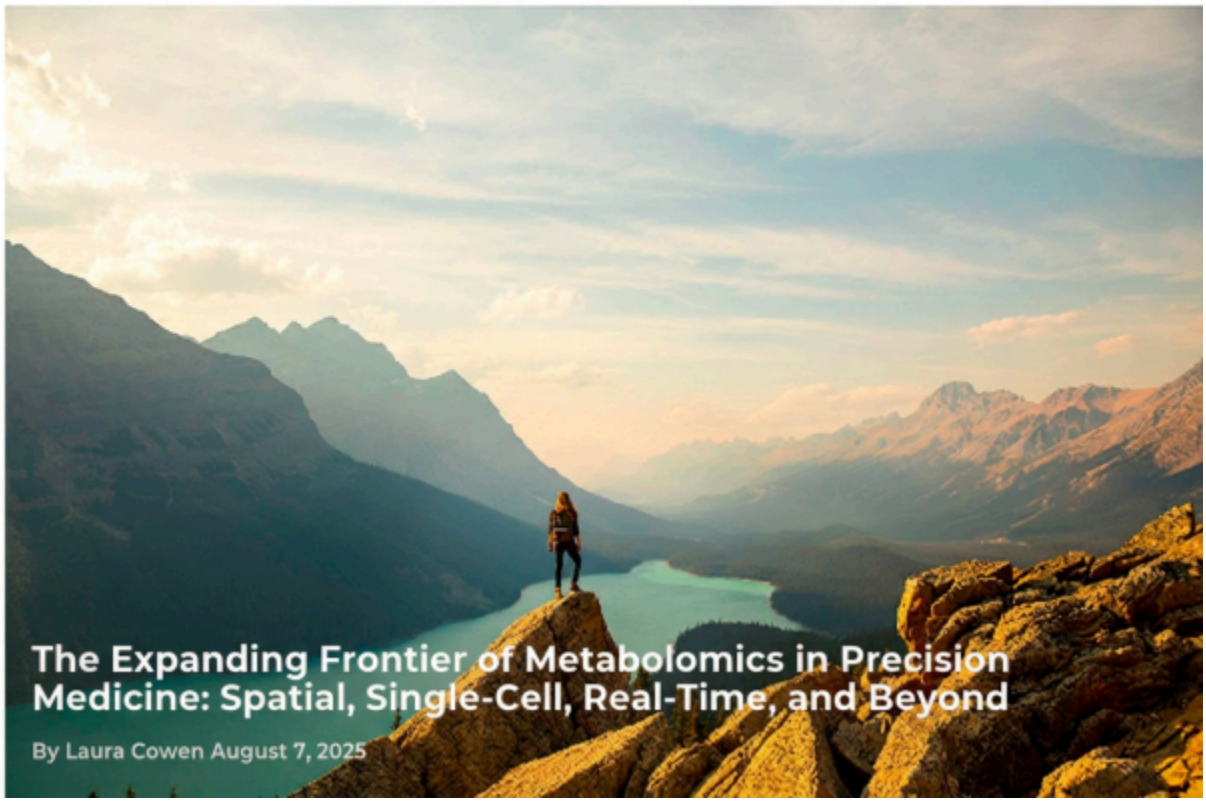

Credit: Jordan Siemens / Getty Images

The study of metabolism is not new. Researchers have been investigating the role of small-molecule metabolites such as sugars, lipids, and amino acids within cells and tissues for decades. In the 1920s, Otto Warburg, MD, PhD discovered that cancer cells alter their metabolism to increase glucose uptake for energy generation. The Warburg effect, as it became known, underpins modern imaging methods like positron emission tomography scans, which use radiolabeled glucose analogs to detect tumors.

The term metabolomics was coined more recently. Unlike genomics or transcriptomics, which describe potential or intermediate states, metabolomics reflects the real-time biochemical activity of cells.

Theodore Alexandrov, PhD
Assistant Professor
University of California, San Diego

"We need readouts of metabolites to really understand the almost instantaneous state of the cells, organisms, and ourselves," said Theodore Alexandrov, PhD, an assistant professor at the departments of pharmacology and bioengineering at the University of California, San Diego. "That's why metabolites are so important, because they're closest to the phenotype and to the function of the cells."

