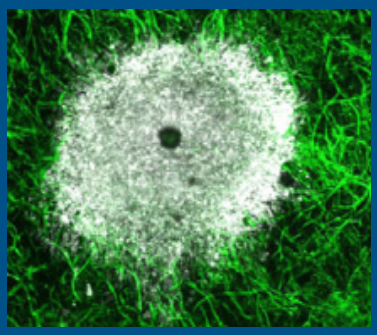
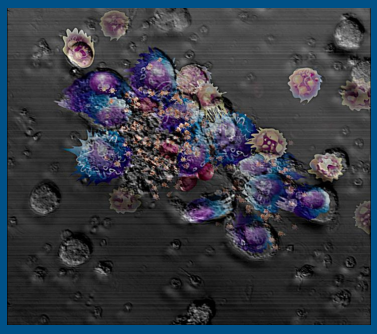
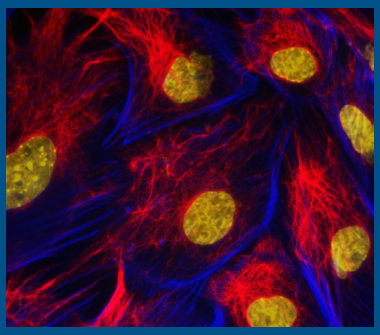
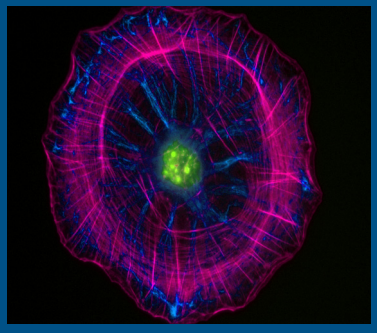


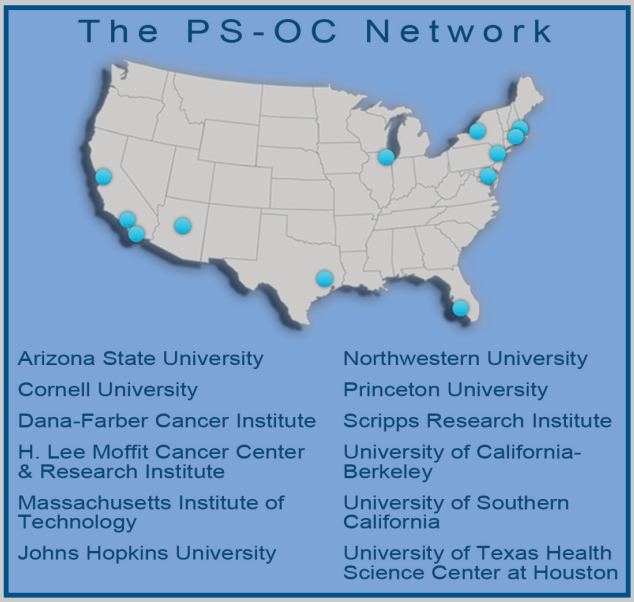






NCI Perspective: Tackling Cancer's Complexity

The complexity of cancer has persevered despite the fact that countless researchers tirelessly strive to discern the underpinnings of this disease. Physical sciences have illustrated successes in comprehending complex problems. With this in mind, the National Cancer Institute (NCI) convened three strategic "think tanks" to assess major barriers in cancer research, including cancer's complexity, which could be addressed using the principles and methods from the physical sciences. Four thematic areas emerged from these initial meetings, which include: Physics (Physical Laws and Principles) of Cancer; Evolution and Evolutionary Theory of Cancer; Information Coding, Decoding, Transfer, and Translation in Cancer; and De-convoluting Cancer's Complexity.

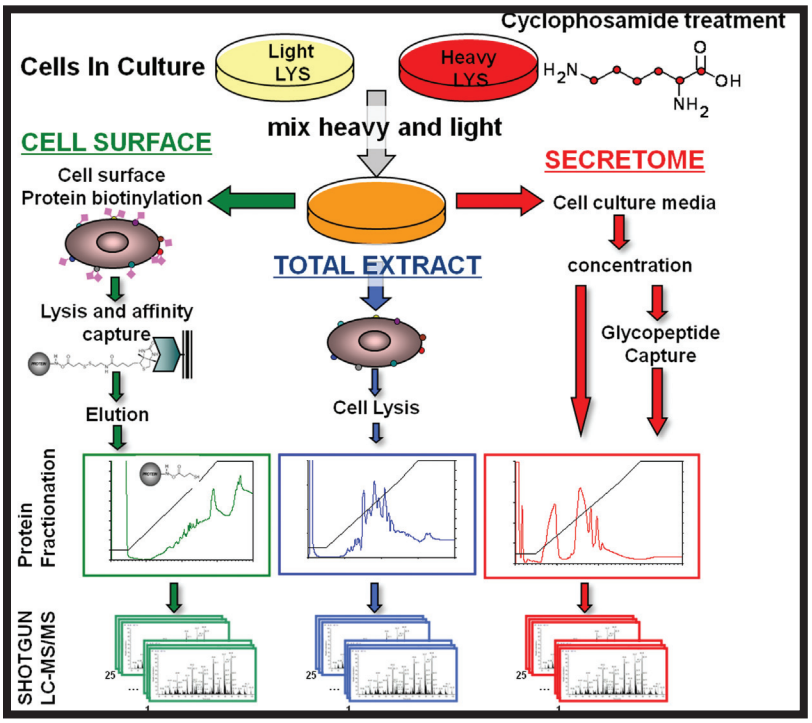


In late 2009, the NCI launched the Physical Sciences-Oncology Centers (PS-OC) Program by awarding twelve leading institutions from across the United States in an effort to more effectively engage and integrate the physical science community in cancer research as well as bring a fresh perspective and understanding to the issues of cancer. This inaugural issue of the PS-OC Perspectives, which was generated by the efforts of the PS-OC Scientific Outreach and Dissemination Working Group, will showcase three of the twelve PS-OCs that have undertaken the task of De-convoluting Cancer's Complexity. Moreover, we begin our newsletter with the Trans-Network Perspective that highlights collaborations between trans-disciplinary researchers across the PS-OC Network, the cornerstone of the PS-OC Program.

Measuring Therapeutic Response with a Suspended Microchannel Resonator: A Trans-Network Collaboration

By Parag Mallick and Scott Manalis

Changes in cell growth kinetics are a hallmark of cancer cells. In particular, many tumor suppressors and oncogenes can affect cellular homeostasis, growth and division. Accordingly, anti-cancer therapies often attempt to reverse or perturb these effects. In our project we attempt to de-convolve the complexity of cancer cell growth, division kinetics and homeostasis by relating these properties to molecular processes and ultimately therapeutic intervention. By investigating therapeutically responsive and resistant lymphomas, we hope to illuminate fundamental biology of cancer cell growth and therapeutic response. Furthermore, we hypothesize that growth kinetics and cell surface protein expression may be used as predictive surrogates for patient therapeutic response.



The Proteomic work flow utilized by the Mallick Laboratory at the USC PS-OC.

To investigate growth and response we are integrating four novel tools within the USC and MIT PSOCs: an exceptional cell model of Burkitt's Lymphoma (Lowe); a novel platform for measuring the rate at which single cells accumulate mass together with the intensity of fluorescent reporters (Manalis); a high throughput proteomics platform for measuring protein dynamics (Mallick); and rigorous computational modeling of cellular dynamics and regulation (Bonneau).

