

MALDI-TIMS-MS

timsTOF *flex*

MALDI Guided SpatialOMx[®]

MALDI 2 *micro*
TECHNOLOGY **GRID**

Innovation with Integrity

Why choose Bruker?

timsTOF fleX – The best 4D-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 microGRID technology down to 5 µm.

More than 25 Years of MALDI



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



microGRID: Accurate and robust high-resolution imaging made simple

A new powerful MALDI stage technology for timsTOF fleX

Consistency and robustness in sampling below 10 μm lateral spatial resolution has eluded commercial MALDI imaging instruments. Bruker now offers a unique solution to eliminate data striping, fading, or over-sampling effects, making the possibility of sub-cellular imaging within reach.



The microGRID option allows high-resolution measurements using laser position correction for making advancements beyond mechanical limitations. Bruker's commitment to innovation continues to push the boundaries of the ordinary.

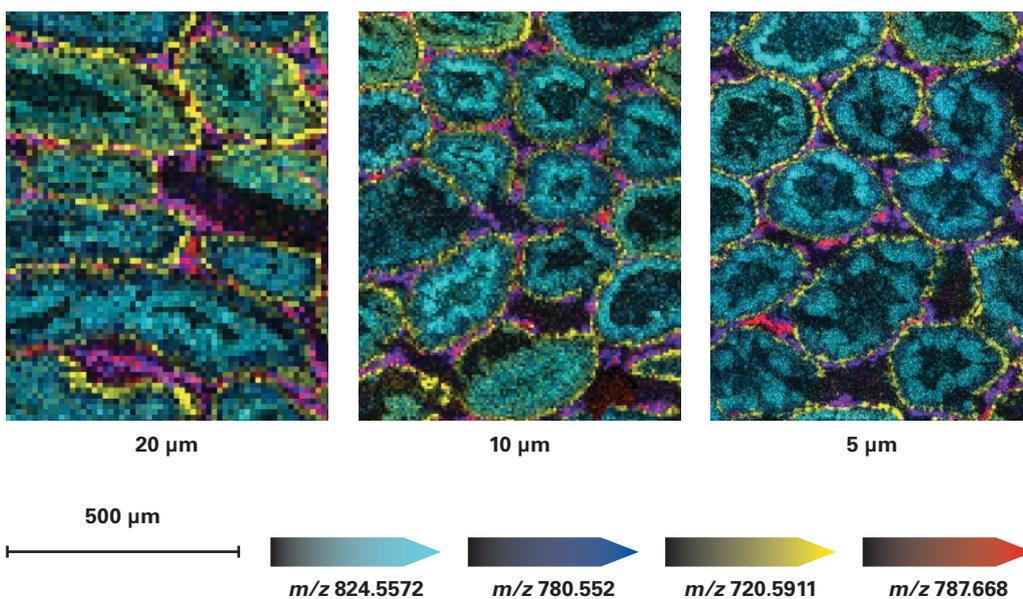
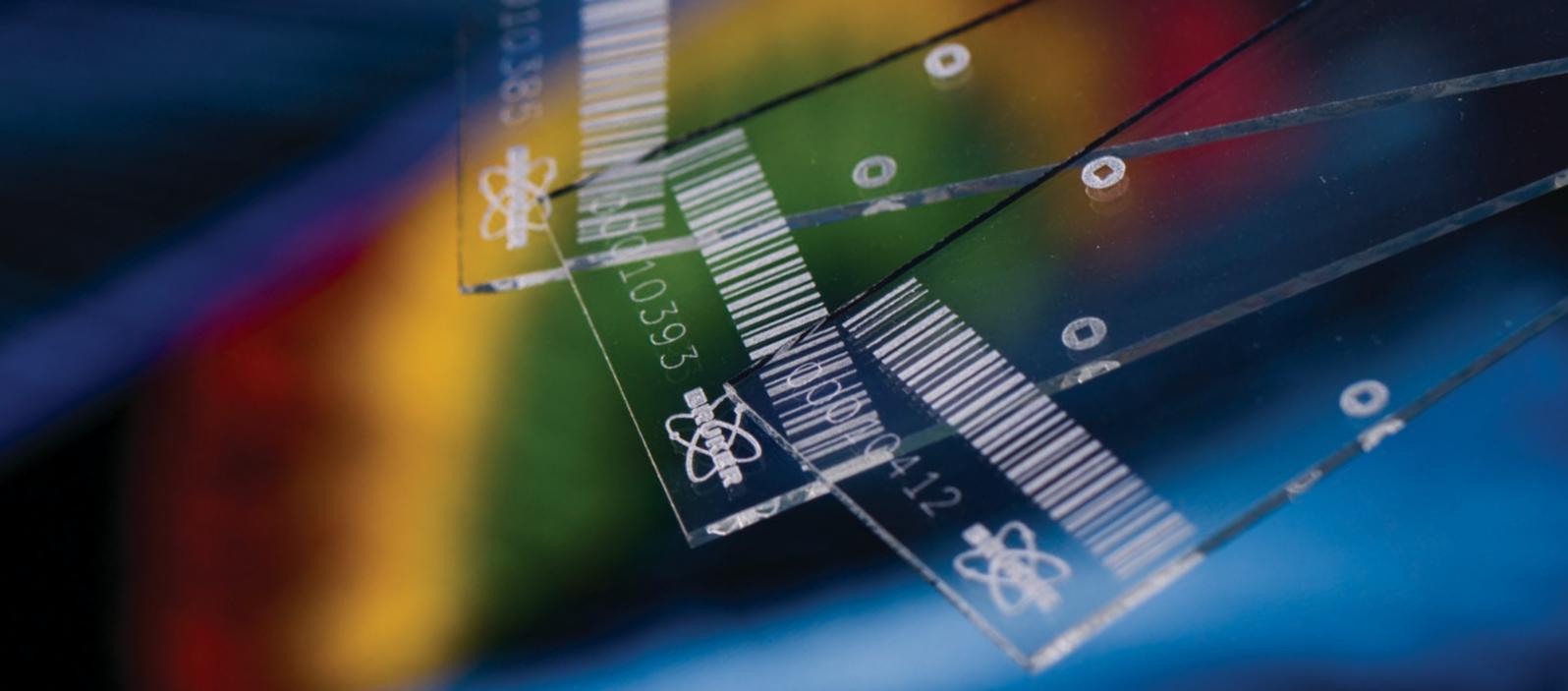


Figure 1

Enhanced resolution of rat testis images from 20 μm to 5 μm with microGRID imaging allows to access fine tissue structures of the organ.