This proximity affords metabolomics immense potential in identifying biomarkers for disease diagnosis, therapeutic monitoring, and drug development.

One of the earliest and most impactful clinical applications of metabolomics has been in screening newborns for inborn errors of metabolism. Introduced in the 1960s, this test now relies on tandem mass spectrometry (MS) to detect dozens of treatable metabolic conditions from a single blood spot, saving thousands of lives globally each year.

Foundations of metabolomics

At the foundation of metabolomics lies technology. Early work relied on nuclear magnetic resonance (NMR) spectroscopy, which allows for the non-destructive analysis of metabolites in biologic samples. Pioneers such as Jeremy Nicholson, PhD, now an emeritus professor of biological chemistry at Imperial College London, used NMR to study pharmaco-metabolomics, looking at pharmacodynamic responses to drugs over time. In an interview from 2013, Nicholson described how he once used NMR to measure his own response to paracetamol in urine.

NMR is still used for metabolomics today, particularly in epidemiologic studies, where large-scale reproducibility across sites is important. However, in the

mid-to-late 2000s, MS became the dominant technology, largely due to advances in sensitivity, resolution, and coverage that continue to improve.

Metabolomics employs two major analytical strategies: targeted, which quantifies a limited number of predefined metabolites with high sensitivity and is suitable for clinical diagnostics; and untargeted, which aims to profile as many metabolites as possible without prior selection, enabling the discovery of novel biomarkers or metabolic pathways.

MS has taken both approaches beyond bulk analyses toward spatial mapping, single-cell resolution, and real-time monitoring.

Spatial metabolomics: Mapping metabolites in tissue

Spatial metabolomics focuses on mapping the distribution of metabolites, lipids, drugs, and other small molecules within cells. Alexandrov describes it as "finally seeing what used to be invisible." It is made possible through MS imaging (MSI) techniques such as matrix-assisted laser desorption/ionization (MALDI) and desorption electrospray ionization (DESI)-MSI.

Alexandrov's lab combines software engineering and experimental science to process and visualize spatial and single-cell metabolic data using MSI. His work on METASPACE, an open-space cloud-based platform for MSI data annotation and sharing, has enabled high-throughput and reproducible interpretation of spatial metabolomics datasets across various tissue types and experimental conditions.

Spatial metabolomics is already producing progress in areas such as oncology, neuroscience, and kidney disease and helping researchers understand drug–target and microbiome–host interactions.

For instance, a 2022 study used spatial metabolomics to identify tumor- and stroma-specific metabolic subtypes in gastric cancer to guide treatment selection. The previous year, another study used DESI-MSI to accurately distinguish tumor, normal tissue, and surgical margins in oral cancer.

"Spatial metabolomics changes pretty much everything with respect to how biology works," said Alexandrov. "You are almost obliged to include it in your studies now, because otherwise you are lagging behind."

With technological advances like including three-dimensional MSI, high-resolution MALDI-Fourier transform ion cyclotron resonance imaging, and machine learning-based image analysis, the accompanying improvements in resolution, sensitivity, and throughput will allow researchers to map molecular processes at single-cell or even subcellular scales and analyze spatial metabolomics data with greater precision.

Single-cell metabolomics: Decoding cellular heterogeneity

Single-cell metabolomics allows researchers to study the metabolic activity of individual cells, revealing crucial details about cellular heterogeneity and function that are often masked in traditional bulk analyses. This level of detail is vital for understanding complex, dynamic biological systems like cell differentiation, disease progression, and responses to environmental changes.

One approach often used in other omics disciplines is to physically isolate single cells via microfluidics, laser capture microdissection, or fluorescence-activated cell sorting. But these strategies do not work well for metabolomics because metabolites degrade during the separation processes. A much more attractive option is to measure metabolites directly from tissues using MSI.

However, achieving single-cell resolution remains a major challenge as it depends on the diameter of the laser beam used to desorb and ionize molecules from the tissue surface. Typical MALDI laser spots (10–100 µm) often exceed the size of individual cells (~6–100 µm), leading to oversampling which masks cell-specific features.