We have initially chosen to focus on an Eμ-myc model of Burkitt's Lymphoma developed by the Lowe lab. This model was one of the first transgenic strains produced and has been widely used to identify cancer genes (via insertional mutagenesis and RNAi screens) and inform other aspects of cancer biology. Importantly, this model closely resembles human Non-Hodgkin's lymphomas. In addition, the response of disparate genotypes to conventional chemotherapy (e.g. cyclophosphamide (CTX) can be varied; specifically, lymphoma lacking the tumor suppressor gene ARF respond well to cyclophosphamide whereas lymphoma lacking p53 respond poorly. In combination with CTX treatment this system is exceptional for studying cell growth and how it is altered by treatment. CTX alkylates groups of DNA bases, resulting in the DNA fragmentation, which prevents DNA synthesis and RNA transcription. CTX also causes DNA damage via the formation of cross-links (bonds between atoms in the DNA), which prevents

"We hope to illuminate fundamental biology of cancer cell growth...and hypothesize that growth kinetics and cell surface protein expression may be used as predictive surrogates for patient therapeutic response."

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DNA from being separated for synthesis or transcription. Together, these effects lead to inhibition of replication and slowing or stopping cell growth as well as apoptosis.

At USC, we have been broadly investigating the molecular response of both sensitive and resistant lymphoma to chemotherapy by first measuring the dynamic changes in the proteome and transcriptome and then developing dynamic networks models of cellular regulation that incorporate time constants and rates for molecular binding and regulatory interactions.

Though characterization of therapeutic response from both a molecular and cellular level has been broadly performed both in large cell populations, and to some extent on single cells, the relationship between molecular processes and cell growth on a per-cell basis remains poorly elucidated. This is partially a result of a lack of platforms to concurrently measure molecular and cellular growth properties.

At MIT, our approach for measuring the buoyant mass of a cell is based on a novel sensing technology known as the suspended microchannel resonator (SMR), which is capable of weighing a mammalian cell with a precision near 0.1%. To measure growth, a cell is repeatedly weighed as it flows back and forth through the vibrating suspended microchannel. By carefully controlling the fluid flow at various junctions on the device, a growing cell can be continuously weighed for periods in excess of 24 hours without perturbing the division time. For lymphoblast cell lines, this has enabled subtle changes in growth properties to be observed over multiple generations. By concurrently measuring protein indicators of pathway activity developed at USC with cell growth dynamics, we are becoming able, for the first time, to truly relate the cellular properties of growth to molecular characterizations of homeostasis and therapeutic response.

Parag Mallick is the Director of Clinical Proteomics at the Center for Applied Molecular Medicine at USC and a project leader for the USC PS-OC.

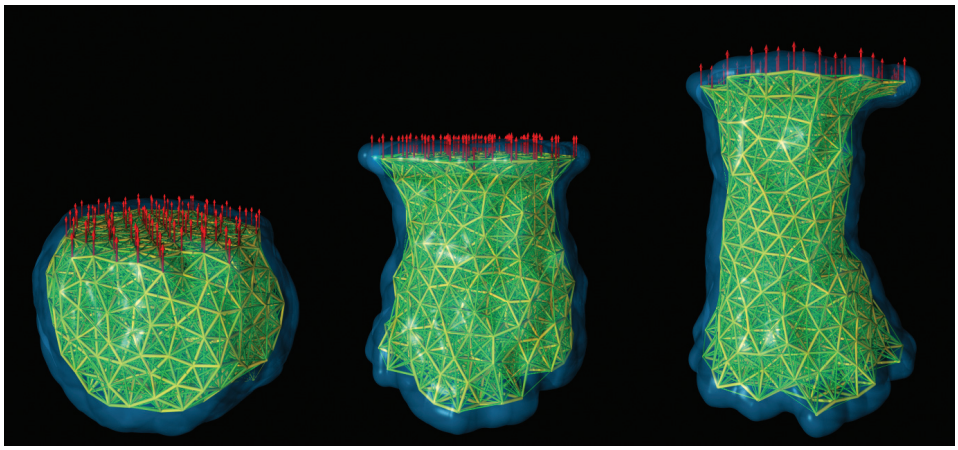
Scott Manalis is a member of the Koch Institute for Integrative Cancer Research, Professor of Biological Engineering at MIT, and a project leader for the MIT PS-OC.

"Our approach for measuring the buoyant mass of a cell is based on a novel sensing technology known as the suspended microchannel resonator, which is capable of weighing a mammalian cell with a precision near 0.1%....It has enabled subtle changes in growth properties to be observed over multiple generations. By concurrently measuring protein indicators of pathway activity developed at USC with cell growth dynamics, we are becoming able...to relate the cellular properties of growth to molecular characterizations of homeostasis and therapeutic response."

Investigating Cancer Cells as Physical Objects: The Arizona State University PS-OC

By Pauline Davies

Our PS-OC is organized around collaboration between Arizona State University, the Fred Hutchinson Cancer Research Center and the Mayo Clinic in Scottsdale. We have a strong physics and engineering slant, and our broad theme is to determine how progression from dysplasia to full malignancy is reflected in the physical properties of cancer cells, across a range of size scales from chromatin to tumor tissue.



The figure above shows the progressive extension of a cell by the application of an external force. The large strain shown is only possible with the inclusion of adaptive cytoskeletal rearrangement.

Three Cores

Core 1: Core 1, led by Center PI, Paul Davies, is hosted by The Beyond Center in the form of a "Cancer Forum." Brainstorming workshops, typically involving 20 participants, aim to push the envelope and challenge conventional thinking about the nature and properties of cancer.

Core 2: Core 2, the Materials Core, is split between the Fred Hutchinson Cancer Research Center and the Mayo Clinic. Led by William Grady, this core provides cells and tissue samples for the experimental program.

Core 3: In Core 3, which focuses on computer modeling of tumors, physicist Timothy Newman and his team are adapting a refined computer simulation model of embryogenesis to apply to tumorigensis. In the simulations, cells are described by a cluster of subcellular elements, each representing a micron-cubed region of cytoskeleton. By "fading in" new elements to highly stressed regions of the cell (and "fading out" elements in low-stress regions so as to maintain an approximately constant cell volume) the simulated cells are able to accommodate very large strains.

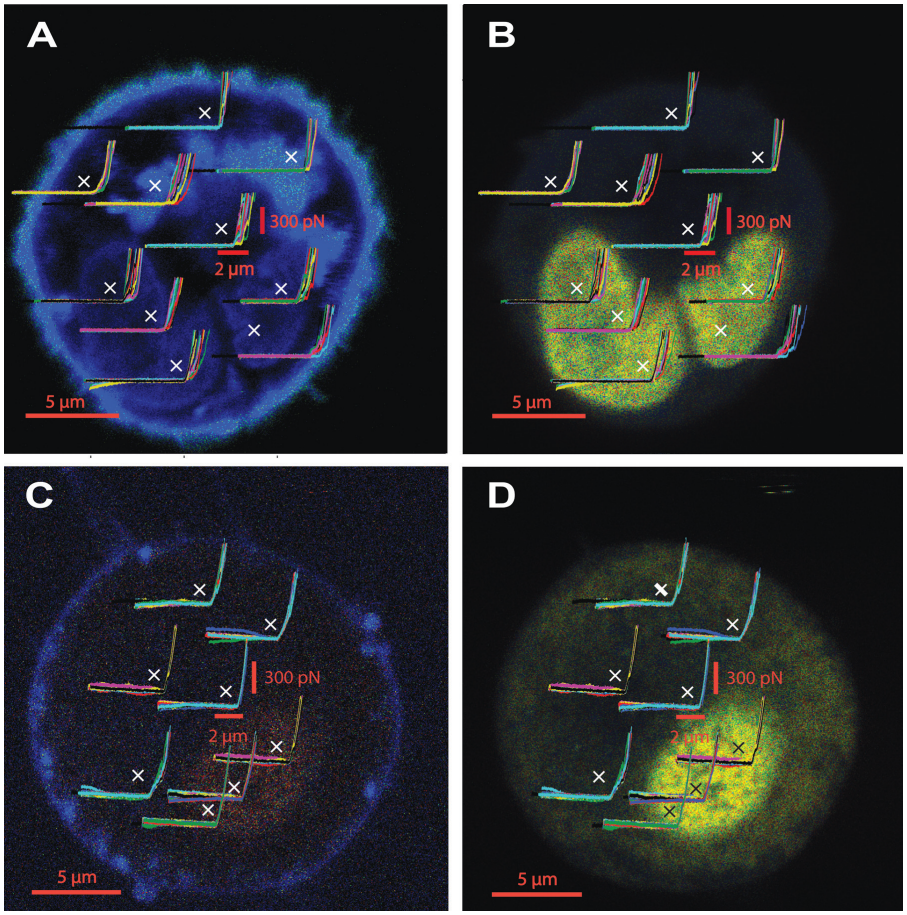
Three Projects

Project 1: In Project 1, a team led by Robert Ros is measuring the elasticity of cancer cells. All cells have a cytoskeleton which provides a mechanical frame that can grow and shift in response to physical and chemical signals. It has been known for some time that cancer cells can modify their overall elasticity, generally becoming squishier and more compliant as a result, but the physical causes are not well understood. To study these changes, Robert Ros of ASU's Physics Department has built a confocal microscope to act in concert with an atomic force microscope. By using the confocal microscope to monitor precisely where the cell is being prodded, a three-dimensional elasticity map can be created.