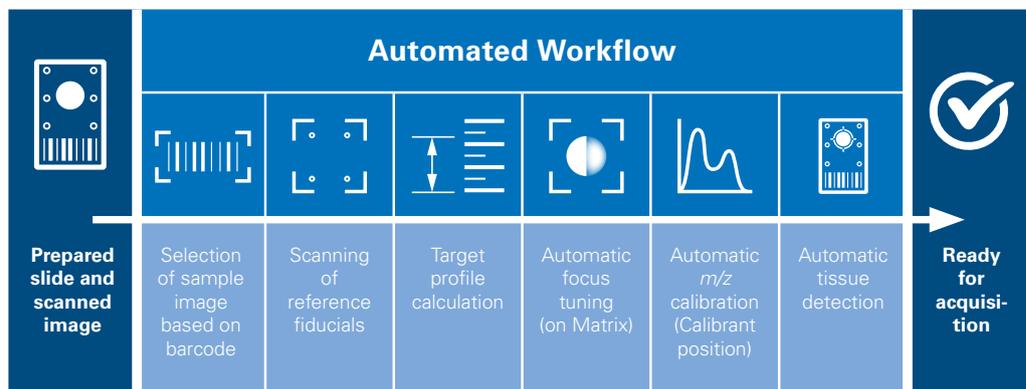


Instruments equipped with microGRID enable out of the box 5 μm imaging without any laborious hardware or software adjustments. Make full use of the unique combination of TIMS and high spatial resolution.



Figure 2

Streamlined software workflow SCiLS™ autopilot provides a simple and controlled way to set-up MALDI Imaging measurements for reliable and robust data acquisitions irrespective of user experience



microGRID seamlessly integrates in Bruker's fully automated SCiLS™ autopilot workflow, making the technology attractive for routine applications. With SCiLS™ Lab Bruker rounds

off the package and delivers a high-performance software to analyze high resolution imaging data.

Robust and reproducible data with all the timsTOF features you rely on

- Seamless integration with existing MALDI imaging workflows
- Easy to use, default application methods available
- The full advantages of MALDI-2 are immediately visible with microGRID technology

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.

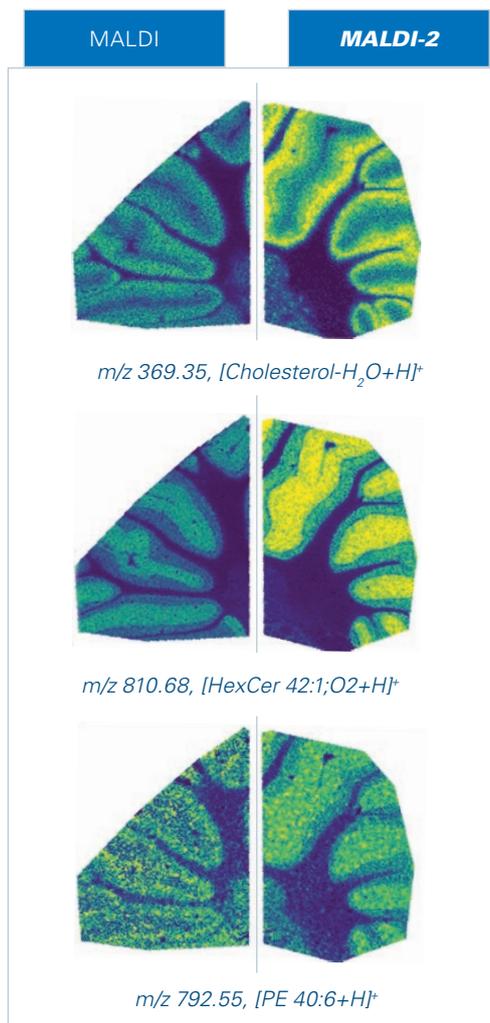


Prof. Dr. Klaus Dreisewerd

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.



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



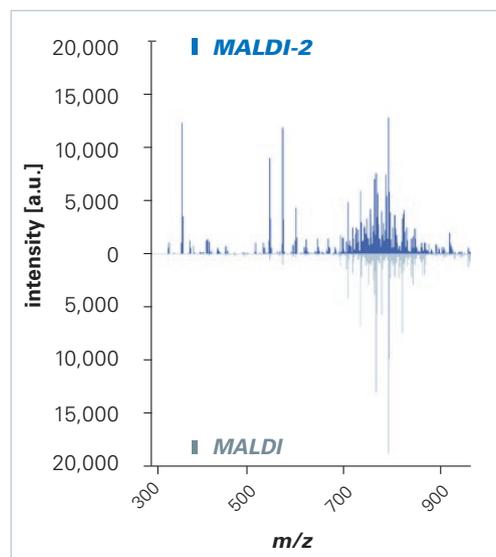
Pharma - Move beyond toxicology to PK/PD and more with the ability to create images from tissue at previously unreachable sensitivity.



