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Guide for writing successful proposals

- for SpatialOMx and timsTOF fleX

Innovation with Integrity

SpatialOMx

Outline

Abstract

MALDI Guided SpatialOMx® represents a quantum advance in how tissue is analyzed. It combines a label-free imaging technique that delivers sensitive distribution mapping for both targeted and untargeted compounds, ranging from metabolites to lipids to proteins with regionally targeted 4D-Omics analysis. The timsTOF fleX is the SpatialOMx tool - integrating a high-speed MALDI Imaging source with the fastest and most sensitive platform for 4D-Omics, from the well-established timsTOF Pro, into a single high-performance mass spectrometer. SpatialOMx improves on conventional solution-based Omics approaches by providing the same molecular depth while also retaining spatial information critical to gaining insight into disease development and treatment. CCS-Aware 4D-Omics, based upon TIMS and PASEF® technologies, provides unmatched selectivity, specificity, and throughput for MALDI. The integrated workflow delivered by the timsTOF fleX provides researchers with the best tool for deep dive into regionally targeted biochemical diversity associated with tissue state, in a single platform offering improved operational efficiency.

Section 1: SpatialOMx and label-free molecular imaging

Introduction

SpatialOMx represents a shift in how tissue is analyzed when studying molecular changes associated with disease development and treatment. Over the past decade, numerous Omics approaches have offered new insight into molecular mechanisms occurring within and across the complex network of cells that make up tissue. Unfortunately, because of their solution-based approach, these techniques are largely unable to correlate specific molecular involvement to distinct cell phenotypes or localized regions within the tissue. Additionally, such approaches tend to dilute signals of interest by analyzing extracts from the tissue rather than extracts from the few cells of interest. What is needed is a new methodology for analyzing tissue that provides the same molecular depth as traditional Omics workflows but which also retains the spatial relationship of those signals within the cellular network. SpatialOMx provides researchers with the ability to map molecular distributions in tissue in situ, identify regions of interest that express the desired molecular profile, and selectively target these subpopulations for 4D-Omics analysis.

Histology is a standard tool in pathology that relies on visual examination of morphological differences between cells of the tissue. Differences in cell count, morphology, and other factors guide the diagnoses of tissue-based diseases. In a sense, histology offers an after-the-fact perspective of cellular transformation and offers very limited information about the molecular mechanisms that fuel cell transformation. As such, histological examinations can be highly subjective and often indeterminant, particularly when used to identify cells at a particular stage of transformation. Conversely, molecular expression can provide a more specific differentiator for cells presenting similar morphology and enables predictive insight into what the cell 'intends' to do, and on what timeframe.

Conventional molecular imaging methods such as immunohistochemistry (IHC) or fluorescence imaging rely on tagging the target molecule to facilitate detection. Antibody or molecular tag selection requires that the target compound be known in advance, thereby limiting the use of these techniques for non-targeted discovery experiments. Antibodies can be specific but have limited applicability beyond visualizing protein distributions, leaving the method blind to many key compound classes such as metabolites and lipids that are active participants in cell signaling and metabolism. SpatialOMx is an unlabeled imaging technique that provides sensitive distribution mapping of both targeted and untargeted molecules, ranging from metabolites to proteins.

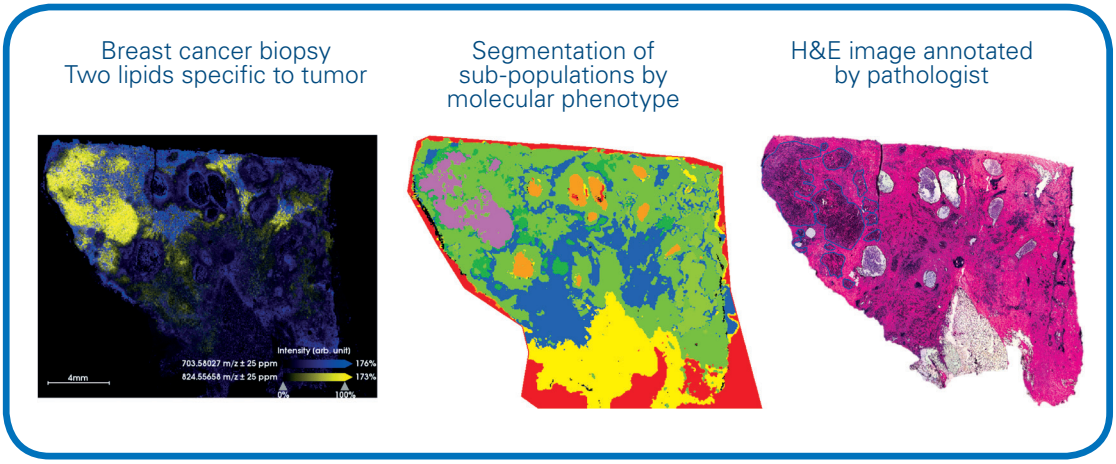

Figure 1: SpatialOMx utilizes MALDI Imaging to map molecular expression across the tissue, and subsequently, segment sub-structure expressing similar molecular phenotype.

Liquid chromatography (LC), in conjunction with tandem mass spectrometry (MS/MS), is the core technique of Omics strategies and yields a higher dynamic range for detection than MALDI Imaging with small sample amounts. A fundamental limitation of LC-MS/MS when analyzing tissue is that sample preparation often involves homogenization and extraction, a process that obscures any information about localized analyte abundance.

SpatialOMx Workflow

SpatialOMx is the integration of MALDI Imaging with 4D-Omics capabilities of timsTOF fleX to create a powerful, novel 4D-Omics workflow targeted to specific regions in the specimen. First, MALDI Imaging is used to identify and categorize sub-populations, or molecular phenotypes, spatially across a tissue. These regions are subsequently targeted for analysis at high dynamic range with LC-MS/MS for compound identification – both known and unknown. This new approach provides researchers with unmatched sensitivity for relevant molecular distributions in tissue and is enabled by the high sensitivity of 4D-Omics. SpatialOMx can therefore increase sensitivity and specificity of any Omics research, including proteomics, lipidomics, and metabolomics.

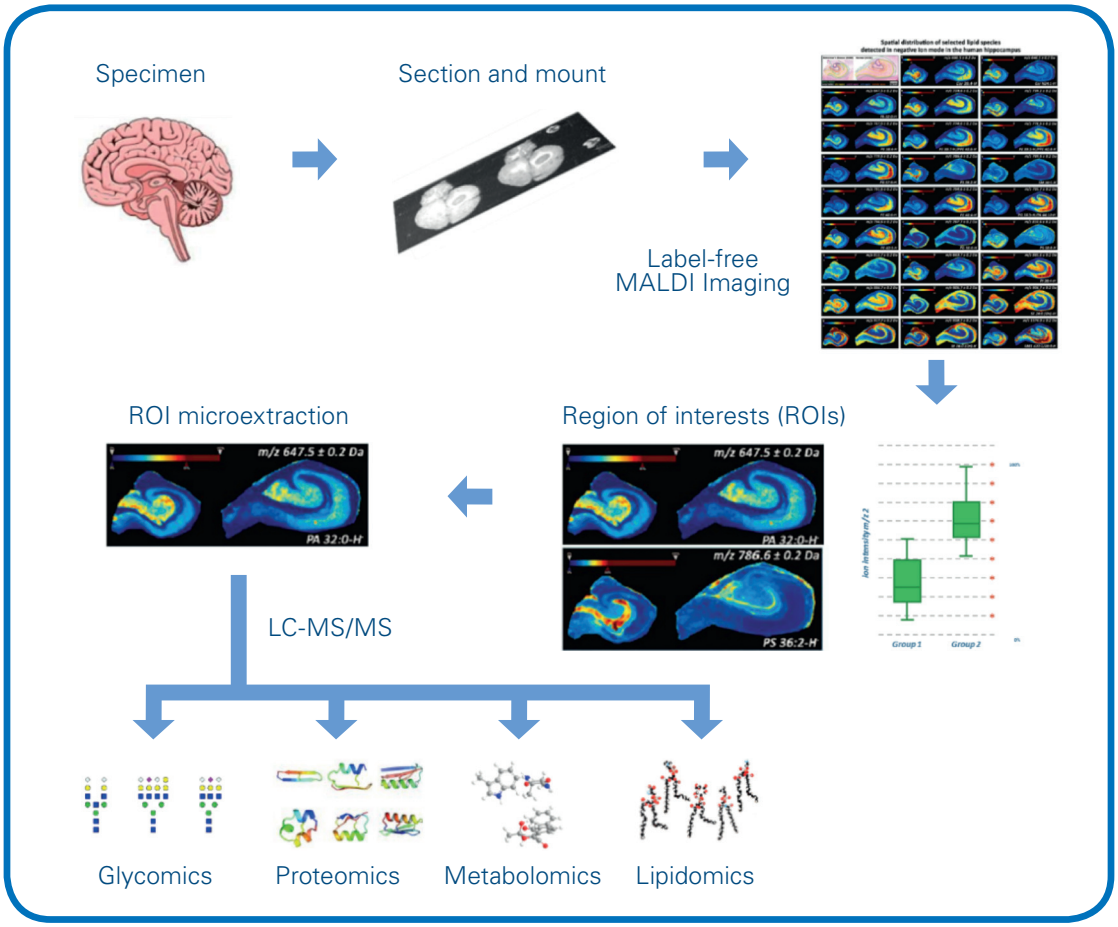

Figure 2: Greater molecular depth and identifications with higher regiospecificity than homogenization

