

Spatial Biology Colors Outside the Lines

High-Plex Fluorescent Labeling
Technology Lets Spatial Biology
Expand Its Boundaries

A SPONSORED PUBLICATION FROM

GEN

Genetic Engineering
& Biotechnology News

You want to go spatial. We'll help you get there.

Breaking into spatial biology is easier than you may think

Spatial biology gives you the power to better understand how cells interact within tissue and how those interactions influence biological processes in healthy or diseased tissue.

Regardless of what platform you're currently using, Thermo Fisher Scientific will collaborate with you to help get you started faster and more easily. Our offerings comprise high-quality, easy-to-choose, and easy-to-use reagents and antibodies, including Invitrogen™ Alexa Fluor dyes, all designed to help you save time and complete complicated experiments confidently.

Break into spatial biology today.

 Go to thermofisher.com/spatial-biology



Spatial Biology Colors Outside the Lines

High-Plex Fluorescent Labeling Technology Lets Spatial Biology Expand Its Boundaries

04 Introduction

05 GEN Interview with Oggie Golub, PhD,
Oggie Golub, PhD, is a Staff Scientist in Cell Biology R&D at Thermo Fisher Scientific

10 Spatial Biology:
How It's Transforming Single-Cell Genomics

15 Organ Mapping Antibody Panels (OMAPs)

23 Spatial Biology and AI Combined by Noetik to Develop Immuno-Oncology Drugs

26 Slide-Seq Hits the Spatial Biology Market as Curio Seeker

30 Super-Resolution Microscopy Can Be Super-Accessible

A new spatial biology is in sight—literally. It is a more vivid, more detailed, and ultimately more informative spatial biology. It can distinguish between cell types that were once indistinguishable, and it can do so while capturing their spatial context. It can even pinpoint the subcellular locations of individual molecules. And it can accomplish these tasks with unprecedented precision because technology is now available that opens a new dimension beyond the usual three spatial dimensions. This new dimension may be called the “plex” dimension.

Plex refers to the number of fluorescence markers that are used with microscopy and other cell analysis platforms. Conventional platforms may accommodate just a handful of markers, constraining investigations of complex biological phenomena. But such investigations may require many markers.

Unfortunately, using more and more markers—and thereby shifting from low-plex to high-plex spatial biology—has been too difficult for most laboratories. They’ve balked at the need for special expertise, complicated workflows, and instrument upgrades. Fortunately, these difficulties can be overcome with new multiplex imaging technologies. For example, there are antibody panels that are compatible with streamlined workflows and automated imaging systems.

To learn more about these technologies, consult the articles in this eBook—especially the article describing organ mapping antibody panels. Also, be sure to read the articles that describe the kinds of spatial biology applications that are bound to become more common as high-plex technology becomes more accessible. Indeed, this technology is democratizing spatial biology.

The logo for ThermoFisher Scientific, featuring the company name in white text on a red rectangular background.

GEN Interview with Oggie Golub, PhD,

Staff Scientist, Cell Biology R&D at Thermo Fisher Scientific

OGGIE GOLUB is a cell biologist and microscopist with a doctorate degree in cellular and molecular biology from the University of Oregon. He has over eight years of R&D experience in advanced microscopy methods, including high resolution and high plex imaging using spectral detection. He leads a technical team working on the development of next-generation cellular analysis tools and workflows, with a focus on labeling and detection solutions for FFPE & cryopreserved tissue samples in spatial biology.



GEN: *What first piqued your interest in spatial biology and when did that happen?*

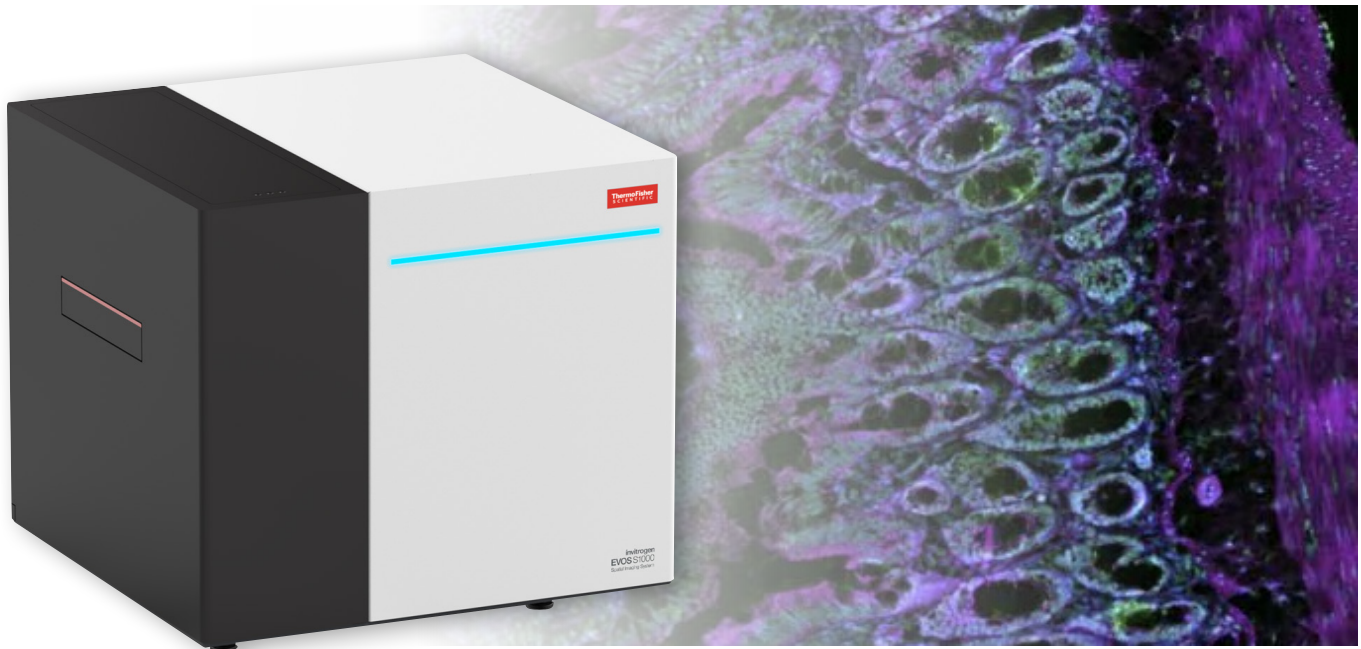
Golub: At Thermo Fisher Scientific, we've been collectively working in earnest on spatial biology. But spatial is something in which I've personally always been interested. During my dissertation, I studied how asymmetric cell division gives rise to the cellular diversity that's found in fully developed tissues, and that's basically the fundamental theme of spatial biology—the complexity in cellular diversity of intact tissues.

On the technical front, fluorescence microscopy is a commonly used detection modality in spatial biology because it naturally lends itself to revealing the spatial organization of complex tissues. It was really the culmination of those two

personal passions that really piqued my interest in the field.

GEN: *Building on that, what do you focus on, in terms of spatial, in your role at Thermo Fisher Scientific?*

Golub: I am a staff scientist and R&D lead for spatial biology labeling and detection technologies. The focus of our entire group is to enable broader access to spatial biology through simplified workflows all the way from a tissue on a slide to an image that comes off an instrument. This includes sample preparation and labeling reagents, such as directly conjugated antibodies. We also have branched DNA technology with our ViewRNA kits that supports mRNA labeling, as well as our newest multiplex spectral detection platform—the EVOS S1000 spatial imaging platform.



EVOS S1000 spatial imaging platform

GEN: *What's the state of spatial biology today in your view?*

Golub: It's an interesting question because spatial biology is still rather new and rapidly evolving. There's a lot of focus on plex and detecting the highest number of markers or targets possible. But when we speak to scientists who are using these technologies, they describe how the data itself is often quite overwhelming and it's quite a struggle to do anything useful with that many markers.

The increase in plex also increases the complexity, scanning throughput, time to data, and overall cost of these experiments both on the reagent and instrument fronts. Therefore, we focused most of our efforts on specific areas to improve the workflows associated with high-plex fluorescence labeling and detection.

We also want to address existing pain points to eliminate barriers for new users, most of whom

may not have the budgets or expertise to do these experiments today. Due to these barriers, spatial has still largely remained exclusive to relatively well-funded labs and core facilities with dedicated staff, extensive expertise, sample prep, and data collection and analysis capabilities.

Our aim really is to democratize this technology by taking away as many of these complexities as possible to make the technology accessible for all. It's something that we internally refer to as the EVOS ethos. We try to simplify and remove complexity across the entire workflow, but specifically in labeling and detection.

GEN: *What areas of biology do you think will most benefit from spatial in the next five years?*

Golub: Immuno-oncology is the obvious answer because that's where spatial is the primary application today. I think that will continue to increase, which should come as no surprise given that spatial provides invaluable insights into how

cancerous tissues are organized, as well as how the immune system responds to different treatments and disease progression. I also see a lot of potential in basic science applications, for example, in developmental biology to answer questions about transcriptomic and proteomic expression profiles during specific phases of development.

Spatial has a lot of potential to answer the question of where these markers are being expressed and at which times in development. As a result, I do see spatial biology being widely adopted and accessible for mid-plex protein or mRNA transcript on sectioned tissues. Cutting edge users will try to advance more towards 3D models and delve deeper into areas like metabolomics and maybe even epigenomics.

GEN: *How might spatial change the course of cancer research in future?*

Golub: Looking at the 2024 AACR conference topics, there was a strong focus on spatial technologies, and that's presumably driven by the potential of spatial to inform molecular mechanisms of disease progression in cancer at the tissue level. But informing treatment potential is a much more challenging task because these assays will need to become a lot more standardized and more robust to be clinically validated. That's where the big challenge lies but there are also some issues on the detection side with instruments needing to comply with specific types of regulatory standards.

The potential is certainly there for these technologies to make it into the clinic and inform treatments. I believe that low to mid plex fluorescence-based spatial platforms will soon

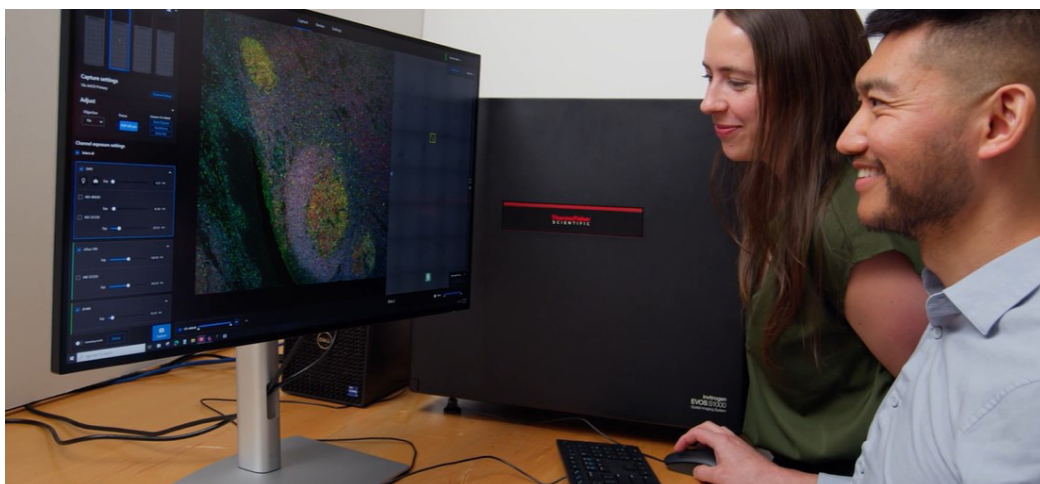
supplement traditional histology techniques such as H&E staining but is very unlikely that they will supplant them. It will be another tool in the toolkit that could be used to inform personalized medicine at the molecular and cellular level.

GEN: *What are some other examples of spatial research that excite you?*

Golub: Spatial biology lends itself to the study of many biological processes because it's really the study of the biological organization of intact tissues. I am excited for spatial to go beyond cellular interactions to elucidating potential microbe interactions as they pertain to both development and disease. We've learned a lot over the past decade, about the importance of the microbiome but it seems we still forget how dependent our biology is on microbial signaling cascades that inform both disease and development. That's another potentially exciting area where spatial is likely to break into in the coming years.

GEN: *What do you think users prioritize in a spatial platform?*

Golub: Even though I'm a cell and molecular biologist by training, I'm really a technologist and microscopist at heart. I've spent a lot of time thinking about the technical aspects of these different platforms and which ones matter. Plex is certainly an important component of spatial and is generally the first spec that gets mentioned because traditional microscopy platforms that are capable of three to four plex are simply not sufficient in this regard. You need more markers to elucidate the variety of cells that are present in the tissue. And there are certain applications that truly do require a high number of markers—



The EVOS family of products empowers researchers, allowing them to focus on gathering data instead of instrument upkeep.

