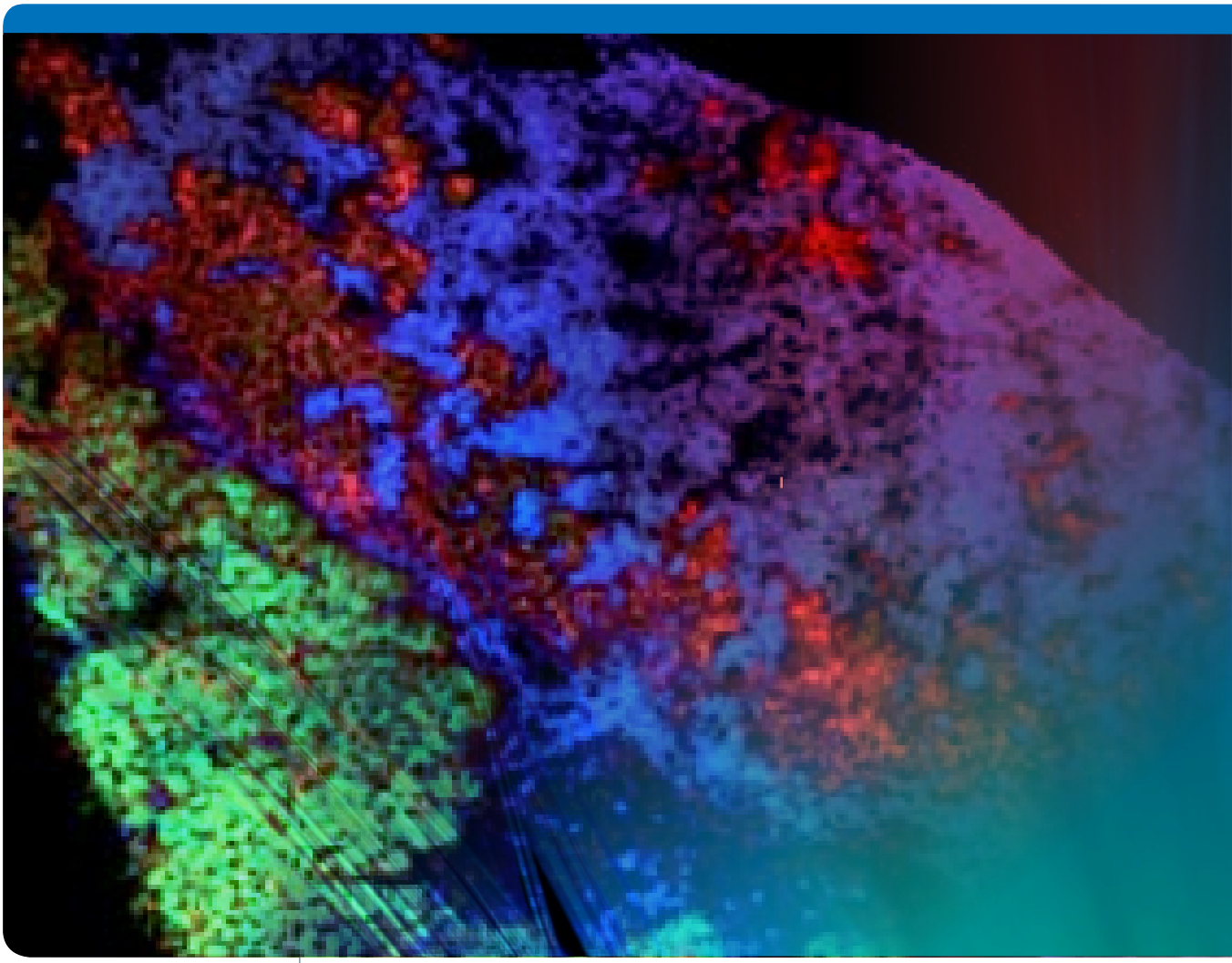
BRUKER



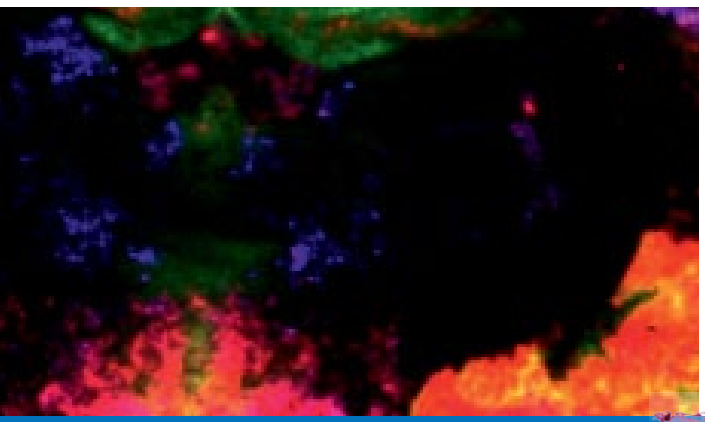
MALDI Imaging –
Mass Spectrometric Imaging