“Although we are not there yet, spatial resolution is improving every year,” said Alexandrov. His work on SpaceM, an open-source method for in situ single-cell metabolomics, has enabled the detection of more than 100 metabolites from more than 1,000 individual cultured cells per hour. It generates spatially resolved metabolic profiles from MALDI-MSI and links them with light microscopy-derived phenotypic data like cell shape and fluorescence. His team used the method to show that stimulating human hepatocytes with fatty acids produces two coexisting subpopulations with distinct cellular metabolic states, a finding that could help understand the function of bioactive lipids in liver disease.

Another area in which single-cell metabolomics is making an impact is the study of tumor resistance. Researchers have used single-cell MS to profile metabolic adaptations in glioblastoma and pancreatic cancer, revealing survival pathways only activated in specific cell clusters. "This is important because we want to predict drug responses in every cell of a patient. If even one subpopulation of cells resists therapy, it can cause relapse," Alexandrov remarked.

Real-time metabolomics: Toward dynamic monitoring

Real-time metabolomics focuses on tracking rapid, dynamic changes in cellular metabolism—which often occur within seconds—to understand biochemical responses during physiological or pathological events. It is challenging to obtain the real-time read outs, but several groups are developing real-time metabolomics tools to bring this capability to clinical settings.

One of the most well-known tools is the intelligent knife, or iKnife, a surgical tool that utilizes rapid evaporative ionization MS (REIMS) technology for real-time tissue analysis during surgery. The technology, developed by Zoltán Takáts, PhD, and his team at Imperial College London, combines an electrosurgical knife, which uses an electrical current to rapidly heat and cut through tissue, with REIMS, which then identifies metabolites in the vaporized tissue. It can accurately discriminate tumor tissue from healthy tissue, which is important for ensuring complete tumor removal and improving surgical outcomes.

Another device designed for intraoperative real-time metabolomics is the MasSpec Pen, developed by Livia Eberlin's, PhD, group at the University of Texas at Austin. The handheld device delivers a microdroplet of sterile water to the tissue to extract metabolites. The droplet is then transferred via a capillary line to a high-resolution MS, where it is analyzed within seconds. Early studies showed that the MasSpec Pen could differentiate tumor tissue from normal tissue in real time with an accuracy of more than 96%.

SpiderMass, from Isabel Fournier, PhD, and her team at the University of Lille, is a real-time, minimally invasive, in vivo technique based on ambient ionization MS (AIMS). It uses a mid-infrared laser to excite water molecules in the tissue, creating a fine plume of ions without the need for biopsy or sample preparation. The ionized molecules are transported through a flexible transfer

line to a remote mass spectrometer, where they are rapidly analyzed. One study showed that the method could diagnose glioblastoma with 90% accuracy.

While MS dominates real-time metabolomics, NMR remains a valuable tool for continuous, non-destructive metabolic monitoring. Alongside its uses in the measurement of drug responses over time, real-time NMR has been employed to monitor live cells in culture, vaccine production, and glucose and lactate turnover in live tissues and perfused organs.

Beyond metabolites: A multi-omics approach

Gary Patti, PhD
Senior Director
Washington University in St. Louis

It is becoming increasingly important to take an integrated approach to precision medicine by combining genomics, transcriptomics, proteomics, and metabolomics to gain a wider understanding of disease biology and therapeutic response.

“We’re going to find cases where metabolomics provides the smoking gun that leads us to the therapy, and there’s going to be cases where genomics or proteomics does it,” said Gary Patti, PhD, senior director of the Center for Mass Spectrometry and Metabolic Tracing at Washington University in St. Louis. “We need an integrated view, and we should be doing all of these omics analyses together in parallel.”

Obtaining this integrated view is not easy because it involves combining diverse biological data types. Yet companies like AmberGen are addressing this challenge. Their platform enables spatial imaging of proteins, RNA, metabolites, and other small molecules, all within the same tissue section and on the same instrument.

Mark Lim, PhD
Vice President, CSO
AmberGen

A key part of their technology is their patented technique and reagents for spatially imaging proteins and RNA in a mass spectrometer using photocleavable peptide mass tags. The technique was invented by company founder and chief innovation officer Kenneth Rothschild, PhD, executive vice president and CSO Mark Lim, PhD, and director of MS Gargey Yagnik, PhD. It allows each molecular target to be labeled with a unique mass signature, which can be detected by MS on being exposed to ultraviolet light. "Now that we can image proteins, RNA, and small molecules together, we're no longer blind to the full biology of the tissue," said Lim.