15, 20, maybe even 40 and beyond, but we have found that many researchers in spatial require 8–12 markers, which is the range supported by our new EVOS S1000 Spatial Imaging system.

However, with increasing plex comes increased complexity and significant time investment to optimize the labeling conditions. To lower that barrier, we're expanding our menu of primary antibody conjugates that are validated for use on FFPE preserved tissues so that users can have more ready-to-use validated antibodies that are basically available off the shelf.

We've also developed a new line of dyes, the Aluora Tyramide reagents, that are specifically formulated for multiplex immunofluorescence on FFPE, because we understand that validated reagents for labeling are immensely important, and some users don't really have the desire or expertise or budgets to make their own. On the detection front, what we've heard is a clear need to simplify the workflow and make the imaging platforms faster and more approachable.

Existing solutions are either too complex or too expensive, or in some cases both, with some

platforms requiring single-use consumables each time the user runs an assay. We've also heard a need for speed and high-resolution images because the downstream analysis depends heavily on high quality image data to perform accurate cell segmentation and feature extraction.

The newest member of the EVOS family, the S1000, uses high resolution optics and is built on top of a fast and flexible platform with an immensely intuitive user interface. Our hope is that the intuitive user interface will eliminate the need for extensive training and post-installation support and enable this technology to be basically accessible to every lab.

Our goal is to empower scientists with our products so they can spend more time collecting valuable data rather than maintaining and calibrating their instrument.

GEN: *What are the current challenges in the field and how do you believe the field will get around them?*

Golub: We've talked about the labeling challenges and complexity but there's a big data

problem. Since spatial biology deals with large areas of tissues across many, many markers, the size of the image datasets can easily approach tens and hundreds of gigabytes per experiment. And by the time you get done processing an entire study, you can easily end up with terabytes of data. It's a logistical challenge for a lot of departments, both in terms of storage and organization, in addition to the computational power needed to work with those images.

The other piece is that the term "data" takes on multiple forms. Image data isn't really quantitative by design so extracting high quality quantitative features from images remains immensely challenging. This too is partially limited by reagent availability because for segmentation, there are no dedicated pan-membrane markers to accurately elucidate cellular boundaries.

A lot of segmentation algorithms rely on nuclear counter stains, and these algorithms are only as capable as the information they have

available—the stains and labels on the tissue. Artificial intelligence is a commonly prescribed panacea provided to the model, but AI models require training, and that training is only as good as either the user in supervised training or the labels that are available to it in unsupervised training.

How will the field get around these challenges? A part of the solution honestly lies in our hands. At Thermo Fisher Scientific we're aware that the problem needs to be addressed and with any new technology, a lot of investigation and planning goes into that process.

For the data question, this is where advances in data science and applications of AI are likely to be the most needed. Addressing this problem requires really close and effective collaboration between biologists and data scientists in order to arrive at approaches that can enable efficient extraction of biologically meaningful data from immensely complex spreadsheets of raw measurements. ■

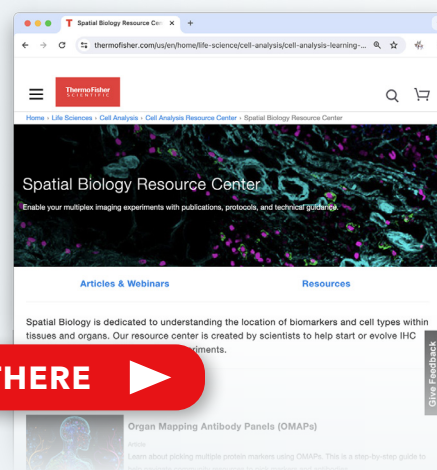
ONLINE RESOURCES

Spatial Biology Resource Center

Enable your multiplex imaging experiments with publications, protocols, and technical guidance.

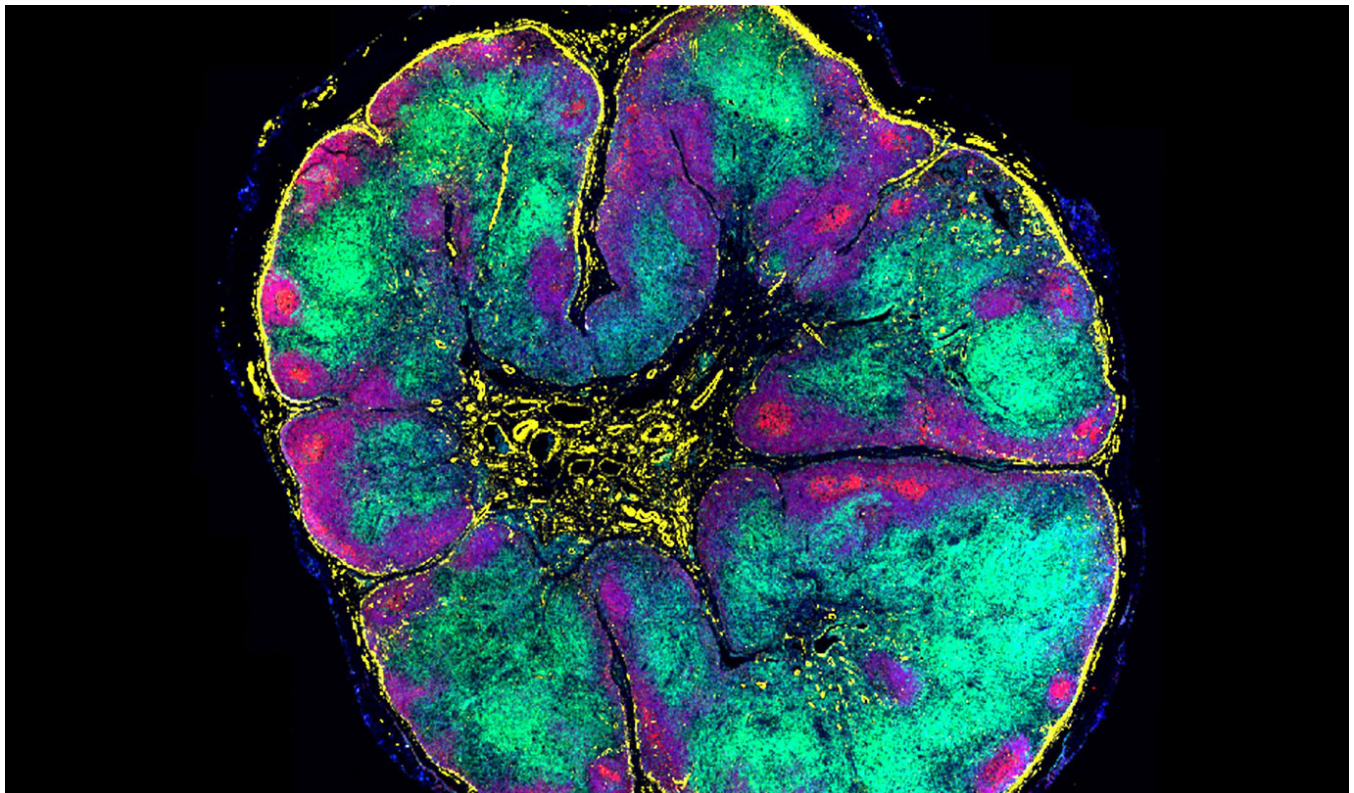
Spatial Biology is dedicated to understanding the location of biomarkers and cell types within tissues and organs. Our resource center is created by scientists to help start or evolve IHC experiments into multi-biomarker experiments.

GO THERE



Spatial Biology: How It's Transforming Single-Cell Genomics

A GEN-led panel conversation on spatial biology technology highlights the importance of community—not just among cells, but also among scientists



Rong Fan, PhD, and Archie Ennifur

Spatial biology has all the hallmarks of the next omics revolution. For example, a key spatial biology technology, spatial transcriptomics, was named Method of the Year by Nature Methods in 2021. More generally, spatial biology is moving in a multiomics direction. Shown above is a 52-plex immunofluorescence image of a whole human lymph node. Selected markers: DAPI (blue), CD20 (red), CD3e (green), collagen IV (yellow). These markers identify nuclei, B cells, T cells, and vascular cells, respectively.

BY JULIANNA LEMIEUX, PHD

When the sober-minded describe spatial biology, they use words such as *fledgling*, *growing*, and maybe even *exciting*. But perhaps more enthusiasm—and words such as *revolutionary* and *transformative*—are in order. Yes, the basic idea behind spatial biology has been around for *decades*. Put simply, this is the idea that single-cell analysis should reflect how cells are organized in tissues. As familiar as this idea may seem, the toolkit to explore tissues spatially has been limited. But new technologies are making spatial biology a reality, ushering the field into a whole new era. Doesn't that justify words that might otherwise seem hyperbolic?



Rong Fan, PhD, Yale University and Nicholas Banovich, PhD, TGen

To convey the passion surrounding spatial biology, *GEN* hosted a panel discussion titled, “How Spatial Biology is Revolutionizing Single-Cell Genomics.” It originally aired on October 18 as an episode of *GEN Live*, our science talk show. And it is still available to anyone who wants to hear about the current status of spatial biology, the impact the technology is making on research (and perhaps one day, patients), and the challenges it faces as it moves forward.

GEN revisits the panel discussion here. Moreover, in sympathy with the basic idea behind spatial biology—that is, the idea of contextualization—we provide some additional background and commentary. We include direct quotes from the two panelists, namely, Rong Fan, PhD, professor of biomedical engineering at Yale University, and Nicholas Banovich, PhD, associate professor in the Integrated Cancer Genomics Division at the Translational Genomics Research Institute (TGen). Our aim is to highlight ongoing trends, challenges that are being overcome, and future possibilities that inspire people to drive spatial biology forward.

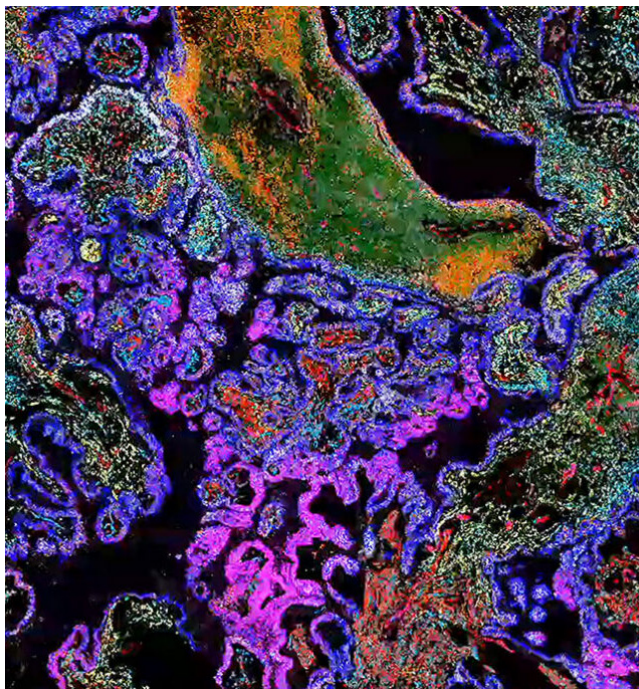
Then and now

The discussion kicked off with a broad view of spatial biology’s evolution. To understand how the technology arrived at where it is today, Fan started with a historical perspective, going back to the work of Robert Hooke (in the 1600s) and his early descriptions of how cells appeared when viewed through the microscope. Then, Fan moved to more modern localization techniques used to investigate proteins and nucleic acids spatially in tissues, including immunostaining and fluorescence in situ hybridization. Now, with the newest wave of spatial technologies, researchers have the ability to dig into mechanisms and function.

Fan noted that analyzing gene expression is currently the centerpiece of spatial. One reason for that, Banovich noted, is the digitization of the signal. The analysis of data in a way that uses the existing genomic tool set seems to be what allowed spatial biology—particularly spatial transcriptomics—to become broadly useful.

“Whether you’re talking about imaging-based platforms or sequencing-based platforms, being able to churn these through the genomics infrastructure has really led to this broad adoption of the technologies,” Banovich remarked. And because the transcriptomics signal is straightforward to digitize—compared to proteomics or epigenomics signals—it was the first to emerge as the dominant technology.

Fan added that there will be more access to investigate above (proteins and metabolites) and below (the genome and epigenome) the RNA in the cell. And that will generate a new question: Can we link all of them together?



