Visualizing Cancer

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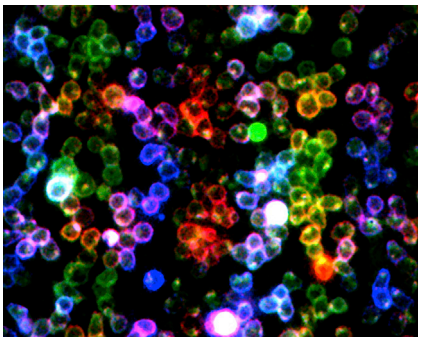
Voices

Visualizing Cancer

Imaging has had a profound impact on our ability to understand and treat cancer. We invited some experts to discuss imaging approaches that can be used in various aspects of cancer research, from investigating the complexity and diversity of cancer cells and their environments to guiding clinical decision-making.



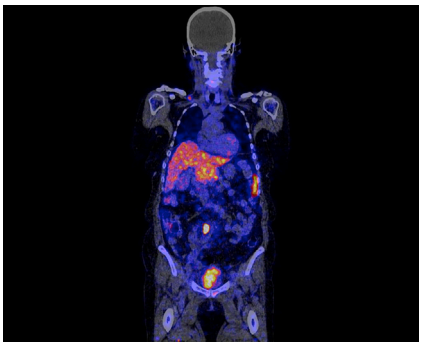
Matthew Krummel and Kenneth Hu
University of California, San Francisco

Linking Single-Cell RNA-Seq and Live Imaging

The tumor microenvironment represents significant heterogeneity in space, with the localization of a given cell being just as important as its transcriptional state in determining tumor progression and response to therapy. We recently introduced ZipSeq, a method for on-demand spatial barcoding of live cells for mapping scRNA-seq data. Following a series of spatially modulated illuminations which uncage DNA for hybridization, combinations of surface-bound barcodes define multiple regions of interest for transcriptomic analysis.

Doing so allows us to overlay scRNA-seq information on top of imaging data, revealing genes whose expression varies in space within a given cell population. For example, we recently described the gradients in cell composition and gene expression in a lymph node, while application to a tumor model revealed a progression of myeloid and lymphoid cell differentiation correlated with tumor infiltration depth.

Extending this approach to patient biopsies will shed light on mechanisms that result in non-responsiveness to immunotherapy. Designating tumors as immunologically hot or cold may not capture the whole story; tumors can consist of varied regions of immune activity. ZipSeq will allow us to dissect the composition of cell compartments within hot and cold regions, revealing cell-cell interactions that drive formation of this heterogeneity. Moving forward, increased spatial resolution of this approach combined with other spatial transcriptomic techniques will augment tissue imaging, answering a question any microscopist has encountered: "The biology in that area looks really interesting; I wonder what's going on there?"



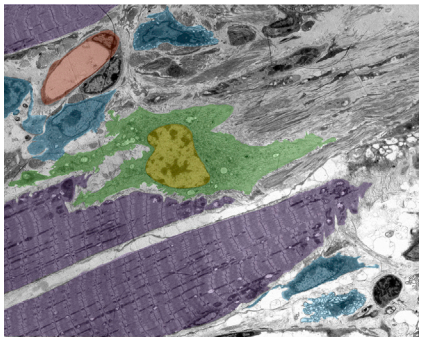
Elisabeth G.E. de Vries
University of Groningen

Molecular Imaging and Cancer Drug Development

Numerous cancer medicines are designed to bind a specific target. In general, we only have information about their blood kinetics but not about the tissue distribution of the drugs or the whole-body distribution of their targets. To comprehensively visualize their distribution, drugs can be radiolabeled and imaged non-invasively with single-photon or positron emission tomography (PET), which can be highly informative. Using this approach with trastuzumab, the monoclonal antibody targeting HER2, we detected more tumor lesions than with conventional imaging. Low tracer tumor uptake meant that the patient had a shorter treatment benefit from the trastuzumab drug conjugate T-DM1. Moreover, administering an HSP90 inhibitor, known to lower HER2 tumor expression, reduced radiolabeled trastuzumab tumor uptake.

Increasingly, antibodies are engineered to have new features—for example, bispecific antibodies. These antibodies bind two epitopes, not necessarily with the same affinity, and most are designed to bind tumor and immune cells. PET imaging with the labeled antibody can provide insights into the consequences for drug distribution of targeting distinct epitopes with one compound.