SpatialOMx Instrumentation: timsTOF fleX

Key Features:

- **Dual MALDI Imaging and LC-MS/MS Platform**: combines the established 4D-Omics functionality of the timsTOF Pro with high-performance MALDI Imaging capabilities in a single instrument.
- **TOF Mass Range**: 20-40,000 m/z
- **Mass Resolution**: 60,000 at m/z 1221
- **Trapped Ion Mobility (TIMS) technology**: provides accurate and reproducible CCS values from first principle. Also, allows for a 'preconcentration' of ions, significantly boosting sensitivity and allowing very low sample loading from fewer cells.
- **Parallel Accumulation – Serial Fragmentation (PASEF)**: synchronizes TIMS separation with MS/MS precursor selection, allowing >100 MS/MS spectra/second.
- **Collisional Cross Section (CCS) aware measurements**: provides unmatched selectivity and sensitivity. TIMS separation of isobaric compounds: i) improves detection sensitivity by eliminating chemical background. ii) enables clean MS-MS fragmentation of precursors isolated by CCS and m/z.
- **Smartbeam 3D Laser**: 10 Khz, 10 μm "zoom" mode, "true" square pixels
- **Robust**: Lower sample loadings mean less cleaning and higher reproducibility.

Section 2: Tips to maximize the success of your proposal

Add value of SpatialOMx to the proposed research

SpatialOMx characterization of tissue allows one to directly characterize not only molecules that are changing but map from where those changes originate within tissue. Studies that will benefit from the enhances specificity of SpatialOMx are any tissue-based Omics studies where the goal is to discover and characterize molecular changes in tissue microenvironment, including those associated with cellular transformation or disease pathways.

Benefits to local research environment

SpatialOMx provides new tools for mapping molecular changes within tissues and will have a direct impact on the Omics research of anyone who is studying cellular processes and disease progression at the tissue level. The ability to map, determine, and identify molecular changes in a regionally specific manner will be unique to the local research community. Having a single platform such as timsTOF fleX that delivers both state-of-the-art MALDI Imaging but also LC-MS Omics analysis brings maximum efficiency to the laboratory.

Enable collaboration

Enabling the SpatialOMx workflow in your research environment will provide an opportunity to establish collaborations between disciplines including, but not limited to, pathology, biology, and radiology. The SpatialOMx workflow is unique in bridging the divide between MALDI Imaging and traditional Omics research and provides a valuable pathology context for molecular changes occurring in tissue specimens. Pathological expertise will, therefore, be essential to utilize the full value of this novel method. Acknowledging these collaborative opportunities and providing details on how the working relationship will be enabled can increase the success of your grant application.

Deliver economy of force

No other single platform offers the analytical performance and flexibility of timsTOF fleX at comparatively lower capital cost and space requirements than similar multi-system configurations.

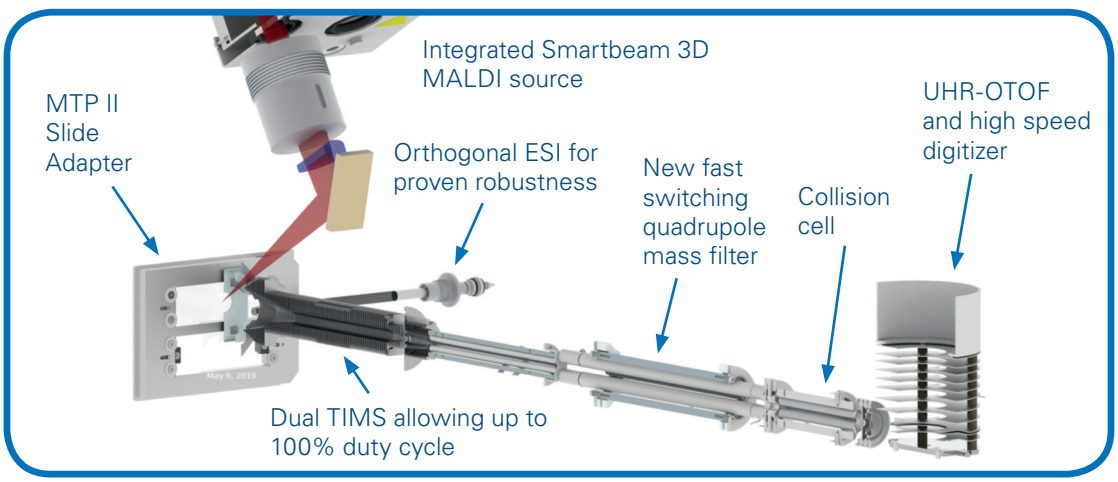

Figure 3: Interior elements of timsTOF fleX shows the integrated ESI and MALDI modes both benefit from TIMS separation and can be switched in seconds.

Section 3: Platform capabilities and performance

CCS Aware 4D-Omics

Just as retention time (RT) is an analyte attribute within the liquid phase, the gas-phase structure of an analyte is also reproducible and intrinsic. The timsTOF fleX measures Collisional Cross Section values using Trapped Ion Mobility Spectrometry, which can achieve IMS resolution up to 200, equivalent to a drift tube length of over 2 meters. Using first principles, CCS can be quickly determined for small molecules, metabolites, lipids, peptides, and protein molecules. Since they derive from first principles, CCS values from TIMS is fully compatible with published CCS determined from drift tube measurements. Further, the CCS can be predicted using Bruker's unique CCSPredict algorithm to accurately predict the CCS of proteotypic peptide ions or lipids. Since CCS is intrinsic to the molecule, accurate measure/prediction increases accuracy and confidence in identification assignments.

This new 4th dimension can also be used to selectively analyze ions of unique interest within the CCS space. Ions having similar or even identical m/z but separated by CCS can be selected independently for MS/MS analysis, adding additional confidence in annotation assignments and quantification completeness.

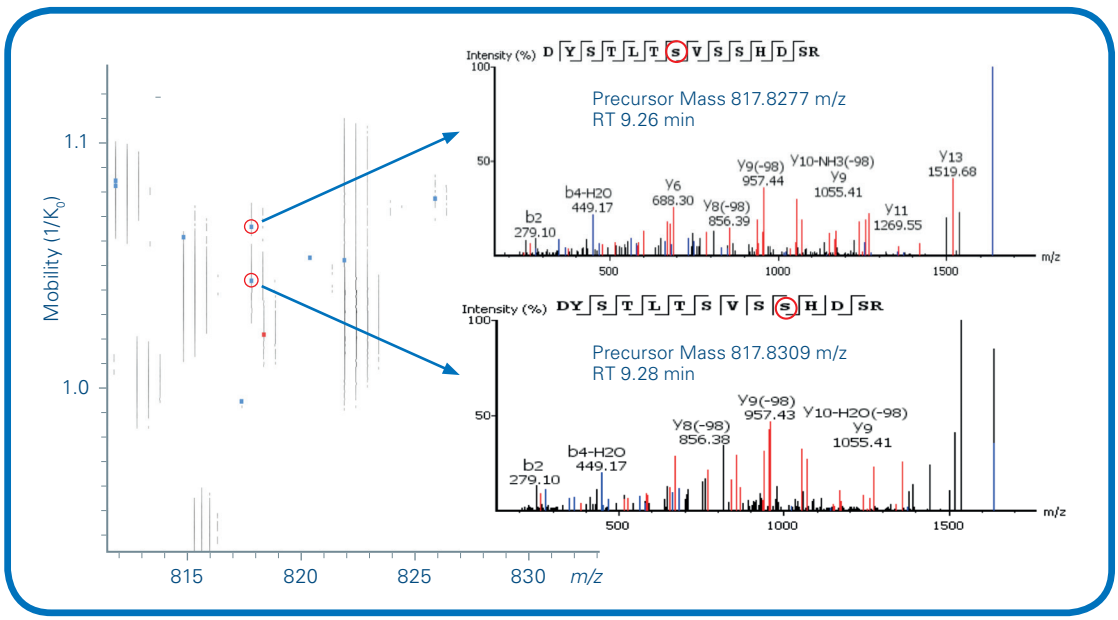

Figure 4: A heatmap of the co-eluting, isomeric mono-phosphorylated peptide DYSTLTSVSSHDSR where mobility offset mass alignment (MOMA) discerns the two positional isomer forms and PASEF acquires non-chimeric and high scoring spectra for each peptide (inset).