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

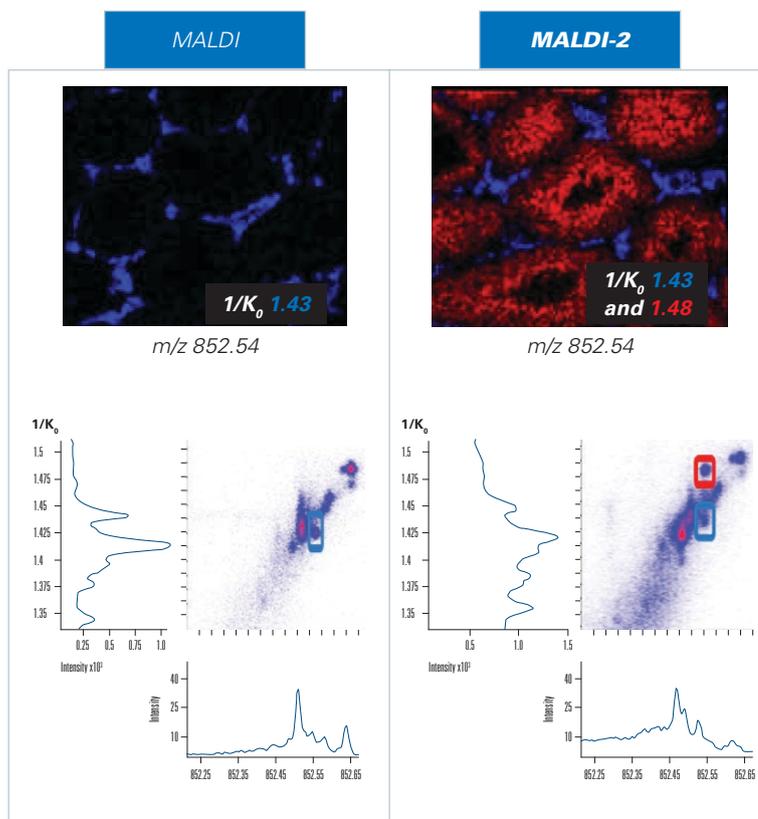
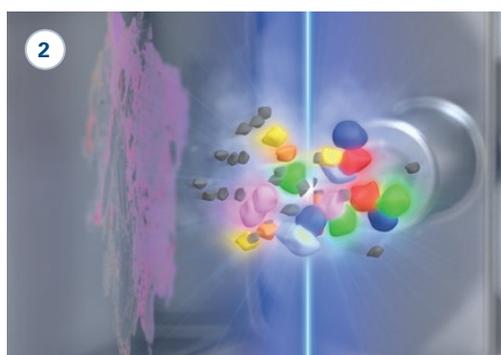
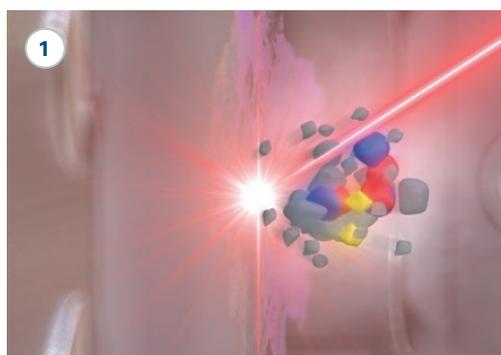
Originally developed by the Klaus Dreisewerd group at the University of Muenster, MALDI-2 uses laser post-ionization to enhance and enrich the MALDI experiment providing access to chemical classes typically opaque to MALDI with sensitivities never seen before on any platform*.

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.

MALDI-2 and microGRID

A powerful combination making the possibility of sub-cellular imaging within reach

Sensitivity in high resolution imaging is challenging, as less material is ablated with smaller pixel sizes.

MALDI-2 provides a sensitivity boost up to 2-3 orders of magnitude and provides access to analytes not detectable by MALDI. Hence, the combination of MALDI-2 and microGRID imaging provides highest information depth at the best resolution.

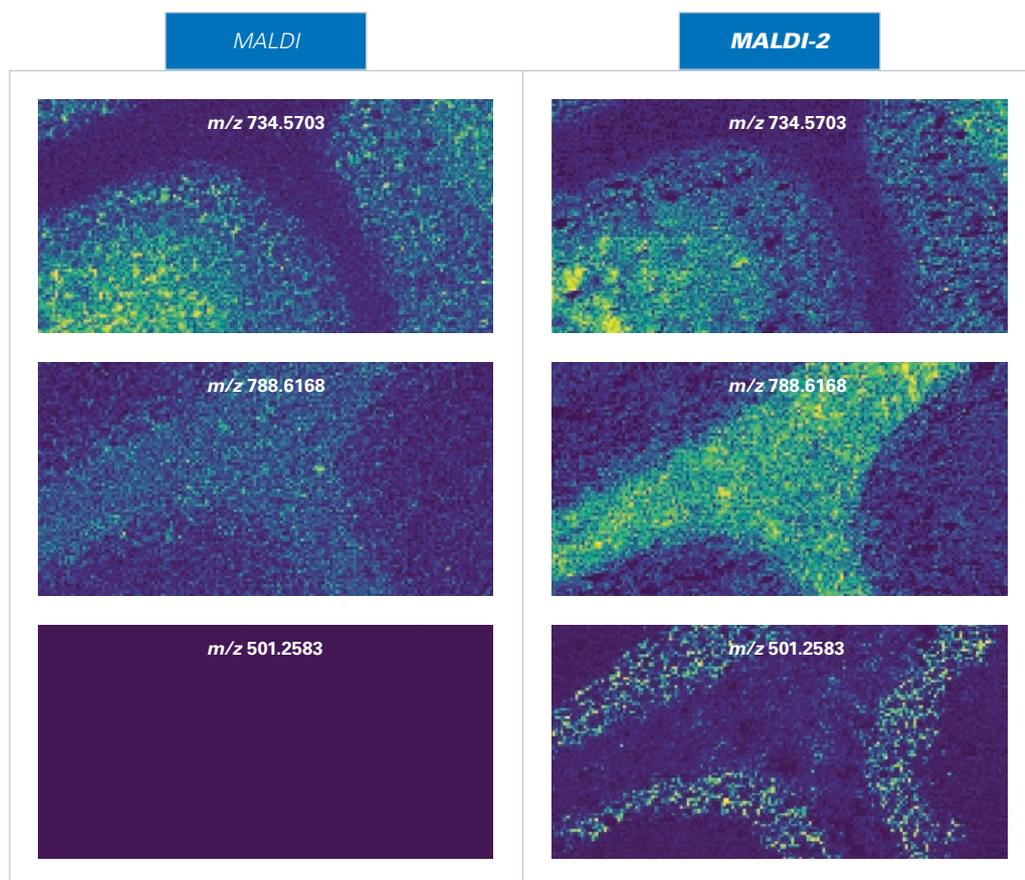


Figure 1

Complementing microGRID imaging with MALDI-2 post-ionization enhances the sensitivity detecting even more substances.

