milestone

THE DEVELOPMENT OF Imaging Mass Cytometry

BY YUNING WANG, PHD

Imaging mass cytometry is a harmonious convergence of several distinct technologies. It merges mass spectrometry's high throughput protein detection with flow cytometry's cellular analysis precision and microscopy's detailed tissue imaging. This creates a transformative tool for single cell proteomic analysis, made possible through interdisciplinary collaboration and innovation.

2007 A meeting of minds

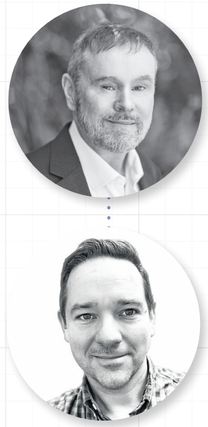
As Tanner led mass cytometry innovations, Sean Bendall, now a biochemist at Stanford University, was completing his PhD studies at Western University. He had been using mass spectrometry to screen proteins from stem cells but was frustrated by the limited information at the single cell level.

"Every screen involved squishing hundreds of thousands of cells and profiling thousands of proteins. Then we had to figure out what one protein was doing on one cell," Bendall said. "Making that connection was really difficult. So, I was determined in my postdoctoral work to get more experience on sophisticated assays that could analyze individual cells."

Bendall set his sights on working with Garry Nolan, an immunologist at Stanford University who had pioneered single cell profiling with flow cytometry. "I had been pushing the use of flow cytometry for studying signaling networks inside cells, but we were limited on the number of parameters," Nolan said.

In May 2007, Bendall and Nolan met at the Symposium on Enabling Technologies for Proteomics in Toronto. Also in attendance was Tanner, who presented his newly developed mass cytometry technology and was on the lookout for potential collaborators. "While I was talking to Garry Nolan, Scott Tanner came up to talk to us," Bendall recalled.

In this fortuitous meeting, Tanner's vision of how mass cytometry could potentially overcome flow cytometry's limitations and enhance single cell analysis resonated with Nolan and Bendall. This led to the convergence of their interests and expertise. "About nine months later, I ended up in Garry's lab working to merge the mass cytometry technology that Scott and his team had created with single cell assays and informatics going on in Garry's lab," Bendall said



CREDIT: GARRY NOLAN AND SEAN BENDALL

Garry Nolan (top) and Sean Bendall (bottom) led the way in applying mass cytometry to profile cells in the human hematopoietic system.

2011 The pioneering project

Together, Nolan, Bendall, and Tanner aimed to examine human bone marrow, the primary source of immune and blood cells. To capture a comprehensive view of the diverse cell types, they developed a series of metal-tagged antibodies, each targeting a specific cellular marker and exhibiting a distinctive mass spectrum. "We worked with chemists to make chelating polymers that had multiple abilities so every chelator tagged onto an antibody could bind 20, 30, or 40 metal ions," Nolan said. "This probably took us two or three years."

Using these metal-tagged antibodies and Tanner's mass cytometer, Nolan and Bendall were able to simultaneously measure 31 cellular markers in hundreds of thousands of bone marrow cells. These markers ranged from cell surface antigens for identifying cell types to cellular epitopes indicative of intracellular signaling states.

They then used a novel data analysis method called spanning-tree progression analysis of density-normalized events (SPADE) to organize these bone marrow cells based on their phenotypic similarities and transitions during differentiation. The SPADE method allowed them to create a tree-like structure that mapped the complex multidimensional relationships of various cell types throughout hematopoietic development in the bone marrow.

In 2011, Nolan and Tanner jointly published this study (2). "We introduced the mass cytometer to the world and showed what we could do with it," Nolan said. "For the first time, we could take a snapshot of the entire immune system."

The publication soon sparked widespread enthusiasm about mass cytometry among researchers. "Everyone was excited and wanted to try it," Bendall said. "A lot of big medical centers started to invest in the technology, and it was like wildfire at Stanford."

2000s From flow to mass

In the 2000s, flow cytometry, the standard method for cell analysis, could manage only a handful of measurements at a time. Flow cytometry uses fluorescent tags to label proteins on cells, but the overlapping emission spectra of different fluorochromes could disrupt the measurements. While many researchers were working on creating new fluorescent reagents, Scott Tanner, a then chemist at the University of Toronto, saw the potential of mass spectrometry to overcome these limitations.