TIMS Sensitivity Boost

SpatialOMx experiments typically involve very small amounts of sample that demand the most sensitive mass spectrometer. Samples from laser capture microdissection, small organs, and even single cells all require very high sensitivity. The dual-TIMS funnel in the timsTOF fleX series significantly improves sensitivity while maintaining unprecedented sequencing speeds. Whether eluting from a column or resulting from multiple laser pulses, ions enter TIMS1 as a diffuse ion cloud of mixed m/z and CCS and are accumulated over a duration of 20-100 ms by the opposing forces of constant gas flow and an electrical gradient. The accumulated ion packet is then transferred to TIMS2 where they are trapped against a similar electrical gradient based on their collision cross sections (CCS), while TIMS1 is simultaneously filled with a new ion cloud. Ions are eluted from TIMS2 towards the downstream TOF analyser by sequentially lowering the electric field. Thus, the ion cloud enters TIMS1 over a 20-100 ms accumulation time but elute from TIMS2 as discrete ion packets separated by CCS and compacted into 2-5 ms segments before entering the TOF. This temporal preconcentrating effect of dual-TIMS funnel results in up to a 30x signal-to-noise improvement compared with other continuous acquisition instruments. Simultaneously filling TIMS1 while eluting from TIMS2 delivers 100% duty cycle.

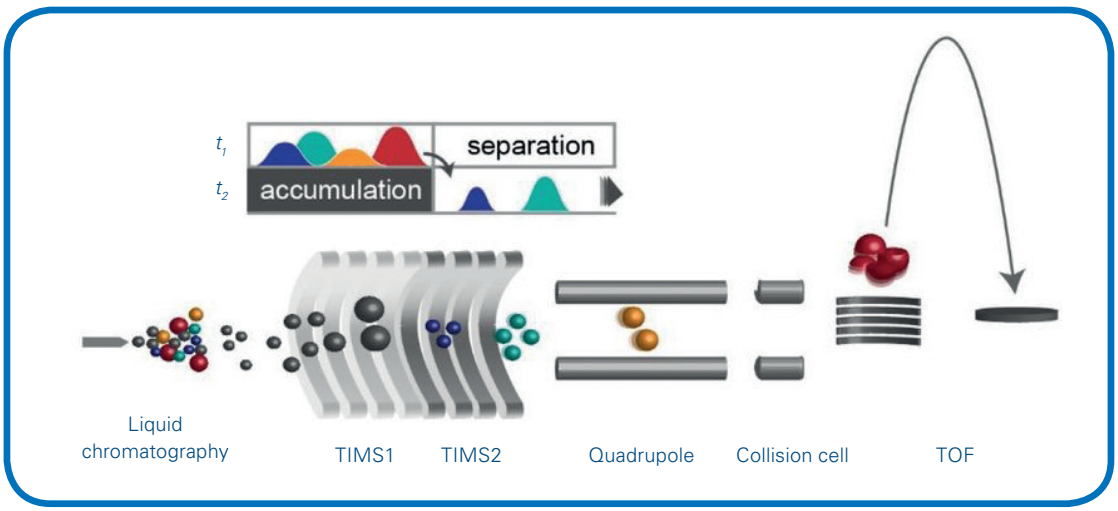

Figure 5: Ions separated by CCS elute from TIMS cell at different times rendering fragmentation spectra specific to ions of a specific CCS and m/z.

Parallel Accumulation - Serial Fragmentation (PASEF):

The patented PASEF acquisition mode is only available on the timsTOF Pro and timsTOF fleX series of instruments. PASEF synchronizes MS/MS precursor selection by the quadrupole with the elution of ions from the TIMS cell to maximize the number of fragmented precursors per TIMS scan, thereby increasing the sequencing speed several-fold. The standard PASEF method acquires on average 120 MS/MS scans with a duty cycle of 1.1 s, achieving an MS/MS acquisition rate of >100Hz. The sensitivity boost provided by the TIMS time-focusing combined with 100% duty cycle, allows PASEF to overcome the traditional problem of sampling fewer ions as acquisition rate increases.

4D-Omics

Traditional Omics analysis provides three dimensions of data: retention time, m/z and MS/MS spectra. The timsTOF platform provides accurate and reproducible CCS values derived from first principles, enabling 4D-Omics.

4D-Proteomics [§]

Fundamental challenges in MS-based proteomics include the high sample complexity, the large dynamic range in protein concentration, and the resulting big-data computational analysis. 4D-Proteomics™ on the timsTOF platform address these challenges. PASEF enables MS/MS acquisition at >100 Hz, making high-throughput measurements using short gradients possible while maintaining deep proteome coverage or high protein depth measurements in less time. The time-focusing benefit of dual-TIMS funnel technology increases sensitivity thereby allowing for loading of lower sample amounts and simpler sample preparation techniques. Co-eluting peptides can be Mobility Offset Mass Aligned (MOMA), where the mobility difference allows positional isomers to be uniquely assigned. MOMA allows the acquisition of non-chimeric spectra and the unambiguous identification and quantitation of PTMs (including PTM-positional isomers). CCS-aware analysis software increases the confidence of identification and quantitation, while increasing data completeness. timsTOF fleX 4D-Proteomics solutions capitalize on a range of proprietary tools that address additional challenges, including the CaptiveSpray nanoBooster that can increase ionization efficiency of glycopeptides, to the use of variable collision energies to provide fragmentation information on both the peptide and modification moiety (especially for Glycopeptides).

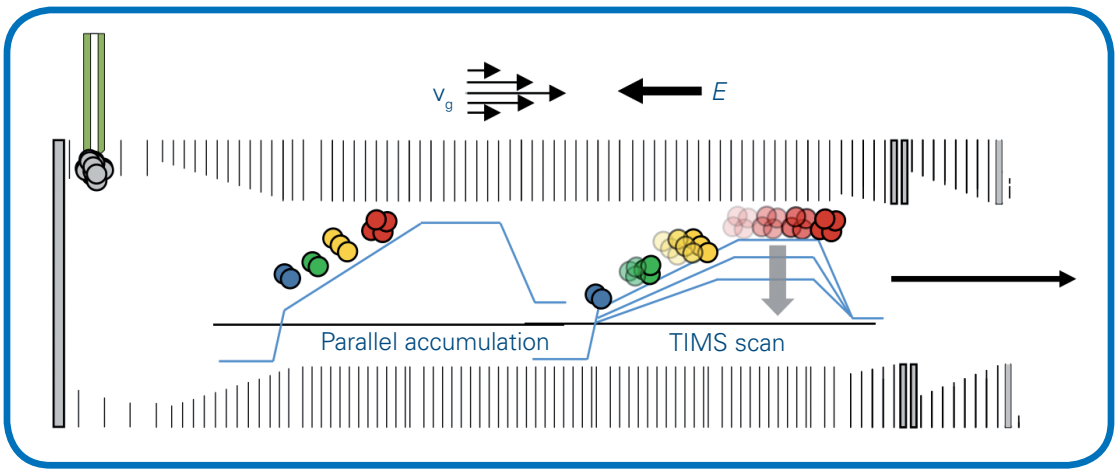

Figure 6: The dual-TIMS cell accumulates and separates in TIMS1 while the previous packet of ions elutes from TIMS2 to provide 100% duty cycle.

In addition to standard data-dependent acquisition based on PASEF, the timsTOF fleX also supports data-independent acquisition (DIA) and parallel reaction monitoring (PRM). dia-PASEF® exploits the correlation between molecular weight and ion-mobility to sample up to 100% of the precursor ion current. The extremely high ion sampling fully translates to higher sensitivity, over 3000 proteins can be identified by dia-PASEF from just 10 ng of HeLa digest or the complete yeast proteome can be acquired in 30 min without compromising reproducibility and quantitative accuracy. prm-PASEF® maximizes the number of precursors that can be targeted per unit time while preserving the increased sensitivity, specificity, and inherent robustness of the timsTOF Pro.

[§] *See* 'Four reasons to switch to 4D-Proteomics on the timsTOF platform' *for more information.*

4D-Lipidomics and 4D-Metabolomics

The metabolome is the final manifestation of biochemical pathways and encompasses an extremely large variety of structural classes produced from the breadth of cellular processes. Changes in the metabolite composition reflect the outcome (phenotype) of interactions at the genomic, transcriptomic, and proteomic levels. Therefore, studying the metabolome cornerstone to a deeper insight into cellular processes e.g., diseases, therapeutic interventions, or environmental influences.

MetaboScape® software is the ideal tool for 4D-Lipidomics™ and 4D-Metabolomics™ using timsTOF fleX. Even for smaller molecules, PASEF delivers high sample throughput and sensitivity. Further, the ability to separate isobaric compounds that have similar retention time in the 4th dimension, CCS, and to extract and identify key molecular feature differences between sample groups is invaluable for clinical research. Even at shorter LC run times, TIMS separates co-eluting isobaric and isomeric compounds. Further, CCSPredict accurately confirms lipid structures with high confidence. Table 1 shows one example of up to 1040 automatically detected lipid assignments from *C. elegans* using MetaboScape alongside the timsTOF fleX.