On the next page, the figure shows two indented cells. Using cells supplied by Thea Tlsty's Lab at UCSF, Ros' team confirmed that metastaic cancer cells (MDA-MB-231) are markedly softer—by a factor of 2.5—than non-tumorgenic cells (MCF-

"Our broad theme is to determine how progression from dysplasia to full malignancy is reflected in the physical properties of cancer cells, across a range of size scales from chromatin to tumor tissue."

(continued from previous page) 10A). However, this difference emerges only for deep indentation, (greater than 400 nm); gentler probing does not seem to reveal much difference, suggesting that the changes in elasticity lie deeper in the cytoskeleton than the actin cortex. Surprisingly, these experiments found that the elasticity of the nucleus and the cytoplasm did not differ significantly. Perhaps more importantly, the cancer cells graphs also displayed strange sawtooth features, reminiscent of catastrophic mechanical failure, exemplified by the large "kink" in the curve shown in panel D at right. They are presumably caused by structural instabilities of the cytoskeleton.



(A, B) Metastatic cancerous breast cell and (MDA-MB-231) (C, D) non-tumorigenic breast cell (MCF-10A), stained for plasma membrane (A, C) and DNA and RNA (B, D). The cells were indented with the AFM tip at the X's. Force-distance curves are superimposed beside the X's. Because the cancer cells displayed more sawtooth features, the superimposed curves for these cells are noticeably fanned out relative to the more uniform responses of the non-tumorigenic cells.

Project 2: Project 2 is an epigenetics investigation designed by Stuart Lindsay, a biophysicist at ASU, and Steve Henikoff, an oncologist at the Fred Hutchinson Cancer Research Centre in Seattle. This work examines how the physical organization of chromatin can be affected if variant histones become incorporated in nucleosomes. One consequence might be that a tumour-suppressor gene gets mistakenly switched off, and this defect may then propagate to the next generation of cells. Lindsay is adapting an atomic force microscope to "recognize" specific molecules within the chromatin. By correlating structural changes at the packing level with various stages of cancer progression, he and Henikoff hope to identify the relevant epigenetic markers that signal cancer.

Project 3: Led by Deirdre Meldrum, Project 3 focuses on microfluidics and three-dimensional tomography. Meldrum and her team are using microfluidics to study metabolic activity in cancer cells. They trap and suspend live cells in a culture medium, sealed inside a microscopic well in which the physical

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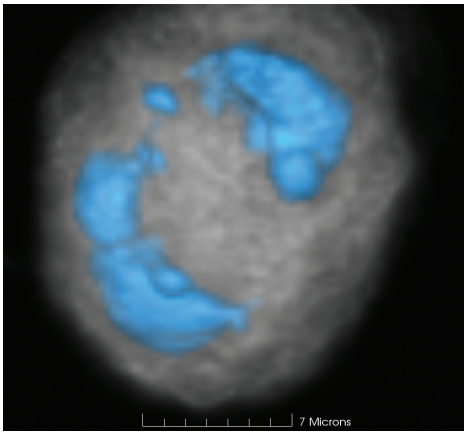

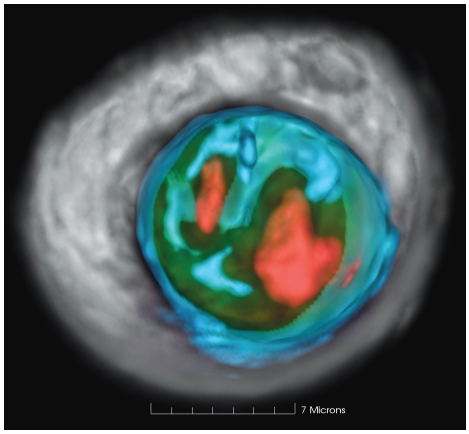

Left: *Pseudo-color volume rendering of MDA-MB-231 cell imaged using cell CT. The image illustrates what appear to be multiple nclei (depicted in blue) within an intact cell membrane. The distorted shape of the nuclei are notable. The cytoplasm is shown in gray.* ***Right:*** *Pseudo-color volume rendering of a slice through an MCF-10A cell imaged using the cell CT. Gray represents cytoplasm and blue represents nuclear membrane. Increasing chromatin density is color coded from green to red, with nucleoli shown in red. The image illustrates anvil-shaped nucleoloi and infoldings in the nuclear membrane: two cytopathological indicators of malignancy commonly observed in non-tumorigenic MCF-10A cells.*

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and chemical environment can be precisely controlled and monitored, revealing any differences between the metabolic behavior of healthy and cancer cells. Meldrum's team has also built a CT scanner for individual cells which can create three-dimensional images of single cells held in a gel-like suspension. By combining CT scanning with microfluidics, it is possible to correlate morphological changes in cells with alterations in their chemical, physical and genetic properties, as a function of cancer progression.

The potential of this new technology is well exemplified by the recent trans-network project in which all the PS-OC's used their specialized techniques to examine the same cell lines, MDA-MB-231 (metastatic BRCA cells) and MCF-10A (non-tumorigenic mammary epithelial cells) supplied by UCSF.

Education and Outreach

We have also developed a vibrant outreach and education unit. In the past nine months, the unit has organized three large public events at Arizona State University that together attracted approximately 800 people. The presentations of two of our terrific speakers, Dr. Carlo Maley and Dr Lee Hartwell, can also be viewed on our website, http://cancer-insights.asu.edu/. We host regular seminars that attract scientists from different disciplines at ASU, the Mayo Clinic, local biomedical research companies and community colleges. The seminars are webcast so they can be viewed in real time or as a videocast by anyone interested. Bringing a flavor of the brainstorming cancer workshops organized by PI Paul Davies to the wider community, videorecorded interviews with participating scientists are also available for viewing on our website.

Future Directions

The central ethos of the Cancer Forum is to find and test fundamentally new ideas about cancer, especially those that derive from novel conceptual schemes. For example, upcoming workshops will explore links between cancer biology and quantum mechanics, and between cancer biology and astrobiology.

Pauline Davies is a Professor in the Hugh Downs School of Human Communication at Arizona State University and directs the ASU PS-OC Education and Outreach Unit.

Creating a Virtual Cancer Model: The University of Southern California PS-OC

By Parag Mallick and Yvonne Suarez

Clinical tools to accurately describe, evaluate and predict an individual's response to cancer therapy are a field-wide priority. For example, only 10-20% of patients with advanced cancer show clinical benefits from intervention, yet we treat the entire population.