- Direct Imaging of proteins, peptides, lipids, metabolites and drugs in tissues

Innovation with Integrity

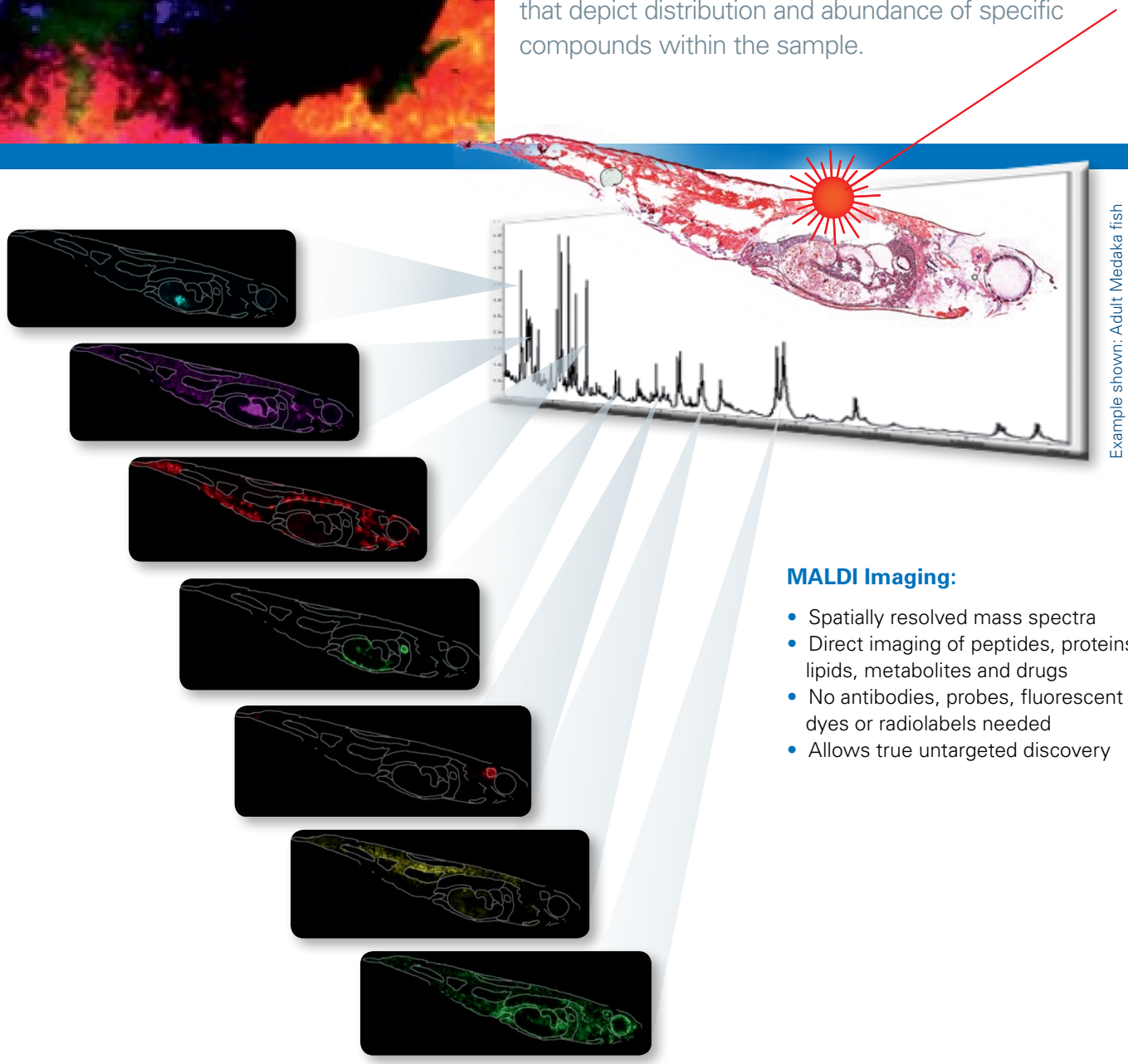
Mass Spectrometry

What is MALDI Imaging?



In MALDI Imaging, a laser scans tissue samples and a full MALDI spectrum is recorded at regular intervals.

Displaying the intensity of selected mass signals at each measurement point generates images that depict distribution and abundance of specific compounds within the sample.



Example shown: Adult Medaka fish

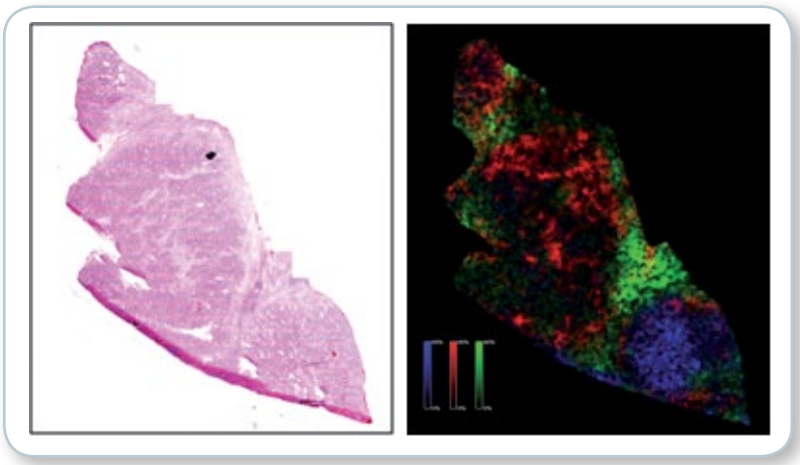
MALDI Imaging:

- Spatially resolved mass spectra
- Direct imaging of peptides, proteins, lipids, metabolites and drugs
- No antibodies, probes, fluorescent dyes or radiolabels needed
- Allows true untargeted discovery