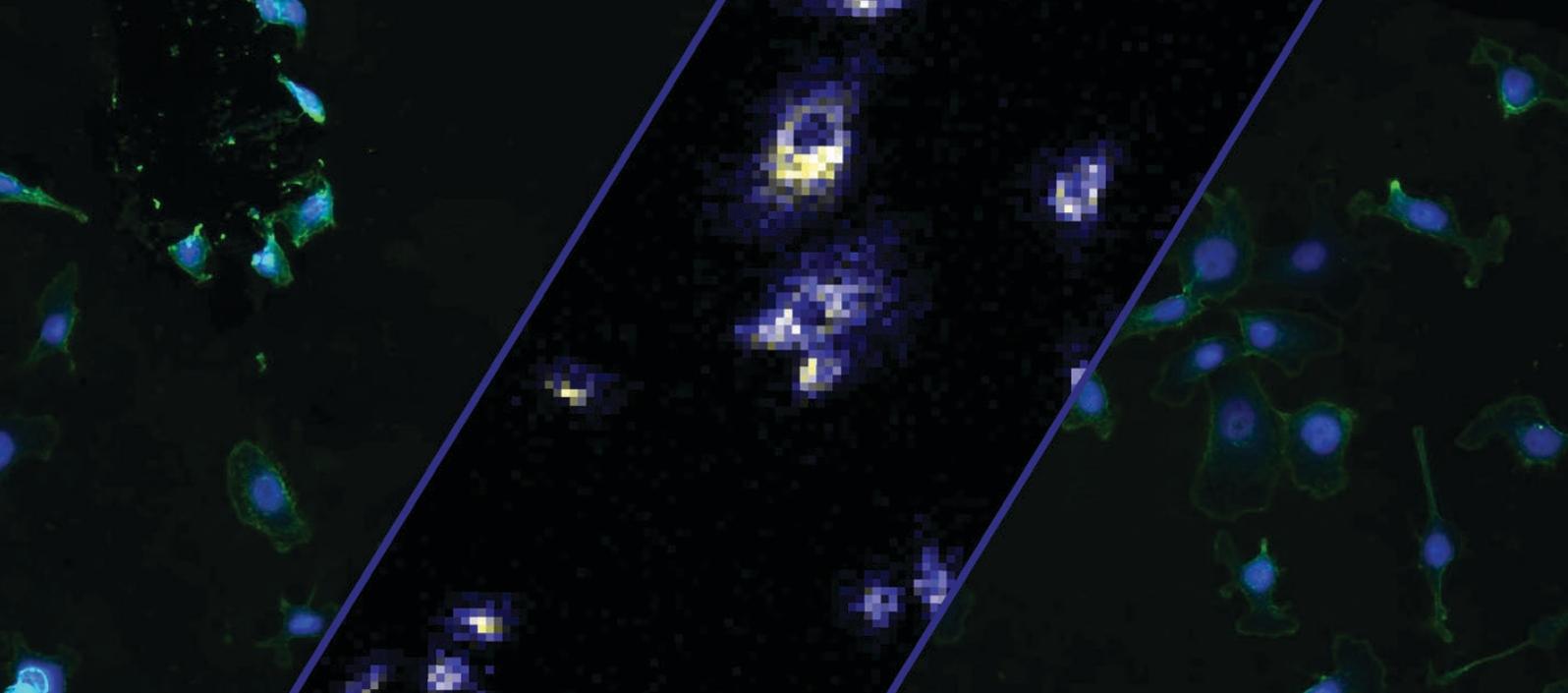


Prof. Dr. Ron Heeren

M4I Maastricht MultiModal Molecular Imaging Institute

Since we work closely with pathologists and people who are used to looking through microscopes, we cannot afford artifactual data in our images, but we also need to cover a large field-of-view. Bruker's microGRID helps us to effortlessly achieve both without limiting the area of the slide we can process for creating multiomic and multimodal images of diseased and homeostatic pathology.





Combining microGRID and MALDI-2 provides molecular insight into fine tissue structures. Moreover, high spatial resolution and sensitivity allows for imaging on single cell level.

The elongated structures of Caki-2 cell can be clearly depicted by MALDI-2 without deprivations in MS dimension.

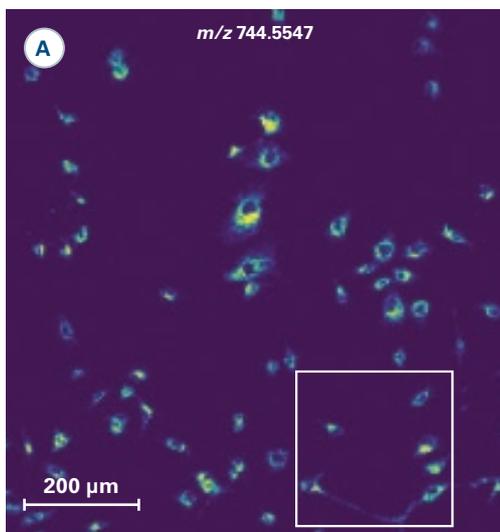


Figure 2A
MALDI-2 ion images of Caki-2 cell culture at 5 µm spatial resolution enabled by microGRID Imaging. Ion distribution of a PE 36:2 at m/z 744.5547 showing the localization of Caki-2 cells, with a zoom in the outlined area (**fig. 2B**).

