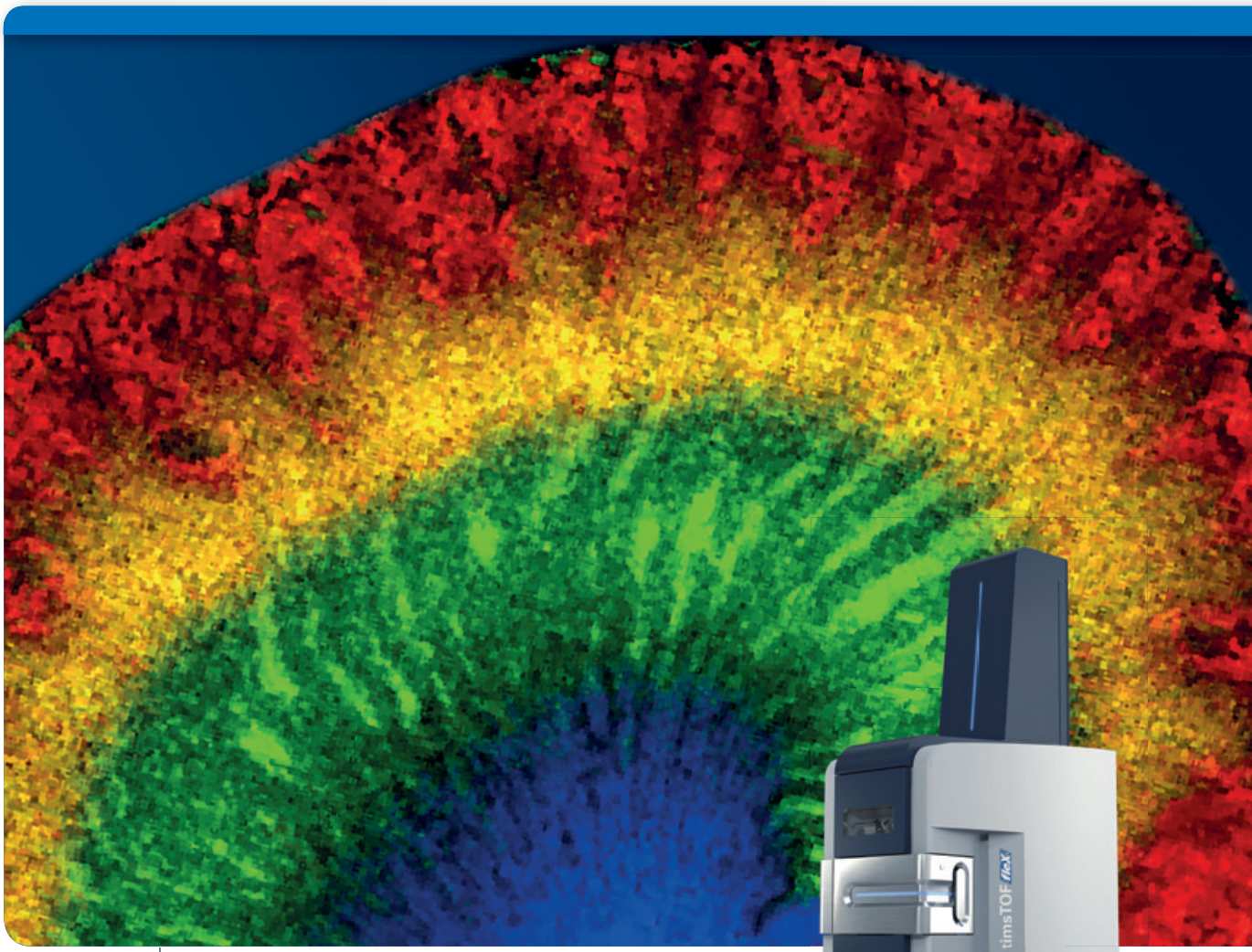


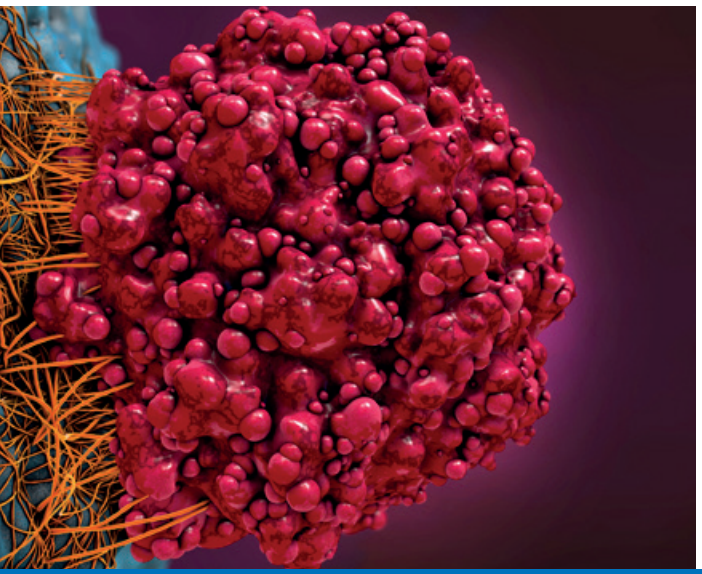
timsTOF flex

- MALDI Guided SpatialOMx

Innovation with Integrity

TIMS-MALDI MS

MALDI Guided SpatialOMx

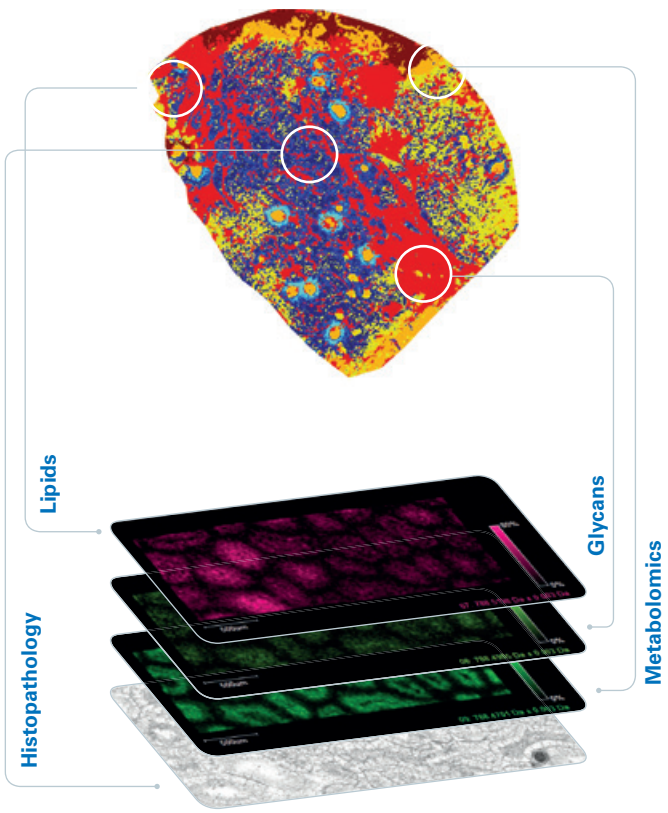


The tumor microenvironment is a highly variable ecosystem, *giving it an intrinsic temporal character. De-coding the cellular communication within the tumor microenvironment via label-free MALDI Imaging and x-omics promises to improve the understanding of the mechanisms responsible for drug resistance and augment the precision of histological diagnoses.*

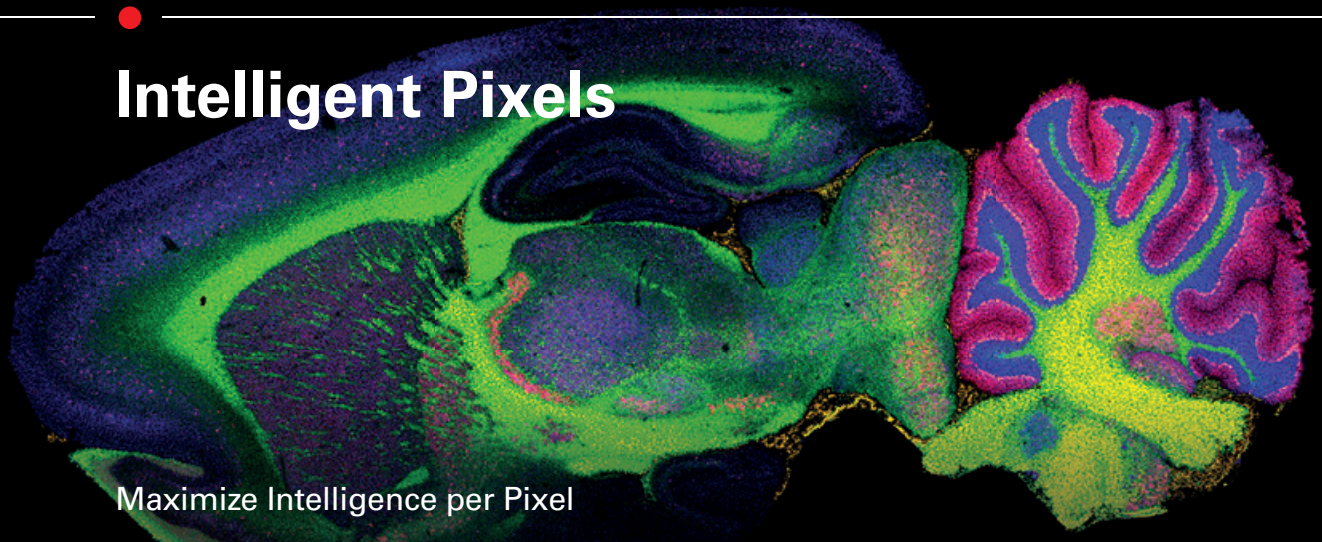
Label-free MALDI Imaging monitors more molecules than antibody or tag-based imaging techniques. *Map a wide range of expressed molecules such as lipids, glycans, metabolites, and peptides to discover spatially significant expression. What if you could then deploy a directed multi-omics approach to decipher the signaling network at specific sites within the tumor's cellular ecosystem?*

Use the timsTOF fleX to dive deeper into the tumor microenvironment and beyond. *timsTOF fleX combines the best x-omics platform with a MALDI source designed for imaging. Intelligence derived from MALDI Imaging can guide x-omics analysis of select cell populations to deliver greater cellular specificity of LC-MS approaches and establish a new SpatialOMx benchmark for the future of pathology.*

timsTOF fleX harnesses the power of SpatialOMx to deliver maximum intelligence per pixel.