This Vizgen MERSCOPE image, which displays spatial transcriptomics data, shows part of a human ovarian tumor tissue section. In the full section, 174 million transcripts from 500 genes were detected.

Where are we now?

“This feels to me very much like the single-cell world of 2016 or 2017,” Banovich said, “where everybody is ‘out in the woods,’ trying to figure out exactly what they can eke out of these technologies.” He explained that there are a lot of researchers wrapping their heads around what they can do with these data. Getting relational information, Banovich continued, is the gold mine of spatial data. But there is a lot of work going into understanding how to use the relational information, figuring out what tools are available to answer those questions, and designing the computational tools that still need to be built.

In the early days of single-cell biology, the sharing of methods and experiences was commonplace. And Banovich expressed his hope that

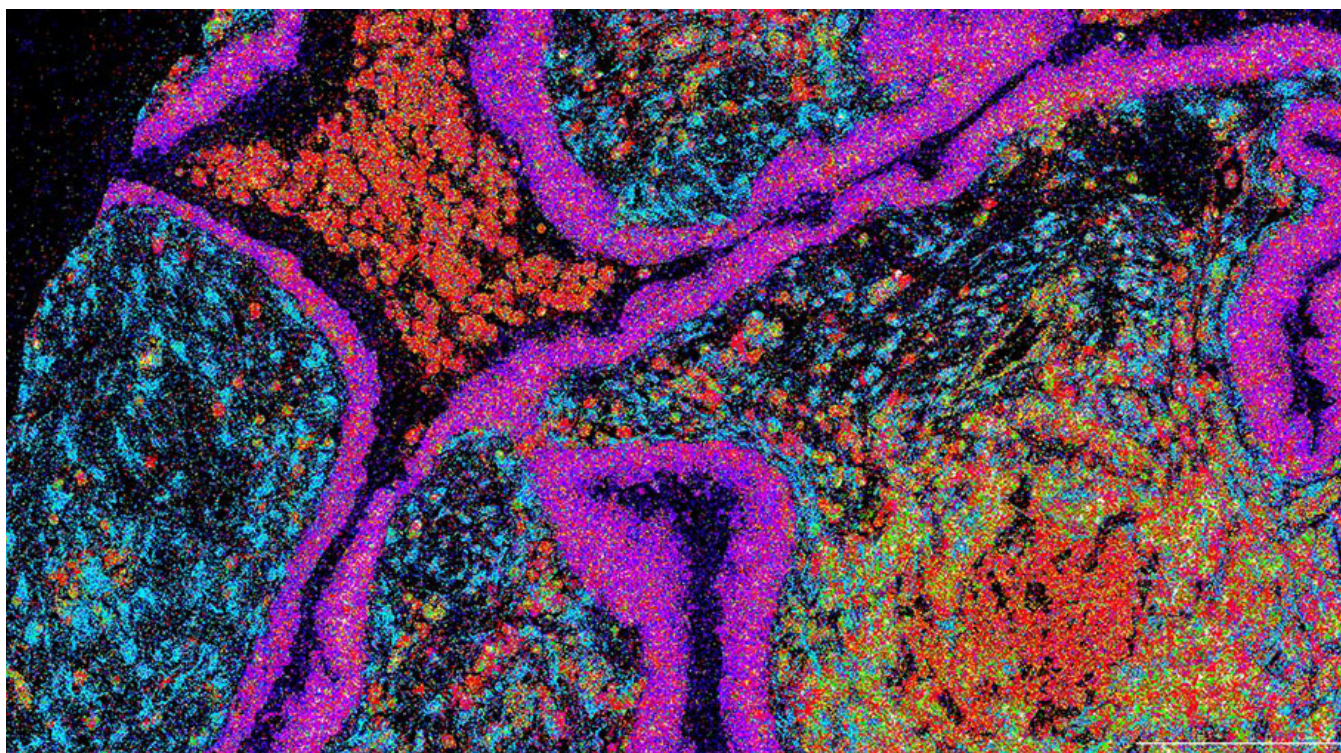
this kind of sharing would be the norm in spatial biology, too. One major difference, he observed, is that single-cell biology was heavily dominated by a single technology. As a result, single-cell biology was given to uniformity. In contrast, there is a huge diversity of spatial technologies, even with respect to a single modality such as transcriptomics.

What they are constantly thinking about in Fan’s laboratory is not just how to standardize, but how to integrate data from different modalities. And even when the data from different modalities is there, he said, there will still be a lot of work in understanding how to put them together. But people are moving in this direction now. He noted that computational biologists are playing a key role in this aspect of spatial.

Collaboration is key

Given the diversity in technologies, there is an increased need among researchers to share openly, making community driven initiatives incredibly valuable. One community that has developed over the past few months is the Global Alliance for Spatial Technologies (GESTALT). It was started by Ioannis Vlachos, PhD, co-director of the Bioinformatics Program, Cancer Research Institute, Beth Israel Deaconess Medical Center; Luciano Martelloto, PhD, associate professor at the University of Adelaide; and Jasmine Plummer, PhD, associate member, St. Jude Faculty and director, Center for Spatial OMICS.

GESTALT holds meetings online that allow spatial users from around the world to come together, share knowledge and challenges, and collaborate. Over 100 people attended the first online



The Banovitch lab at TGen specializes in multiomics, with a particular focus on diseases, and identifying genetic variation associated with changes in gene regulatory phenotypes. One area of research is uncovering the molecular mechanisms underlying pulmonary fibrosis. Here, spatial transcriptomics examines fibrotic lung disease using the 10X Genomics Xenium platform. Each point is a transcript, and they are colored by the cell type typically associated with that gene.

meeting. The founders hope that it will be a catalyst in advancing spatial technologies. The focus is on collaboration, knowledge sharing, and innovation that will serve to amplify the impact of spatial technologies across sectors.

GESTALT is a space to foster productive dialog for everyone, provide valuable feedback for active users, advocate for best practices for presenting data, and pool growing expertise to address challenges and foster innovations. The group plans to establish working groups to push the needle on specific topics such as standards and best practices, assay optimization, and data analysis.

The spirit of open science is a terrific part of the single-cell community, Fan stressed. And because the single-cell community comprises many of the

researchers moving to spatial, that collaborative spirit will be brought into the spatial world as well.

Bringing it all together

One thing that the spatial community would benefit from is a reference. Fan suggested that the spatial community might follow the example set by the human genome project. Once a reference genome was built, researchers could map everything to the reference genome. In the spatial community, an analogous effort would help researchers make sense of observations and data.

Banovich indicated that he appreciates the efforts of the atlas community over the past couple of years. These efforts include the building and unification of cell atlases across different organs. Once a certain point is reached, researchers could map

spatial data to single-cell reference data, unifying datasets and minimizing batch effects. But that, he admitted, poses a huge challenge.

Moving from the collection of spatial data to the generation of insights and knowledge is a long way to go, Fan said. And practical challenges arise from spatial biology's multidisciplinary nature. To illustrate this point, Fan offered the following example: A pathologist who has decades of experience in tissue embedding knows how to process tissue specimens but is unlikely to know the omics side of the technology. This intrinsic challenge requires people to work together. If people want to succeed, Fan maintained, they need people with extremely diverse backgrounds working together to overcome those barriers.

Magic wand

If given a magic wand, what would Fan and Banovich wish for to move spatial forward? An increase in plex, sensitivity, or resolution? Multiomics in one run? Or more sophisticated bioinformatics?

Banovich answered, laughing, that if the wand is really magic, he would change them all! The reason it is so hard to pick one, he explained, is because it is too early to know which of these things will really elevate spatial to the next level. He also contributed this brief self-dialogue: "Would I love an increase in plex? Absolutely. Does that come with a hit to sensitivity? Probably."

He said that he would be incredibly excited about a 10,000-plex spatial transcriptomics maps. But he added that if such maps were to come with a 75% sensitivity drop, he would immediately feel less excited. A 10,000-plex capability today, with no drop in sensitivity, would change the game for the spatial platforms.

Fan told *GEN* that he "wants everything, for sure." And that is not too far out of range; it is becoming possible. He emphasized that the field should embrace artificial intelligence and machine learning for computational data. The real revolution of the field, he asserted, will come when computer scientists, and artificial intelligence researchers, join the force to write the next chapter. ■

ON-DEMAND WEBINAR

How to pick antibodies for spatial biology

Get information about biomarker panel design, staining protocols, antibody and clone choices, and data interpretation strategies for spatial biology research applications.



Presenter:

Dr. Leticia Montoya, PhD, Chemistry and Biochemistry
Staff Scientist, Cell Biology
Thermo Fisher Scientific

WATCH NOW



Organ Mapping Antibody Panels (OMAPs)



Organ Mapping Antibody Panels (OMAPs) are detailed community-validated resources that give information about healthy organs and the experimental design used for multiplexed antibody-based imaging of healthy organs and tissues. The information from OMAP designed experiments provide better understanding of healthy anatomical structures and cell types.

The goal of a published OMAP:

- Reduce development time and expense for researchers who want to do a similar experiment or panel.
- Standardize panels to increase data reproducibility while providing flexibility for research purposes.
- Provide a starting point for a new panel that is community-validated and peer reviewed.
- Support the development of a Human Reference Atlas.

What is an OMAP?

OMAPs are community validated multiplex antibody panels that identify cell populations and structures within a diverse set of healthy tissues. This project was conceived by Dr. Andrea Radtke and consortium members associated with the Human BioMolecular Atlas Program (HuBMAP). Members within this group selected tissue specific antibodies and rigorously tested them for (1) high signal and low noise, (2) low non-specific interactions, (3) lower autofluorescence, and other attributes. Information about the antibodies is available through HuBMAP [Antibody Validation Reports](#), publications, vendor websites, and other community sites.

OMAPs are developed from tissues acquired from healthy or known disease-free organs. The information from these studies provides: (1) anatomical structure (2) cell types within these structures, (3) spatial location of cells, (4) intensity and size of cells and structures, (5) protein co-localization from conjugated antibody staining patterns.

OMAP information can be found on the [OMAP HuBMAP CCF portal pages](#).

How can I use an OMAP in my research?

OMAPs can be used to design spatial biology experiments with antibodies used to label tissue samples. The design and validation of OMAPs allow researchers to confidently choose the antibody and reagents to generate spatial data without spending as much time re-validating panels or testing antibodies. OMAPs include information on tissue sample preparation, antigen retrieval, and staining methods. OMAP tables will include cell

type markers and potential antibodies that maybe useful for the method used within the OMAP or other multiplexed imaging methods. Researchers may closely follow the panel design and method, or they can use the OMAP as an initial reference, to help guide the development of a multiplex panel for a particular tissue type.

OMAP tables link validated antibodies to cell types, providing a useful starting point for investigators seeking an antibody that will label their cell of interest in their tissue.

Nature Methods Brief Communication: Organ Mapping Antibody Panels (OMAPs): A community resource for standardized multiplexed tissue imaging.

A community resource for standardized multiplexed tissue imaging.

This publication provides information about anatomical structures, cell types in seven human organs, and validated antibodies used in spatial biology experiments.



[Learn about the team behind the work](#)

[Read the Spatial Biology HuBMAP Nature Methods Collection](#)

READ NOW ▶

Researchers may closely follow the established panel or they can use the OMAP as an initial reference to guide development of a multiplexed imaging panel tailored to their questions of interest that is compatible with their sample preparation (FFPE, fixed frozen) and imaging method.

Examples of OMAPs used to understand tissue can be found in two publications included in the HuBMAP Nature Methods Collection . These publications used highly multiplexed imaging panels to evaluate the cellular neighborhoods and tissue organization of the human skin and intestine.

Let's go through an example OMAP from the HuBMAP CCF Portal

Choose organ and multiplexed antibody-based imaging method

OMAP ID ↓	Organ ↓	Tissue Preservation Method ↓	Multiplexed antibody-based imaging method ↓	Download OMAP	Representative Dataset ↓	#AS ↓	#CT ↓	#BP ↓
OMAP-1	Lymph Node	Fixed Frozen	IBEX	CSV XLSX	Link	28	32	39
OMAP-2	Intestines	Fresh Frozen	CODEX	CSV XLSX	Link	30	33	54
OMAP-3	Kidney	Fresh Frozen	CODEX	CSV XLSX		28	38	24
OMAP-4	Skin	FFPE	Cell DIVE	CSV XLSX	Link	22	13	18
OMAP-5	Liver	Fresh Frozen	SIMS	CSV XLSX	Link	24	14	19
OMAP-6	Pancreas	FFPE	CODEX	CSV XLSX	Link	10	10	11
OMAP-7	Lung	FFPE	Cell DIVE	CSV XLSX	Link	28	36	30
OMAP-8	Placenta	FFPE	IMC	CSV XLSX		8	15	26
OMAP-9	Kidney	Fresh Frozen	CODEX	CSV XLSX		29	33	41
OMAP-10	Palatine Tonsil	PFA fixed	MACSima	CSV XLSX	Link	12	16	30
OMAP-11	Spleen	Fixed Frozen	IBEX	CSV XLSX	Link	23	23	24
OMAP-12	Eye Retina	Fixed Frozen	IBEX	CSV XLSX	Link	4	7	14
OMAP-13	Pancreas	4% PFA-fixed frozen	CODEX	CSV XLSX	Link	13	21	27
Totals						259	291	357

Download the spreadsheet with antibody and experimental design information

Click on the spreadsheet link (see red circle in the screenshot below) and download the file. Keep the information regarding how to cite the information for publications, grants, webpages, and other external documents.