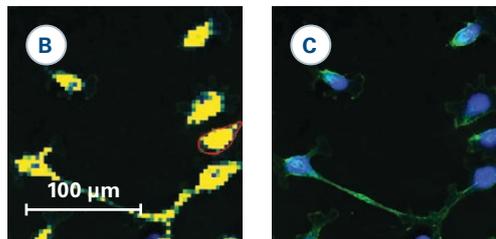
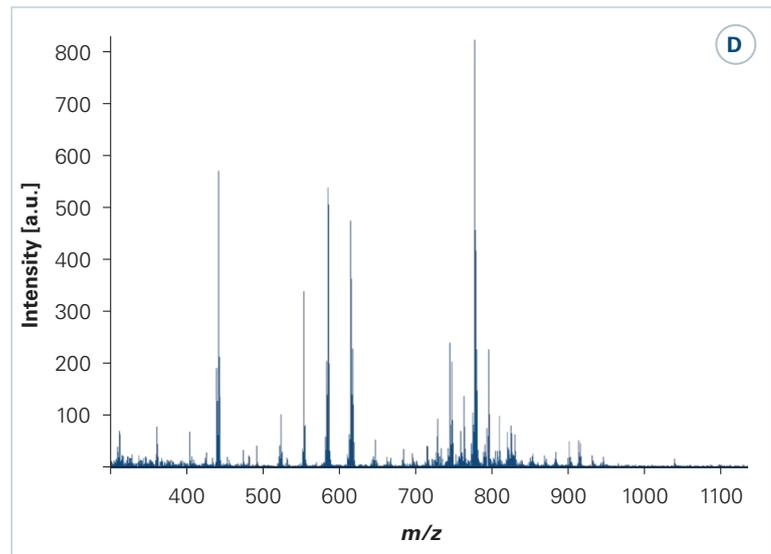
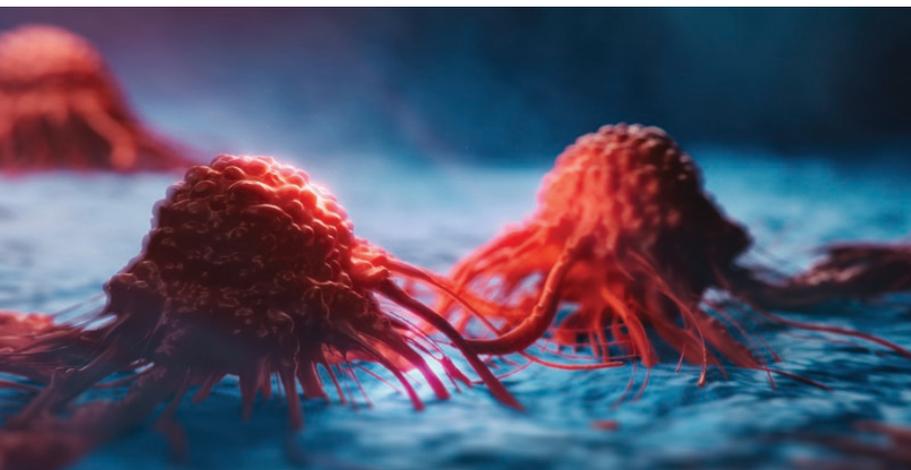


Figure 2B/C
Comparison of the m/z image of the zoomed area (**fig. 2B**) and the respective fluorescence image (**fig. 2C**) shows correlation of the very fine structures.

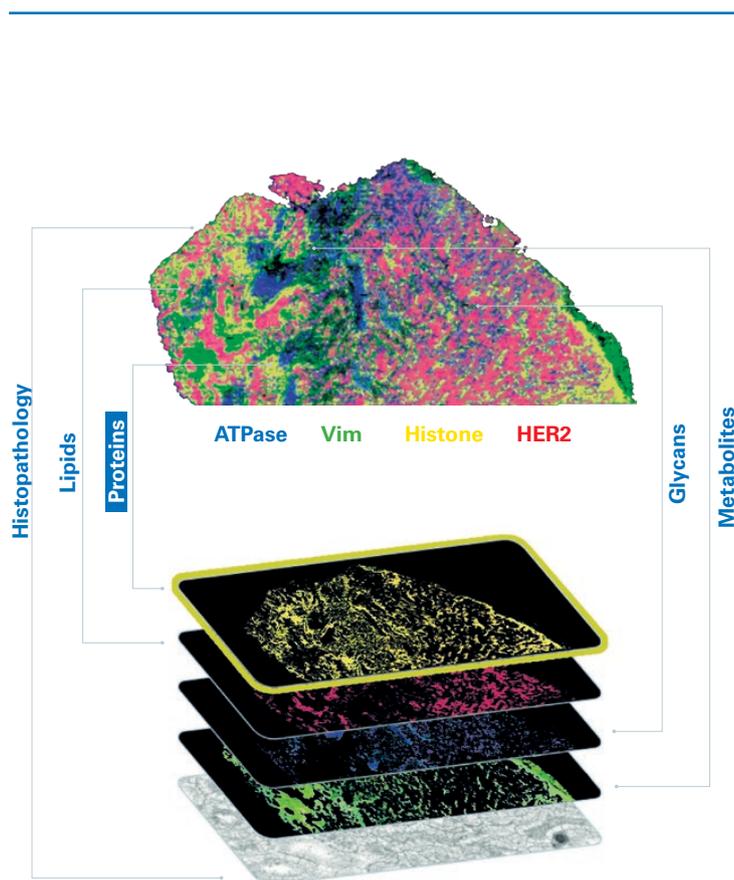
Figure 2D
 m/z spectrum of a single cell, highlighted in (**fig. 2A**).



MALDI Guided SpatialOMx



MALDI-Guided SpatialOMx[®] has been adopted broadly to improve the understanding of the mechanisms responsible for drug resistance and augment the precision of histological diagnoses.



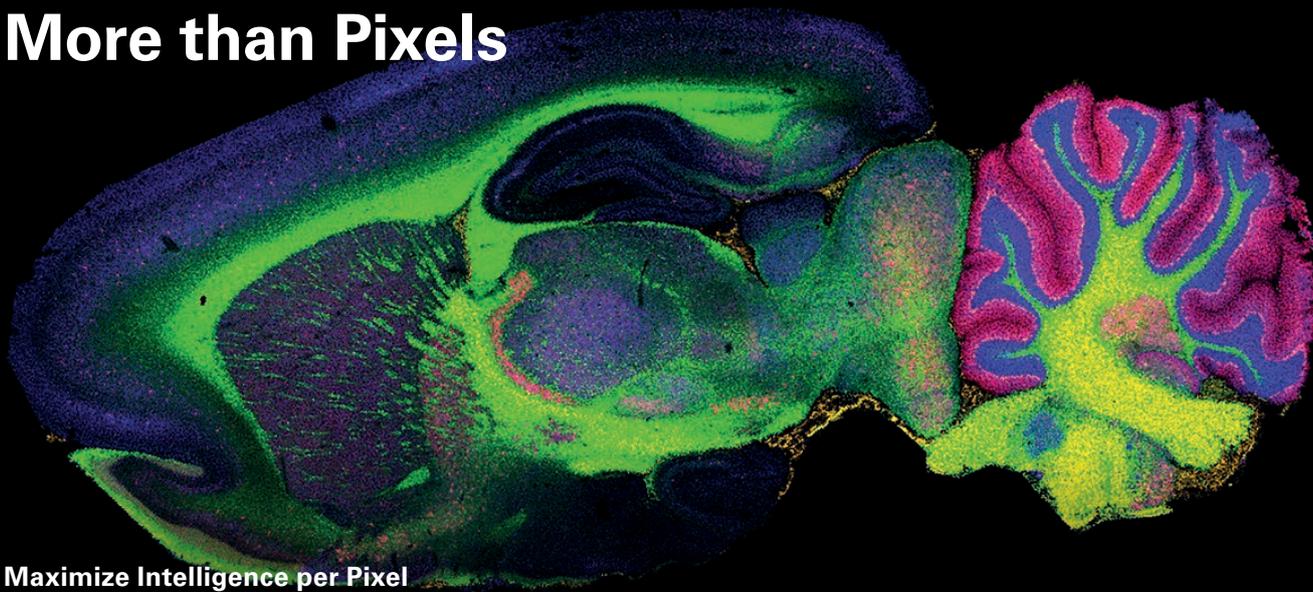
MALDI Imaging is a true multiomic technique, monitoring more molecular classes than other imaging techniques.