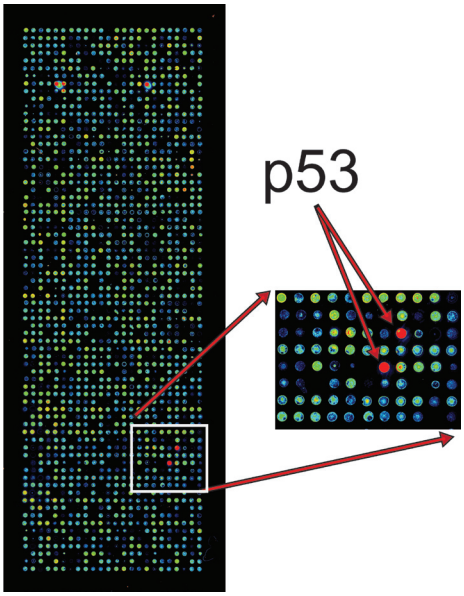
Central to the USC PS-OC's strategy is an integrative, multi-scale approach to develop accurate, useable models to study cancer. We hypothesize that multi-scale measurements integrating genotype, tumor environment and treatment parameters will allow cancer to be modeled with sufficient fidelity to predict treatment outcome. In addition to answering fundamental questions about cancer mechanism, complexity and evolution, this Virtual Cancer Model (VCM) will enable a new paradigm in treatment. Our VCM will employ a small number of measurements taken from a patient at a variety of scales, from genetic to organismic, to simulate that patient's response to therapy. This will allow us to choose the course of treatment most likely to succeed. Hundreds of therapeutics can be tested virtually, preventing unnecessary harm to patients. Additionally, we will be able to identify the changes in a tumor or serum protein that indicate if a patient is truly responding to therapy, radically improving the standard of care.

Under the direction of Danny Hillis, we have assembled a unique team of leading scientists from more than ten disciplines to develop the VCM. Team members come from USC, Stanford, UCLA, CalTech, Arizona State, University of Arizona, Cold Spring Harbor, University of Texas Medical Center, Translational Genomics Institute, Prognosys Biosciences and Applied Minds.

Four Projects

Project 1: Project 1 is predicated on the confounding observations that some molecular level changes are insignificant from the perspective of the tumor while others can produce dramatic, tumor-scale and organism-scale effects. We hypothesize the complexity of the cell's molecular processes should be described as sets of equivalence classes relating alterations in molecular phenomena to alterations in cell physiology relevant to therapeutic response and other larger length-scale phenomena. We have recently tested network inference methods that integrate genetic perturbations with time series data to effectively model cellular networks and relate them to phenotype.

Project 2: Project 2 describes the complex evolutionary processes within tumors. As tumors grow, they naturally acquire varied somatic mutations increasing genetic diversity among cells. This diversity is likely responsible for much of the complex and seemingly adaptive behavior of tumors in response to intervention. We hypothesize that



The 1,728 spot Nucleic Acid Programmable Protein Array (NAPPA) microarray slide shown here as a false color image (red = high fluorescence; blue = low fluorescence) of the whole array; the inset shows duplicate spots of p53 differentially detected by antibodies known to react to p53.

"We will be able to identify the changes in a tumor or serum protein that indicate if a patient is truly responding to therapy, thereby radically improving the standard of care. "

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cytotoxic and cytostatic treatments are specifically altering the selective pressures within tumors, driving the somatic evolution towards resistant cells. Project 2 investigators recently developed models describing how drugs that act against the shared "public goods" products of cancer cells instead of cell-intrinsic targets can be effective while imposing less selection, and can thereby reduce the emergence of acquired drug resistance.

Project 3: Project 3 will add insights on the biophysical mechanisms that link molecular- and cell-scale variations in the tumor and the microenvironment to the tumor's growth; develop techniques to rapidly incorporate cutting-edge experimental data into the multiscale framework; investigate and quantify the spatiotemporal dynamics of tumor response to therapy; improve the *in vivo-in silico* development feedback loop that forms the backbone of true integrative modeling; and so push the frontier of multiscale, integrative cancer modeling. We dynamically couple discrete (cell-scale) and continuum (tissue-scale) models developed in a hybrid, multiscale framework. We integrate this framework with state-of-the-art intravital microscopy time-course measurements of tumor growth, vascularization, and chemotherapy response. Recently, we developed an agent-based model that includes heterophilic cell-cell adhesion. In addition, the 3-D lattice-free vascularization model now includes flow in individual vessels.

Project 4: An integrated and systems approach is paramount to studying the interaction of how both the tumor and host behave over time and in response to various cancer interventions. Project 4 focuses on multiscale measurements of the host response to cancer and its therapy and integrating this information with the tumor responses measured by the other projects into a comprehensive VCM. Our host-level measurements focus on host immune response and cytokines that mediate intercellular communication. Systems-level measurements of the host immune response dynamics are obtained using a novel self-assembling, high-density protein microarray platform, and serum cytokine levels are monitored with a highly sensitive magneto nano protein chip technology.

Intravital tumor and vasculature measurements will be performed using the lymphoma mouse models. In the picture above, the inguinal lymph node is exposed prior to imaging with intravital microscopy.

Education and Outreach

Our center has been extremely active in its education and outreach efforts. In addition to our ongoing seminar series, we recently hosted our first symposium, which attracted nearly 200 attendees and generated discussions about cancer, the physical sciences, and the interface between the two.

Parag Mallick is a USC PS-OC project leader and the Director of Clinical Proteomics at the USC Center for Applied Molecular Medicine.

Yvonne Suarez leads the USC PS-OC Education and Outreach Unit.

Examining Single-Cell Dynamics in Cancer: The Massachusetts Institute of Technology PS-OC

By Alexander Van Oudenaarden

The MIT PS-OC is a collaboration among MIT, the Whitehead Institute, Harvard University, University of California-San Francisco, Boston University, the Hubrecht Institute, and Brigham and Women's Hospital. The overarching goal of this team is to use both theoretical and experimental approaches inspired by physics and engineering to attack important problems in cancer biology by developing novel technology and analytical and computational methods to track the dynamics of cancer at the single-cell level. The center's research revolves around four projects.

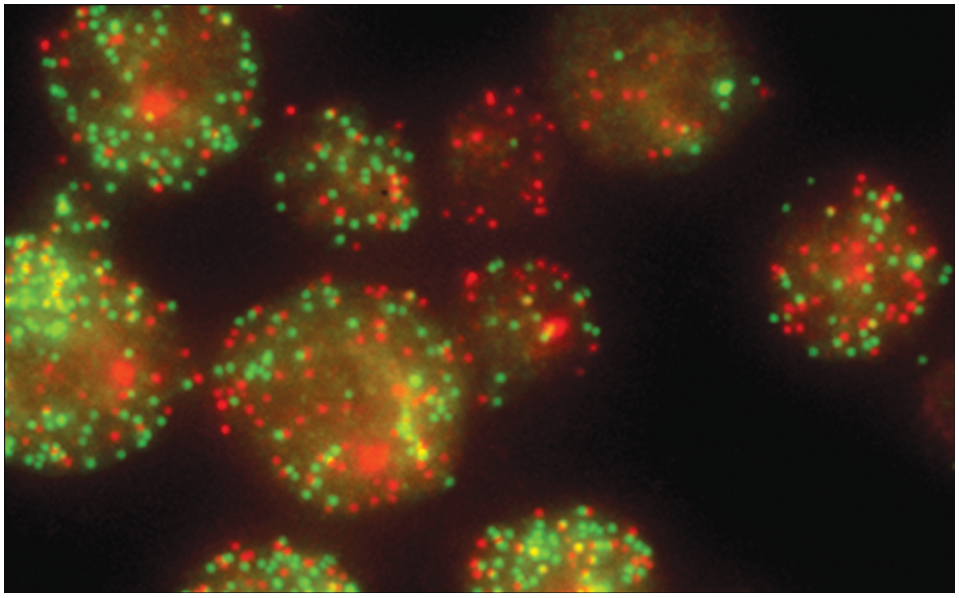
Four Projects

Project 1: The objective of Project 1, led by Dr. Alexander van Oudenaarden (MIT Physics and Biology), is to develop quantitative models of stem cell differentiation and reprogramming by obtaining absolute measurements of the transcript abundance in individual stem cells and their progeny in healthy tissue and cancer. Two complementary experimental systems are explored: the intestinal epithelium and induced pluripotent and embryonic stem cells.