Intelligent Pixels



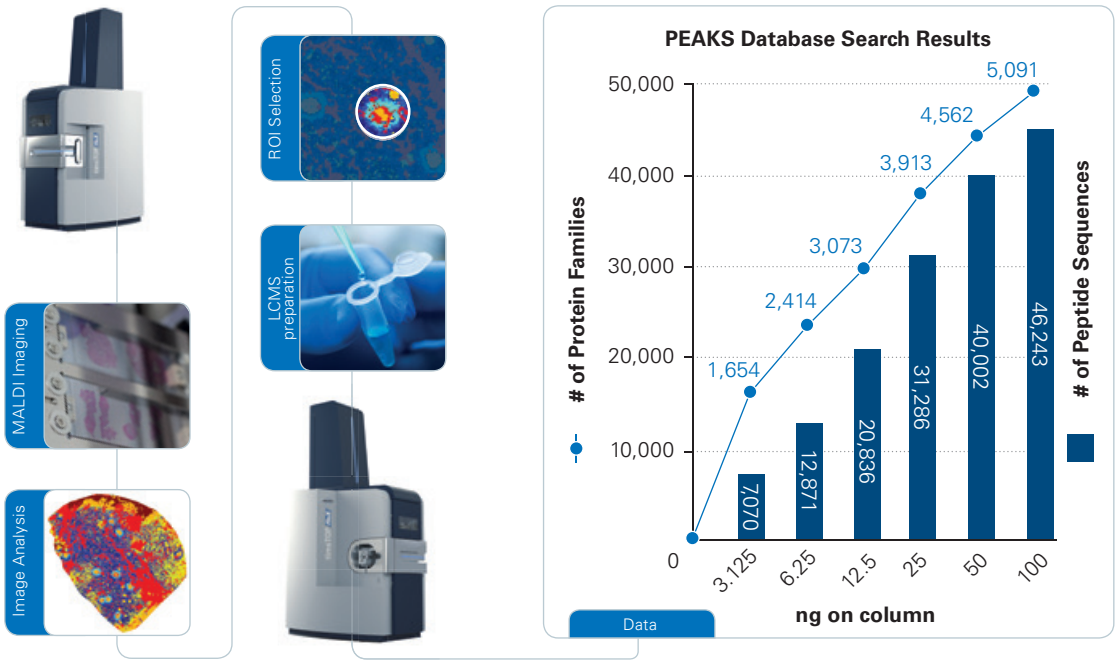
Maximize Intelligence per Pixel

An average cell measures approximately 10 µm in diameter. Using MALDI guided laser microdissection (LCM) for example, a 50 µm LCM tissue section will contain roughly 25 cells; enough for bottom-up proteomics analysis on the timsTOF fleX. One instrument that gives you the capability to do both – high spatial resolution, high speed MALDI and high sensitivity ESI analysis.

Ultimate flexibility and specificity at the click of a software button, comes standard on the timsTOF fleX.

SpatialOMx Workflow

SpatialOMx is the combination of using MALDI Imaging and ESI to unlock a 5th dimension and show the distribution of target compounds. On the timsTOF fleX, use the MALDI source to map the distribution of molecules in your sample and identify regions of interest. After extracting and preparing the sample for LCMS, use the ESI source for the highest level of identifications



Why choose Bruker?

timsTOF fleX – The best 4D x-omics and MALDI Imaging system

Since introducing the refleX MALDI-TOF system in 1992, Bruker has continuously pushed technological boundaries, developed a wide variety of applications, and became the uncontested MALDI market leader. In MALDI Imaging, spectra are collected spatially, creating a mass spectrum at every location which can be projected as a 2D map.

Single datasets may contain hundreds to thousands of unique, label-free ion images, which can be used for molecular marker discovery or investigating molecular content of specific regions.

Bruker has constantly advanced MALDI Imaging from our patented SmartBeam 3D technology to SCiLS Lab analysis software. timsTOF fleX continues this tradition, operating at the industry standard 20 µm spatial resolution with optional Zoom Mode down to 5 - 15 µm.

More than 25 Years of MALDI

2020 – First commercialization of **MALDI-2 technology** for significantly enhanced sensitivity and chemical range

2019 – Pioneering the era of SpatialOMx on the timsTOF fleX

2018 – Bruker launches MRMS instrument scimaX

2016 – Bruker launches MALDI PharmaPulse, enabling one million compound libraries to be screened within a week

2015 – Bruker launches rapifleX

2013 – FDA approves MALDI Biotyper for clinical diagnostic use for microbial identification

2012 – Bruker launches solariX with dual MALDI and ESI ion source

2008 – Bruker launches MALDI Biotyper for microbial ID

2005 – Bruker launches Smartbeam-1 laser on Autoflex and Ultraflex

2002 – Nobel Prize in Chemistry award shared by Koichi Tanaka of Japan for discovery of MALDI

1997 – Richard Caprioli publishes on Tissue Imaging using MALDI-TOF

1992 – Bruker launches reflex MALDI-TOF with world's first MTP target plate

1985 – Bruker introduces reflex, its first commercially available MALDI-TOF system

Karas, M., Bachmann, D., Bahr, U. & Hillenkamp, F. Anal. Chem. 57, 2935-2939 (1985) Matrix-assisted ultraviolet laser desorption of nonvolatile compounds time for analysis to hours

Trust the Experts

Years of defining the leading edge for technology in MALDI imaging gives Bruker the largest imaging customer base packed with reference leaders in a wide variety of research fields. Learn what some of them have to say about how the timsTOF fleX enables SpatialOMx as an essential innovation for molecular imaging.