Tanner had decades of experience developing inductively coupled plasma mass spectrometry (ICP-MS), a method that ionizes samples using high-temperature plasma and separates ions based on their mass-to-charge ratio. Having previously utilized ICP-MS to simultaneously detect numerous elements and isotopes in environmental samples like water and oil, Tanner envisioned adapting this capability for cell analysis.

Instead of using fluorescent tags, Tanner created antibodies conjugated to rare earth metal isotopes such as terbium, holmium, and thulium to label specific proteins on cells. These metals have distinct mass spectra, easily differentiable from one another, and many were readily available for simultaneously labeling multiple proteins.

To analyze labeled cells, Tanner and his team constructed a modified ICP-MS instrument. Within this device, cells are first nebulized into individual droplets, each containing a single cell. These droplets pass through high-temperature plasma, which ionizes the metal tags on the antibodies bound to cells. The ionized metal tags then traverse through a time-of-flight chamber, where they travel at different velocities based on their mass-to-charge ratio. This separation allows for the precise identification and quantification of the metal tags, which reflect the abundance of labeled proteins within individual cells. Tanner named this method mass cytometry, or cytometry by time-of-flight (CyTOF).

In their pilot mass cytometry study, Tanner's team concurrently detected 20 surface antigens in cells from leukemia cell lines and patient samples (1). Recognizing the technique's multiplexing potential, Tanner began actively seeking collaborations to expand its applications.

CREDIT: SEAN BENDALL

Scott Tanner and his team developed mass cytometry, also known as CyTOF, employing metal-tagged antibodies to label and identify proteins on individual cells. In 2009, the first commercial CyTOF instrument by DVS Sciences started operating in Tanner's laboratory.

CREDIT: SEAN BENDALL

In the fall of 2009, Sean Bendall (left) unboxed the first mass cytometer at Stanford University with Dmitry Bandura (right), a research associate in Tanner's team and co-inventor of mass cytometry.

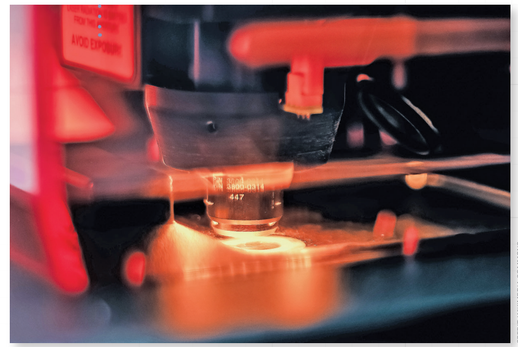
2014 The integration of imaging

Bernd Bodenmiller, currently a cancer biologist at the University of Zürich, joined Nolan's laboratory for his postdoctoral training in 2009. Like Bendall, Bodenmiller had the unique opportunity to be among the first to work with mass cytometry. During his time there, he developed a sample multiplexing method to analyze the signaling network states of human peripheral blood mononuclear cells (3), which significantly enhanced mass cytometry throughput.

In 2012, Bodenmiller became an assistant professor at the University of Zürich, where he focused on investigating tumor ecosystems using mass cytometry. However, the technique primarily analyzed cells in suspension, missing the spatial information crucial for understanding cell-cell interactions within tissue or tumor microenvironments. To bridge this gap, he collaborated with his colleague, chemist Detlef Günther, to integrate imaging with mass cytometry.

To preserve the spatial information, they stained tissue sections, instead of suspended cells, with metal-tagged antibodies for mass cytometry and fluorescently labeled antibodies for immunofluorescence microscopy. They first examined the tissue section under a standard fluorescence microscope to visualize and identify regions of interest. Then, they used a laser ablation system, where a pulsed laser beam vaporized the tissue sample pixel by pixel. This process led to the ionization of the metal tags, which were then introduced into the mass cytometer for analysis.