Cancer Research and Compound Distribution Studies

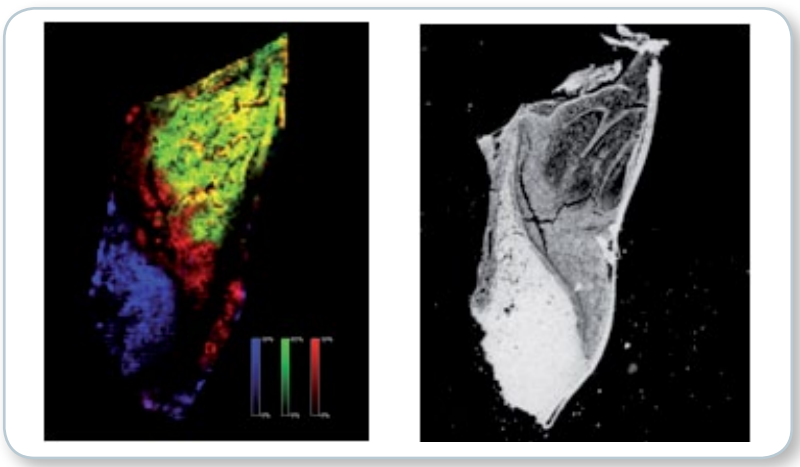
By enabling histological correlation of molecular phenotypes and correlation of molecular signals to clinical end-points, MALDI Imaging is a powerful tool in cancer research.

MALDI Imaging is the only proteomic technology that enables detailed assignment of detected biomarkers to histological features.

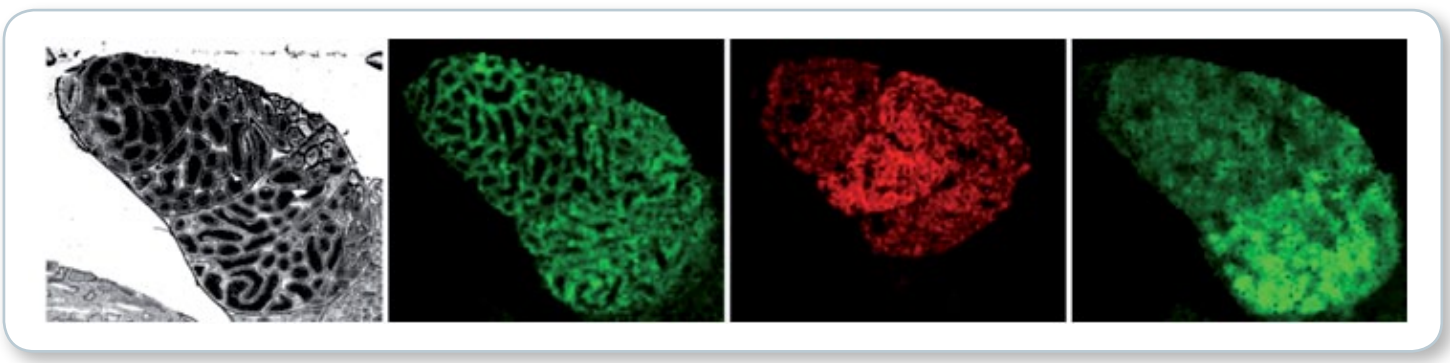


Colon cancer section: H&E stain (left) and MALDI image showing distribution of three proteins (right).

MALDI Imaging is also used to study the distribution of lipids, proteins and secondary metabolites in plants.



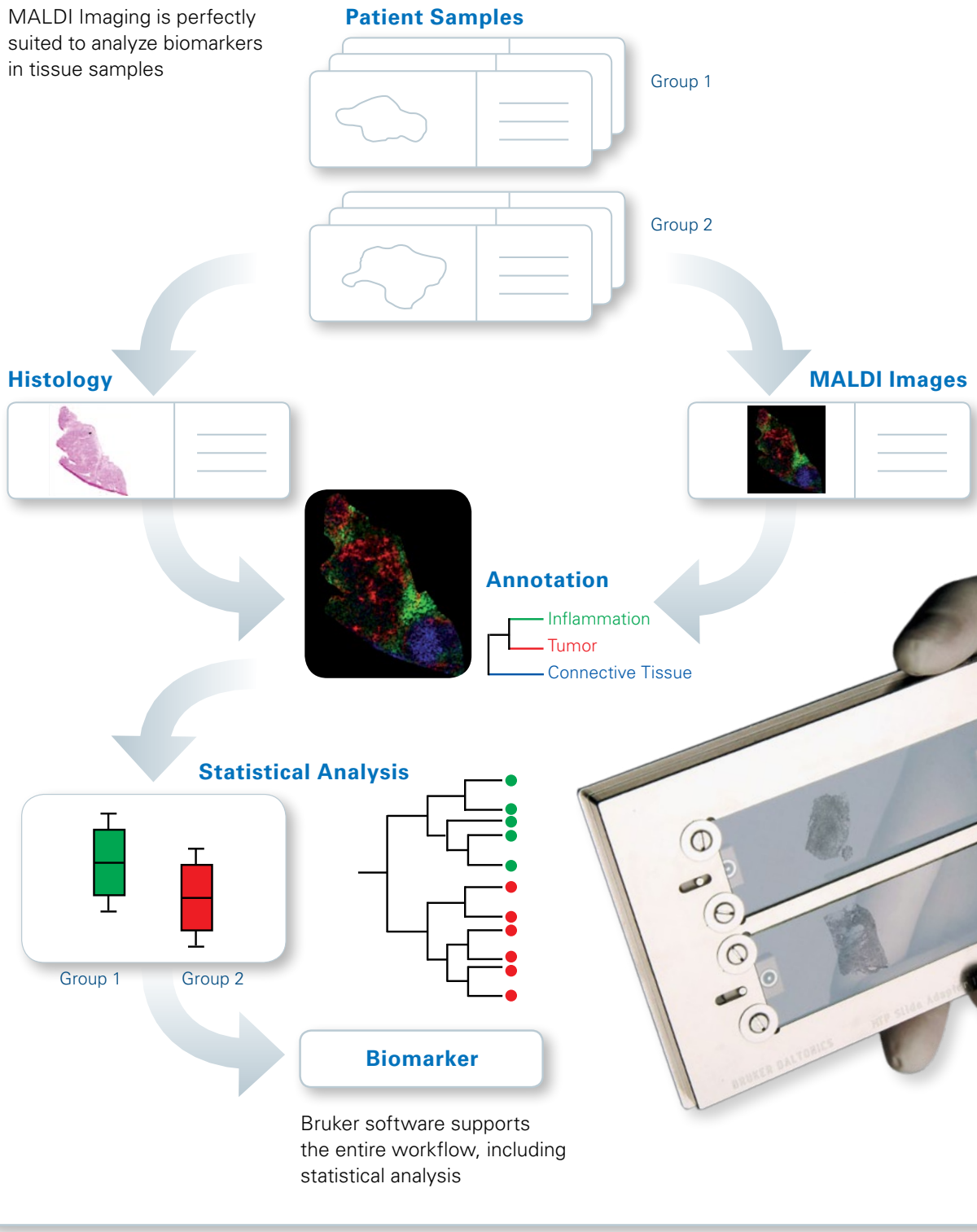
MALDI image showing distribution of three lipid signals in a barley seed (left) and optical image (right).