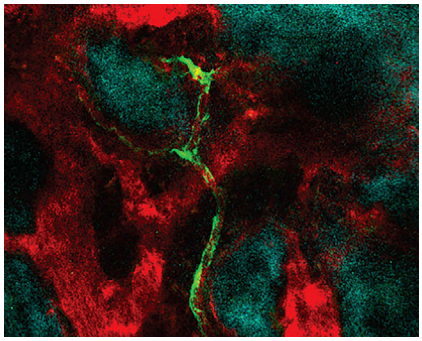
In the field of immunotherapy, molecular imaging can contribute as well. A small study showed that radiolabeled PD-L1 antibody tumor uptake before treatment with PD-1 antibody was related to response and patient survival. Another example is using a tracer against non-tumor cells critically involved in generating the antitumor immune response, such as $CD8^+$ T cells, which may support proper timing and dosing of new immunotherapeutic approaches.



Jacky G. Goetz
Fédération de Médecine Translationnelle de Strasbourg

Resolving Metastasis with Intravital CLEM

Metastases are resistant to multiple therapies and are responsible for the large majority of cancer-related deaths. Yet, the cellular and molecular mechanisms driving the formation of these secondary tumors remain only partially solved. The current challenge is to improve our understanding of metastasis and identify machineries that are ideal targets for anti-metastatic strategies. It is therefore of utmost importance to dissect, at the highest resolution possible, tumor cell behavior *in vivo*. In collaboration with experts in metastasis and high-resolution imaging, we recently designed and applied correlative tools to link the dynamic and functional recordings of tumorigenic events *in vivo* to their most-detailed ultrastructure. This technique, called intravital correlative light and electron microscopy (intravital CLEM), combines the power of intravital imaging with electron microscopy, and it can be applied to several animal models, such as mice or zebrafish. This technique can be used to study tumor growth and invasion, priming of metastatic niches via extracellular vesicles, and mechanisms of arrest and metastatic extravasation. It can capture subcellular features (cellular protrusions, trafficking machineries, and organelles) or nanoscale objects (such as extracellular vesicles) that are "invisible" to classical intravital imaging approaches. It is versatile, complementary to other high-end approaches, and likely to unravel key metastatic programs that could lead to therapeutic targeting in the near future.



Diana Passaro
Université de Paris, Institut Cochin

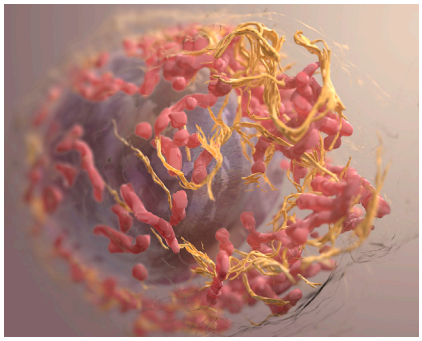
It Takes a Village to Raise a Cancer

Cancer is by definition a genetic disease and has classically been studied from this perspective. In fact, how cancer cells misbehave is largely driven by their genetic alterations. Nevertheless, the surrounding environment is key for keeping cancer cells alive. A wide range of stromal cells, vascular structures, extracellular matrix, nerve fibers, and immune components populate most tissue environments and regulate their homeostatic balance.

The advent of intravital microscopy has revolutionized the way we study the tumor microenvironment. The bone marrow was one of the first tissues to be imaged at high resolution over time, thanks to the easy access to the calvarium through minimal surgery. Our group has used this approach to observe fluorescent hematopoietic cells floating through dark bone cavities. Then, lighting up one niche cell type after another revealed a dynamic and complex multicellular unit supporting normal and malignant hematopoiesis.

The primary application of intravital microscopy is the study of dynamic behavior of cancer cells within their native environment. In parallel, researchers have implemented protocols, probes, and pipelines to study complex features such as metabolite flow, intercellular exchange, and vascular functionality.

This multiparametric map of a tissue can be astonishing and raise a wide range of questions. Reducing the dimensionality of this intricate net is the next challenge of the field, with image processing algorithms and pattern recognition approaches opening novel avenues in our understanding of cancer biology.