Spatially mapping a wide range of biomolecules such as lipids, glycans, metabolites, and peptides accelerates biomarker discovery and elucidates protein activity. Application of a directed multiomics approach using SpatialOMx[®] can decipher signaling networks at specific sites within the tumor's heterogeneous cellular ecosystem and integration with protein expression profiling with MALDI HiPLEX-IHC provide an unbiased overview of key biological processes.

The timsTOF fleX and MALDI HiPLEX-IHC allow you to dive deeper into the tumor microenvironment and beyond.

timsTOF fleX combines the best multiomics platform available with a MALDI source designed for imaging. Intelligence derived from MALDI Imaging can guide omics analysis of specific cellular structures to deliver greater cellular specificity of LC-MS approaches and establish a new SpatialOMx[®] benchmark for the future of pathology. Search for regions of biological interest on tissues and perform deep quantitative analysis of their omics makeup on the same platform.

More than Pixels



Maximize Intelligence per Pixel

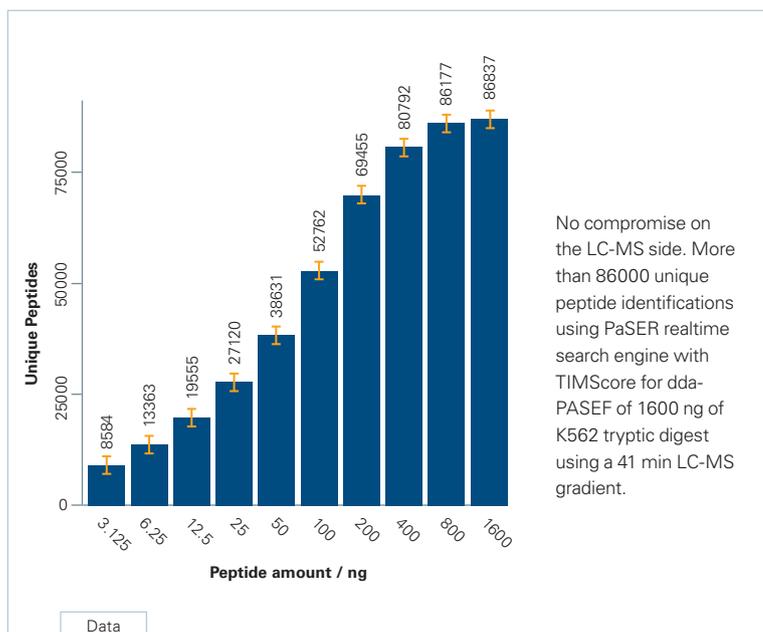
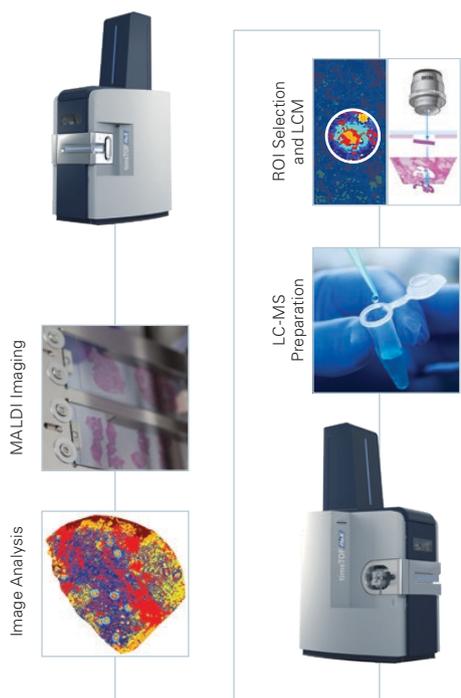
Using MALDI guided laser capture microdissection (LCM) for example, a 50 μm LCM tissue section will contain roughly 25 cells; enough for bottom-up proteomics analysis on the timsTOF fleX. One instrument that gives you the capability to do both – high spatial resolution, high speed MALDI and high sensitivity 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 enhance on CCS-enabled 4D-Omics and unlock a 5th dimension and show the distribution of target compounds. After completing a

typical MALDI Imaging experiment and data analysis, further depth of information can be gained by selecting a population of cells of interest for full LC-MS analysis in the omics method of your choice.



