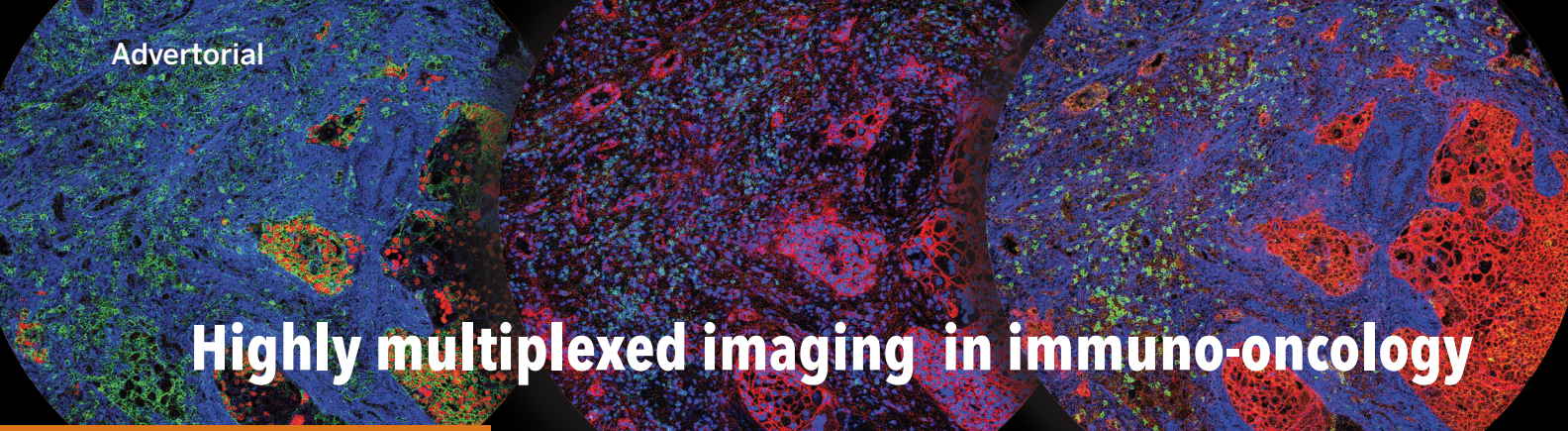


# Highly multiplexed imaging in immuno-oncology

Examples of biomarker images obtained with the Hyperion system.

*With the Fluidigm Hyperion Imaging System, scientists can track up to 37 protein markers at the subcellular level in tumors and surrounding tissue. This analysis reveals, for example, the kinds of proteins found in the tumor microenvironment and their spatial locations in and around cells. Increasingly, scientists see that spatial organization plays a fundamental role in the progression of cancer and understanding how to treat it.*

Immuno-oncology–a relatively new field focused on using the patient's own immune system to fight cancer–already saves some people from this disease, but not everyone. Where cancer resists treatment or turns resistant over time, scientists need to better understand the molecular details of the disease and its interaction with treatments. To do so, they need tools that track many targets at once and in fine detail.

Priscilla Kelly wrote in the March 23, 2018 special issue of *Science* on immunotherapy: "Unraveling the cellular and molecular basis of treatment resistance should facilitate rational design of new mechanism-based studies."[1] A promising method uses Imaging Mass Cytometry (IMC), which provides high-dimensional, subcellular spatial and molecular information that is sufficiently high-resolution to precisely locate crucial molecular interactions in relation to a tumor and its surroundings.

## An imaging advantage

The Hyperion Imaging System focuses a laser beam at 1 micron to capture information at the pixel level from biological tissues that have been stained with up to 37 proprietary metal-tagged antibodies. The platform then transfers this information for analysis with CyTOF, or mass cytometry, a technology that includes readout by time-of-flight mass spectrometry.

"The results from the Hyperion Imaging System are contextual at subcellular resolution," says Kerry Lowrie, director, Marketing, Imaging Mass Cytometry at Fluidigm. "You can see spatial arrangements of cells and look at intracellular markers and markers on cell surfaces." She adds, "You need this level of single-cell data to capture accurate phenotypic information."

With such detailed data, scientists can apply this system to many aspects of medical research. As examples, Lowrie mentions identifying new biomarker signatures to predict therapeutic responses in translational and clinical research as well as drug mechanism, safety, and toxicity studies.

## Exploring the microenvironment

At Cedars-Sinai Medical Center in Los Angeles, California, associate professor of medicine Akil Merchant and his colleagues explore the microenvironment of blood cancers, especially lymphomas, and the role of bone marrow in these diseases. "These cancers provide very unique opportunities," Merchant says. "Hodgkin's lymphoma, for example, is unique among tumors, because 95% of the cells are immune cells–making the cancer a small fraction of the cells in a tumor." These cancers are also very responsive to some forms of immunotherapy, such as checkpoint inhibitors.

Currently, the excitement in immuno-oncology "is around the clinical responses, but not all of them are 100% effective," says Merchant. Consequently, scientists want to know what makes a cancer resistant to these treatments. "Multiplex imaging can get at the mechanism of resistance," he notes. He and his colleagues apply the Hyperion Imaging System platform in prospective and retrospective studies. "We're using the system to drive correlative science," he adds. For example, he's running a prospective clinical trial in Hodgkin's lymphoma to assess the impact of immuno-oncology agents alone or in combination with cellular therapy.



Kurt Schalper, M.D., Ph.D. (left), Akil Merchant, M.D. (right), use the Hyperion system to explore the tumor-immune microenvironment.