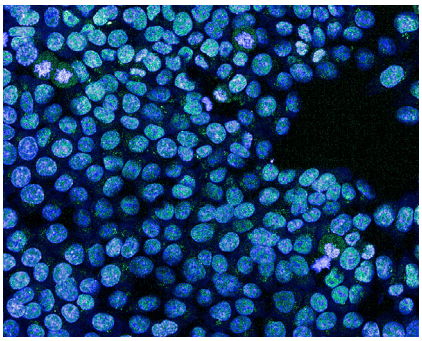


Sriram Subramaniam
The University of British Columbia

Focused Ion Beam Scanning Electron Microscopy

Understanding the hierarchical organization of molecules and organelles within the interior of large eukaryotic cells is a challenge of fundamental interest in cell biology and cancer research. About 15 years ago, we began developing a strategy for 3D imaging of cells and tissue by combining iterative removal of material from the surface of a bulk specimen using focused ion-beam milling with imaging of the newly exposed surface using scanning electron microscopy. We originally coined the phrase "ion abrasion scanning electron microscopy" to describe this method, but we and others switched later to labeling this more simply as focused ion beam scanning electron microscopy (FIB-SEM). The level of detail in the 3D images obtained with FIB-SEM is about an order of magnitude higher than what can be achieved with confocal microscopy. 10 nm-sized gold particles and quantum dot particles with 7 nm-sized cores can also be detected in single cross-sectional images, allowing imaging in conjunction

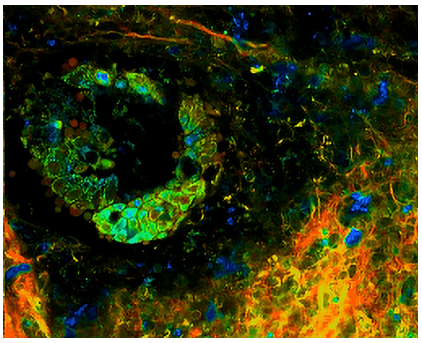
with tagging of specific proteins. Revelations about the nature of organization of membranes have emerged in almost every instance where FIB-SEM has been applied, including insights into the organization of mitochondria in muscle tissue, organization of membranes at virological synapses, and the nature of contact zones between mitochondria and internal membranes such as the endoplasmic reticulum in melanoma cells. FIB-SEM methods can be used to provide a deeper structural understanding of cancer biology, enabling detailed insights into differences in subcellular architecture between normal and cancer cells.



Tom Misteli
National Cancer Institute, USA

High-Throughput Imaging: Seeing More Is Seeing More

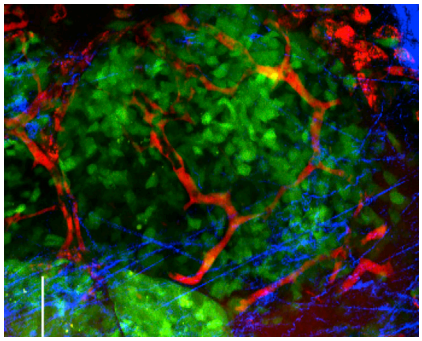
The discoveries made using cellular imaging are countless. Yet, traditional light microscopy methods are limited in that they are typically low-throughput and often require prior knowledge of the interrogated pathways. High-throughput imaging (HTI) is revolutionizing cellular imaging. HTI methods use high-capacity, high-precision, automated microscopes that allow rapid imaging of large numbers of samples, acquisition of complex datasets, and computational image analysis methods to quantitatively capture morphological phenotypes, enabling powerful new experimental strategies for cancer studies. As HTI is inherently a single-cell method and thousands of cells can be observed in a sample, rare events, such as the presence of sparse stem cells, the stochastic behavior of cells in a population, or features of tumor heterogeneity, can be detected. Conversely, large numbers of cellular targets can be interrogated simultaneously in a sample by using sets of hundreds or thousands of antibodies or DNA probes. HTI also makes possible large-scale small molecule, RNAi, or CRISPRi screens, which can use any phenotypic difference between normal and cancer cells as an assay. The full potential of HTI has not been reached; combining high-throughput with super-resolution imaging, developing new types of imaging probes, adapting HTI to tissue imaging, and using artificial intelligence will make previously unseen patterns of cellular and tissue organization visible and measurable. HTI is the next wave of microscopy and has transformed cellular imaging from a descriptive candidate approach into a powerful unbiased discovery tool in cancer research.