Company CEO John Gillespie added: "Before AmberGen, people could look at the distribution of small molecule drugs and metabolites in tissue, but not the drug's protein targets. Now, we can map them together and see whether a drug is changing the metabolic signature of the intended target cells."

AmberGen's technology is being widely used by pharmaceutical companies that want to bring their drugs to market faster. "If you can see whether your drug is engaging with its target in an animal model, it saves a lot of time and money versus waiting for a clinical trial," said Gillespie.

John Gillespie
CEO, AmberGen

The method is also pushing the boundaries of decoding biomolecular pathways. In a poster recently presented at the American Association of Cancer

Research's annual meeting, Lim and colleagues showed that they could detect over 600 multi-omic biomarkers on a single lung cancer tissue microarray using untargeted lipid imaging combined with targeted protein and RNA imaging. This more complete view of tumor tissue biology is expected to lead to future mechanistic studies and ultimately, improved biomolecular signatures for precision medicine, remarked the authors.

Other companies working on multi-omics solutions include Metabolon, Panome Bio, and Sapient. Metabolon specializes in untargeted metabolomics and has developed an extensive reference library to interpret complex metabolic data across disease states, nutrition, and pharmacology. Rather than analyzing each multi-omics dataset separately and stitching them together at the end, Panome Bio takes the unique approach of processing multi-omics data using an integrated pipeline to enable a more holistic understanding of each molecular profile. Sapient, meanwhile, is integrating multi-omics data with machine learning to build one of the largest annotated human multi-omics databases, which they will use to uncover new drug targets and stratify patient p opulations.

In academia, researchers like Julio Saez-Rodriguez, PhD, head of research at the European Bioinformatics Institute, are developing integrative multi-omic computational frameworks that model cellular signaling and predict drug responses, further bridging the gap between omics data and clinical applications.

Impacting precision medicine

In terms of clinical applications, Alexandrov believes that metabolomics could have bigger potential for use in precision medicine than other omics. "It is the fastest and cheapest of all the omics and sample preparation is extremely simple," he said.

Furthermore, there is growing interest in carrying out high-throughput metabolomics to create large-scale metabolic atlases and databases that can be used for diagnostics and prediction. "We've entered a world where metabolomics data can be acquired at such throughput that it's entered the precision medicine arena," said Patti. "Just a handful of years ago, you would struggle to find studies that had more than a few hundred samples but now we're doing untargeted metabolomics experiments on 15,000 samples. That gives statistical power to make important discoveries."

Various research groups have demonstrated how untargeted metabolomics can reveal functional changes in tumors that are undetectable through genomics alone. These include metabolite markers that correlate with tumor aggressiveness, treatment resistance, and survival outcomes. In one study, researchers used liquid chromatography (LC)-MS to show that 2-hydroxyglutarate levels were significantly higher in head and neck squamous cell carcinoma tissue than in normal tissue, suggesting that the compound has potential has a non-invasive biomarker.

In another study, which examined 330 triple-negative breast cancer samples, researchers identified high levels of N-acetylaspartylglutamate as a metabolic signature of the basal-like immune-suppressed subtype, a group with significantly worse overall survival than others.

Moreover, Patti's work has revealed that cancer isn't just a local disease. "What has been surprising to me is how cancer affects healthy tissues. Even if the tumor is in the skin, we've seen altered metabolism in the liver, brain, and beyond." He explained that tumors hijack distant tissues and coerce them to produce metabolites that the tumors can use as fuel. "I think this is a really exciting area, and not only important to understanding our biology of cancer, but also as potential therapeutic targets that we can go after."

Beyond cancer, metabolic biomarkers are showing promise in cardiovascular disease, neurodegeneration, and diabetes, and are increasingly being incorporated into clinical trials for therapy monitoring.

Challenges in metabolomics: Quality control and annotation

Despite major advances, metabolomics face challenges in quality control, standardization, and metabolite annotation. Inconsistent practices across platforms and laboratories hinder reproducibility and quantitation.