Kurt Schalper, assistant professor of pathology and internal medicine at the Yale School of Medicine and director of the Translational Immuno-Oncology Laboratory at the Yale Cancer Center, also explores the tumor microenvironment. "Every tumor-immune microenvironment is quite different, due to patient variation and the tumor," Schalper explains. "It takes complex data and methods to answer complex questions."

In the past, scientists addressed such questions with RNA sequencing (RNA-Seq). It allows scientists to follow maybe half a dozen biomarkers, but that's not enough for a diverse population. Plus, RNA-Seq methodology requires grinding the tissue, which destroys the architecture of the sample. Schalper is showing that structure matters in finding the best treatments.

"The spatial architecture of a tumor is not random," Schalper says. "It's driven by chemical gradients of signals, which define how a tumor is shaped." He adds, "Some indications suggest that spatial context is

PHOTOS: COURTESY OF FLUIDIGM (TOP), DR. SCHALPER AND DR. MERCHANT

relevant, but there are not many good tools to develop that idea." Enter high-order multiplexing.

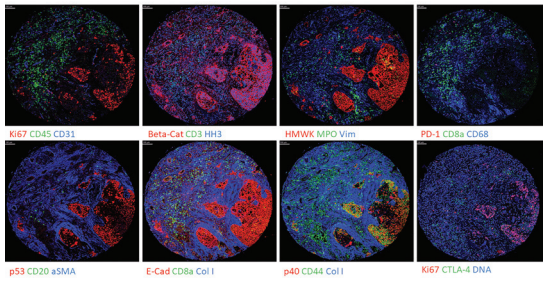
With the Hyperion Imaging System, Schalper has tracked about 35 protein biomarkers in the same sample and collected spatial information. "Identifying the proteins of the tumor and the immune cells seems to tell us about the likelihood of some tumors being more resistant or sensitive to treatment," he says. To make the connection, Schalper and his colleagues used the system's ability to simultaneously track many proteins and their locations in the tumor-immune niches of the microenvironment.

## Expanding sample options

One of the most valuable features of the Hyperion Imaging System is its ability to analyze archival formalin-fixed, paraffin-embedded (FFPE) samples. "Often, analyzing such samples requires special handling at the time of preparation, which is fine for prospective studies," Merchant explains. But the Hyperion Imaging System works with almost any sample. "We can take archival tissue from large annotated clinical datasets and interrogate it," he says.

His team has been working on samples from patients who were cured of Hodgkin's lymphoma and from others who relapsed. "These samples provide a wealth of biomarkers associated with the mechanism of resistance," Merchant says. So far, his work suggests that expression of certain proteins in specific cell subtypes might indicate which treatment would be the most effective. Doing this kind of work requires tracking more than one marker, such as an immunotherapy target like the protein PD-L1, and a cell-related marker, such as a cytokeratin associated with the cytoskeleton. "With the Hyperion Imaging System you can multiplex without worrying about steric hinderance at high coexpression," says Merchant. "That's really hard to do on almost any other platform."

For Merchant's team, it's also important that the Hyperion Imaging System works well with bone marrow. Most other methods of analysis–such as decalcifying the bone marrow in a strong acid–damage or even destroy the DNA and RNA in bone-marrow samples. "People think of the bone marrow as a liquid," Merchant explains, "but it's spatially organized, and we're trying to understand that architectural framework of bone marrow in normal hematopoiesis or during diseases like bone-marrow cancer, or [diseases] that metastasized to bone marrow." Scientists specializing in hematology can do that with the Hyperion Imaging System.







Imaging mass cytomtery (IMC) is made easy with the Hyperion Imaging System from Fluidigm.

PHOTOS: COURTESY OF FLUIDIGM

## Analyzing complex data

Even with so many potential applications, Fluidigm made the Hyperion Imaging System easy to use. "The IMC workflow is very similar to standard immunohistochemistry," Lowrie says. "Plus, you do the staining at one time, followed by scanning all the markers at one time." She adds, "Once you have it running, the acquisition is automated, allowing for scanning to be run overnight."

Although it is relatively easy to collect high-dimensional data, doing so can create data management challenges. Scientists must really pay attention when collecting and analyzing the data. "Collaboration between translational researchers and pathologists to help select the right regions to image benefits the IMC experimental design," Lowrie explains.

To make IMC technology as accessible and easy as possible for scientists analyzing complex data and interactions between molecules, the Hyperion Imaging System includes a license for histoCAT software, which makes it easy to analyze cellular phenotypes and spatial relationships. Additional analysis solutions are now available through other software providers for use with the system's high-multiplex data from the Fluidigm Hyperion Imaging System. Some third-party imaging packages, such as HALO image analysis software from Indica Labs and Phenomap software from Visiopharm, can now be used to analyze IMC data.

## Exploring even more medicine

Scientists already see the value of high-resolution IMC. Yale University pathologist David Rimm and his colleagues studied breast cancer tissue with IMC, and they noted that the technique "appears to uniquely reveal relationships not previously seen using conventional in situ assessment methods."[2]

Eventually, scientists hope to take a biopsy of a patient's cancer and then track the tumor and immune system proteins over time. "That would be very powerful, but quite challenging because it would require integrating so many markers," Schalper explains, "but that's where we're going." And so is IMC.

**References**

1. P. N. Kelly. *Science* **359,** 1344–1345 (2018).
2. D. E. Carvajal-Hausdorf *et al., Clin Cancer Res.,* doi: 10.1158/1078-0432.CCR-18-2599 (2019).

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