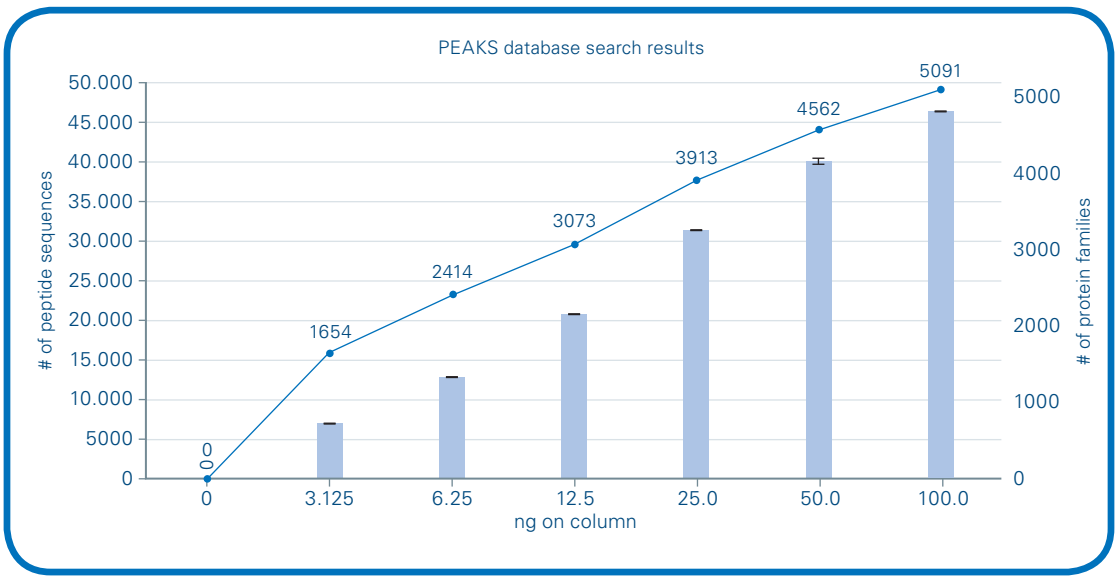

Figure 7: Number of protein groups identified as function of sample loading (HeLa digest) using DDA PASEF

High-speed, high-spatial resolution MALDI Imaging

MALDI Imaging reveals localized molecular fingerprints within specific regions of a sample. It is a label-free imaging tool used to investigate temporal changes in the biochemistry of cell transformation or to correlate molecular expression to a disease state.

A large body of work in scientific literature validates using MALDI Imaging to investigate many molecular classes such as glycans, lipids, metabolites, proteins and products of on-tissue enzymatic digestion or chemical derivatization. The timsTOF fleX is capable of imaging these compound classes at high spatial and mass resolving power, while also offering True Pixel Imaging whereby the cellular origin of detected signals can be unambiguously determined to a discrete AND unique cellular region.

Table 1: Automatic lipid assignments from C. elegans using MetaboScape software and the LipidBlast spectral library [1].

Bucket Table

<filter rules>

	RT [...	CCS...	ΔCCS [...	m/z me...	M meas.	Ions	MS/...	Name	Molecular F...	Annotati...	AQ	Annotation Source
1	0.11	290.5		745.621...	744.614...			SM(d37:1)	$C_{42}H_{85}N_2O_6P$	AL		Celagans_Lipids_po...
2	5.22	289.2		705.554...	704.547...			SM(t33:1)	$C_{38}H_{77}N_2O_7P$	AL		Celagans_Lipids_po...
3	6.59	292.5		735.601...	734.594...			SM(t35:0)	$C_{40}H_{83}N_2O_7P$	AL		Celagans_Lipids_po...
4	6.80	293.2		733.586...	732.579...			SM(t35:1)	$C_{40}H_{81}N_2O_7P$	AL		Celagans_Lipids_po...
5	7.48	288.2		717.591...	716.584...			SM(d35:1)	$C_{40}H_{81}N_2O_6P$	AL SL		Celagans_Lipids_po...
6	8.72	297.0		763.632...	762.624...			SM(t37:0)	$C_{42}H_{87}N_2O_7P$	AL		Celagans_Lipids_po...
7	8.98	298.5		761.616...	760.609...			SM(t37:1)	$C_{42}H_{85}N_2O_7P$	AL		Celagans_Lipids_po...
8	13.29	286.6		731.601...	730.594...			SM(d36:1)	$C_{41}H_{83}N_2O_6P$	AL		Celagans_Lipids_po...
9	13.83	305.1		801.684...	800.676...			SM(d41:1)	$C_{46}H_{93}N_2O_6P$	AL SL		Celagans_Lipids_po...
10	14.05	288.2		742.617...	741.610...			HexCer(d37...	$C_{43}H_{83}NO_8$	AL		Celagans_Lipids_po...
11	14.07	306.9		803.697...	802.689...			SM(d41:0)	$C_{46}H_{95}N_2O_6P$	AL SL		Celagans_Lipids_po...
12	14.12	307.7		815.699...	814.691...			SM(d42:1)	$C_{47}H_{95}N_2O_6P$	AL SL		Celagans_Lipids_po...
⋮												
1032	5.59	290.0		869.519...	870.526...			PI 37:5; PI 1...	$C_{46}H_{79}O_{13}P$	SL		LipidBlast-VS34.msp
1033	8.31	298.7		878.592...	879.599...			PS 43:5; PS ...	$C_{49}H_{86}NO_{10}P$	SL		LipidBlast-VS34.msp
1034	4.24	290.3		879.503...	880.511...			PI 38:7; PI 1...	$C_{47}H_{77}O_{13}P$	SL		LipidBlast-VS34.msp
1035	9.25	299.3		880.608...	881.615...			PS 43:4; PS ...	$C_{49}H_{88}NO_{10}P$	SL		LipidBlast-VS34.msp
1036	9.84	299.6		880.608...	881.615...			PS 43:4; PS ...	$C_{49}H_{88}NO_{10}P$	SL		LipidBlast-VS34.msp
1037	5.55	293.2		883.532...	884.540...			PI 38:5; PI 1...	$C_{47}H_{81}O_{13}P$	SL		LipidBlast-VS34.msp
1038	5.91	293.8		883.533...	884.541...			PI 38:5; PI 1...	$C_{47}H_{81}O_{13}P$	SL		LipidBlast-VS34.msp
1039	6.36	293.2		883.534...	884.542...			PI 38:5; PI 1...	$C_{47}H_{81}O_{13}P$	SL		LipidBlast-VS34.msp
1040	7.30	296.0		897.550...	898.557...			PI 39:5; PI 1...	$C_{48}H_{83}O_{13}P$	SL		LipidBlast-VS34.msp

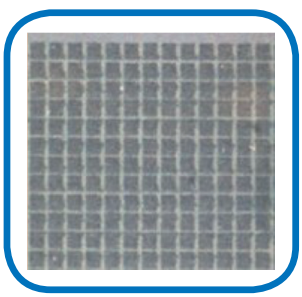

Figure 8: True Pixel imaging - detected signals are unique to the area of sample defined within boundaries of the visualized pixel. Only with true-pixel imaging can one confidently determine that 100% of the detected signal originated from a discrete region of sample. True Pixel Imaging is not possible with constant scanning techniques such as DESI.