Project 2: The central theme of Project 2, led by Dr. Arup Chakraborty (MIT Chemical Engineering, Biological Engineering, and Chemistry), is to employ complementary theoretical and experimental studies at the crossroads of the physical and life sciences to deconvolute the origins of aberrant Ras signaling in a specific T-cell lymphoma observed in the clinic. Investigators will focus on understanding the mechanisms underlying recently observed complex and heterogeneous responses.

Project 3: The replication and segregation of the genome (the cell cycle) and the increase in bio-mass of individual cells (cell growth) must be coordinated in all cells. Many tumor suppressors and oncogenes can alter the normal balance between growth and division and some cancers are characterized by abnormal cell size. Project 3, led by Dr. Scott Manalis (MIT Biological Engineering and Mechanical Engineering), will deconvolve cell growth and the cell division cycle, determine the molecular basis for the coordination of these two processes, and determine how they and their coordination are altered in cancer.

Project 4: The development of cancer can be viewed as an evolutionary process within an organism. During neoplastic progression, cells acquire mutations, compete for re-



Individual Gata3 (green) and Tbet (red) transcripts in cultured T lymphocytes using Single Molecule FISH

"The overarching goal of this team is to use both theoretical and experimental approaches inspired by physics and engineering...to track the dynamics of cancer at the single-cell level."

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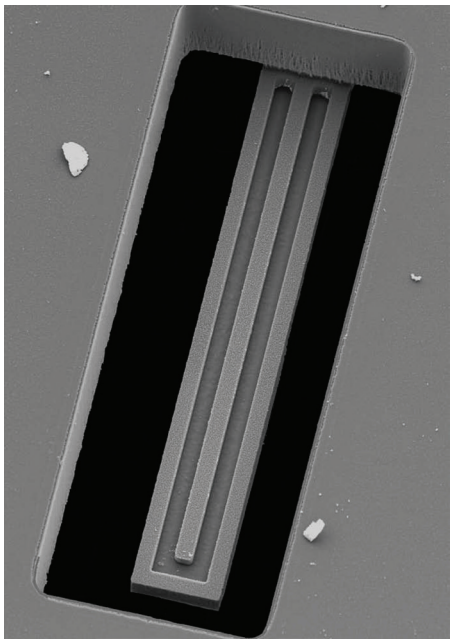
sources, and are subject of selection for ability to grow fast in a complex and dynamic environment. Project 4, led by Dr. Leonid Mirny (MIT/Harvard HST and MIT Physics), will develop a theory of neoplastic evolution informed by cancer genomic and experimental data; use it as a framework for characterizing driver and passenger mutations by original statistical techniques, and test feasibility of pushing a cancer into a population meltdown from elevated mutation load.

Two Research Cores

In addition to this research program the center is running two cores: the single-cell transcript counting core and the Cell Sorting and Physical Measurement Core.

Core 1: The Single-Cell Transcript Counting Core will provide network investigators with the infrastructure to image individual mRNA molecules in single cells, both in culture and in tissue. In addition to the exceptional sensitivity and spatial resolution, superior to other existing mRNA imaging methods, this technique allows measurements of absolute quantities of up to four different mRNAs in a single cell. Custom-designed software, developed by the Core, will computationally detect single RNA molecules and analyze images. Recently this core organized a hands-on workshop that will allow the researchers of the Bay Area PS-OC to implement the transcript counting technology.

Core 2: The Cell Sorting and Physical Measurement Core provides PS-OC investigators with emerging microfluidic technologies for sorting cells and dynamic single-cell measurements of physical properties such as mass and density. The cell sorting system consists of microfluidic technology developed by Innovative Micro Technology (IMT) that utilizes fluorescence-activated sorting, but differs from conventional FACS in three important aspects: i) it can achieve a throughput of a million events per second, which is an order of magnitude faster than existing machines such as the BD FACSAria, ii) it maintains high viability without the need to compromise throughput, and iii) all the key cell sorting elements are microfabricated and are therefore disposable. The single cell measurement platform is based on the suspended microchannel resonant (SMR) mass sensor, which is capable of measuring the size of single cells with a precision that is orders of magnitude better than what can be achieved by optical microscopy. In addition, the SMR measures mass, which – in the context of studying cell proliferation and cancer - is a superior description of cell size than is volume. This core is developing SMR-based technologies that, by monitoring the mass of single cells over time, will measure the rate of single cell growth with unprecedented precision and accuracy.



A suspended microchannel resonant mass sensor, which was invented in the Manalis laboratory, will be used to make precision measurements of cell growth.

Alexander van Oudenaarden is a Professor of Physics and Biology at MIT and the Principal Investigator for the MIT PS-OC.

Cancer is Complex...But Is It Simple?

By Timothy Newman

Timothy Newman (left) talks with PS-OC colleagues Deirdre Meldrum and Robert Johnson at April's PS-OC Network Investigators' Meeting.

What appears to be complex is subjective. A turbulent sea appears as a bewildering chaotic dance to our eyes, but microscopically is nothing more than Newton's laws of mechanics applied endlessly to collections of water molecules. A sunlit leaf on a tree in the forest appears so tranquil in comparison, but it hides a complexity that lies well beyond our current understanding. Turbulence, despite its daunting reputation, is "simple complexity" in that the complex macro-scale is an emergent outcome of relatively simple and well understood microscopic interactions. A leaf, which arises from the multi-scale metabolism of light energy, and intricate networks of gene and protein interactions, is "complex complexity". These simple examples illustrate the fundamental difference between the physical and life sciences, and help explain the increasing lure of biology to physical scientists; quantitative minds in search of a new kind of challenge, which ironically, is not of a strictly quantitative nature.

What makes living systems so different, and so special? One answer is that, in contrast to non-living systems, they don't appear to optimize anything. Many physical systems have succumbed to quantitative understanding because they optimize certain quantities. The arc of a projectile, for example, can be calculated by minimizing the well-defined mathematical quantity known as its "action." The properties of materials, such as magnets and semiconductors, can be revealed, in thermodynamic equilibrium, by minimizing their "free energies." In fact, as shown by Ludwig Boltzmann, the properties of any isolated system in thermodynamic equilibrium can be calculated by maximizing its "entropy". What is an "action" or "free energy" or "entropy"? These are not simple everyday objects, but neither are they mysterious. They were discovered, by a combination of experimental and theoretical insights over the past 150 years, to play a determining role in the behavior of physical systems. Once these quantities are determined for a specific system, scientists can make hard predictive statements and the objects of study become clay in our hands.

For the life sciences, we do not have, as yet, enigmatic quantities to place in quotation marks which, through optimization, would allow us privileged access to biological prediction. Do such quantities exist in biology? This is the heart of the matter. Physicists working in biology, essentially as an article of faith,

"Ironically, cancer may be amenable to physicists. There may be a function to optimize, which will yield a handle on prediction—and that, perhaps, may bring us closer to a cure."

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have to believe the answer is "yes." Why? Because then their role, and the application of their insights and expertise, is on familiar terms, with an unparalleled record of success. If the answer is "no" - and some believe this is so - then the potential insights of physicists are significantly reduced. Their quantitative skills and predilection for simplified models will certainly be useful, but there would be no simple principles to be uncovered by such means.

The elephant in the room is, of course, the role of evolution. Living systems are the result of billions of years of evolution. This process, in which populations of organisms compete for resources and certain subsets, through natural selection, preferentially survive to propagate their genes, is a curious mixture of optimization and contingency. In a statistical sense, one can speak of optimization, as in Darwin's famous phrase "survival of the fittest." But the complication, and it is profound, arises from the ever-changing environment in which natural selection occurs. Fitness landscapes come and go, and populations are forever playing catch-up. The slow wheels of adaptation fold yesterday's "fittest" into tomorrow's "also-rans".