Rat epididymis; optical image (left) and MALDI images showing distribution of three proteins.

A Clinical Discovery Workflow

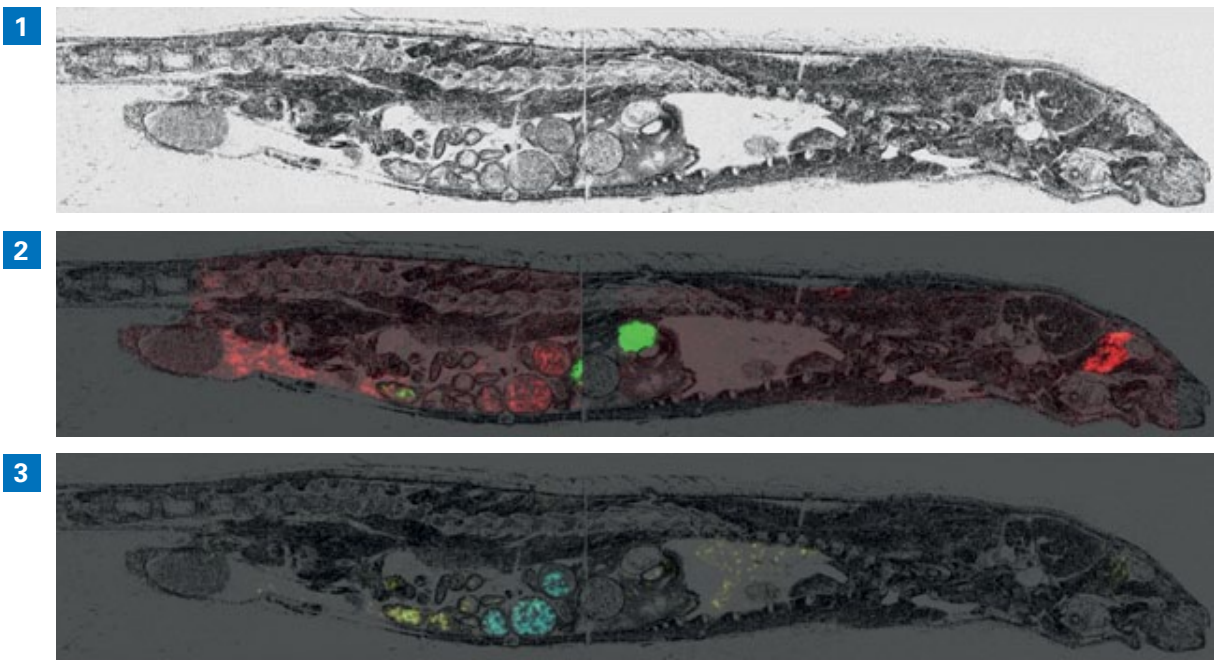
MALDI Imaging is perfectly suited to analyze biomarkers in tissue samples



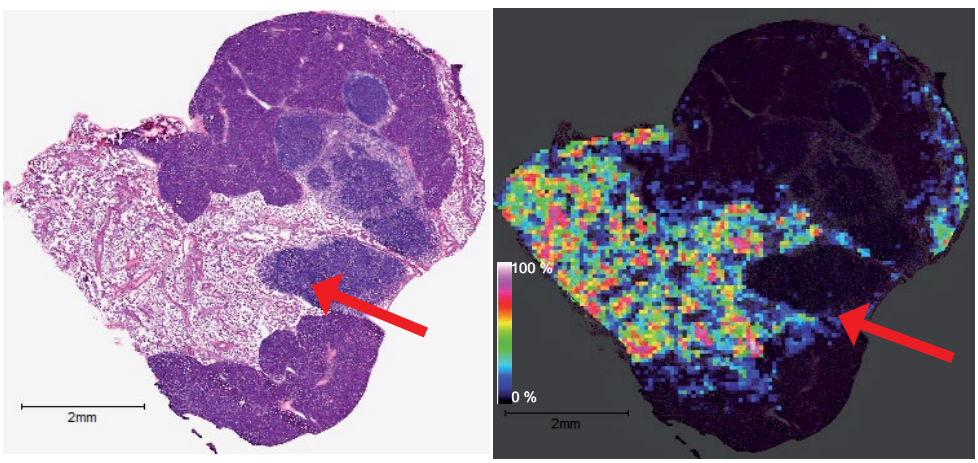
Bruker software supports the entire workflow, including statistical analysis

Drug and Metabolite Localization

MALDI Imaging is the only imaging technique that enables differentiation between pharmaceutical compounds and their metabolites.