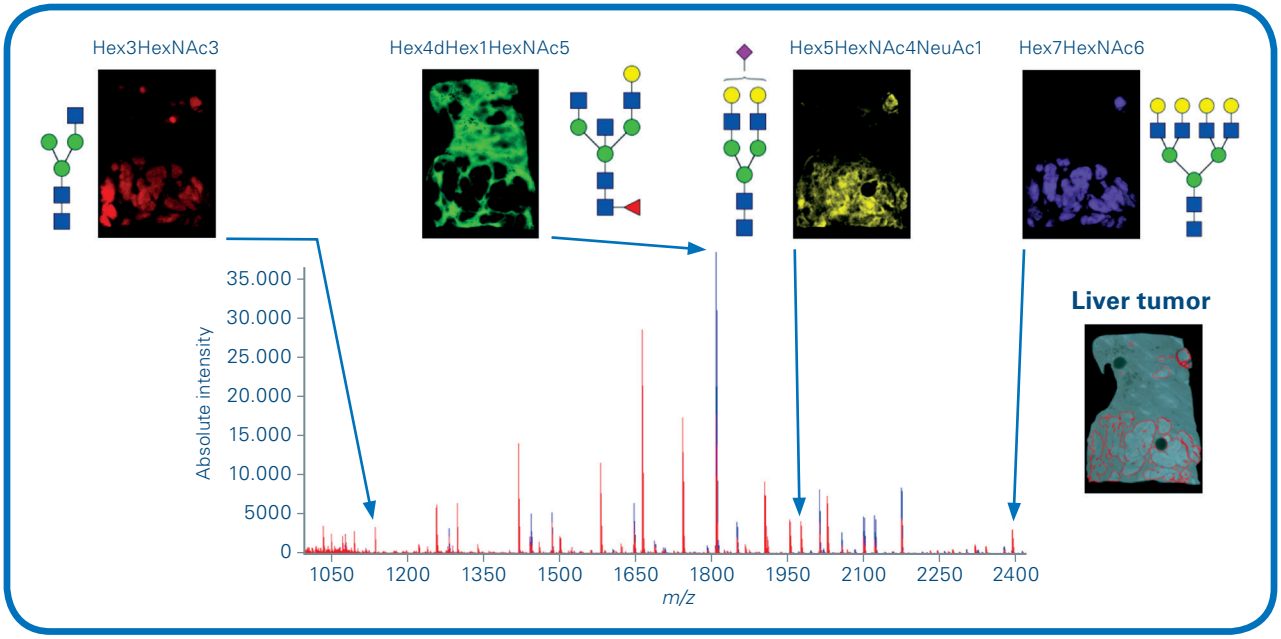

Figure 9: N-Glycan Imaging of prostate tumor illustrating regional specificity of individual glycans. Image courtesy of Dr. Richard Drake, Medical University South Carolina.

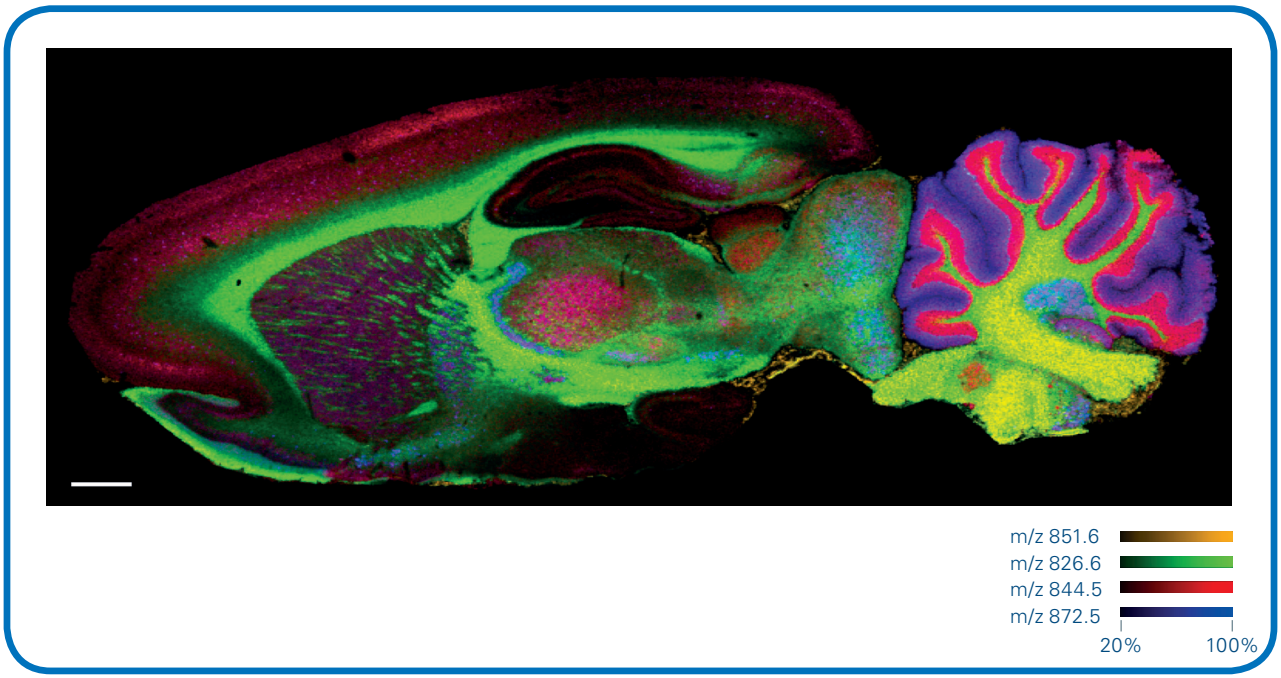

Figure 10: Lipid images in rat brain.

TIMS Imaging

Given the molecular complexity of biological specimens, TIMS separation delivers obvious advantages for MALDI Imaging applications. Unlike solution-based analyses, chromatographic separations are not amenable to MALDI analysis direct from tissue and it is often not possible to separate the multitudes of isobaric and isomeric MALDI ions solely relying on mass resolving power. TIMS provides an orthogonal tool for separating similar isobaric and isomeric ions by the combination of CCS and *m/z*. In fact, with maximum TIMS resolving power of more than 200. TIMS provides the only tool with the capacity to resolve and image many isomeric species.

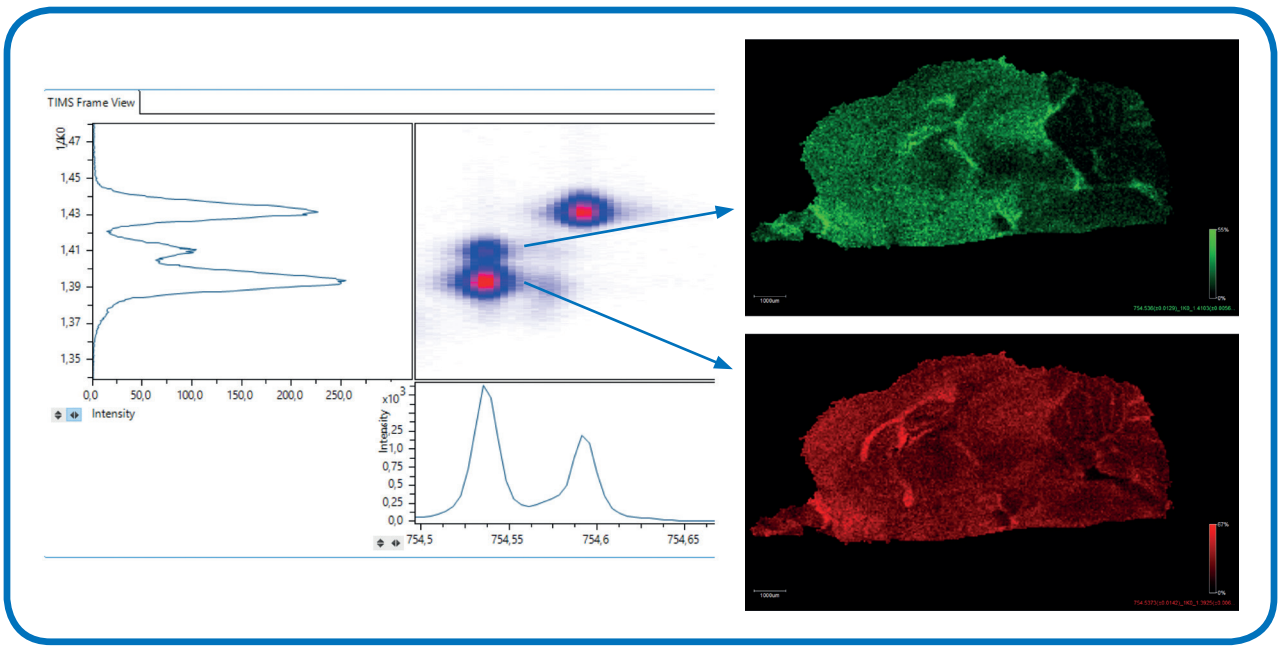

Figure 11: CCS vs m/z separation of two isomeric images.