"There is good reason to think that the complexity of cancer may be of the simple variety. Why so? Because...cancer may not be genetically hardwired.... Cancer breaks down order and architecture. It disrupts genetic programs. It is almost a thermodynamic process, by which disorder is re-established and entropy is ultimately maximized."

Unscrambling the mess, an organism of today, may be an impossible task. But it would be a mistake to be overwhelmed by evolution's incessant scrambling and folding. It is still unknown if simple truths lurk in the cell, truths which will enable us to make direct connections between genotype and phenotype, and have a predictive handle on life.

It would also be a mistake to tar all of biology with the same brush - it may not all be complex complexity. Perhaps some simple complexity is in the mix. There is good reason to think that the complexity of cancer may be of the simple variety. Why so? Because cancer may not be genetically hardwired unlike, for example, embryonic development. In development, a fertilized egg runs ancient genetic codes to transform itself into an organism replete with beautiful architectural details with which we are familiar. It is the ultimate antithesis of maximum entropy, channeling massive energy fluxes to build systemic order from nothing. By contrast, cancer breaks down order and architecture. It disrupts genetic programs. It is almost a thermodynamic process, by which disorder is re-established and entropy is ultimately maximized.

So, ironically, cancer may be amenable to physicists. There may be a function to optimize, which will yield a handle on prediction—and that, perhaps, may bring us closer to a cure.

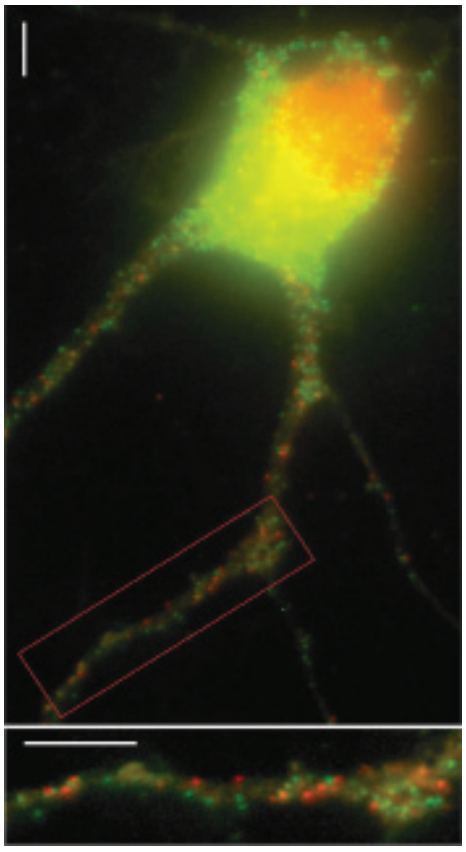
Timothy Newman is a Physics Professor at Arizona State University and a core leader for the ASU PS-OC.

No Molecule Left Behind: The Importance of Counting Single mRNA Molecules

By Alexander van Oudenaarden

As it is becoming increasingly apparent that gene expression in individual cells deviates substantially from the average behavior of cell populations (Raj and van Oudenaarden, 2008), new methods that provide accurate integer counts of mRNA copy numbers in individual cells are needed. Ideally, such methods should also reveal the intracellular locations of the mRNAs, as mRNA localization is often used by cells to spatially restrict the activity of RNA binding proteins.

The MIT PS-OC is developing and utilizing technologies that allow quantification of gene expression at the single-cell level by counting endogenous mRNA molecules. We recently applied this technology to explore gene expression fluctuations during embryonic development in C. elegans embryos (Raj et al., 2008; Raj et al., 2010). To examine the consequences of gene expression variability, we explored intestinal development in C. elegans, in which wild-type cell fate is invariant and controlled by a small transcriptional network. In contrast, cell fates in embryos with mutant skn-1, the first gene expressed in this network, are variable: while most mutant embryos fail to develop intestinal cells, some embryos nevertheless produce intestinal precursors. By counting the number of transcripts in individual embryos, we showed that mutations in skn-1 resulted in large variability in expressing the downstream gene end-1 that are subsequently thresholded during a critical time window to produce an ON/OFF expression pattern of elt-2, the master regulator of intestinal differentiation. The loss of skn-1 activity eliminates redundancy in the network, making elt-2 activation particularly sensitive to variability in end-1 expression, which stems partly from misregulation of chromatin remodeling. Although end-3 can also activate elt-2, deleting end-3 in wild-type animals results in variability in levels and timing of elt-2 expression, suggesting that robust expression of the downstream target requires multiple transcriptional activators and also hinting at subtle differences in the roles of putatively redundant elements in the network. Thresholds and redundancy are common features of developmental networks and their results show that mutations in such networks can expose otherwise buffered stochastic variability in gene expression leading to pronounced phenotypic variation.



Expression of Actb (green) and Mtap2 (red) mRNAs in rat hippocampus neurons in a dissociated neuron culture. Enlarged and contrasted image of a segment of a dendrite enclosed by the red box. All scale bars are 5 µm long.

Another application of this technology is to utilize transcript counting to develop detailed models of transcriptional regulation. For example, my lab recently applied the transcript counting technology to better understand the transcriptional regulation of ribosomal RNA (rRNA) genes in yeast (Tan and van Oudenaarden, 2010). Most eukaryotes

"My lab..focuses on developing and utilizing technologies that allow quantification of gene expression at the single-cell level by counting endogenous mRNA molecules."

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contain many tandem repeats of rRNA genes of which only a subset is transcribed at any given time. Current biochemical methods allow for the determination of the fraction of transcribing repeats (ON) versus non-transcribing repeats (OFF) but do not provide any dynamical information and obscure any transcription activity at the single-cell level. In this work we used transcript-counting, which allowed the detection of single RNA molecules in individual yeast cells. We complemented this method with theoretical modeling to determine the rate of switching from OFF to ON (activation rate) and the average number of RNA molecules produced during each transcriptional burst (burst size). We explored how these two variables change in mutants and different growth conditions and demonstrated that this method resolves changes in these two variables even when the average rDNA expression is unaltered. These phenotypic changes could not have been detected by traditional biochemical assays.

Currently we are applying the transcript counting technology to quantify transcript activity in adult stem cells of the mammalian intestine. We are applying three-color single molecule fluorescent *in-situ* hybridization to obtain integer transcript counts for a comprehensive panel of putative intestinal stem-cell markers and determine their single-cell co-expression patterns within their niche. Our analysis uncouples niche-dependent and independent components of transcript co-expression to uncover cell-intrinsic correlations. We will soon use this approach to identify molecular stem cell signatures and characterize putative cancer stem cells in tumors.

"We are applying three-color single molecule fluorescent in-situ hybridization to obtain integer transcript counts for a comprehensive panel of putative intestinal stem-cell markers and determine their single-cell co-expression patterns within their niche. In the near future this approach will be used to identify molecular signatures of stem cells to characterize putative cancer stem cells in tumors."