Prof. Richard R. Drake, Director, Proteomics Center, Medical University South Carolina, USA

"The timsTOF fleX is an innovative instrument that synergizes multiple analytical capabilities to allow development of novel omics workflows. For imaging MS, it may be potentially transformative, especially for tissue metabolomics and glycomic applications."

Dr. Kristina Schwamborn, Senior Physician, Institute of Pathology, Technical University Munich, Germany

"MALDI imaging mass spectrometry goes far beyond microscopy and enables the assessment of a multitude of analytes in parallel in spatial molecular arrangements in tissue sections without the need of target specific reagents. Since the sample remains intact throughout the analysis, it can be stained or even used for DNA-analysis afterwards. The analysis is fast, has been proven to be reproducible and no more expensive than other standard pathology techniques like immunohistochemistry. Thus, it has the potential to revolutionize pathology."

Dr. Marten Snel, Head of SAHMRI
Mass Spectrometry Core Facility, Australia

"In my opinion the MALDI enabled timsTOF fleX is a big step forward in this field. I am confident that timsTOF fleX imaging will have a very positive impact on our biomedical and clinical research at SAHMRI, especially in small molecule, lipid and drug imaging"

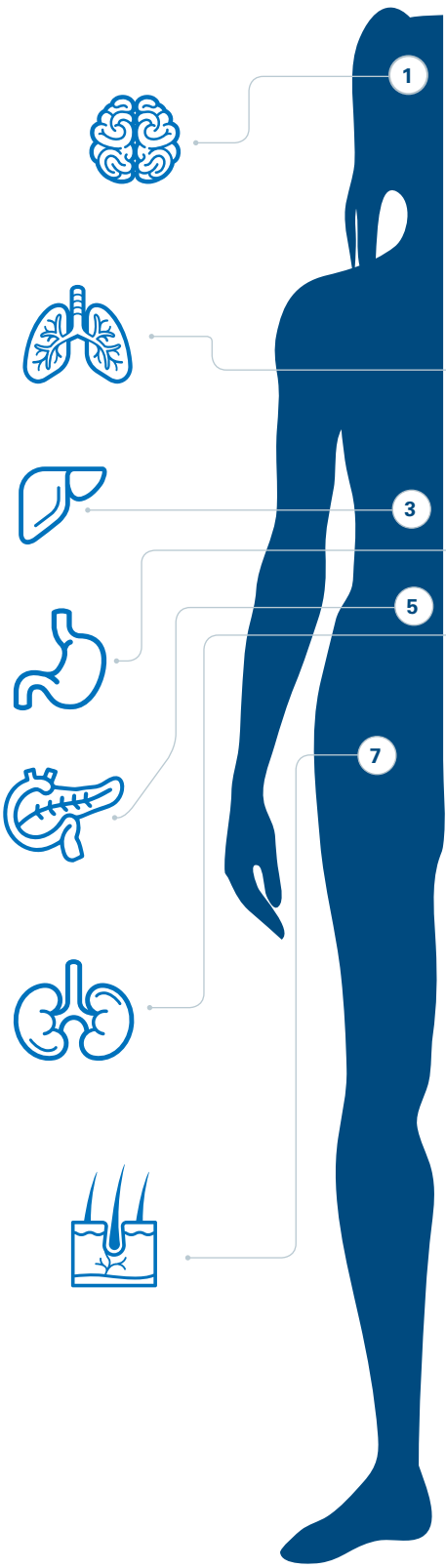
Bridging the gap between 4D X-Omics and Pathology

Within Tissues
Strict tissue-specific protein expression is uncommon, however, some types of proteins are predominant - higher molecular weight motor proteins in muscle, smaller neuropeptides within the brain, digestive enzymes within the gastrointestinal tract, transport proteins within the kidney and barrier function-related proteins within the skin. The proteome of each tissue or organ points to its primary function.

For Regulation of Protein Expression and Celluar Processes
Within any given cell, the processes that regulate life, growth, functional changes, and death are directed by peptides and proteins. Transcription factors (generally between 50 - 100 kDa) drive or halt protein expression from genetic templates. These expressions create the proteomic means necessary for cell proliferation, differentiation, or apoptosis, whether in common healthy cellular cycle function or in response to stress. Similarly, the enzymes that produce post-translational modifications (e.g., phosphorylation, glycosylation, methylation) can regulate protein localization, functional activity, and stability.

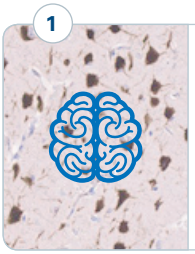
For Housekeeping Processes
Housekeeping proteins are expressed at similar levels throughout the body. "Powerhouse" organelle proteins, such as those in mitochondria which convert food energy into ATP required by cells, and high molecular weight scaffolding proteins, such as tubulins and actins, are required for both maintenance of cellular structure and regular cellular function.

And the Druggable Proteome
Pharmaceuticals target many different types of proteins; greater knowledge of protein localization, form, and function could improve drug design and efficacy. Alternative, high(er) affinity binding partners can more effectively modulate enzymatic activity. Examples include NSAIDs which reduce pain and inflammation by decreasing production of prostaglandins by cyclooxygenase (COX) enzymes, or statins which competitively bind to HMG-CoA reductase to reduce cholesterol. Commercialized biologics, such as mAbs, often have higher molecular weight and structural complexity and target specific cell surface proteins.