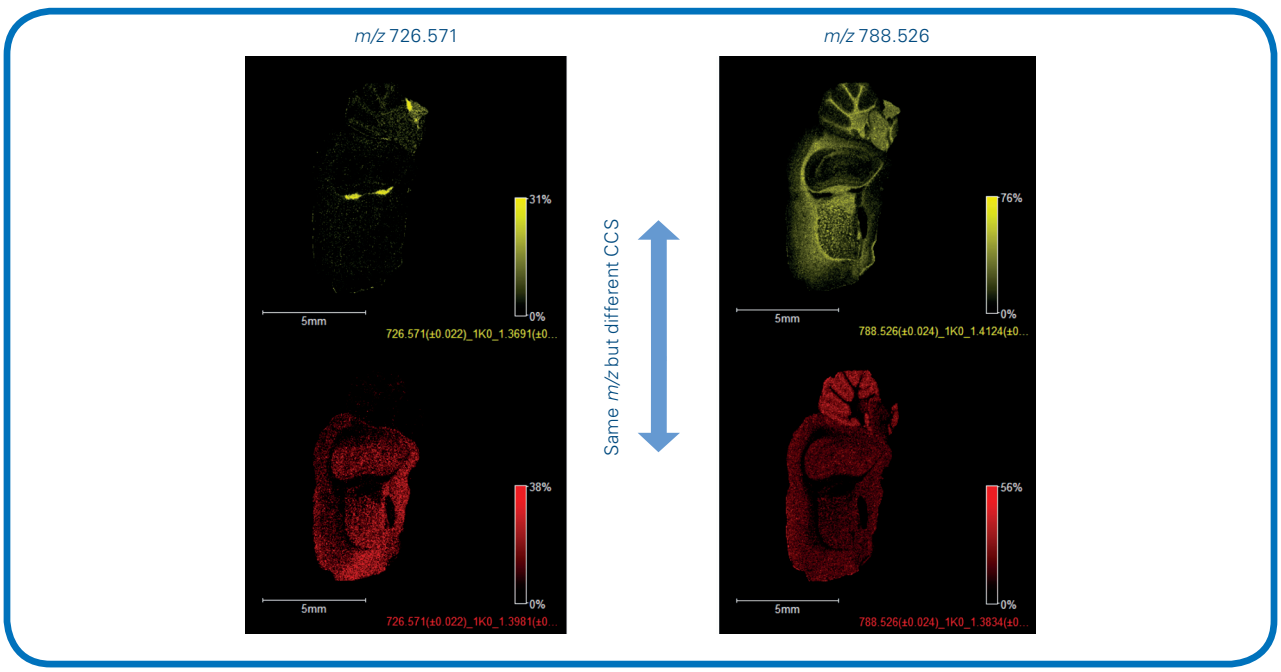

Figure 12: CCS separation reveals molecular distributions not observable with solely using high m/z resolution.

In addition to being uniquely suited for uncovering the widest range of molecular distributions, even of isomeric species, TIMS separated ions can be selectively probed by MS/MS to achieve clean, unambiguous fragmentation patterns, leading to higher confidence in identification assignment.

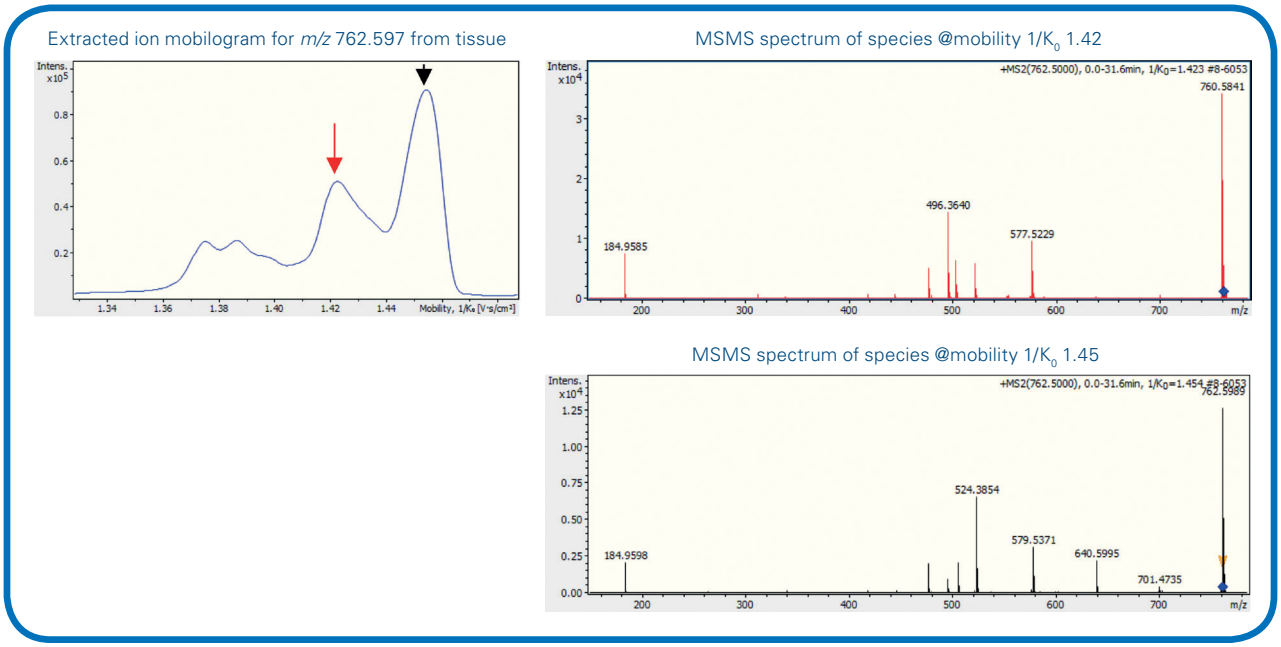

Figure 13: TIMS separation allows clear differentiation of m/z 762.59 from the A+2 isotope of m/z 760.58.

timsTOF fleX reproducibility and robustness

The timsTOF fleX exhibits excellent robustness and reproducibility for both LC-MS Omics and MALDI Imaging of large sample cohorts, a quality essential for long-term clinical research. The extremely sensitive orthogonal LC-MS ion optics of the timsTOF fleX requires less sample throughput. The contamination of the instrument is, therefore, minimized – resulting in stable and reproducible results.

Below are images of three superimposed phospholipid images from six serial mouse brain sections at 20 µm pixel resolution. Each image is composed of approximately 125,000 pixels (~2 hours measurement time) for a total of 12 hours of continuous MALDI measurement. The distinct similarity of the lipid images demonstrates the robustness of timsTOF fleX to matrix contamination and reflects the reproducibility of MALDI performance.

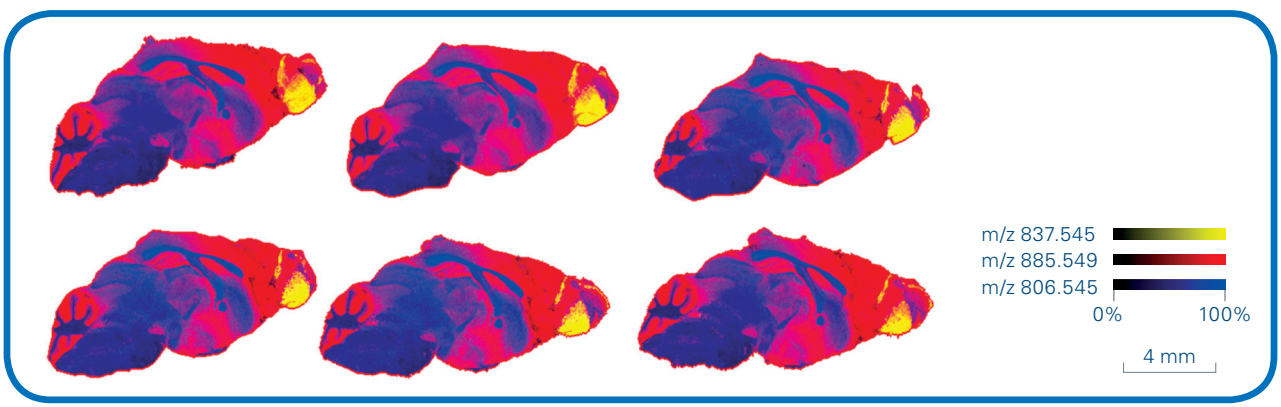

Figure 14: Lipid images from 6 rat brain sections acquired in sequence shows high stability of signals over time.

The Figure below illustrates the high number of protein IDs obtained throughout 40-weeks usage. The only drop-in sensitivity observed was due to LC column decay rather than instrument contamination.