Whole body section of a rat dosed with a pharmaceutical compound.
1) Optical image.
2) Drug (green) localized mainly in the stomach and metabolite 1 (red) localized mainly in the Harderian gland.
3) Metabolite 2 (blue) and metabolite 3 (yellow) localized in the intestines.



Mouse pancreas tumor tissue:
A comparison of histology (left) and drug distribution (right) shows that a drug is contained in the fatty tissue and does not enter the targeted tumor tissue (arrowed).

Integrating Virtual Microscopy – Gaining Biological Insights

While MALDI Imaging is usually not performed at cellular or sub-cellular resolution, the nature of the underlying tissue can only be determined by detailed microscopic analysis. For instance, the shape of cells and nuclei can be used to differentiate between cancerous and epithelial cells.

Bruker has integrated the use of digital slides with flexImaging software, giving instant access to full microscopic resolution and allowing true untargeted molecular histology.

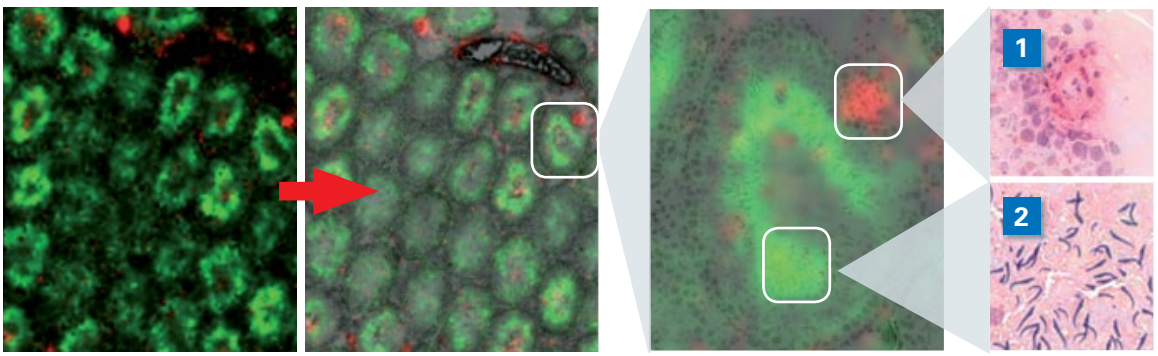
Molecular histology with MALDI Imaging

MALDI Imaging and histology are fully compatible – tissue sections can be stained after MALDI measurement. Bruker flexImaging software allows direct overlay of MALDI Imaging data onto high-resolution virtual slides, providing full access to molecular and histological information.

See a video demonstration at:

http://www.youtube.com/watch?v=YV2wzVXEvqg

Rat testis at 20 µm spatial resolution



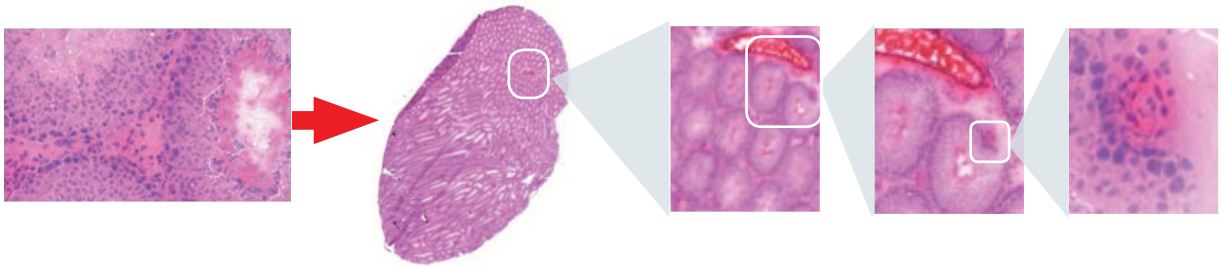
MALDI image of rat testis at 20 µm spatial resolution. Two molecular signals that highlight different features are selected.

Bruker flexImaging software allows cross-fading between the MALDI image and the superimposed virtual slide.

At higher resolution, histological features become visible in the virtual slide image.

Full resolution reveals (1) a capillary vessel and (2) nuclei of mature spermatids and demonstrates correlation of histological features and molecular signals.

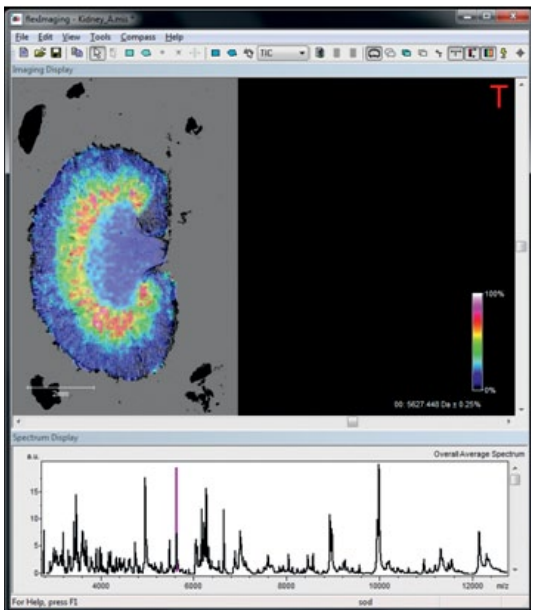
Virtual microscopy



Digital microscopy enables detailed evaluation of high-resolution histological images on a computer screen.