LABEL	VALUE
Creator(s):	Andrea J. Radtke
Creator ORCID(s):	0000-0003-4379-8967
Project Lead:	Andrea J. Radtke; Katy Börner; Neil Kelleher; Ronald N. Germain
Project Lead ORCID:	0000-0003-4379-8967 ; 0000-0002-3321-6137 ; 0000-0002-8815-3372 ; 0000-0003-1495-9143
Reviewer(s):	Elaine Jaffe ; Stefania Pittaluga; Ronald N. Germain; Ellen M. Quardokus; Christopher Werlein; Diane C. Saunders; Anna Martinez Casals; Presha Rajbhandari
Reviewer ORCID(s):	0000-0003-4632-0301 ; 0000-0001-7688-1439 ; 0000-0003-1495-9143 ; 0000-0001-7655-4833 ; 0000-0002-7694-4257 ; 0000-0002-8849-6746 ; 0000-0003-2722-1965 ; 0000-0003-2184-7238
Creation Date:	2023-06-15
License:	Creative Commons Attribution 4.0 International (CC BY 4.0)
Publisher:	HuBMAP
Funder:	National Institutes of Health
Award Number:	OT2OD026671
HuBMAP ID:	HBM458.NSWR.957
Data Table:	OMAP-1 Organ Mapping Antibody Panel (OMAP) for Multiplexed Antibody-Based Imaging of Human Lymph Node with IBEX v1. CSV Excel
DOI:	https://doi.org/10.48539/HBM458.NSWR.957
How to Cite This Data Table:	Andrea J. Radtke. Organ Mapping Antibody Panel (OMAP) for Multiplexed Antibody-Based Imaging of Human Lymph Node with IBEX v1.3. https://doi.org/10.48539/HBM458.NSWR.957 . Accessed on June 23, 2023.
How to Cite OMAP Tables Overall:	Hickey, John W., Elizabeth K. Neumann, Andrea J. Radtke, Jeannie M. Camarillo, Rebecca T. Beuschel, Alexandre Albanese, Elizabeth McDonough, et al. 2021. "Spatial Mapping of Protein Composition and Tissue Organization: A Primer for Multiplexed Antibody-Based Imaging." <i>Nature Methods</i> , November. https://doi.org/10.1038/s41592-021-01316-y .

ADVANCING SPATIAL BIOLOGY THROUGH MULTIPLEXED APPROACHES

The downloaded spreadsheet contains the information required to choose antibodies. It describes antibody name, clone, vendor, and conjugate type if applicable (e.g., fluorophore). Experimental design details include protocol method, tissue preservation method, and antibody dilution amount. In this example, the authors used [IBEX](#), which is a multiplexed immunostaining protocol that employs iterative

bleaching and cyclic antibody staining for high-parameter imaging. This open source method is supported by the [IBEX Imaging Community](#), an international group of scientists committed to sharing knowledge related to multiplexed imaging in a transparent and collaborative manner. This open, global repository is a central resource for reagents, protocols, panels, publications, software, and datasets. .

Sheet Mapping Antibody Panel (OMAP-1) for Multiplexed Antibody-Based Imaging of Human Lymph Node with IBEX																								
1	Author: J. Radtke																							
2	CD138																							
3	CD138																							
4	CD138																							
5	CD138																							
6	CD138																							
7	CD138																							
8	CD138																							
9	CD138																							
10	CD138																							
11	CD138																							
12	CD138																							
13	CD138																							
14	CD138																							
15	CD138																							
16	CD138																							
17	CD138																							
18	CD138																							
19	CD138																							
20	CD138																							
21	CD138																							
22	CD138																							
23	CD138																							
24	CD138																							
25	CD138																							
26	CD138																							
27	CD138																							
28	CD138																							
29	CD138																							
30	CD138																							
31	CD138																							
32	CD138																							
33	CD138																							
34	CD138																							
35	CD138																							
36	CD138																							
37	CD138																							
38	CD138																							
39	CD138																							
40	CD138																							
41	CD138																							
42	CD138																							
43	CD138																							
44	CD138																							
45	CD138																							
46	CD138																							
47	CD138																							
48	CD138																							
49	CD138																							
50	CD138																							
51	CD138																							
52	CD138																							
53	CD138																							
54	CD138																							
55	CD138																							
56	CD138																							
57	CD138																							
58	CD138																							
59	CD138																							
60	CD138																							
61	CD138																							
62	CD138																							
63	CD138																							
64	CD138																							
65	CD138																							
66	CD138																							
67	CD138																							
68	CD138																							
69	CD138																							
70	CD138																							
71	CD138																							
72	CD138																							
73	CD138																							
74	CD138																							
75	CD138																							
76	CD138																							
77	CD138																							
78	CD138																							
79	CD138																							
80	CD138																							
81	CD138																							
82	CD138																							
83	CD138																							
84	CD138																							
85	CD138																							
86	CD138																							
87	CD138																							
88	CD138																							
89	CD138																							
90	CD138																							
91	CD138																							
92	CD138																							
93	CD138																							
94	CD138																							
95	CD138																							
96	CD138																							
97	CD138																							
98	CD138																							
99	CD138																							
100	CD138																							
101	CD138																							
102	CD138																							
103	CD138																							
104	CD138																							
105	CD138																							
106	CD138																							
107	CD138			</																				

Where can I find a new OMAP?

OMAP ID	OMAP Name	Tissue type
OMAP-1	Organ Mapping Antibody Panel (OMAP) for Multiplexed Antibody-Based Imaging of Human Lymph Node	Lymph node
OMAP-2	Organ Mapping Antibody Panel (OMAP) for Multiplexed Antibody-Based Imaging of Human Intestine	Intestines
OMAP-3	Organ Mapping Antibody Panel (OMAP) for Multiplexed Antibody-Based Imaging of Kidney	Kidney
OMAP-4	Organ Mapping Antibody Panel (OMAP) for Multiplexed Antibody-Based Imaging of Human Skin	Skin
OMAP-5	Organ Mapping Antibody Panel (OMAP) for Multiplexed Antibody-Based Imaging of Human Liver	Liver
OMAP-6	Organ Mapping Antibody Panel (OMAP) for Multiplexed Antibody-Based Imaging of Human Pancreas	Pancreas
OMAP-7	Organ Mapping Antibody Panel (OMAP) for Multiplexed Antibody-Based Imaging of Human Lung	Lung

Examples of Thermo Fisher Scientific antibodies in OMAP publications.

OMAP for multiplexed antibody-based imaging of human lymph node with IBEX

Creation date	May 6, 2022
HuBMAP ID	HBM624.PJSR.685
Citation	Andrea J. Radtke. Organ Mapping Antibody Panel (OMAP) for Multiplexed Antibody-Based Imaging of Human Lymph Node with IBEX. https://doi.org/10.48539/HBM624.PJSR.685
Products	- CD20 Monoclonal Antibody (L26), Alexa Fluor 488, eBioscience (Cat. No. 53-0202-82) - Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 700 (Cat. No. A21038) - CD4 Monoclonal Antibody (RPA-T4), Alexa Fluor 532, eBioscience (Cat. No. 58-0049-42) - FOXP3 Monoclonal Antibody (236A/E7), eFluor 570, eBioscience (Cat. No. 41-4777-82) - Alpha-Smooth Muscle Actin Monoclonal Antibody (1A4), Alexa Fluor 488, eBioscience (Cat. No. 53-9760-82)

OMAP for multiplexed antibody-based imaging of human intestine with CODEX v1.0

Creation date	May 6, 2022
HuBMAP ID	HBM373.HQCB.363
Citation	John Hickey. Organ Mapping Antibody Panel (OMAP) for Multiplexed Antibody-Based Imaging of Human Intestine with CODEX. https://doi.org/10.48539/HBM373.HQCB.363
Products	• Anti-FAP antibody (Cat. No. 13-0300) • Anti-α-SMA antibody (Cat. No. 14-9760-82) • Anti-OLFM4 antibody (Cat. No. MA5-29457)

OMAP for multiplexed antibody-based imaging of human skin with Cell DIVE v1.0

Creation date	May 6, 2021
HuBMAP ID	HBM467.LRKZ.884
Citation	Liz McDonough. Organ Mapping Antibody Panel (OMAP) for Multiplexed Antibody-Based Imaging of Human Skin with Cell DIVE v1.0 https://doi.org/10.48539/HBM467.LRKZ.884
Products	• Cytokeratin Pan Type I Monoclonal Antibody (AE1), eBioscience (Cat. No. 14-9001)

OMAP for multiplexed antibody-based imaging of human intestine with CODEX v1.0

Creation date	May 6, 2021
HuBMAP ID	HBM395.LXKX.734
Citation	Anna Martinez Casals, Frida Björklund. Organ Mapping Antibody Panel (OMAP) for Multiplexed Antibody-Based Imaging of Human Pancreas with CODEX v1.0. https://doi.org/10.48539/HBM395.LXKX.734
Products	• C-Peptide Monoclonal Antibody (C-PEP-01) (Cat. No. MA1-19159) • E-cadherin Monoclonal Antibody (Cat. No. 33-4000)

OMAP for multiplexed antibody-based imaging of human intestine with CODEX v1.0

Creation date	May 6, 2022
HuBMAP ID	HBM366.RQZK.248
Citation	Christine Surrette, Lisa Lowery, Gloria Pryhuber, Gail Deutsch, Fiona Ginty. Organ Mapping Antibody Panel (OMAP) for Multiplexed Antibody-Based Imaging of Human Lung with Cell DIVE v1.0 https://doi.org/10.48539/HBM366.RQZK.248
Products	• Zenon Rabbit IgG Labeling Kits, Alexa Fluor 555 (Cat. No. Z25305) • anti-COX2 (Cat. No. 35-8200)

References

- Quardokus EM, Saunders DC, McDonough E, Hickey JW, Werlein C, Surrette C, Rajbhandari P, Casals AM, Tian H, Lowery L, Neumann EK, Björklund F, Neelakantan TV, Croteau J, Wiblin AE, Fisher J, Livengood AJ, Dowell KG, Silverstein JC, Spraggins JM, Pryhuber GS, Deutsch G, Ginty F, Nolan GP, Melov S, Jonigk D, Caldwell MA, Vlachos IS, Muller W, Gehlenborg N, Stockwell BR, Lundberg E, Snyder MP, Germain RN, Camarillo JM, Kelleher NL, Börner K, Radtke AJ. Organ Mapping Antibody Panels: a community resource for standardized multiplexed tissue imaging. *Nat Methods*. 2023 Jul 19. PMID: 37468619.
- Radtke AJ, Quardokus EM, Saunders DC. SOP: Construction of Organ Mapping Antibody Panels for Multiplexed Antibody-Based Imaging of Human Tissues. DOI 10.5281/zenodo.5749882
- Hickey JW, Neumann EK, Radtke AJ, Camarillo JM, Beuschel RT, Albanese A, McDonough E, Hatler J, Wiblin AE, Fisher J, Croteau J, Small EC, Sood A, Caprioli RM, Angelo RM, Nolan GP, Chung K, Hewitt SM, Germain RN, Spraggins JM, Lundberg E, Snyder MP, Kelleher NL, Saka SK. Spatial mapping of protein composition and tissue organization: a primer for multiplexed antibody-based imaging. *Nat Methods*. 2022 Mar;19(3):284-295. PMID: 34811556.
- Radtke AJ, Chu CJ, Yaniv Z, Yao L, Marr J, Beuschel RT, Ichise H, Gola A, Kabat J, Lowekamp B, Speranza E, Croteau J, Thakur N, Jonigk D, Davis JL, Hernandez JM, Germain RN. IBEX: an iterative immunolabeling and chemical bleaching method for high-content imaging of diverse tissues. *Nat Protoc*. 2022 Feb;17(2):378-401. PMID: 35022622.
- Du Z, Lin JR, Rashid R, Maliga Z, Wang S, Aster JC, Izar B, Sorger PK, Santagata S. Qualifying antibodies for image-based immune profiling and multiplexed tissue imaging. *Nat Protoc*. 2019 Oct;14(10):2900-2930. PMID: 31534232.
- Andrea J. Radtke. Organ Mapping Antibody Panel (OMAP) for Multiplexed Antibody-Based Imaging of Human Lymph Node with IBEX ([link](https://doi.org/10.48539/HBM624.PJSR.685)). <https://doi.org/10.48539/HBM624.PJSR.685>
- Scalia CR, Boi G, Bolognesi MM, Riva L, Manzoni M, DeSmedt L, Bosio FM, Ronchi S, Leone BE, Cattoretti G. Antigen Masking During Fixation and Embedding, Dissected. *J Histochem Cytochem*. 2017 Jan;65(1):5-20. Epub 2016 Oct 23. PMID: 27798289.