Uhlén M et al, 2015. Tissue-based map of the human proteome.
Science. 2015 Jan 23;347(6220):1260419. DOI: 10.1126/science.1260419.
Human Protein Atlas available from www.proteinatlas.org

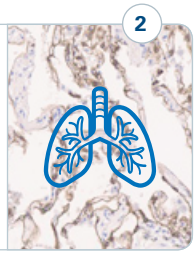
1



Brain A highly complex and energy-intensive organ, coordinated higher functions, e.g. motion, perception, and cognition, are received, processed and executed in the brain. Neural proteins show specific expression patterns among cells and structures, as well as in subcellular structures such as axons, dendrites, and synapses.

2

Lung The lungs are primarily responsible for respiration: the gaseous exchange of O_2 and CO_2 between air and blood occurs in ~300 million alveoli. Pneumocytes, bronchial epithelium, and the endothelial cells facilitate O_2/CO_2 exchange, while alveolar macrophages protect against potential infection from inhaled microbes.



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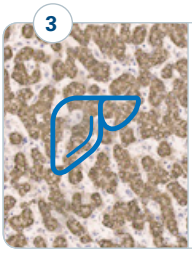
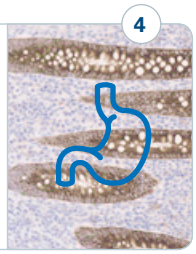


Liver Composed of parenchymal cells (hepatocytes and bile duct cells) and non-parenchymal cells (sinusoidal endothelial, Kupffer, and hepatic stellate cells), the liver is the largest internal organ. Liver-specific proteins include plasma and bile proteins, and proteins associated with metabolic processes, glycogen storage and detoxification.

4

Gastrointestinal tissues The gastrointestinal tract (GIT) – the esophagus, stomach, small and large intestines, and rectum – absorbs nutrients and water, maintains the balance of beneficial microorganisms and protects against pathogens. GIT proteins are mostly involved in nutrient breakdown, transport and metabolism, immune response, and tissue morphology maintenance.



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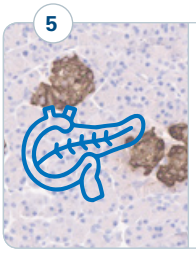
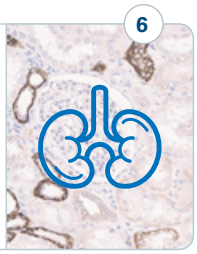


Pancreas The pancreas has both exocrine and endocrine functions. Glandular cells in the exocrine compartment secrete digestive enzymes into the gastrointestinal tract, while the islets of Langerhans execute the pancreatic function, secreting insulin and other hormones. Many pancreatic mRNAs encode specialized secreted proteins.

6

Kidney Primary functions of the kidney include maintaining body homeostasis by regulating blood composition and eliminating waste. Different cell types are organized into sub-anatomical structures with distinct functions, showing elevated levels of essential proteins, e.g. proteins required for blood filtration are elevated in the glomerulus.



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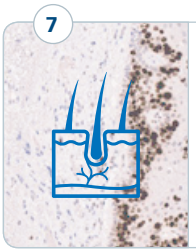


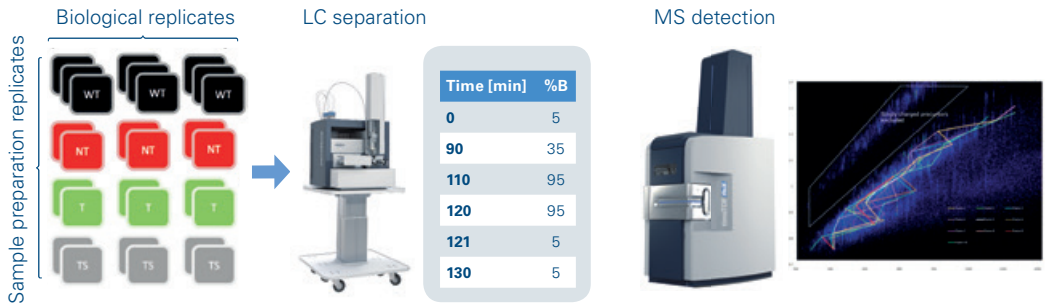
Skin The skin (epidermis, dermis and subcutaneous layer) is a sensory organ and a protective barrier. The epidermis is mostly keratinocytes which protect against physical, chemical and biological insults. Most protein functions are related to squamous cell differentiation and cornification, pigmentation, and hair development.

4D X-Omics Workflows – Just a Click Away

Label-free quantitation on the timsTOF fleX with PASEF: investigation of proteomic changes in tissue samples of mouse gastric carcinoma.

The timsTOF fleX offers a combination of two unique technologies, Trapped Ion Mobility Spectrometry (TIMS) to enhance ion separation and Parallel Accumulation Serial Fragmentation (PASEF) to improve ion utilization efficiency and data acquisition speed. The performance of the timsTOF fleX mass spectrometer with PASEF for label-free quantitation of proteins can be demonstrated on proteins extracted from sectioned mouse tissue. In brief, more than 5000 protein groups could be reliably identified and quantified from 240 ng protein per sample using 90-minute gradients. The optimized PASEF method used on the timsTOF fleX gave very high technical reproducibility which is an important prerequisite for label-free quantitation (LFQ).