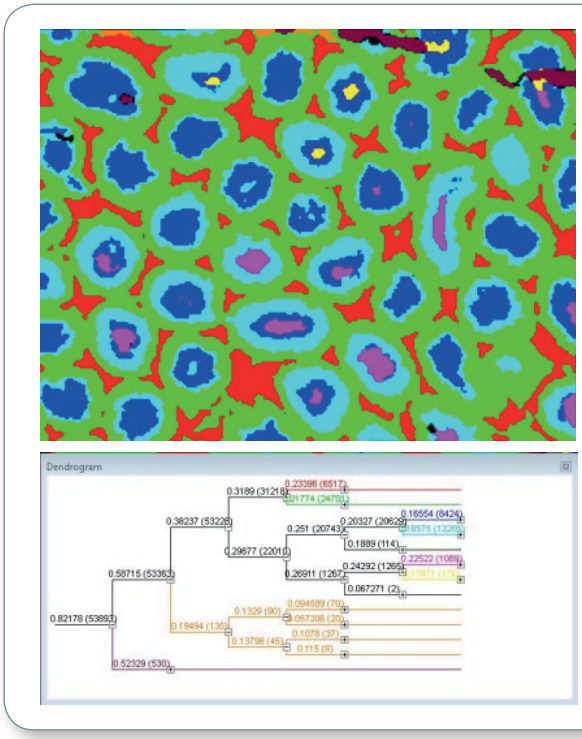
flexImaging – Leading Software for Acquisition and Visualization

- Wizard driven user-friendly set up of new imaging experiments
- Explore datasets interactively by selecting masses in spectrum view
- Direct access to single spectra
- Superimpose digital slides and optical images
- Smart data compression for FTMS spectra
- Advanced normalization options
- Easy comparison of spectra in different regions of interest
- Comparison of different datasets
- True 64-bit application supports large datasets
- Batch acquisition capabilities



Explore datasets by integrated hierarchical clustering

- Hierarchical clustering groups the mass spectra (molecular phenotypes) according to similarity
- Interactive dendrograms allow a simple and concise segmentation and annotation of complex datasets
- Additional feature extraction methods such as principal component analysis (PCA) and probablistic semantic analysis (pLSA) are available through integration with statistical software (e.g. SCiLS Lab, ClinProTools or ProfileAnalysis)



Cluster analysis of a rat testis sample with ~50k pixels. The interactive dendrogram allows a detailed and interactive segmentation into functional regions.

SCiLS Lab – Statistical Analysis Software

SCiLS Lab is the advanced software for statistical analysis of MALDI imaging data. From analysis of large individual datasets to the comparative analysis of sample cohorts for biomarker discovery, SCiLS Lab turns data into knowledge.

SCiLS Lab is the software of SCiLS, Bremen, and is a part of Bruker's MALDI Molecular Imager solution. SCiLS Lab is exclusively available for Bruker data and seamlessly integrates with Bruker's flexImaging software. It reads annotated data from flexImaging and can export results back into flexImaging.

SCiLS Lab implements the following features for mining MALDI imaging data:

Preprocessing of data

- Baseline correction
- Normalization
- Peak picking
- Edge-preserving spatial de-noising

Hierarchical structuring of large data

- Definition of "regions of interest"
- Definition of cohorts by grouping regions from multiple datasets

Univariate analysis

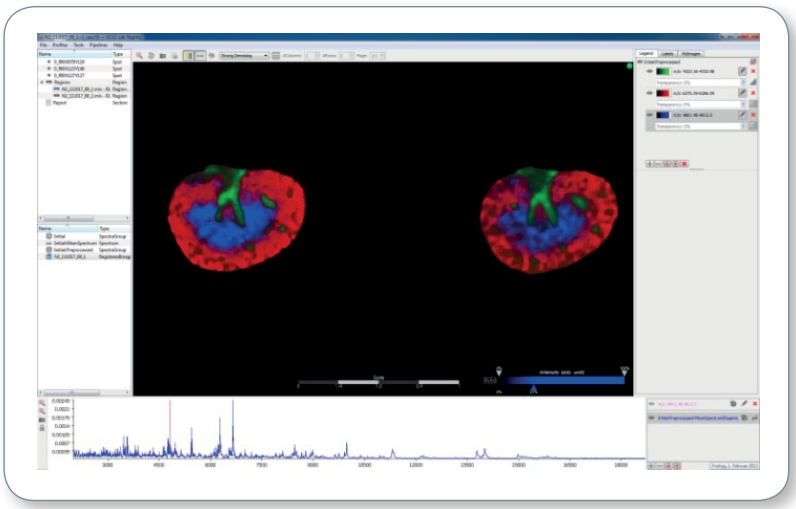
- Mean and standard deviation of peak intensities
- ROC analysis and univariate hypothesis tests
- Discovery of co-localized m/z values

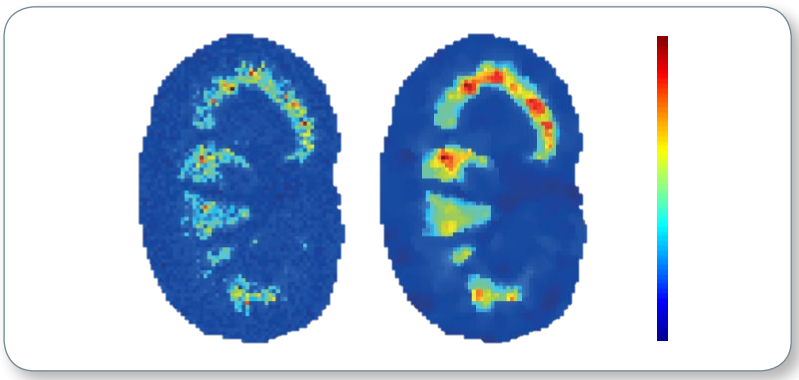
Unsupervised multivariate analysis

- Spatial segmentation of very large datasets
- Component analysis for extraction of underlying trends