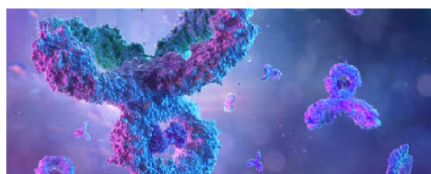
Databases

- [HuBMAP CCF Portal: Organ Mapping Panels \(OMAPs\)](#)
- [Human Protein Atlas](#)

Resources

- [Basic IHC steps](#)
- [Considerations for selecting IHC antibodies](#)

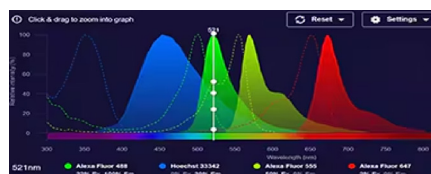
WEB TOOLS



Antibody search tool

Easily search for high-quality primary, secondary, and conjugated antibodies for IF, IHC, and other imaging applications.

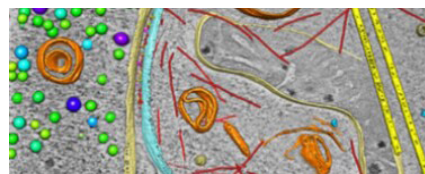
ACCESS TOOL ▶



SpectraViewer

Supports all levels of experimental complexity, use the tool to compare excitation and emission spectra of fluorophores and reagents.

SEE FLUOROPHORES ▶

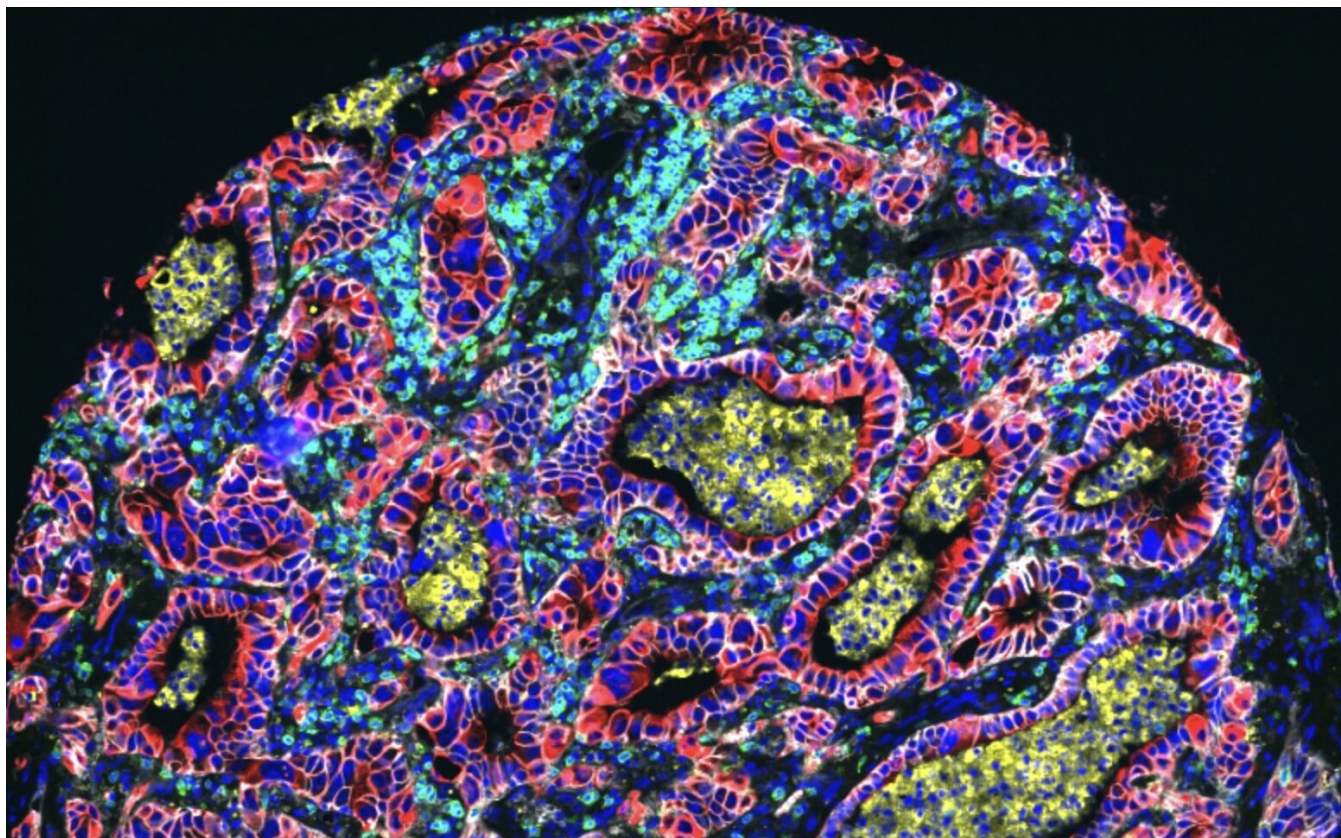


Amira Software

2D–5D solution for visualizing and analyzing data from many image modalities, including optical and electron microscopy, CT, MRI and other imaging techniques.

START YOUR TRIAL NOW ▶

Spatial Biology and AI Combined by Noetik to Develop Immuno-Oncology Drugs



Noetik

Human lung cancer specimen demonstrating tumor and immune interactions with a subset of Noetik protein panel.

When asked about the focus of his new company, Ron Alfa, MD, PhD, begins with an epistemological explanation, and the definition of disease (he is a trained physician, after all.) In some cancers, he explained, a tumor type is defined by a genotype. This allows for the development of precision therapeutics and the stratification of patients. But these lines have yet to be drawn in immuno-oncology. “We haven’t been able to define patient populations by the underlying immune biology in a clear and simple way,” he told GEN. To support his assertion, he points to the dearth of new immuno-oncology molecules developed over the past decade or two (with the exception of PD-L1) with promising preclinical data.

Why hasn’t there been more success in this area? Alfa cites a lack of understanding of which tumors are most likely to respond to certain mechanisms. Because, he said, we don’t have a system that helps us understand that some tumors are inhibiting the immune system by

mechanism A, some by mechanism B, and others by mechanism C. Once that information is uncovered, immuno-oncology can be pushed into precision oncology, and tumors can be classified based on their mechanisms of immune resistance and immune biology.



Ron Alfa, MD, PhD, and Jacob Rinaldi, PhD

But immune biology does not cleanly map to genomics. So, Alfa and Jacob Rinaldi, PhD, co-founder and current CSO at Noetik—who met during their graduate school days at Stanford University—have founded their company to solve this problem.

First, they sought to understand what are the best data to 1) understand tumor biology and 2) use in machine learning models. Enter spatial biology.

Not your normal spatial customer

Noetik is not a spatial company, Alfa asserted. But they use a lot of spatial. They are, according to Alfa, the biggest users of two new platforms from two different spatial companies—one for proteomics and the other transcriptomics.

For Noetik, spatial is the right dataset for machine learning to help them achieve their goal, Alfa said. But the spatial going on at Noetik is different

from how other researchers are using the new technology. “What we’re doing doesn’t look much like what [academics are] doing.” Because Noetik is using spatial through the lens of building a dataset for machine learning. To that end, they need a lot of data.

“I’ve literally told both of [the spatial companies] that we are not your normal customer.” Academics have a totally different set of requirements than we do. Noetik needs to scale, run the machine 24 hours a day, get the data onto the cloud, have QC metrics, and other industrialization requests.

They have, in six months, generated hundreds of patients worth of data. For each patient, they have genomic data, H&E staining, proteomics, and transcriptomics.

The goal is for the models to learn from that information. They are training the models to do

something that humans can't do: to classify these tumors into tumor immune subsets in ways that are therapeutically important. To do this, they use self-supervised learning, allowing the models to learn internal representations of the data themselves. Eventually, they hope to learn tumor biology from the models.

The company's work points in the direction of building a portfolio of molecules that are specifically directed at tumor subsets—though there is no pipeline yet. But they will start from the patient, using reverse translation. Noetik's platform does not help predict a molecule or design a drug. Hopefully, it uncovers novel biology, and in doing so, informs what drug they should make.

Driven by frustration

Over the course of Ron Alfa's lifetime, he has pursued things that he finds frustrating. "It's always been very frustrating that we don't have better cancer drugs. It's one of these things that [you think], this is ridiculous! We know so

much about biology, why don't we have better cancer drugs?"

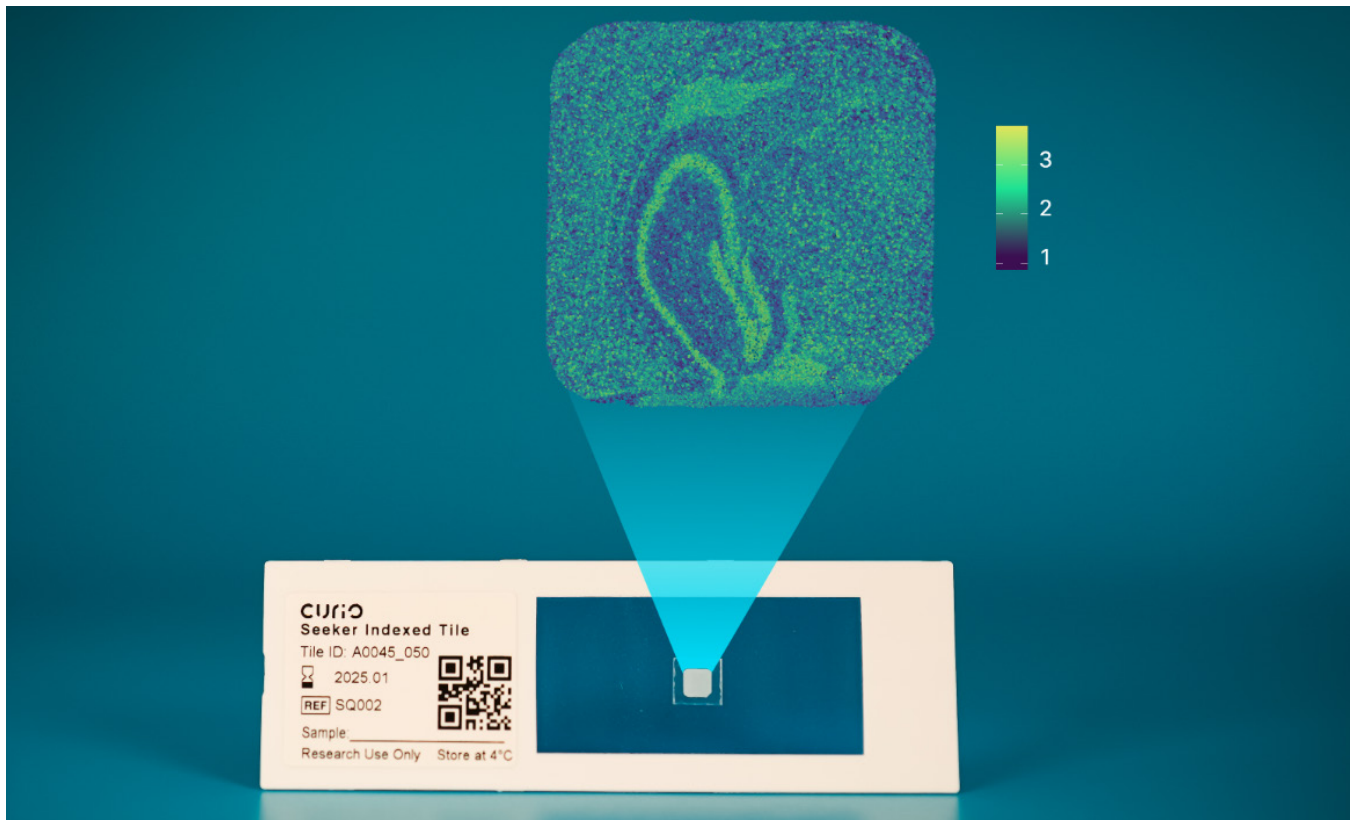
Alfa and Rinaldi left their previous posts at Recursion in February of last year, and Noetik was incorporated in June of the same year—just 15 months ago. They built the company remotely but have the largest cluster in South SF and the second largest group in Boulder. The company of 18 employees announced last week that it closed an oversubscribed \$14M seed financing round led by DCVC.