Furthermore, the complete process (including tissue preparation, digestion, and data acquisition) was highly reproducible, which is critical in the application of proteomics to clinically relevant specimens. When comparing the proteome composition of tumor and non-tumor tissue, gene ontology analysis indicated the enrichment of the minichromosome maintenance protein complex (MCM-complex) in tumor over non-tumor samples. The MCM-complex has been shown as an essential component of the pre-replication complex (pre-RCs), which is involved in DNA replication initiation and the recruitment of DNA-Polymerases. In various studies a malfunction of the MCM-complex has been linked to genomic instability, increased cell proliferation, and a variety of carcinomas.



1. Cryosections of mouse stomachs (WT, NT, T, TS)
2. Sample lysis
3. Digestion

Column:	C18 (25 cm x 75 µm, 1.6 µm, IonOpticks, Australia)
Flow rate:	400 nL/min
Oven temp.:	50°C

Source:	CaptiveSpray
Acquisition method:	PASEF
Mass Range:	100 – 1700 m/z
Total cycle time:	1.1 s
Number of MS/MS ramps:	10 PASEF scan at 100 ms (each MS/MS PASEF scan contains 12 MS/MS)

Workflow for the analysis of isolated mouse stomach tissue using label-free quantitation

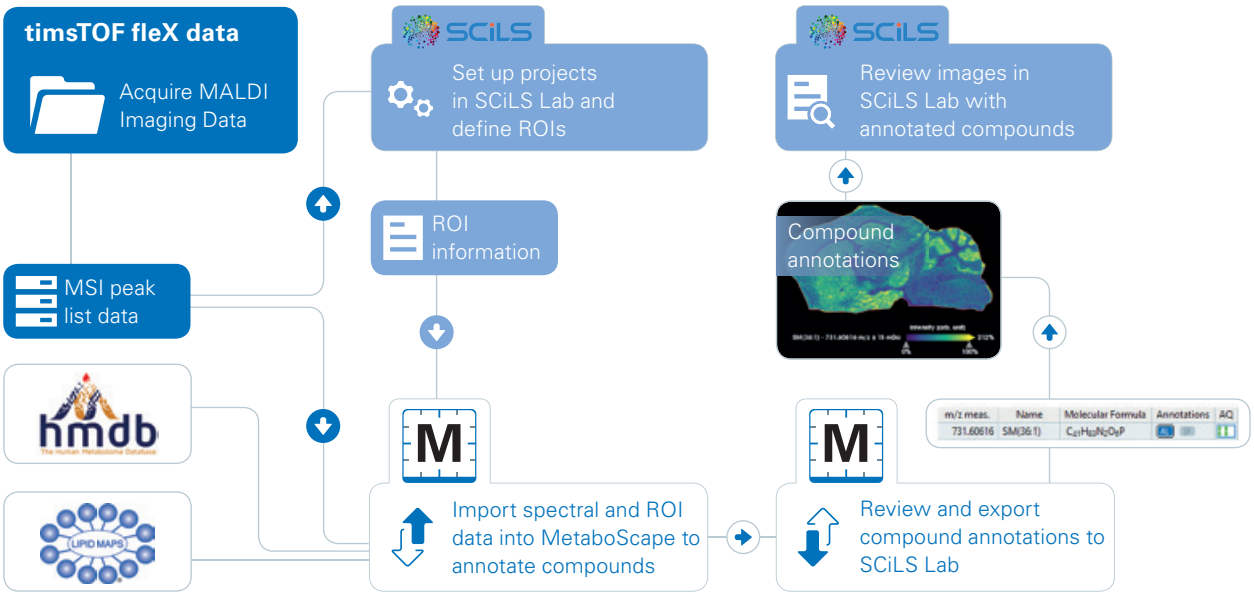
SpatialOMx Automated Molecular Annotation Workflow

SCiLS Lab – Industry Leading Imaging Software

For SpatialOMx a new mass spectrometry imaging workflow with automatic metabolite annotation will be supported by SCiLS Lab and MetaboScape: regions of interest can be transferred from SCiLS Lab to MetaboScape and the annotated peak lists can be loaded into SCiLS Lab for visualization of spatial compound distribution.

- Vendor-neutral analysis and visualization
- Quantify target molecules directly from tissue
- New SpatialOMx workflow for automatic metabolite annotation

Novel Mass Spectrometry Imaging Workflow



Novel mass spectrometry imaging workflow: Automated annotation of metabolites and lipids from tissue
**Lipid Maps and HMDB are not Bruker products.*

Bruker's MALDI Imaging solutions consists of established sample preparation protocols, covers fully integrated hardware and software control, all the way to analysis workflows.

- Discover regionally specific molecular markers and biochemical changes
- Wide range of applications including proteomics, lipidomics, glycomics, inorganic compounds and clinical research
- Localize and quantify for drug discovery
- Visually explore metabolic pathways
- Correlate molecular changes to disease

MALDI 2 TECHNOLOGY

- MALDI-2 enables access to chemical classes typically prone to ion suppression in MALDI
- Sensitivity boost by up to 2-3 orders of magnitude compared to MALDI, depending on sample, matrix and analyte
- No physical hardware changes needed, switch between MALDI and MALDI-2 by one click in the software
- User friendly software solution, easy instrument calibration and application scientist tested methods to start measurements immediately

Pharma - Move beyond toxicology to PK/PD and more with the ability create images from tissue at previously unreachable sensitivity.