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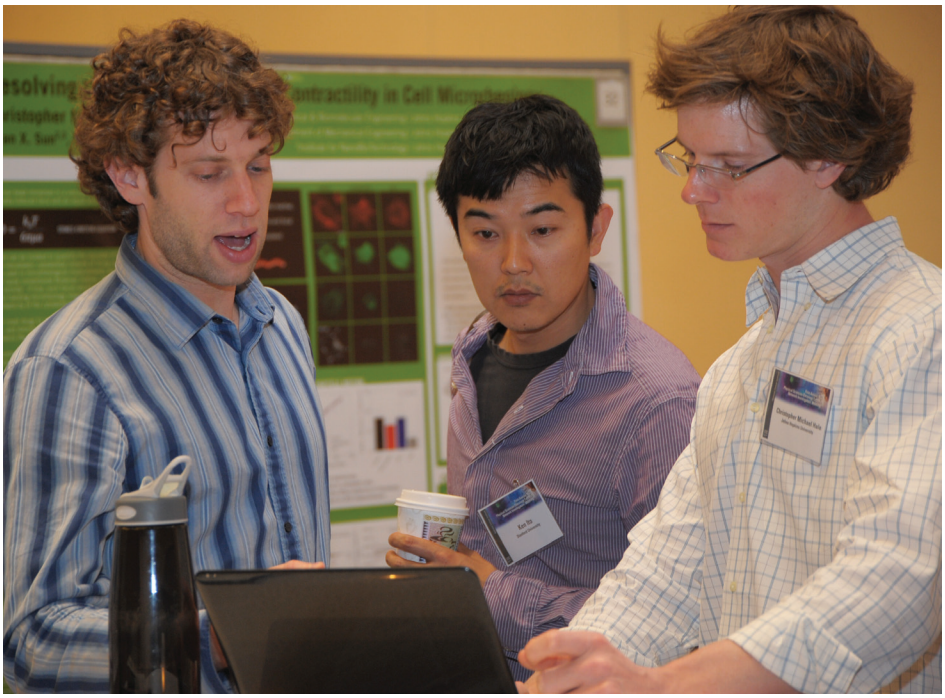
Alexander Van Oudenaarden is a Physics and Biology Professor at MIT and the Principal Investigator for the MIT PS-OC.

Connecting Scientists from Coast to Coast: The Young Investigators' Trans-Network Award Program

By Bryan Smith

The importance of cross-disciplinary collaboration and improved interactions between scientists is increasingly obvious for the advancement of science and medicine. The National Cancer Institute has directly and intimately interwoven this belief into the fabric of cancer eradication through the Physical Sciences-Oncology Center consortium, judiciously wagering significant sums of funding and effort that communication between vastly diverse physical and biological scientists will lead to truly breakthrough leaps in cancer research.



PS-OC Young Investigator Trans-Network Award winners Bryan Smith (left) and Chris Hale (right) discuss their collaborative research project with postdoctoral scholar Ken Ito of Stanford University (center).

In January of this year, I was encouraged by my advisor to attend a small PS-OC meeting hosted by Thea Tlsty at the University of California-San Francisco campus. The assembly consisted of Bay-area PS-OC participants, a group which comprised representatives from five of the twelve newly established Physical Sciences-Oncology Centers. The purpose of the meeting was to understand more about each other's Centers, the idea being to keep one another in mind for future collaborations. I was intrigued as Val Weaver and Jan Liphardt of the Berkeley PS-OC introduced their ideas on the mechanical underpinnings of cancer, pestering them with many questions. Dr. Jerry Lee, Acting Director of the NCI Office of Physical Sciences-Oncology, evidently perceived my interest in the mechanics of cancer. During the meeting he subtly passed along several suggested articles in the field published by himself and the laboratory of Denis Wirtz at Johns Hopkins University. Thus were the roots of a collaboration born.

I am a postdoctoral fellow in Sanjiv Sam Gambhir's laboratory at Stanford University. Much of my work involves fundamentally understanding how diagnostic and/or therapeutic nanoparticles interact within tumors in living animals using intravital microscopy; we anticipate that this will lead to improved nanoparticle design and quicker regulatory approval. Although I am now in a highly biological molecular imaging laboratory, my origins remain in physics and mathematics. Probing the basis of cancer through application of state-of-the-art physical science techniques consequently struck me as a familiar and essential path in cancer research; thus, I read with great in-

"We seek to translate multiple-particle tracking microrheology, which non-invasively quantifies intracellular mechanical properties, into a technique to track the intracellular properties of cancer cells in a real tumor."

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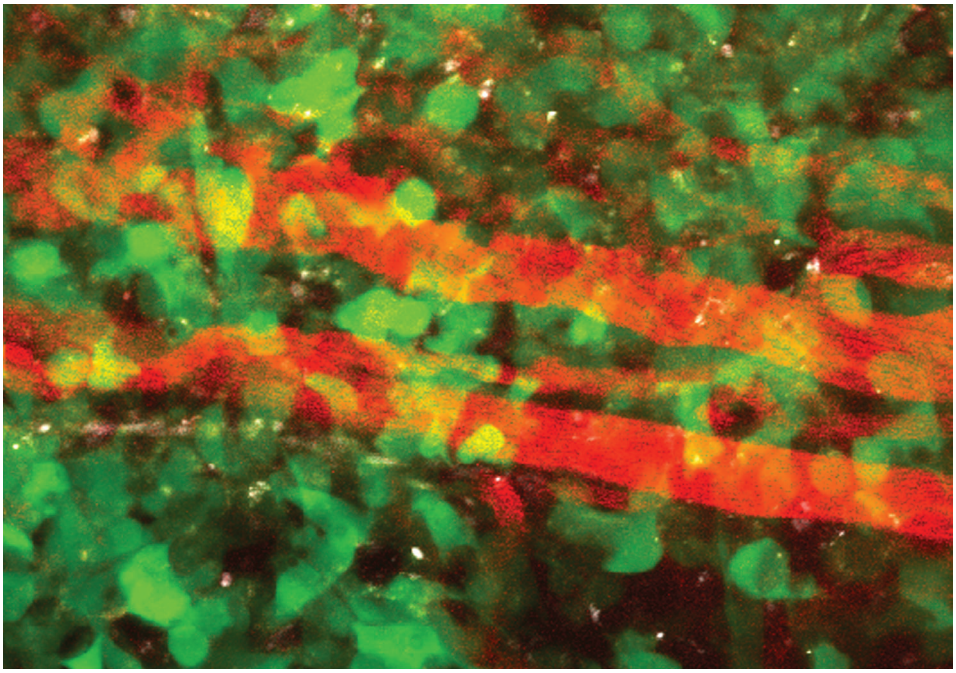
terest and curiosity the Wirtz lab's articles describing methods to interrogate the intracellular mechanical properties of cells, including cancer cells. The mechanics of cancer has been of broad interest recently, with numerous studies implicating the mechanical properties of the microenvironment, the vasculature, and even the cells themselves in the initiation and progression of disease. I realized that little, however, has thus far been done in living animals, and that a non-invasive technique that could be applied in living animals could become a vital tool in investigating cancer mechanics. It therefore seemed an obvious partnership with JHU given (1) the JHU approach applying nanoparticles in vitro to quantify intracellular mechanical properties, (2) my expertise in developing methods to image nanoparticles in living subjects, and (3) my natural tendency to seek out quantitative physical approaches to understand cancer.

Excited about the potential for this collaboration, I soon spoke with Professor Wirtz about fleshing out these ideas as a Young Investigator's Trans-Network Proposal. Our conversation encouraged me further as he was enthusiastic about the possibilities and suggested that I contact his student, Christopher Hale. A proposal was quickly wrought from these concepts and we developed our presentation for the Trans-Network Program panel when we met at the April PS-OC meeting in National Harbor, MD.



The image above reveals a tumor (green) with its blood vessels (red) in a living mouse. Also visible are nanoparticles (grayscale) in the tumor. High-resolution intravital microscopy was used to visualize the tumor in its native state with sufficient spatial and temporal resolution to visualize the nanoparticles in and around tumor cells.

After receiving the award, Chris and I are now completing the first quarter of this proposal. We seek to translate multiple-particle tracking microrheology, which non-invasively quantifies intracellular mechanical properties, into a technique to track the intracellular properties of cancer cells in a real tumor inside living mice. We have also begun to leverage this collaboration by forming a group with several other centers in the network, including the Johns Hopkins PS-OC, to submit a broader Trans-Network proposal to analyze the microrheology of circulating tumor cells.

Bryan Smith is a Postdoctoral Fellow in the Molecular Imaging Program at Stanford University and works in the laboratory of Sanjiv Sam Gambhir.