Bridging the gap between 4D-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 Cellular Processes

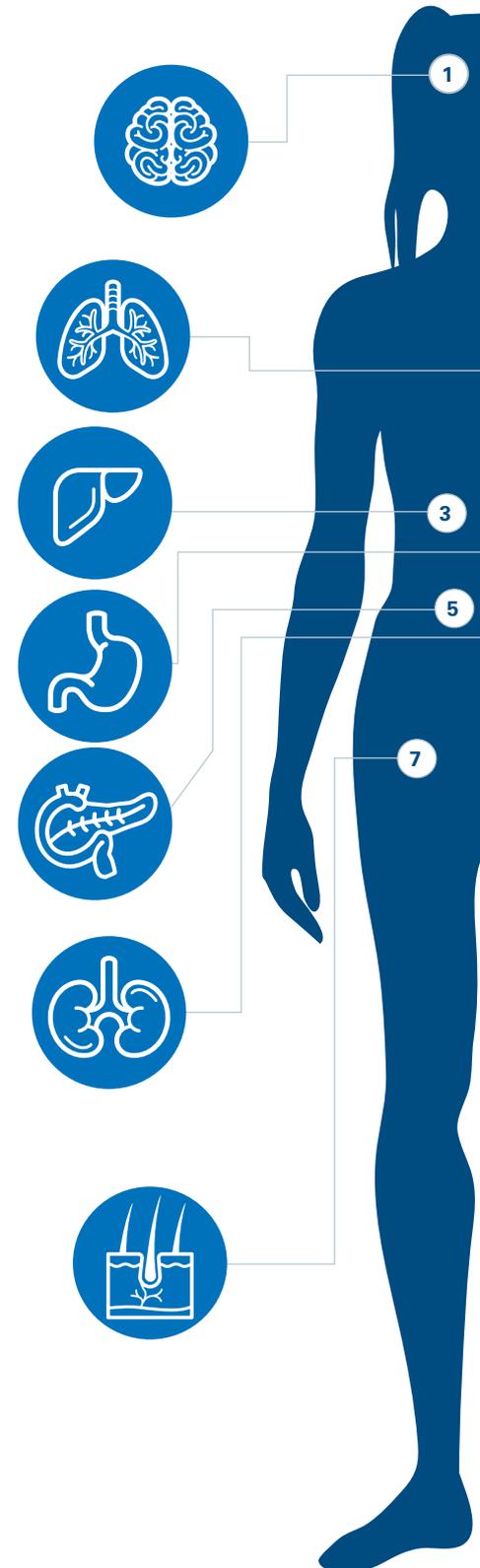
Within any given cell, the processes that regulate life, growth, functional changes, and death are directed by peptides and proteins. Transcription factors (generally between 50 - 100 kDa) drive or halt protein expression from genetic templates. These expressions create the proteomic means necessary 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.

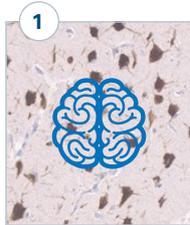
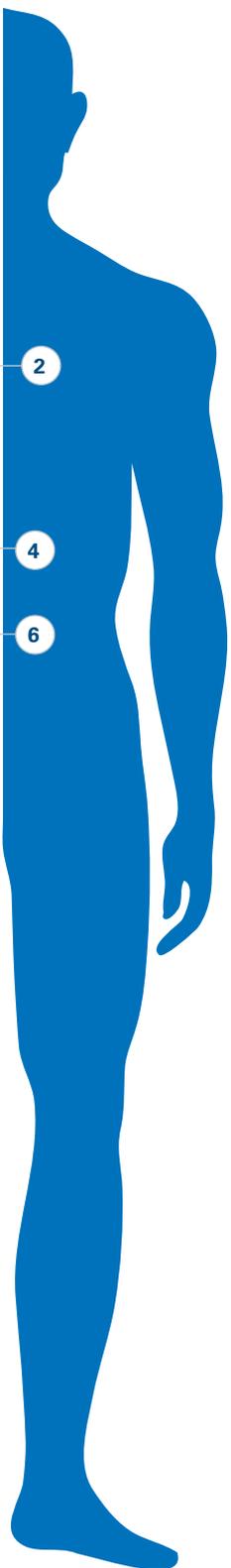
For Housekeeping Processes

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

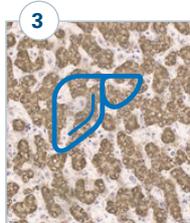
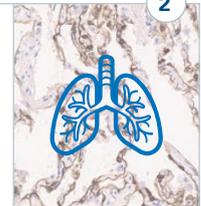
Pharmaceuticals target many different types of proteins; greater knowledge of protein localization, form, and function could improve drug design and efficacy. Alternative, high(er) affinity binding partners can more effectively modulate enzymatic activity. Examples include NSAIDs which reduce pain and 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.





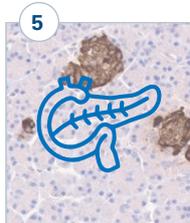
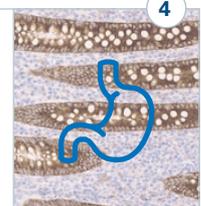
Brain A highly complex and energy-intensive organ, coordinated higher functions, e.g. motion, perception, and cognition, are received, processed and executed in the brain. Neural proteins show specific expression patterns among cells and structures, as well as in subcellular structures such as axons,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

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.



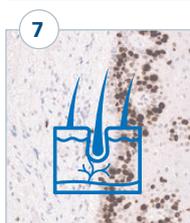
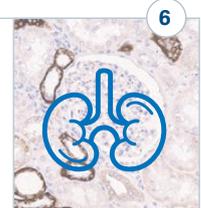
Liver Composed of parenchymal cells (hepatocytes and bile duct cells) and non-parenchymal cells (sinusoidal endothelial, Kupffer, and hepatic stellate cells), the liver is the largest internal organ. Liver-specific proteins include plasma and bile proteins, and proteins associated with metabolic processes,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

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.



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.

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.



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-Omics Workflows – Just a Click Away