Metabolites - Image metabolic classes and pathways previously undetectable by MALDI alone.

Prof. Klaus Dreisewerd, Leader Section Biomedical Mass Spectrometry, University of Muenster, Germany

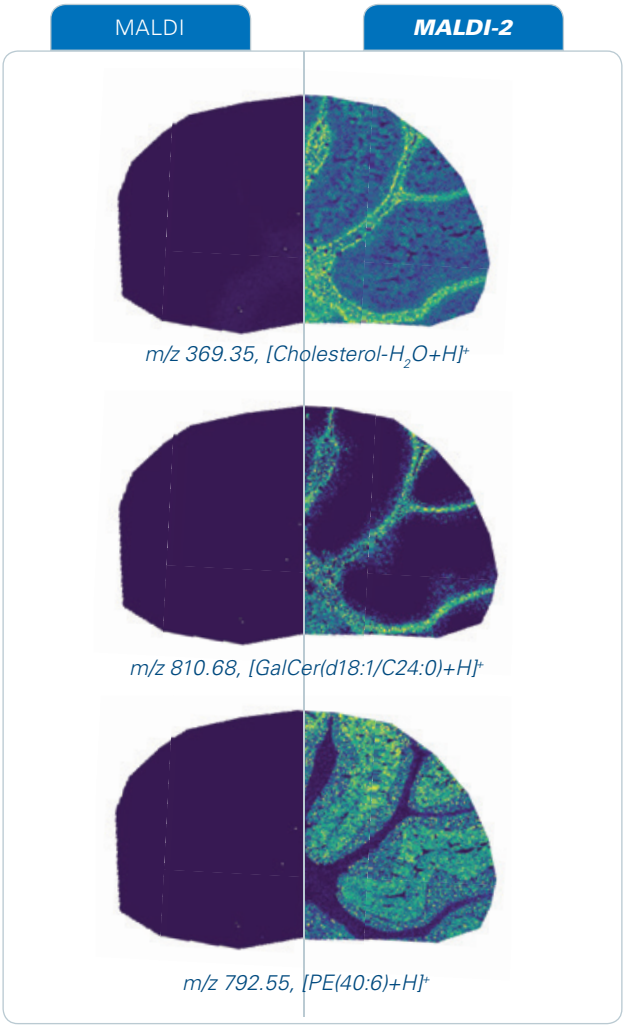
"In the last 35 years, MALDI has become a unique and rapid analytical tool for a wide variety of applications. We developed MALDI-2 to significantly extend the technique by providing much higher sensitivity for small molecules and inclusion of chemical classes that didn't traditionally ionize. With an extensive set of unique features, the MALDI-2 empowered timsTOF fleX will take MALDI to new frontiers previously not available."

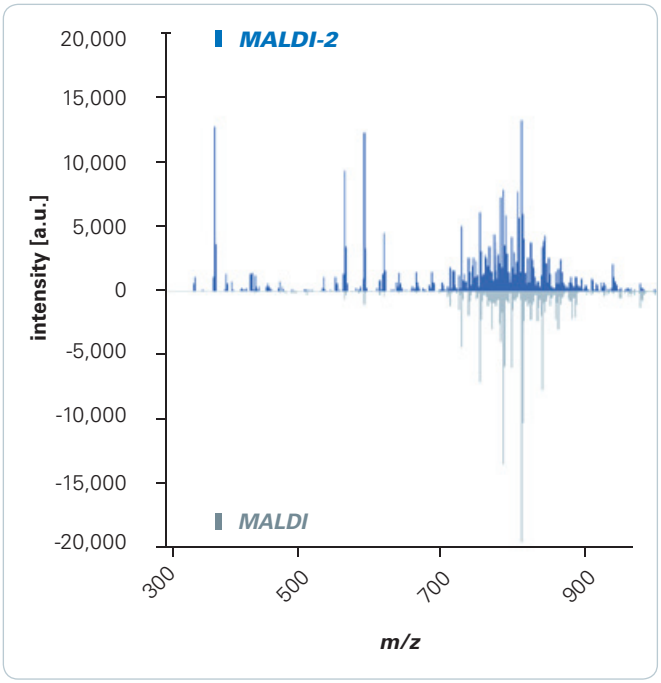
Bringing Enhanced Depth and Sensitivity

The SpatialOMx enabled timsTOF fleX represents an entirely unique solution for adding biological context to routine OMICS or pharma studies.

While many researchers can utilize spatialOMx "out of the box", customers with challenging workflows asked for more. Research centered around small molecules and lipids typically test the limits of MALDI sensitivity and molecular coverage.

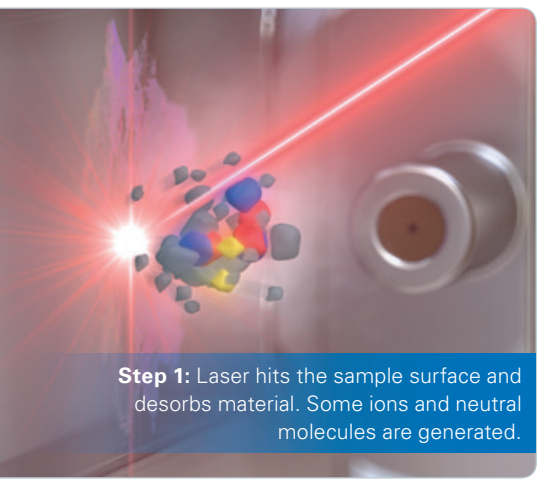
The answer is MALDI-2.