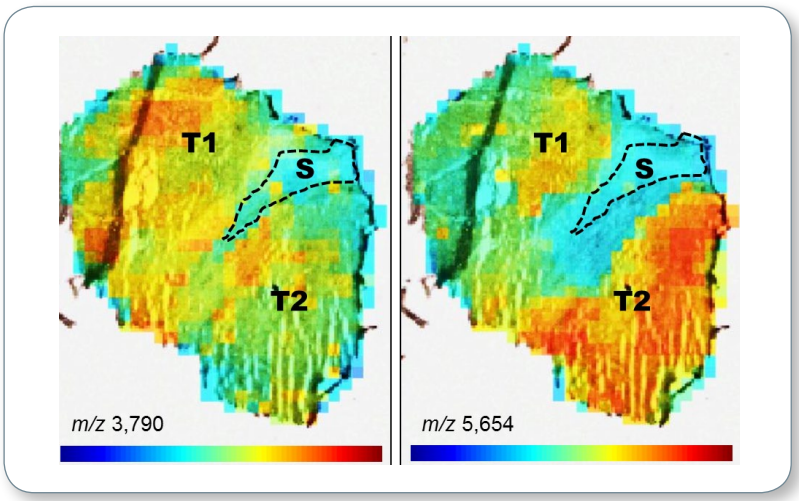
Supervised multivariate analysis

- Calculation of classifiers based on training data
- Classification of samples





Imaging data before (left) and after (right) edge-preserving de-noising.



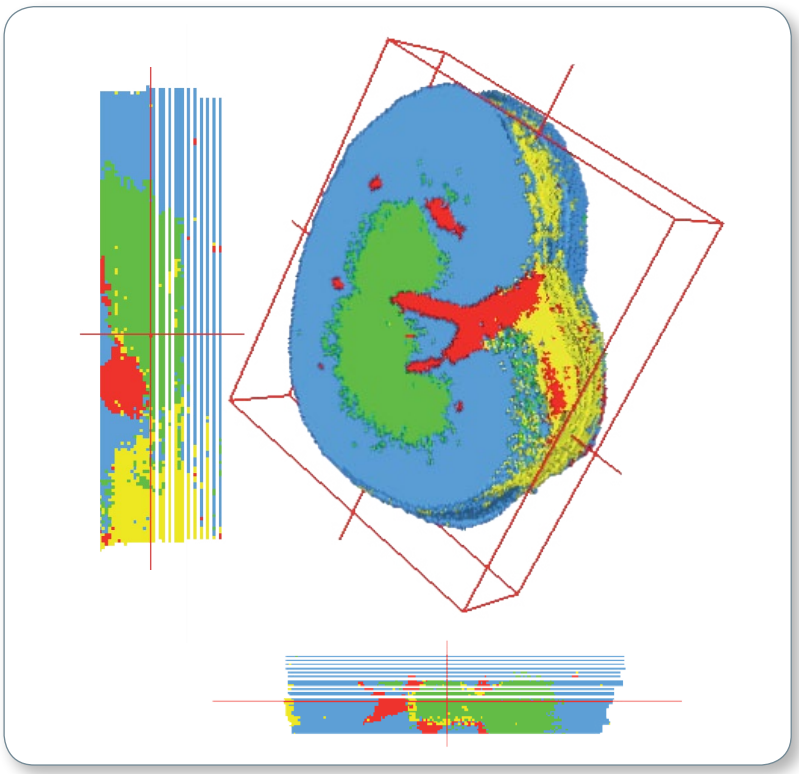
Biomarker discovery of hepatocellular carcinoma with microvascular invasion [Pote et al., Hepatology, 2013, doi: 10.1002/hep.26433].

Coming soon!

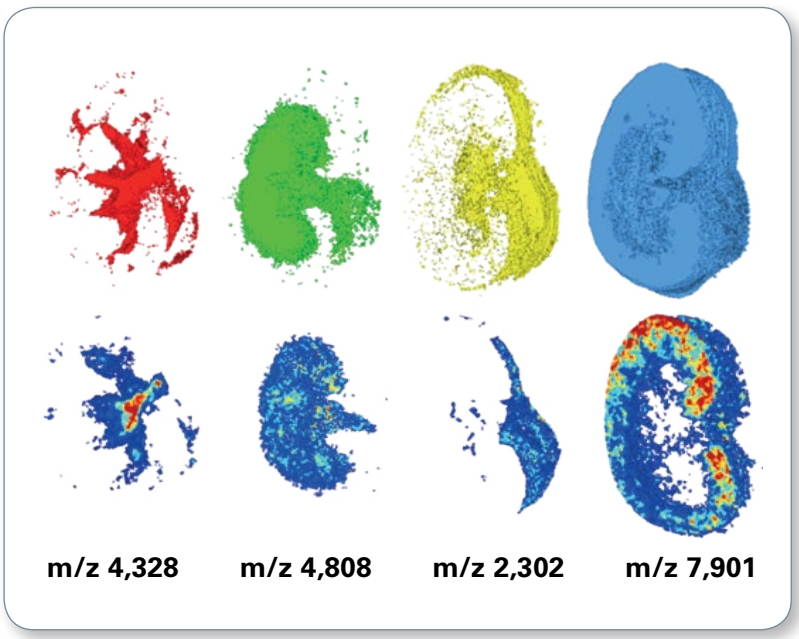
SCiLS Lab 3D – MALDI Imaging Goes 3D

SCiLS Lab 3D is the dedicated software allowing for the reconstruction of spatial 3D models from serial MALDI Imaging datasets.