The challenge Noetik faces is the same one facing many biotech and pharma companies: developing better cancer drugs. But Noetik's approach uses different data to try to solve that problem.

The name Noetik is derived from a Greek word for intellectual. They chose the name for two reasons: one, the relationship between the word intellectual and artificial intelligence. Two, the hope that artificial intelligence is smart enough to understand patient groups, which will lead to better cancer therapeutics. ■

Slide-Seq Hits the Spatial Biology Market as Curio Seeker

The spatial technology developed in 2019, known as Slide-seq, is now commercially available as the Seeker, by Curio Bioscience.

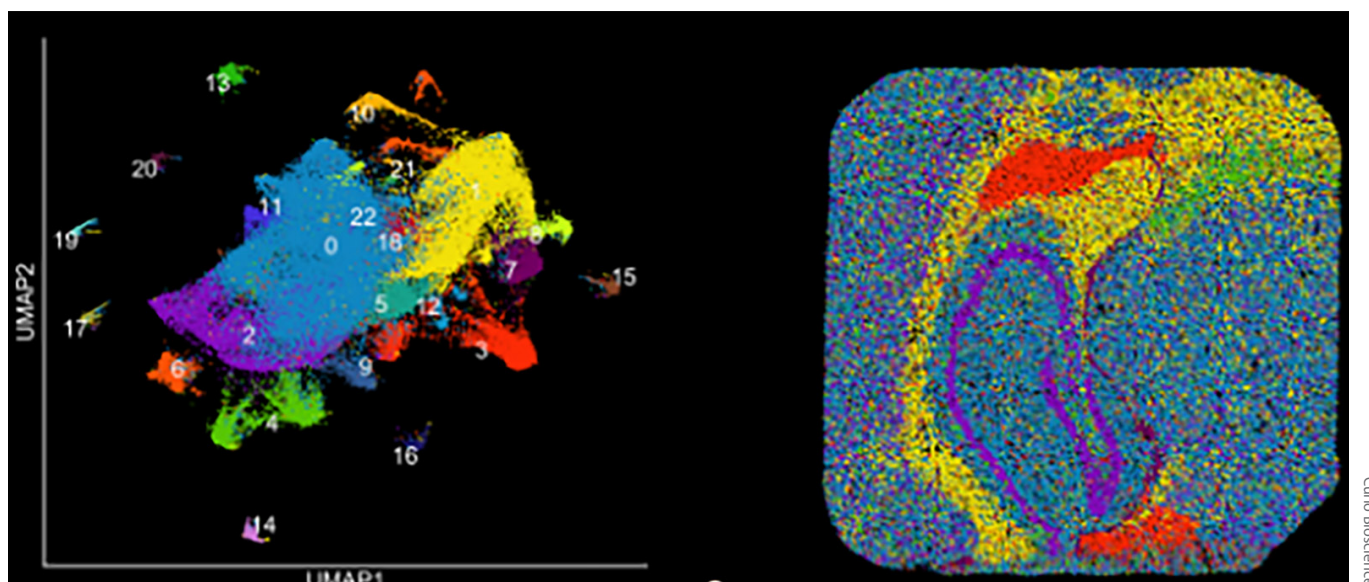


Curio Seeker tile with example data from mouse hippocampus.

In 2019, spatial transcriptomics was creating a lot of buzz in the genomics world. Technologies like MERFISH and FISSEQ were the foundation of new companies. That same year, Fei Chen, PhD, Sam Rodriques, PhD, and Evan Macosko, MD, PhD, colleagues at the Broad Institute, published a new spatial technology that doesn't need any equipment, other than a sequencer, called Slide-seq. In addition, Slide-seq offers high resolution due to the platform's minute (10 microns) barcoded beads and limited diffusion.

Immediately after the paper was published, calls and requests from collaborators came rolling in. Chen told GEN that they were overwhelmed supporting other folks, some of whom were far outside of Chen's core scientific focus.

Chen's goal was to get the tool into the hands of other researchers. The impact of developing technologies, he says, is when people use them. But they needed a company to do it. They tried to do it themselves and sought interest from venture



Example output from the Curio Seeker bioinformatics pipeline includes results from unbiased clustering and dimension reduction (left) as well as a spatial map of identified clusters (right).

capital firms. But selling tools to researchers is a somewhat non-traditional business model for a VC. They were more interested in using it internally to discover drugs. And that wasn't what Chen had in mind.

Then the pandemic hit, other priorities took over, and Slide-seq stayed in the lab. All the while, Chen's group continued to work on Slide-seq, making improvements along the way.

In early 2021, Chen, Macosko, and Rodriques were connected to Steve Fodor, PhD, genomics pioneer and co-founder of Affymetrix, through a VC incubator. Chen called it a "very chance meeting." Fodor liked the technology and liked the idea of distributing it to the world.

The partnership, which includes co-founders Ari Chaney (COO) and Christina Fan, PhD (CTO), was formed in early 2021 and today, less than two years later, Curio Bioscience is launching the Slide-seq technology under a new name, Seeker. The kit includes eight Seeker tiles (which are

pucks in Slide-seq), reagents, and text files with bead barcodes and spatial locations.

Chen said that, in retrospect, 2019 probably would have been a little early to the game. The additional two years meant that the technology matured to a place where it was easy to manufacture and turn into a tool. And the excitement for spatial grew considerably over those two years. All this probably served the technology's developers well in the end.

Two other non-imaging-based spatial methods have been developed. The highest resolution is Stereo-seq technology, developed by BGI-Research, which is not commercially available yet. One complication with Stereo-seq is that it requires an MGI sequencer. The other technology is 10X Genomics's Visium—the most widely used spatial technology currently.

Chen is excited to see Slide-seq launching as a product, in particular for researchers in the single-cell community who want to ask questions about

tissue biology. Another key group are researchers working in the tumor microenvironment, developmental biology, and non-model organisms—an area that is not as heavily focused on but is uniquely enabling.

Weird critters

“We work on weird critters,” said Anoja Perera, director, sequencing and discovery genomics at the Stowers Institute for Medical Research in Kansas City. Instead of human samples, they have model organisms like planaria and axolotls. “You name it,” said Perera. When they started using spatial technologies, about three years ago, they used Slide-seq (in collaboration with Chen) and Visium from 10X Genomics.

Chen involved Perera with the product even before the company had a name. Early on, Perera started comparing the Seeker prototype with Slide-seq and noted that it “worked beautifully.” So, now they have completely switched over to Seeker. “We are loving it,” she said.

That said, they are considering all the spatial platforms in her facility. For example, they are currently testing samples using stereo-seq. The in situ-based spatial techniques are, Perera said, less applicable to the work at Stowers because they are geared to human and mouse. In addition, she likes the flexibility of Curio. One day, she can do an experiment on zebrafish, another she can do planaria. To use Seeker, all she needs is for the organism to have a genome sequence. And she doesn’t have to worry about highly expressed genes saturating the images. But she added that, as with any small company, she worries about the production—will they be able to keep up?

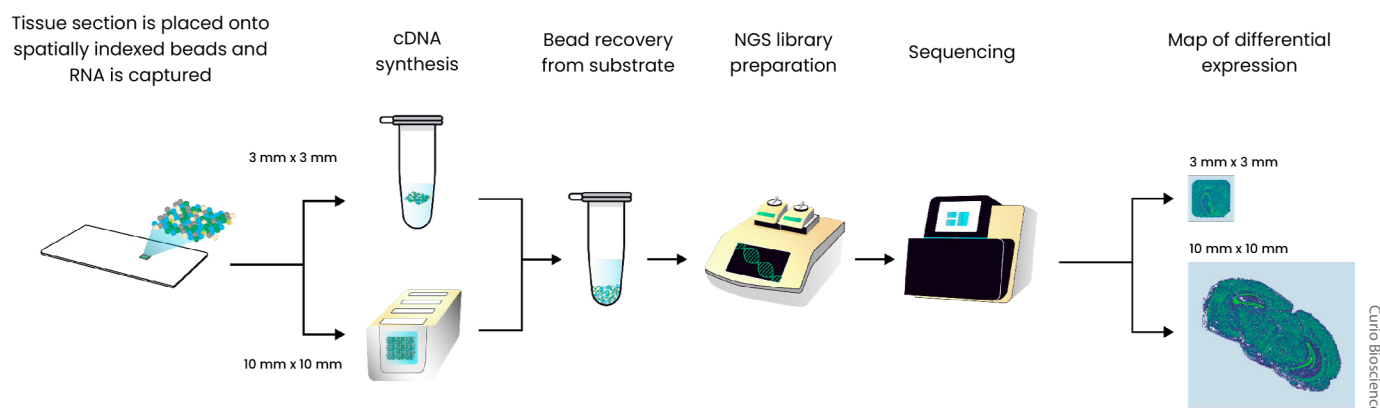
Because of their familiarity with Slide-seq, Perera said moving to Seeker was an easy transition. But in a lab that is not as familiar, she thinks that Curio may need to visit customer labs and do more training. Spatial is almost an art, she said; you need a great histology group and a skilled molecular biology group. If there is a good histology lab and someone who is skilled in molecular biology protocols, she said, someone who has never done spatial before could use Seeker.

Back to humans

Angela Pisco, PhD, associate director of data science, quantitative cell science at the Chan Zuckerberg Biohub, leads a project to build a reference atlas. The first version of Tabula-sapiens, which was built on dissociated single cells—half a million cells in 23 tissues—was published last year.

Although the project started with dissociated samples, Pisco and colleagues have been obtaining spatial data since the beginning of 2021. They have been using Vizgen’s platform, the Merscope, but jumped at the chance to use the Seeker, primarily because they were interested in the unbiased approach it offered. Now, they are about to embark on building a reference using Curio. Pisco said that Curio’s signal-to-noise ratio is a key reason why she is using it. The quality of the signal is a priority because, she says, a reference that is going to be used by the community must be as high quality as possible.

One downside is that the current version of the Seeker works only on fresh frozen samples, not formalin-fixed paraffin-embedded (FFPE) samples. Moreover, unlike some of the other



The Curio Seeker offers a simple, single-day workflow without specialized instrumentation.

platforms, there is no point at which a picture of the tissue is taken. So, there is no way to recover the morphology, thus making a comparison to an H&E stained image, which may be important in a clinical setting, very difficult. Curio may need to reconcile the lack of a way to image the tissue. Without it, researchers need to place a lot of trust in the technology. And some scientists may be less willing to do that than others.

The only option

Kelvin Chen, PhD, is a researcher in Shimon Sakaguchi's lab at Osaka University—an immunology lab that relies heavily on single-cell RNA-seq and ATAC data to profile human tissue samples. Spatial would be very useful to profile cell-to-cell interactions. But the previous spatial technologies were not options for Chen. Either the resolution was too low, or they were prohibitively expensive.

Although Chen wanted to use Slide-seq, he didn't know how to access it. He doesn't know Chen, Macosko, or Rodriques. But when Bertrand Yeung,

PhD, director of business development at Curio, reached out to him about the Seeker, he was eager to try it.

Kelvin Chen said he finds the Seeker easy to use, but quickly added that he has a lot of experience in this type of work, especially in library prep. As an immunology lab, the histology has been the biggest challenge for him. And, he added, the tiles are small, which is tricky. It's difficult to mount a large sample onto a small tile. And "it's computationally heavy." Indeed, the cell resolution was so high, he said, that the department's supercomputer ran out of memory.

Fei Chen is excited to see what people do with the Seeker. It's "a little bit into the unknown." He's also looking forward to redirecting the ongoing requests for Slide-seq to a company. As for himself, he'll continue making arrays, and building on top of the Slide-seq technology, in his lab. And he'll likely become a customer of Curio's as well.