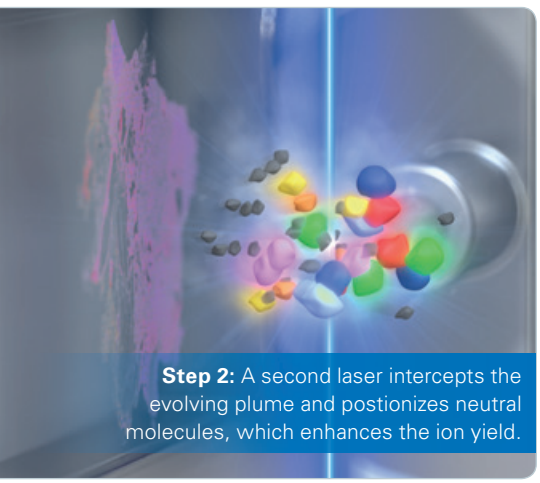


Originally developed by the Klaus Dreisewerd group at the University of Muenster, MALDI-2 uses laser post-ionization to enhance and enrich the MALDI experiment providing access to chemical classes typically opaque to MALDI with sensitivities never seen before on any platform*.

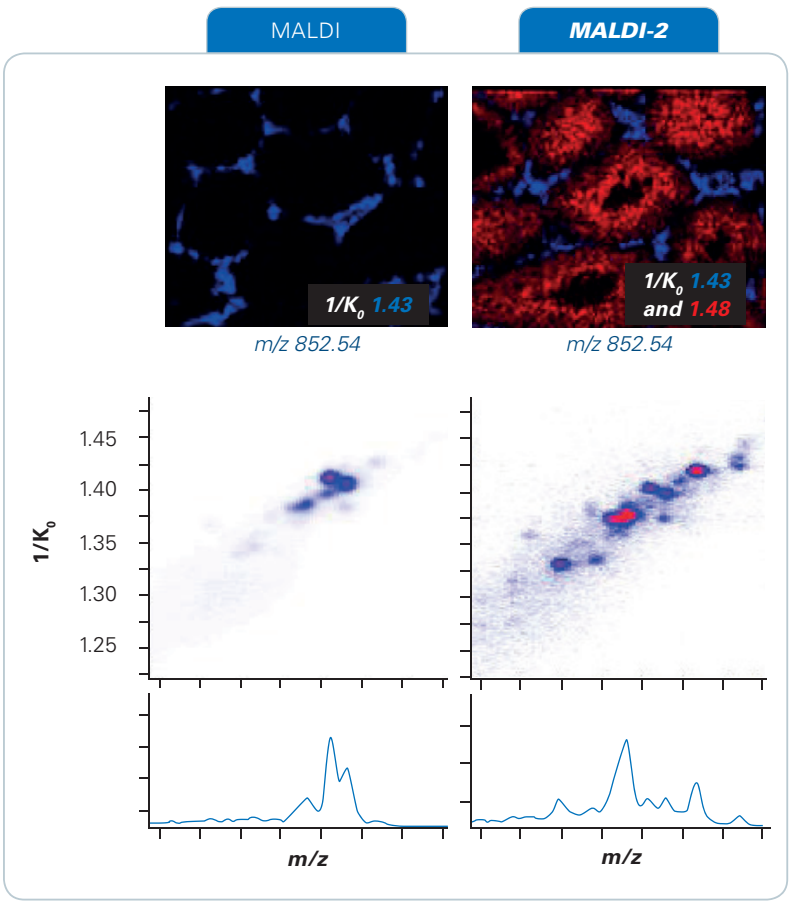
Post-ionization leads to a significant boost in ion yields and a reduction of ion suppression effects, resulting in increasingly complex spectra. In this context, de-convoluted feature assignment in the TIMS mobilogram becomes increasingly useful. Next to finding a larger number of features, they are also described by not one but two independent measures, enabling confident identification.



Step 1: Laser hits the sample surface and desorbs material. Some ions and neutral molecules are generated.



Step 2: A second laser intercepts the evolving plume and postionizes neutral molecules, which enhances the ion yield.



* 1. Soltwisch, J. et al. Mass spectrometry imaging with laser-induced postionization, Science, 2015, 348, 211-215.
2. Barré, F. P.Y. et al. Enhanced Sensitivity Using MALDI Imaging Coupled with Laser Postionization (MALDI-2) for Pharmaceutical Research, Anal. Chem., 2019, 91, 10840-10848.

timsTOF fleX

MALDI Guided SpatialOMx

timsTOF fleX uniquely enables SpatialOMx

PASEF powered LC-MS/MS identification matched with spatial localization identifies and locates multilevel genomic expression in tissue without labels

timsTOF fleX provides results without compromise

All the 4D-Omics power that you demand from proven PASEF workflows with fast, software-controlled changeover to MALDI for rapid molecular imaging

timsTOF fleX allows you to work smarter

Label-free mapping of metabolites, lipids, glycans, peptides and more can efficiently direct your deep 4D-Omics studies with laser guided precision so that you focus on the tissue regions that matter.

For research use only. Not for use in clinical diagnostic procedures.

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