Melissa Skala
Morgridge Institute for Research

Imaging Cellular Metabolic Diversity in Cancer

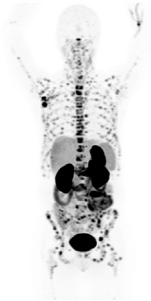
The autofluorescent redox cofactors reduced nicotinamide adenine dinucleotide (phosphate) (NAD[P]H) and oxidized flavin adenine dinucleotide (FAD) are label-free indicators of metabolic activity in cells. Optical metabolic imaging (OMI) uses two-photon fluorescence lifetime microscopy of NAD(P)H and FAD to quantify cell redox state and enzyme-binding activity within intact 3D samples. This approach is advantageous because fluorophores that are already present in the cells can be used to monitor metabolism with single-cell resolution. OMI has been developed with image analysis tools and population density models to quantify cellular heterogeneity and correlate it with tumor growth and treatment. These tools predict treatment response at early time points *in vivo* and in patient-derived tumor organoids by monitoring dynamic changes in cell subpopulations that are responsive and resistant to treatment. Recently, these technologies have quantified metabolic diversity within distinct cell populations including tumor cells, fibroblasts, and immune cells. OMI can provide new insights into metabolic differences between responsive and resistant cells within the same tumor, changes in the relative abundance of responsive and resistant cells during treatment, and spatial distributions of responsive and resistant cells within the tumor. OMI methods are important for testing and refining drugs to target treatment-resistant minority cell subpopulations that can ultimately result in tumor recurrence and to promote an anti-tumor microenvironment.



Jose Javier Bravo-Cordero
Icahn School of Medicine at Mount Sinai

Intravital Microscopy—A Window into Metastasis

Conventional microscopy only provides static images at the time the sample was collected, not capturing their dynamic behavior, which is required to fully understand complex processes like metastasis. The last 20 years have been a revolution in our understanding of tumors, and the use of intravital microscopy (IVM) to image living animals has contributed to understanding the dynamic behavior of cancer cells in real time. IVM of small animals (from zebrafish embryos to mice) has been used to study different aspects of tumor metastasis, including invasion, intravasation, and extravasation, and to define tumor phenotypes present *in vivo* (i.e., motile versus non-motile cells) and how they relate to one another. The development of new fluorescent proteins as well as biosensors has allowed investigators to interrogate interactions between different cell types, expanding the possibility of studying the contribution of multiple cellular compartments to metastasis. The combination of mouse models with implanted anatomical imaging windows has improved the temporal and spatial resolution of IVM, facilitating the study of the same tumor region for several days and tracking tumor cells for long periods of time, a technique that has now been extended to metastatic organs. The next challenge will be to further develop preclinical imaging approaches used in models and translate them into a clinical setting to be able to acquire real-time information of tumor features in patients. This approach could potentially guide future clinical decisions to better treat primary and metastatic cancer based on direct observation of tumors in patients.



Julie L. Sutcliffe
University of California, Davis

Molecularly Targeted Cancer Imaging via $\alpha_v\beta_6$

With the recognition that personalized medicine can dramatically impact patient outcomes, there is an obvious need for novel positron emission tomography (PET) molecular imaging agents that measure clinically relevant targets that are surrogates or predictors of disease. A rapidly growing body of literature identifies one such target as the integrin $\alpha_v\beta_6$, an epithelial-specific cell surface receptor that is generally undetectable in healthy adult epithelium but significantly upregulated in a wide range of epithelial-derived cancers. $\alpha_v\beta_6$ is also recognized as a prognostic indicator for several challenging malignancies and correlates with metastatic phenotype. Therefore, $\alpha_v\beta_6$ is an attractive molecular target for both detection and treatment of cancer.

We and others have developed radiolabeled peptides to non-invasively image integrin $\alpha_v\beta_6$ expression *in vivo* using PET. Our group has developed a ^{18}F-$\alpha_v\beta_6$-binding-peptide (^{18}F-$\alpha_v\beta_6$-BP) with high affinity and selectivity for the integrin $\alpha_v\beta_6$ and favorable pharmacokinetics in tumor-bearing mice and non-human primates, and we have translated this imaging agent into patients with breast, colon, lung, and pancreatic cancer. ^{18}F-$\alpha_v\beta_6$-BP was well-tolerated, with PET/CT images showing low background uptake in common sites of metastatic disease and demonstrating significant uptake in primary lesions and metastases, including sub-centimeter lesions. Beyond the immediate clinical impact of ^{18}F-$\alpha_v\beta_6$-BP PET/CT on pretreatment molecular imaging, this ligand may also serve as a therapeutic delivery platform for some of the most lethal cancers facing patients today.