Matthew Lewis, PhD
Vice President, Bruker

“Many scientific fields are facing crises of reproducibility, and we have a duty of care to ensure metabolomics isn’t one of them,” said Matthew Lewis, PhD, vice president of metabolomics at Bruker Daltonics. To address this, the company has developed QSee™, a software suite that supports longitudinal quality control monitoring and instrument performance tracking, which Lewis says is essential for scalable and reproducible metabolomics.

Initiatives such as the Metabolomics Quality Assurance and Control Consortium, the National Institute of Health’s Multi-Omics for Health and Disease Consortium, the Metabolomics Society, and the U.K.’s National Phenome Centre are also working to address these gaps, but more widespread adoption is needed.

Both Lewis and Patti described metabolite annotation as another bottleneck. “You collect a metabolomics dataset and see thousands of signals, but the majority can’t be identified. Even today, we’re only able to name a few percent,” said Patti.

He suggests that there are two potential reasons for this: one is that they represent small molecules that have not been seen before and therefore cannot be identified; and the other is that the signals come from non-biologically relevant background noise such as contaminants and artifacts that have formed inside the mass spectrometer.

“I do think there are new metabolites, I don’t want to miscommunicate that, but I also believe that a lot of the datasets that we struggle with identifying, at least in our lab, represent the complexities of the mass spectrometry data,” Patti remarked.

Bruker’s timsMetabo™ Mass Spectrometer

Bruker's solution is trapped ion mobility spectrometry (TIMS). "It separates isomers and interferences, and generates cleaner MS/MS spectra more rapidly, with higher sensitivity, which is key for annotation," said Lewis. Their timsMetabo™ platform combines TIMS with ultra-high-resolution LC-MS and has been specifically adapted for small molecule identification.

In addition, community-driven tools like the Human Metabolome Project are providing reference spectra to support identification and classification. The resulting Human Metabolome Database now contains over 220,000 entries, including around 20,000 confirmed endogenous human metabolites, and has become an important tool for identifying metabolites, discovering biomarkers, and linking metabolic features to clinical phenotypes.

Future directions

You often hear metabolomics described as the final frontier in the omics cascade. Yet upon speaking to the experts, it has become clear that metabolomics goes further. One theme that comes up repeatedly is that of exposomics—the study of environmental exposures and their biological effects over a lifetime. Metabolomics is uniquely suited to study the exposome because it can directly detect both exogenous chemicals and their metabolic effects.

As Patti noted: "Every blood sample we test contains PFAS [per- and polyfluoroalkyl substances]. We're all exposed, and we don't yet understand the long-term health effects. That's where exposomics comes in."

Large-scale projects such as EXPOsOMICS and HELIX are already using untargeted metabolomics to define exposomic signatures linked to disease.

For Lewis, the holy grail would be something that can measure the exposome in real-time. His suggestion of at-home monitoring via accessible MS may sound far-fetched, but a non-invasive device that samples urine daily to see if a person is deficient in vitamins or experiencing inflammation, for example, could benefit long-term health.

More realistically, Lewis says that democratizing the technology by making it more accessible and increasingly deployable at the point of care will be exciting future endpoints. "But bottom line, we are pushing on all fronts. We're pushing upward on scale aiming to measure billions of samples in biobanks, we're pushing downward in scope to measure metabolites at the subcellular level, and

we're pushing laterally to carry out multi-omics. It's a multidimensional frontier that we're working to aggressively tackle," he said.

Artificial intelligence (AI) will also play a central role. Gillespie sees it as a critical partner to the next generation of metabolomics. "When you're collecting hundreds of biomarkers per sample, AI will help interpret and contextualize those data," he said.

Alexandrov agreed: "The impact of metabolomics will depend on how we train and apply AI models." As Lewis concluded: "The world around us is rapidly changing, there will be no final frontier. There will be new environmental challenges, new toxins, new pollutants, new food products, and the confluence of a lot of those is going to be in small molecule analysis, but I don't see finality in our future."

Laura Cowen is a freelance medical journalist who has been covering healthcare news for over 10 years. Her main specialties are oncology and diabetes, but she has written about subjects ranging from cardiology to ophthalmology and is particularly interested in infectious diseases and public health.

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