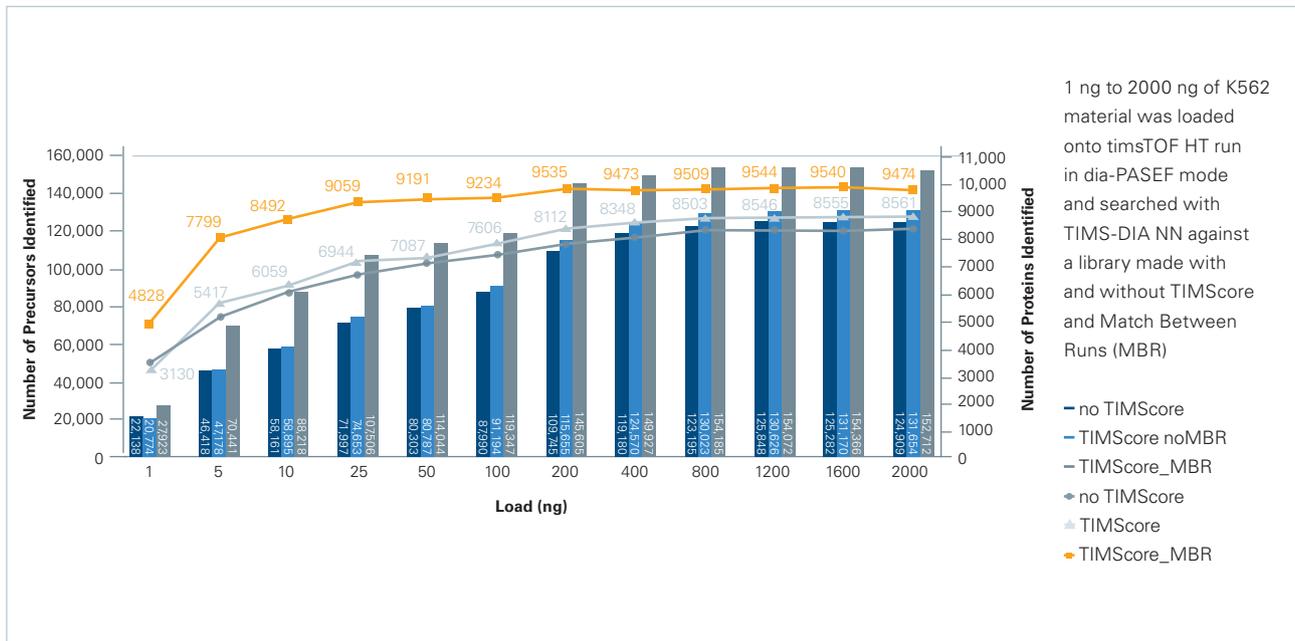
The power of 4D-Proteomics on the timsTOF fleX

The timsTOF fleX is capable of all CCS-enabled 4D-Proteomics workflows bringing you comprehensive results in high-throughput analyses with PASEF, dia-PASEF and prm-PASEF. The unique synergy of hard- and software combines sensitivity and acquisition speed resulting in unprecedented depth of proteome coverage and unmatched data completeness in quantitative proteomics.

Search results in real-time on the timsTOF fleX are brought to you by PaSER 2022. The GPU-powered Parallel Search Engine in Real-time delivers results as soon as the run is done, for both data-dependent and data-independent acquisition strategies. The proteome and sequence coverage are maximized by applying TIMScore, a CCS-prediction model in PaSER 2022 based on machine learning to

extend FDR calculation to the mobility dimension. dia-PASEF workflows are processed by a customized version of the DIA-NN software from the Lilley, Rasler and Demichev labs. TIMS DIA-NN provides stringent statistical control, automatic parameter optimization and allows to easily build spectral libraries from TIMScore powered DDA search results.

The proteomics capabilities of the timsTOF platform can be demonstrated by the analysis of 1200 ng K562 lysate in 60 min gradient, processed with TIMS DIA-NN against a library made with TIMScore and without Match Between Runs (MBR). More than 8,000 protein groups can be identified, while the number of precursors exceeds 120,000. With reducing the sample amount to 10 ng, still about 4,000 protein groups and nearly 60,000 precursors can be identified, showing the strength of dia-PASEF and TIMS DIA-NN for low sample amounts.



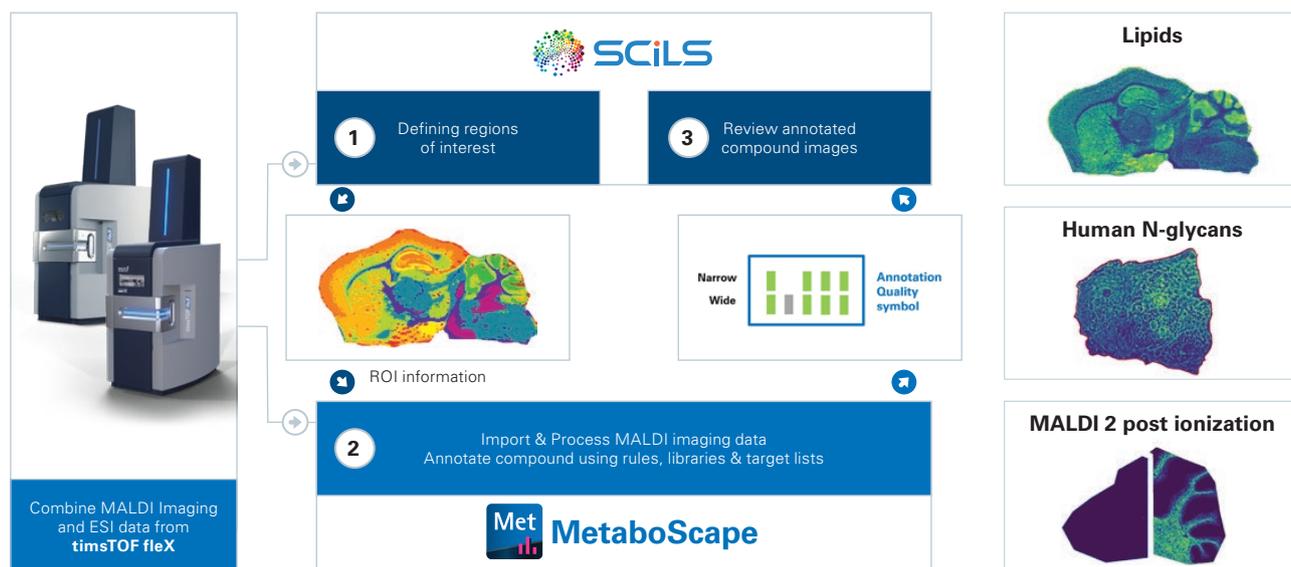
SpatialOMx Automated Molecular Annotation Workflow

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

SpatialOMx® – Automatic annotation workflow combining SCiLS™ and MetaboScape®



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

timsTOF *flex*

MALDI Guided SpatialOMx®



microGRID for sub-cellular spatial resolution

Pairing microGRID and MALDI-2 technology on the timsTOF flex to unlock out-of-the-box sub-cellular imaging capabilities.

timsTOF flex uniquely enables SpatialOMx®

PASEF powered LC-MS/MS identification matched with spatial localization identifies and locates multilevel multiomic 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.

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