"He was pulsing laser clouds of the tissue and using the mass cytometer as the sensor," Nolan explained. By plotting the metal ion signals using their coordinates, Bodenmiller and Günther were able to reconstruct the spatial distribution of the target proteins. Aligning this data with immunofluorescence microscopy images enabled them to generate spatially resolved maps of protein expression across tissues. In 2014, Bodenmiller and Günther published this innovative method, demonstrating the imaging of 32 proteins at subcellular resolution in breast cancer samples (4).



CREDIT: VANMEDIA.COM/ADOBE

Bernd Bodenmiller incorporated laser ablation into mass cytometry to enable spatially resolved analysis of tissue samples, leading to the development of imaging mass cytometry.

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Bodenmiller developed an imaging mass cytometry method to measure and visualize mRNA and protein simultaneously in tissue samples.

2018 Bridging proteomics and genomics

Following the development of imaging mass cytometry, Bodenmiller continued to explore the technique's potential and versatility. He recognized that a comprehensive understanding of cellular functions and interactions in tissues would require both genetic and proteomic information. At the time, there were no available approaches that could simultaneously measure mRNA and proteins in a spatially resolved manner.

Bodenmiller and his team turned to the widely used RNAscope technique for inspiration. The RNAscope technique, known for its high specificity and sensitivity, involves the *in situ* hybridization of specialized fluorescent-labeled oligonucleotide probes to target mRNA sequences in tissues (5).

To make the RNAscope technique compatible with imaging mass cytometry, Bodenmiller conjugated the oligonucleotide probes with metal-chelated polymer reporters, which enabled mRNA detection by mass cytometry. An additional staining step using metal-tagged antibodies allowed for the co-detection of mRNA and proteins on the same tissue image.

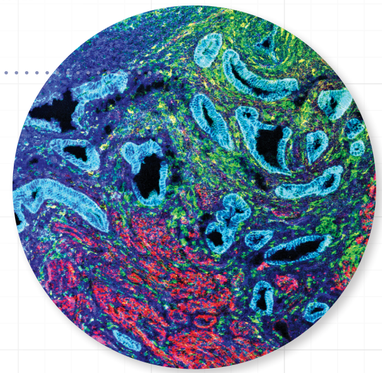
In 2018, Bodenmiller's team applied this integrated method to measure and visualize three mRNA transcripts and 16 protein markers simultaneously in breast cancer tissue samples. The findings of the study highlighted mRNA-to-protein correlations of key cancer-associated genes such as human epidermal growth factor receptor 2 (6). The team further expanded the method's multiplexing capability by 2022, achieving the measurement of 12 mRNAs and 39 proteins in a single experiment (7).

2018–Present Expanding applications

Since the commercialization of imaging mass cytometry in 2017 by Standard BioTools, researchers have rapidly adopted this technology to approach previously challenging questions. One promising application of this technology is correlating imaging mass cytometry data with clinical information to predict how patients respond to treatment. In 2021, David Rimm, a pathologist at Yale University, and his group applied imaging mass cytometry to characterize the immune-related protein expression patterns of patients with melanoma undergoing immune checkpoint blockade therapy (8). They identified potential biomarkers that could serve as predictive indicators of patient survival, offering new avenues for precision cancer treatment strategies.

"Imaging mass cytometry shines in areas where tissues are difficult to measure with light-based microscopy. This includes highly pigmented tissues and tissues with cholesterol like the brain," Bendall said. In 2023, a team led by Logan Walsh at McGill University utilized imaging mass cytometry to analyze 1.1 million cells across more than 200 brain tumors from patients (9). This analysis enabled the profiling of the cellular composition and spatial organization of the tumor microenvironment, revealing significant differences in immune landscapes between primary tumors and brain metastases originating from various solid cancers.

From its inception as the original CyTOF method, "the technology has taken on a life of its own," said Bendall. Today, imaging mass cytometry is deeply integrated with a wide range of research areas, from immunology and stem cell research to cancer biology and neuroscience, demonstrating its versatility and potential to drive advancements across scientific fields.



CREDIT: BRICE GAUDILLIERE, STANFORD UNIVERSITY

Using imaging mass cytometry, researchers unravel the spatial and molecular complexity of tissues and their microenvironments, uncovering insights into issue heterogeneity, biomarker discovery, and treatment response.

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