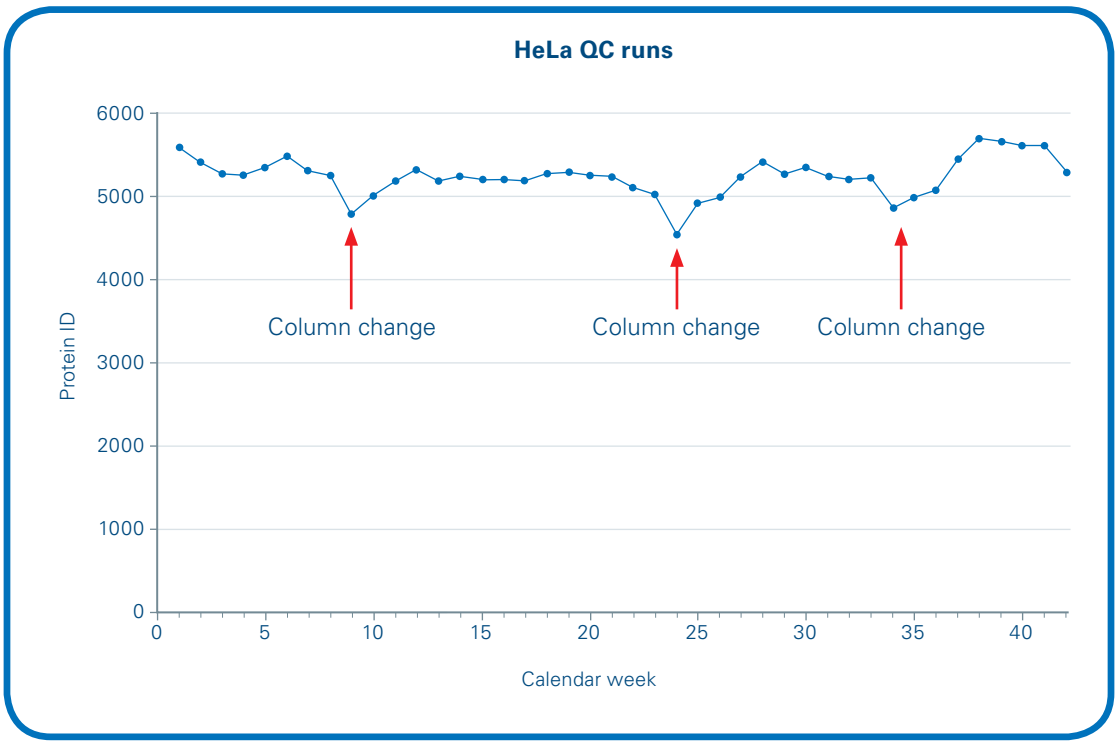

Figure 15: timsTOF Pro instrument performance over 40 weeks. On a weekly basis, a 200 ng Hela digest sample was measured using a 90-minute gradient. Observed drops below 5000 protein IDs were related to column aging and immediately after exchanging the column instrument performance was above 5000 protein identifications.

Section 4: SpatialOMx example

SCiLS Lab Software

A single SpatialOMx dataset can contain hundreds of thousands of mass spectra. Manual evaluation of data is not feasible, and successful interpretation of such large data sets requires computational data mining strategies. Bruker provides sophisticated statistical imaging software, SCiLS™ Lab, specifically for this purpose. SCiLS Lab offers numerous statistical tools such as comparative analysis of multiple samples (classification, Co-localization analysis, PCA, ROC, and spatial segmentation for automatic data analysis) and interactive 2D and 3D visualization. SCiLS Lab also integrates with Bruker's MetaboScape software for annotating MALDI Images with high-confidence compound IDs from MetaboScape. SCiLS Lab is available as Multiple Vendor Support (MVS) version, enabling users to combine MALDI Images from different platforms using the universal imzML data format.

Segmentation mapping

The basis of SpatialOMx is to analyze a series of molecular images and categorize regional similarities, or molecular phenotypes, often where histology is unable to differentiate. SCiLS Lab examines the lipid fingerprint within each pixel of data and clusters them into 'segments' based on their molecular similarity. A color-coded dendrogram is then created, with each branch representing populations of each segment. A color-coded spatial map is also generated, with each segment overlaid onto the digital image of the tissue. The automatic segmentation pipeline is fast and requires only a few mouse clicks and offers a clear molecular histology view of the section.

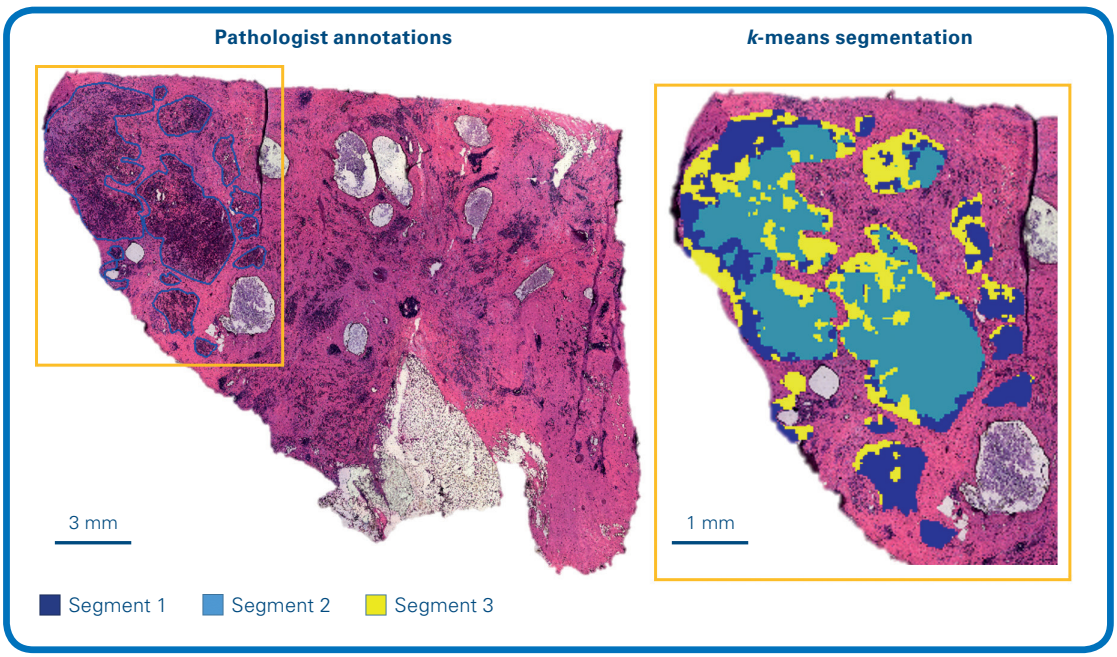

Figure 16: Image of the H&E stained breast cancer section with tumor area marked in blue by a pathologist (left side). Zoom on the tumor area after segmentation analysis using k-means clustering, which produces three segments representing tumor subpopulations (right side).

Regions of the desired molecular phenotype are identified and located in the segmentation image. In the example illustrated below, ~2000 cells were removed from each of three selected regions using laser capture microdissection. The excised tissue was then extracted and digested for bottom-up 4D-Proteomics analysis on timsTOF fleX. Rather than deconvolve signals from complex homogenate into the regional origin, the resulting identified proteins unambiguously originated from the excised regions, providing a window of very high cellular specificity for the pathways each protein participates, only possible using SpatialOMx.

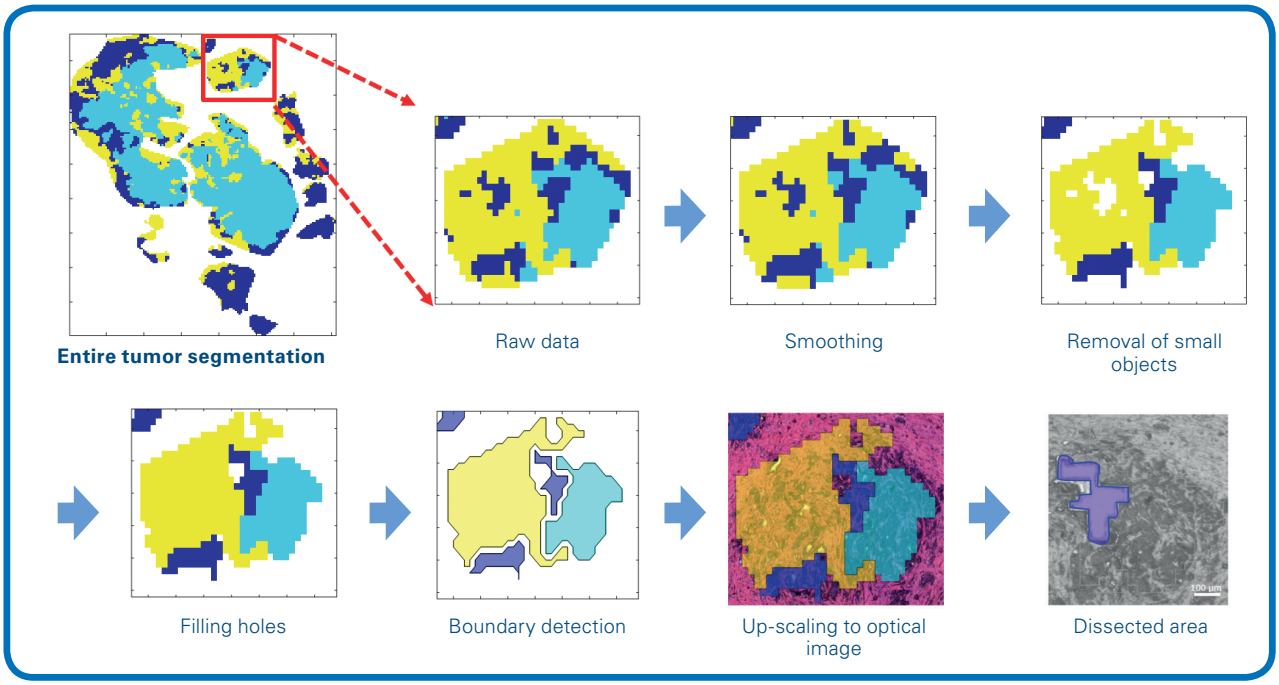

Figure 17: Image processing pipeline to produce ROI boundary information as x,y-coordinates for laser capture microdissection from segmentation raw data. The workflow includes smoothing, removal of small objects, holes filling, boundary detection and up-scaling to the optical image.