But he might have to get in line. ■

Super-Resolution Microscopy Can Be Super-Accessible

To bring single-molecule localization microscopy to everyday researchers, laboratories can upgrade existing systems or deploy all-new platforms

BY JULIANNA LEMIEUX, PHD

A decade ago, the Nobel Prize in Chemistry was awarded to a trio of researchers for the development of super-resolved fluorescence microscopy. The announcement at the time stated that the researchers' work had "brought optical microscopy into the nanodimension." Indeed, their work broke the diffraction-limited resolution barrier. In conventional light microscopy, the diffraction limit, or the physical limit for the maximum resolution, is 200 nm.

One of the two techniques recognized by the prize was single-molecule localization microscopy (SMLM). Actually, SMLM is a group of techniques. It includes stochastic optical reconstruction microscopy (STORM), DNA-based point accumulation for imaging in nanoscale topography (DNA-PAINT), and photoactivated localization microscopy (PALM). The three approaches vary in how they carry out the random activation of a subset of fluorescent molecules—the cornerstone of SMLM. The data analysis leads to the resolution of structures at the nanoscale level.

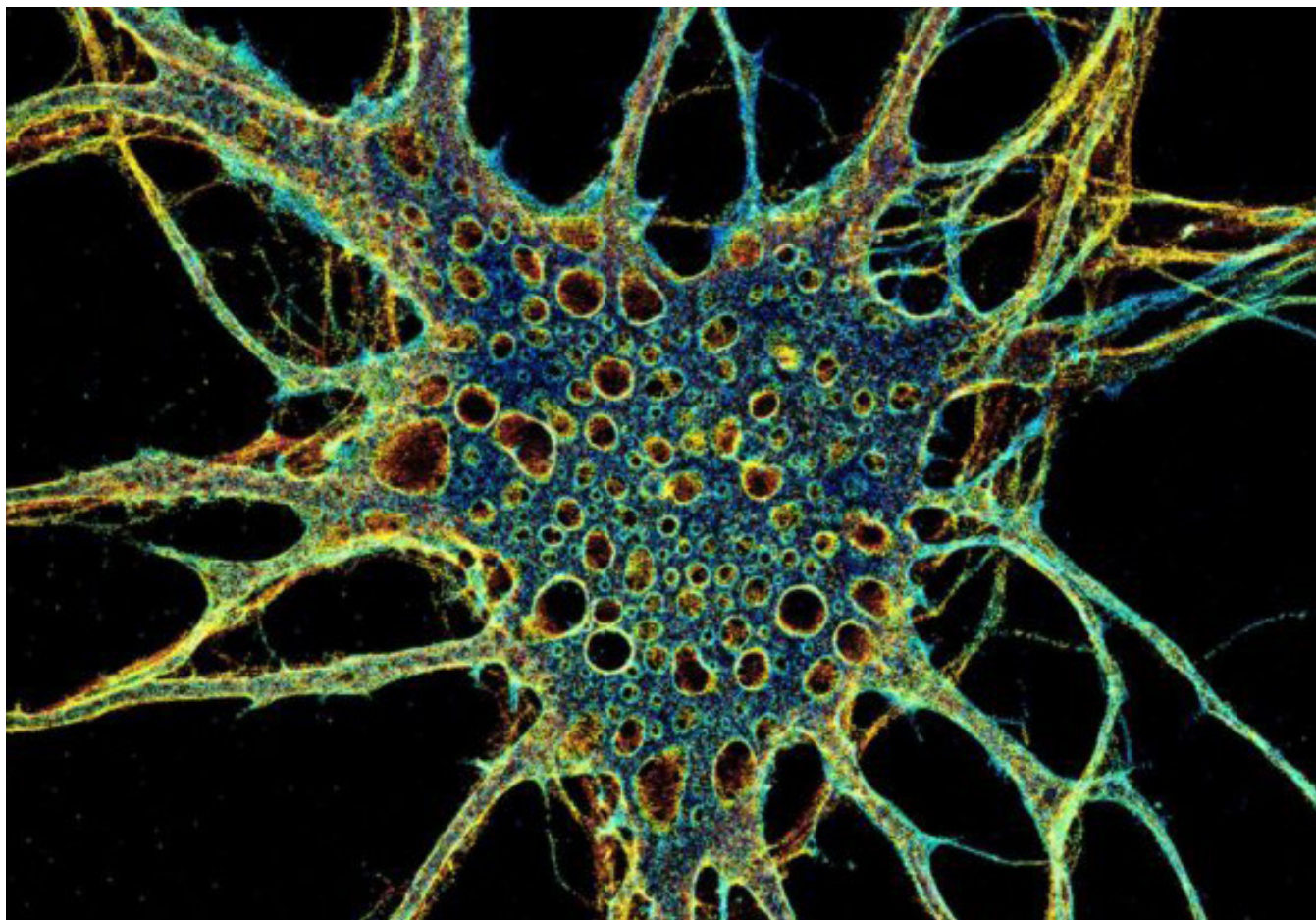
SMLM was developed by two of the Nobel Prize winners, Eric Betzig, PhD, and William E. Moerner, PhD. Betzig is a senior fellow at the Janelia Research Campus, a professor of molecular and cell biology and a Howard Hughes Medical Institute Investigator at the University of California, Berkeley. Moerner is a professor of chemistry and a professor of applied physics at Stanford University.

Also recognized in 2014 was stimulated emission

depletion (STED) microscopy, invented by Stefan W. Hell, PhD, director of both the Max Planck Institute for Multidisciplinary Sciences in Göttingen and the Max Planck Institute for Medical Research in Heidelberg.

The discoveries instigated progress on two fronts. On the scientific front, it became possible to visualize molecules (and the pathways of individual molecules) inside living cells. On the technological front, it became possible to develop commercial nanoscopy instruments. Developments on each of these fronts are reinforcing each other. Technology providers are introducing increasingly accessible microscopy systems, removing the need for highly expert users, and bringing high-powered microscopy to more and more biologists. And a growing community of users is encouraging more commercial activity.

Between the years 2016 and 2017, several companies launched or expanded into this space. Here, GEN spoke with some of these players to learn how they are making super-resolution



This image of cultured hippocampal neurons was obtained by Karoline Friedl, PhD, at the NeuroCyto Lab. She employed a direct stochastic optical reconstruction microscopy (dSTORM) system that combined an Olympus IX83 inverted microscope and an Abbelight SAFe360 nanoscope. With dSTORM, a single-molecule localization microscopy (SMLM) technique, consecutive images are acquired as different molecules fluoresce, and the accumulated raw data are processed in real time to localize all the fluorescing individual molecules with nanometer precision.

microscopy easier, cheaper, and more accessible than ever.

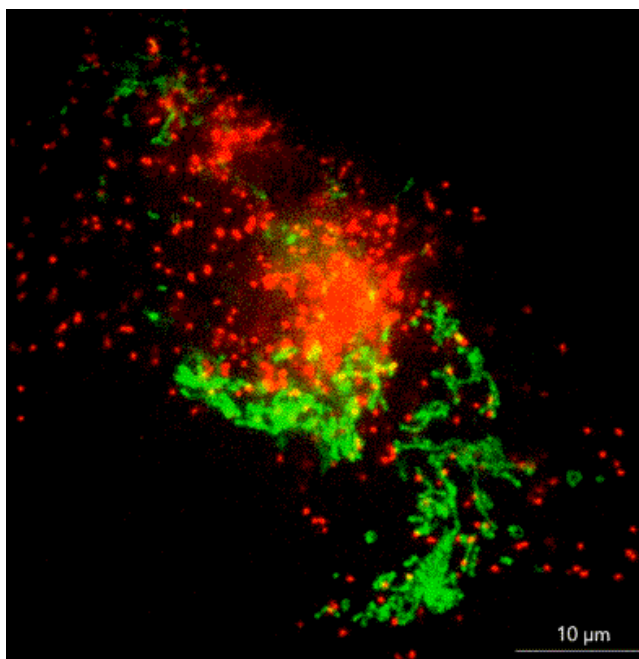
ONI's black box breaks barriers

When the Nobel Prize in Chemistry was awarded in 2014, STORM started enjoying wider recognition. But according to Jason Jell, PhD, vice president of marketing at ONI, the technology "wasn't particularly accessible to anyone." Users needed to be highly specialized, with expertise in physics, chemistry, and biology. In 2016, ONI was started to bring the technology to researchers who work in traditional

laboratories and aren't microscopy experts.

ONI was spun out of academic research led by Bo Jing, DPhil, at Oxford University. Besides founding ONI, Jing served as the company's CEO until he left to pursue other interests in 2023. ONI's current CEO is Paul Scagnetti, PhD, formerly Illumina's vice president of corporate and business development. Now, at about 100 people strong, the company remains in the United Kingdom but has recently opened a U.S. office in San Diego.

What does it take to bring SMLM to non-microscopists? Jell suggests removing barriers such as



Contact sites between mitochondria and endoplasmic reticulum are of interest because they influence many cellular functions. However, these sites are hard to study because they involve brief periods of apposition (at distances of 5–100 nm) rather than stable fusions. To visualize these contact sites during live cell imaging, Xufeng Wu, PhD, deputy director of the NIH's Light Microscopy Core, used single-particle tracking technology from ONI.

the need for a darkroom, temperature control, a special table, or a technician to tweak or align the instrument. In ONI's instrument, the nano-imager, a microscope floats within an enclosure with stable optics that are easy to maintain. The company has swapped out commercially available lasers for ones that it has designed in house, and it has built flow cells to allow multiple lanes on a single chip.

Jell suggests that SMLM will push microscopy to overcome assumptions. For example, that microscopy is only qualitative. SMLM doesn't just produce images. It also generates data. In a sense, the images are data. The rows and rows of points that constitute an image are actually coordinate-based datasets that can be used not only to

pinpoint the 3D position of molecules, but also to determine how proteins interact.

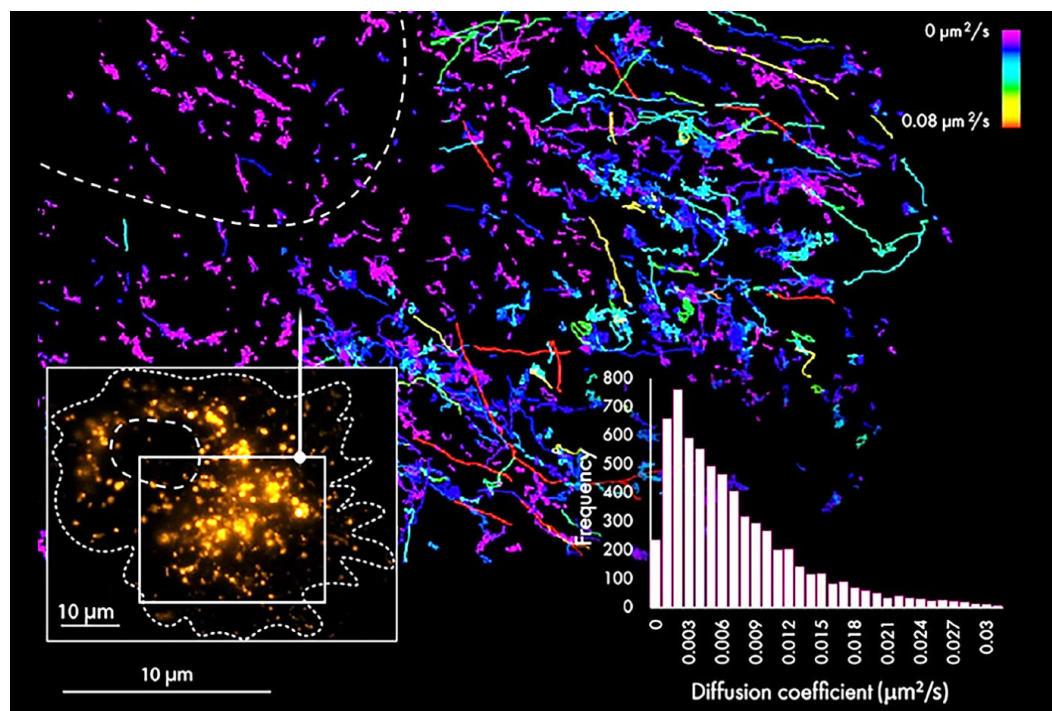
Microscopy has also driven assumptions about biology, notes Jell, based on the resolutions that could be captured using the microscopes that have been available to date. But techniques like SMLM offer a new granularity and allow researchers to change their fundamental thinking of how organelles are arranged and how molecular structures are set up.

Une entreprise de microscopes

Many researchers already have microscopes. And many of those scientists are already doing fluorescence microscopy. But they want better resolution because they are tired of hitting the "diffraction barrier" and being unable to see anything smaller than 200 nm.