Helping Unravel the Complexity of Cancer: The Role of the Advocate

By Carole Baas

Cancer advocates fill many positions: we counsel patients, educate the public, raise money for local and national programs, lobby for research funding, and serve on grant review panels and advisory committees. But one of our most important roles is to work with the scientific community to represent the patient's perspective—to provide a human context for the research. It's easy to do this when the studies are clinically-focused, it's a bit more difficult with translational work, and it's incredibly challenging when the research involves basic science. But that's exactly when an advocate is most useful, for many of these scientists have never worked alongside someone who has a personal connection to the very disease they're researching.

Cancer patient advocate Carole Baas (center, in red) discusses the need for an advocate's perspective in the PS-OC network with (from left to right) Jeff Allen, Executive Director of Friends of Cancer Research; patient advocate Susan Samson; Anna Barker, NCI Director of the Center for Strategic Scientific Initiatives; and Carlo Maley of the Princeton PS-OC at the spring Network Investigators' Meeting in National Harbor, Maryland.

The Physical Sciences in Oncology initiative is designed as a systematic convergence of the physical sciences with cancer biology: experts from diverse fields are forming collaborative networks to provide insight into some of the most critical issues in cancer research. These scientists are learning each other's language —the engineering terminology, the medical jargon, the mathematical expressions—and how their spheres of expertise can coalesce into effective cross-disciplinary teams.

Research advocates are a critical part of this process, for not only do we add yet another language—that of the end user—but we also have a much broader knowledge of cancer than some scientists. And many of us come to cancer advocacy from technical fields and are therefore able to help form these collaborations as well as strengthen them.

The NCI values the opinions and expertise of the cancer advocacy community and involves advocates in their programs in many ways. At the First Annual PS-OC Network Investigators' Meeting, held last April in National Harbor, Maryland, there were just two advocate-participants out of 224 attendees: Susan Samson from the University of California-Berkeley and me. There is ample room for more advocates, however, and the need exists for their participation at each of the twelve PS-OC sites.

"One of our most important roles is to work with the scientific community to represent the patient's perspective—to provide a human context for the research."

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So how can a PS-OC find qualified advocates who have the skills and interest to contribute to its program? You can start by contacting the NCI's Office of Advocacy Relations (OAR). The CARRA (Consumer Advocates in Research and Related Activities) program, which is administered by OAR, is a pool of highly qualified research advocates comprised of individuals representing many different cancer types, age ranges, and ethnic groups across the nation. You can also inquire about advocacy programs within your own institution and request contacts from Susan G. Komen for the Cure's Advocates in Science (AIS) Program. And don't overlook the possibility of meeting potential advocates at scientific conferences and meetings as well as through educational opportunities such as the Scientist-Survivor Program sponsored by the American Association for Cancer Research.

Advocates have the ability to strengthen any cancer research program, but our role within the Physical Sciences-Oncology Center network is unique. We can serve as translators, helping communicate complex ideas to other team members as well as the public. We can provide a different perspective on cancer, which is, after all, one of the fundamental objectives of the Physical Sciences in Oncology initiative. We can represent those whose lives have been affected by cancer and serve as a reminder of the importance of the research. And we can tell our own cancer stories, serving as motivation for why we are all pursuing a common goal: to better understand the physical and chemical forces that govern the emergence and behavior of cancer in hopes of finally unraveling its complexities and finding an eventual cure.

"Advocates can serve as translators, helping communicate complex ideas to other team members as well as the public. We can represent those whose lives have been affected by cancer and serve as a reminder of the importance of the research. And we can tell our own cancer stories as motivation for why we are all pursuing a common goal: to better understand the physical and chemical forces that govern the emergence and behavior of cancer in hopes of finally unraveling its complexities and finding an eventual cure."

Carole Baas, Ph.D., is a five–year breast cancer survivor, breast health educator, medical writer and biomedical engineer. Drawing upon her background in research and academia as well as from her personal experience with cancer—Carole has a Ph.D. in Biomedical Engineering from Texas A&M University and prior to her diagnosis was a medical researcher studying high altitude physiology for USAF and NASA—she is currently an NCI Advocate for the PS-OC program in addition to working directly as an advocate with Mauro Ferrari, Ph.D., at the University of Texas Health Science Center at Houston PS-OC.

Job Openings and Upcoming Events

Postdoctoral Positions

In Vivo Microscopy & Cancer Metastasis: The Cornell PS-OC invites applications for a postdoctoral position that will help advance a collaborative effort to visualize the interaction of circulating tumor cells with the endothelium of cerebral blood vessels and improve understanding of the physiological mechanisms of metastasis. Ph.D. in a relevant discipline and an exceptional publication record is required. A complete description of this position and application instructions can be found here: http://www.nbtc.cornell.edu/cmm/positions.htm.

Single-Cell 3D Computed Tomographic Imaging: The Center for Biosignatures Discovery Automation in Arizona State University's Biodesign Institute seeks to fill a postdoctoral research fellow position in single-cell CT imaging research. This position will focus on further development of a new method, cell CT, for 3D imaging of biological cells. The Center seeks an energetic scientist with significant depth and experience in image reconstruction from projections, preferably for micro-CT. A PhD in engineering, physics, mathematics, computer science, or other related discipline is required. The successful candidate will have the demonstrated ability to conceive and implement standard and novel image reconstruction algorithms. A much more extensive job description can be found on the Biodesign Institute's website: http://www.biodesign.asu.edu/jobs/postdoctoral-research-fellow-2010-09-20-11-03.

Physics of Cancer: The Johns Hopkins University PS-OC has an opening for a postdoctoral researcher specializing in cancer nanomedicine. Candidates should have a Ph.D. in the physical sciences or engineeering with expertise in one or more of the following areas: microfabrication, microfluidics, bioconjugation, and cell culture. Interested parties can read more about this position here: http://www.jhu.edu/searson/.

Cancer Nanomedicine: A postdoctoral position in cancer nanomedicine is available immediately as part of the NIH-funded training program in Nanotechnology for Cancer Medicine (NTCM) at the Johns Hopkins University Institute for Nanobiotechnology (inbt.jhu.edu). Candidates should have a Ph.D. in the physical sciences or engineering with expertise in one or more of the following areas: nanoparticle synthesis, microfabrication, materials characterization, bioconjugation, cell culture, and in vivo imaging. Please go to http://www.jhu.edu/searson/ for additional details.

Upcoming Events

Memorial Sloan-Kettering Symposium
Nanobiotechnology and Cancer
Date: November 12th
More Information: http://www.mskcc.org/mskcc/html/95742.

Northwestern University Symposium
The Coding, Decoding, Transfer and Translation of Information in Cancer
Date: November 30th
More Information: http://www.psoc.northwestern.edu

On the Cover

Figure 1: The image shows a dorsal wound chamber placed in a SCID mouse. A matrigel disc was inserted in the chamber with RFP-labelled tumor (MDA-mb-231) cells in the central circle of the disc and then GFPlabelled endothelial buds placed in a peripheral circle around the tumor cells. This image was obtained 8 days after implantation and shows the endothelial buds have formed vessels that are growing into the tumor and have linked with the native mouse vasculature allowing blood flow. Submitted by the H. Lee Moffitt PS-OC.

Figure 2: This image shows a cluster of circulating tumor cells and platelets, possibly representing a tumor microembolus, isolated from the peripheral blood of a patient with metastatic prostate carcinoma. DIC imaging provides the textural definition while the cartoonized overlay is derived from fluorescent immunostaining with markers for cytokeratin and DAPI. Image generated by the 4DB Center's RP1: Cytophysics and RP2: Topology.

Figure 3: Confluent wildtype mouse embryonic fibroblasts stained for actin (blue), alpha-tubulin (red), and nuclear DNA (DAPI-yellow). Submitted by Shyam B. Khatau, Johns Hopkins University PS-OC.

Figure 4: Vimentin knockout (vim-/-) mouse embryonic fibroblast stained for actin (purple), alpha-tubulin (blue), and nuclear DNA (DAPI-green). Submitted by Shyam B. Khatau, Johns Hopkins University PS-OC.

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