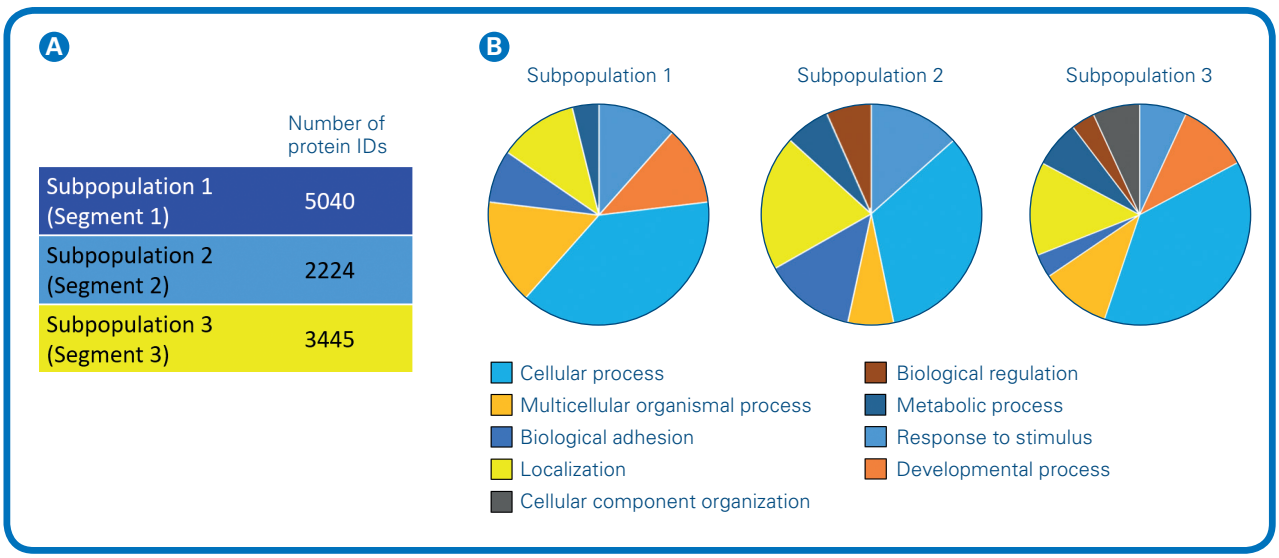

Figure 18: Proteomics of the three excised tumor subpopulations (segmented from Imaging data) reveal different biological process characterization based on the identified proteins. Only SpatialOMx delivers such a high degree of regional specificity for biological activity. Proteins from microdissected tissue (approx. 160 ng) were extracted, digested with trypsin and peptide extracts were run on the timsTOF fleX using PASEF. Number of protein IDs per tumor subpopulation segment (A) and biological process characterization per segment as revealed by PANTHER (B).

Summary: The timsTOF fleX is *the* tool for SpatialOMx

SpatialOMx is the next-generation method for in-situ characterization of tissue, and the ability to map molecular distributions label-free, determine regional bioactivity, and annotate morphological properties will be unique to the local research community. Such an innovative approach will directly impact the research of anyone who is studying cellular processes and disease progression at the tissue level and enable them to lead their fields. Label-free MALDI Imaging is capable of mapping the distribution of more biologically relevant molecules and molecular classes in a single tissue section than immuno-histochemistry or other tag-based imaging techniques. From this data, it is possible to discover cell phenotypes invisible to histology and target selectively for deeper 4D-Omics analysis. The timsTOF fleX is the only tool for SpatialOMx - delivering the fastest and most sensitive platform for 4D-Omics workflows, with a seamlessly integrated high-speed and high spatial resolution MALDI Imaging source. The next-generation step for anyone currently doing Omics research is SpatialOMx.

Relevant Publications:

[1] Spraggins JM, Djambazova KV, Rivera ES, Migas LG, Neumann EK, Fuetterer A, Suetering J, Goedecke N, Ly A, Van de Plas R, Caprioli RM (2019). *High-Performance Molecular Imaging with MALDI Trapped Ion-Mobility Time-of-Flight (timsTOF) Mass Spectrometry.* Analytical Chemistry, **91**, 14552-14560, https://doi.org/10.1021/acs.analchem.9b03612

[2] Boughton BA, Thomas ORB, Demarais NJ, Trede D, Swearer SE, Grey AC (26 October 2019). *Detection of small molecule concentration gradients in ocular tissues and humours.* Journal of Mass Spectrometry, https://doi.org/10.1002/jms.4460

[3] Finotello F, Eduati F (05 Oct 2018). *Multi-Omics Profiling of the Tumor Microenvironment: Paving the Way to Precision Immuno-Oncology.* Frontiers in Oncology, https://doi.org/10.3389/fonc.2018.00430

[4] Tellez-Gabriel M, Ory B, Lamoureux F, Heymann M-F, Heymann D (2016). *Tumour Heterogeneity: The Key Advantages of Single-Cell Analysis.* International Journal of Molecular Sciences, **17**, 2142, https://doi.org/10.3390/ijms17122142

[5] Hänela V, Pendleton C, Witting M (2019). *The sphingolipidome of the model organism Caenorhabditis elegans.* Chemistry and Physics of Lipids, **222**, 15-22, https://doi.org/10.1016/j.chemphyslip.2019.04.009

[6] Meier F, Brunner A-D, Koch S, Koch H, Lubeck M, Krause M, Goedecke N, Decker J, Kosinski T, Park MA, Bache N, Hoerning O, Cox J, Räther O, Mann M (2018). *Online parallel accumulation – serial fragmentation (PASEF) with a novel trapped ion mobility mass spectrometer.* Molecular & Cellular Proteomics, https://doi.org/10.1074/mcp.TIR118.000900

[7] Meier F, Beck S, Grass N, Lubeck M, Park MA, Raether O, Mann M (2015). *Parallel Accumulation–Serial Fragmentation (PASEF): Multiplying Sequencing Speed and Sensitivity by Synchronized Scans in a Trapped Ion Mobility Device.* J. Proteome Res. 2015, **14**, 12, 5378-5387, https://doi.org/10.1021/acs.jproteome.5b00932

[8] Dewez F, Oejten J, Henkel C, Hebeler R, Neuweger H, De Pauw E, Heeren RMA, Balluff B (2020). *MS Imaging–Guided Microproteomics for Spatial Omics on a Single Instrument.* Proteomics, 2020, https://doi.org/10.1002/pmic.201900369

Appendix

SpatialOMx Protocol:

1. A thin section of tissue is mounted onto an IntelliSlide, and a digital image of the slide is acquired using the TissueScout scanner.
2. A thin layer of fleXmatrix is applied to the slide using the TM sprayer, which is also available from Bruker.
3. The IntelliSlide is loaded into the timsTOF fleX for MALDI Imaging.
4. User defines portions of the sample to image.
5. MALDI Images are analyzed using SCiLS Lab software, which will aid in identifying spatially relevant molecular changes.
6. Microextractions are collected from the spatially relevant regions of the tissue.
7. Extracted solutions are analyzed on timsTOF fleX for 4D-Omics.
8. 4D-Omics data is analyzed and parsed through appropriate databases for compound identifications.
9. Spatially relevant signals identified by SCiLS Lab are matched to the identified compound list using accurate m/z and collisional cross-section (CCS).
10. SCiLS Lab presents user with a list of annotated images.

Required Instrumentation to fully equip a SpatialOMx lab

Execution of a SpatialOMx workflow requires the timsTOF fleX and accessories for both LC-MS/MS and MALDI Imaging workflows. MALDI Imaging sample preparation requires the following accessories: TM Sprayer from HTX Imaging to apply matrix; SpatialOMx Starter Kit, which includes SCiLS Lab Core analysis software, TissueScout slide scanner, IntelliSlides and fleXmatrix. A cryostat and/or microtome is needed for sectioning specimens. For LC-MS/MS analyses, the timsTOF fleX is optimized for use with Bruker's Elute family of LC systems. Most common LC systems are also compatible. Available 4D-Omics software such as PEAKS, MaxQuant, or MetaboScape can be used and depends on the specific area of study.

Instrument training and support

Bruker's extensive network of worldwide Service and Applications teams are available to provide support. Upon installation of the system, an engineer will qualify the system by demonstrating that the system meets all specifications and provides a familiarization training so that users can begin measuring straightaway. Included with each system are certificates for users to attend one of the many training sessions held throughout the year at Bruker's demo facilities. These sessions cover in greater depth components of the SpatialOMx workflows. At any time, Bruker scientists or engineers are available for phone or online support at no charge.

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