This limitation is especially vexing when signals from individual fluorophores interfere with each other. To distinguish between closely packed fluorophores, researchers may resort to a temporal separation technique such as STORM, where fluorophores are stochastically activated. Different fluorophores emit light at different times so that signals do not overlap. Such techniques can involve a lot of repetition, with 1,000 to 100,000 frames being needed to reconstruct an image at nanoscale.

To help scientists overcome the diffraction barrier, the French company Abbelight offers a solution: SAFe, a multimodal imaging platform that is designed to fit with the camera port of any inverted microscope. Two of the available SAFe configurations support SMLM, accommodating



This image summarizes a study in which single-particle tracking technology from ONI was used to analyze the internalization of extracellular vesicles in living Burkitt's lymphoma cells. The image, from Maria Panagopoulou, PhD, and Margaret Paterson, PhD, in the laboratory of Christopher D. Gregory, PhD, at the University of Edinburgh, measured, tracked, and extracted information on diffusion coefficients to illuminate intercellular communication processes.

researchers who want to break the diffraction barrier and prefer to do so by upgrading their existing microscope as opposed to replacing it. Essentially, these configurations incorporate super-resolution add-ons that can transform a microscope into a fluorescence super-resolution microscope.

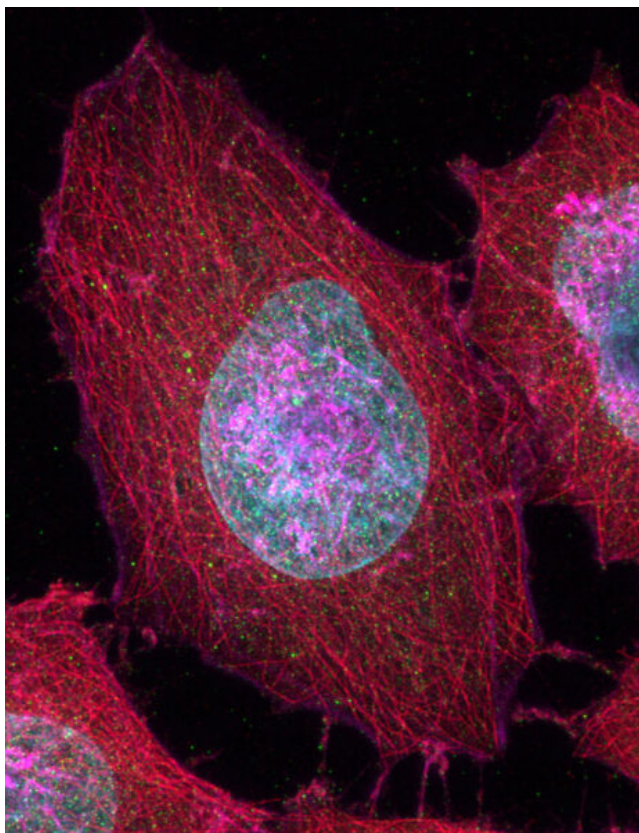
Abbelight's technology is based on optical engineering research that was conducted at Paris-Saclay University by Nicolas Bourg, PhD, when he was earning his doctorate. He co-founded Abbelight in 2016 and has since served the company as CTO.

According to Bourg, a super-resolution journey needn't be difficult, especially since Abbelight can help with the journey's most difficult part—getting started. Also, Abbelight understands that

it may need to accommodate different itineraries as users' priorities change. According to Bourg, users were focused on resolution. But now, they're beginning to pay more attention to ease of use, reliability, robustness, and the ability to image several biological structures at nanoscale.

Bourg points out that the key for SMLM applications in biology is the ability to obtain an image of one protein and to correlate that image with one of another protein. He adds that an image of just one structure is great, but that it is useless unless you can compare it to another one.

Some researchers prefer a full microscope. To accommodate these researchers, Abbelight typically works with Evident (a former Olympus subsidiary that became an independent corporation in 2022). Evident has been in the



Super-resolution microscopy can incorporate multiplexing capabilities, as demonstrated by this image of HeLa cells obtained by Sara R. Roig, PhD, and Alexia Ferrand, PhD, advanced microscopy specialists at the Imaging Core Facility, Biozentrum, University of Basel. In the image, which was obtained with Evident/Olympus technology, different proteins are labeled to highlight different cytoskeletal features: pericentrin (centrosome, green); α -tubulin (microtubules, Alexa 568; red), and phalloidin (actin, Alexa 647; magenta).

super-resolution space since 2017. In addition to working with Abbelight, Evident has its own SpinSR system, which utilizes the SoRA spinning disk confocal system from Yokogawa Life Sciences to enable super-resolution imaging down to 120 nm. Evident also has the Fluoview point scanning confocal system, which can image down to 110 nm using super-resolution algorithms.

Academics show a lot of interest in moving into SMLM, Bourg notes. However, Abbelight is also working with biotechnology companies and with pharmaceutical companies such as Sanofi.

Bruker joins the SMLM mix

In 2014, back when the pioneers of SMLM were receiving their Nobel prize, Bruker acquired Vutara—a University of Utah spin-off company that had developed an SMLM microscope. Vutara's microscope was added to Bruker's existing portfolio, which already included confocal fluorescence and atomic force microscopes.

Vutara's microscope also had capabilities for acquiring 3D Z-stack images of tissue samples. Indeed, it incorporated bi-plane technology developed in the laboratory of Joerg Bewersdorf, PhD, professor of cell biology, biomedical engineering, and physics at Yale School of Medicine.

In 2020, Bruker released an improved version of the microscope, the Vutara VXL. This instrument supports multiplexing applications such as DNA-PAINT and spatial multiomics imaging.

Bruker's Vutara super-resolution microscopy product manager, Winfried Wiegäbe, PhD, says that SMLM technology currently appeals to cell biologists and neuroscientists. The former are most interested in using super-resolution technology to understand the structures and interactions of subcellular organelles. The latter are most interested in using the technology to study synaptic and cytoskeletal structures.

Wiegäbe notes that multiomics applications are another area where localization microscopy is used. Chromatin tracing applications explore the 3D structure of the chromatin in individual cells, and smFISH-based imaging explores the spatial relationship between subcellular structures within specialized cells such as myocytes.

SMLM experts peer into the future

Bourg thinks that SMLM will expand the role that microscopy plays in drug discovery. In addition, he predicts that SMLM will improve nanoparticle characterization, both externally (showing particle shapes) and internally (showing particle contents). Extracellular vesicles are a rapidly growing field and, Bourg says, SMLM is “perfect for them.” Currently, the characterization of extracellular vesicles and lipid nanoparticles is done with electron microscopy, but when it is done with SMLM, the addition of fluorescence

information enables a more targeted approach. The next big challenge, Bourg observes, will be SMLM in living cells.

The market for super-resolution systems is still in its infancy even though it is based on technologies that were developed roughly 20 years. For example, SMLM emerged in 2006, and STED microscopy goes back to the mid-90s. Nonetheless, with the technological improvements that have been made over the past 10 years, the market is poised for robust growth. According to Future Market Insights, the market is growing at

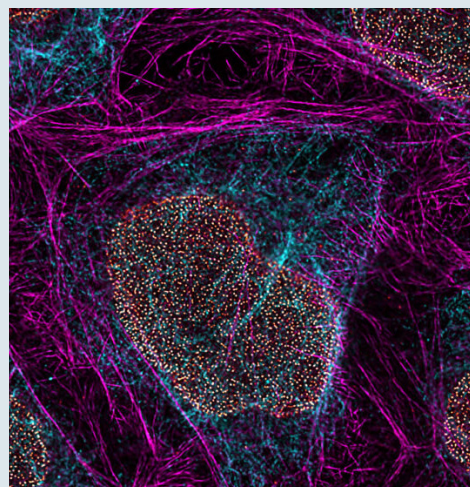
Carcinotech

The super-resolution microscopy market is attracting new entrants. However, Leica has been in the space for 20 years. In 2004, the company introduced the TCS 4PI imaging system. Shortly thereafter, it introduced the first commercially available stimulated emission depletion (STED) microscope. As a result, 2024 marks Leica’s 20-year anniversary in the market.

More recently, Leica launched TauSTED Xtend, a STED system that works with the company’s STELLARIS confocal platform. TauSTED Xtend makes use of the changes in fluorescence lifetime during the depletion process (instead of straight intensity depletion).

Leica asserts that the future will bring more use of live sample imaging to study dynamic interactions of biological molecules. Concepts like fluorescence lifetime are actively used in functional imaging, as well as in standard imaging, to improve resolution, study molecular interactions, or separate/incorporate unwanted fluorescence signals.

Ultimately, Leica notes, more biological questions will be answered when imaging is done with multiple colors to study interactions with the highest spatial and temporal resolution. Improvements in technology are slowly tackling the challenges in the field, and the hope is that the future will bring molecular-scale imaging at biologically relevant speed.

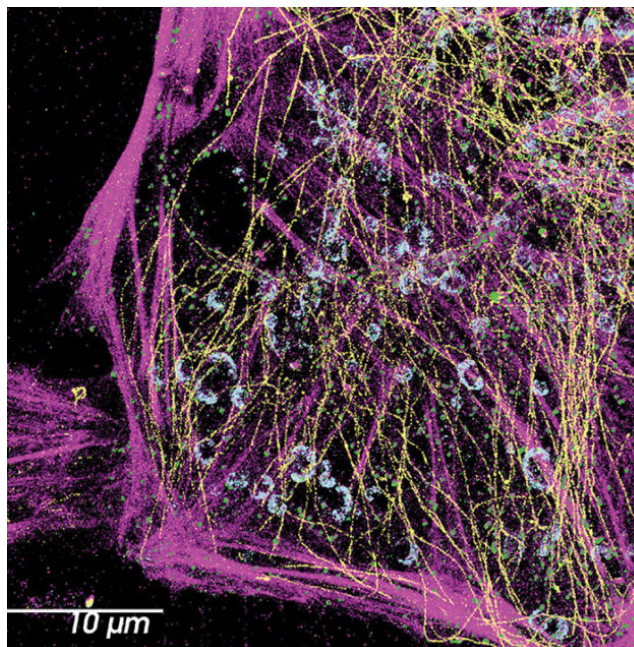


This image of a multicolor fixed sample was taken using Leica’s TauSTED Xtend instrument (Vimentin AF 594 (cyan), Phalloidin ATTO 647N (magenta) and NUP107 CF680R (glow)). The image was obtained at the Core Facility Bioimaging, Biomedical Center, Ludwig Maximilians University, and is provided courtesy of technician Brigitte Bergner, scientist Mariano Gonzales Pisfil, and head of the core facility Steffen Dietzel, PhD.

an annual rate of 10.2% and will reach a value of \$9.5 billion by 2033.

Avi Smith, Associate Product Manager at Evident, notes that as super-resolution microscopy becomes more accessible, users are showing more interest in multimodal systems, such as systems that combine confocal laser-scanning microscopy and SMLM. With these systems, imaging data between modalities can be correlated. The company also sees the integration of super-resolution imaging into high-content screening systems to automate the analysis and quantification of results.

Wiegand anticipates that “scientists will discover the power of combining omics and super-resolution approaches.” He also expects a range of improvements in SMLM: DNA-PAINT-based technologies will be extended by techniques such as resolution enhancement by sequential imaging; multiplexed labeling will dramatically increase the number of targets imaged in one sample;



A multiplexed DNA-PAINT image obtained with Bruker technology. SRX software combined Vutara VXL micrographs of different targets (F-actin: magenta; mitochondria: cyan; microtubules: yellow; clathrin: green). Labeling involved the use of secondary antibodies that had orthogonal docking strands. Using this technique, a potentially unlimited number of targets is possible.

and improved photoswitchable proteins and live cell-compatible organic dyes will improve live cell imaging. ■

ON-DEMAND WEBINAR

Introduction to harnessing single cell-resolution data

Watch this webinar for a primer for how to get started handling single-cell resolution data and discuss how powerful big data techniques can be for discovery.

Presenters:

Michelle S. Massaquoi, PhD

Product Owner, R&D, Protein and Cell Analysis, Thermo Fisher Scientific

Dr. Emily Friebe, Ph. D.

Developmental Editor, Wiley Interdisciplinary Reviews (WIREs), Wiley



WATCH NOW



Access the ultimate compilation of imaging protocols.

The Imaging protocol handbook will help you accelerate your research by helping you plan and set up your imaging experiments. The guide covers main procedural steps to achieve your best results.

Protocols included:

- Cell proliferation
- Cell viability
- Cell structure
- Live cell assays
- Immunolabeling
- Cell painting

 Go to thermofisher.com/imaging-protocols