- Easy computer-assisted alignment of serial sections
- Rotate the dataset in all directions
- Low-intensity voxels can be made transparent to reveal a look inside 3D spatial distribution
- 3D spatial segmentation including search for co-localized mass signals
- All other beneficial features from SCiLS Lab 2D



Spatial segmentation of a 3D mouse kidney dataset in SCiLS Lab 3D. The dataset consists of 122 serial sections measured at a spatial resolution of 50 µm. The dataset consists of approximately 2 million spectra. Acquisition was done on a MALDI-TOF instrument in linear mode. For more details, see: Oetjen et al., *Journal of Proteomics*, 2013, doi: 10.1016/j.prot.2013.03.013.



Individual clusters of the 3D mouse kidney dataset and co-localized mass signals for: renal pyramids (red), medulla, pelvis and ureter (green), sinus (yellow) and cortex (blue). For more details, see: Oetjen et al., *Journal of Proteomics*, 2013.

About SCiLS: SCiLS is an R&D company with the mission to bridge the gap between data and knowledge. Built on innovation and enabled by the broad expertise from the bench to software engineering, SCiLS develops and supports software solutions making cutting-edge research accessible.

For more information, see www.scils.de

Quantinetix – Dedicated Software for Drug Quantification

Quantinetix™ is a **Quantitative Imaging Mass Spectrometry Software** that provides quantitation of target compounds (taking into account the ion suppression effect) following Mass spectrometry imaging experiments.

Quantinetix is developed by ImaBiotech, a leading service provider for MALDI Imaging.

Quantinetix™ supports Bruker MALDI-TOF and FTMS data. It normalizes and quantifies molecules in several ways:

- On-tissue dilution (Nilsson et al., PLoS One, 2010)
- Isotopic labeling compound (Stoeckli et al., Int. J Mass Spectrom, 2007)
- Ion suppression calculation (Hamm et al., ImaBiotech, Journal of proteomics Sept 2012)

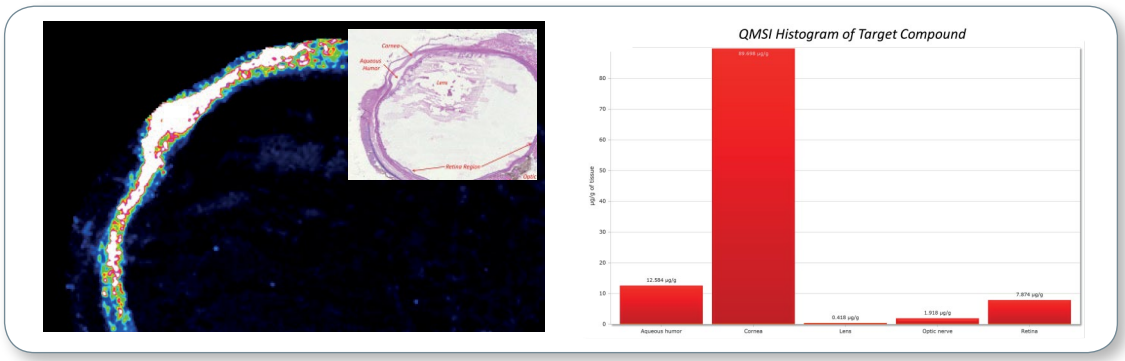
Dedicated to research, Quantinetix™ is a **user-friendly imaging program** that offers "**quantitative images**" and **quantitation of your target compounds in over 25 organs in Whole body distribution, as well as in smaller organs** such as the eye or brain via the calculation of normalized ion suppression.

About ImaBiotech: ImaBiotech is a biotechnology company specialized in molecular analysis (identification and quantification).

Founded by a team of experts in MALDI Imaging Mass Spectrometry, ImaBiotech offers services and products in the healthcare field in order to accelerate the launch of medications on the market, and to strengthen the precision and applications for new medical applications.

For additional information see: www.imabiotech.com

imabiotech
accelerating discovery, driving innovation



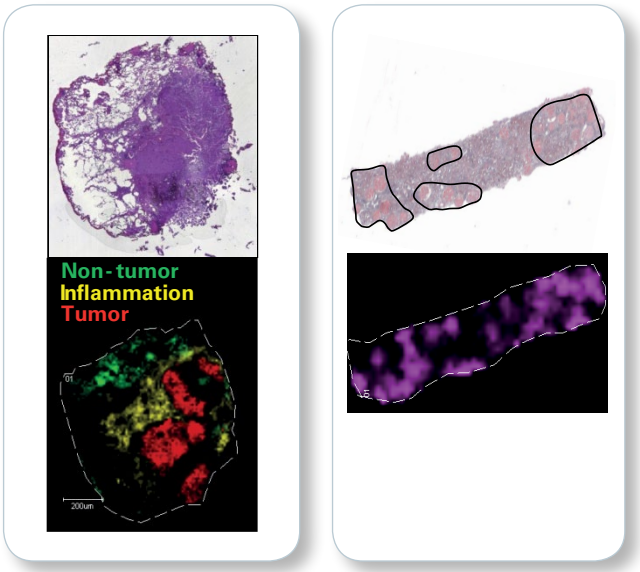
Example: Quantification of a drug compound in rabbit eye.

Formalin Fixed Tissue – ImageID

Formalin fixation leads to crosslinking of the proteins in the tissue. To analyze such samples an antigen retrieval step followed by a spatially resolved tryptic digest is necessary. This digest releases tryptic peptides that can be measured by MALDI Imaging. This tryptic digestion can be done with Brukers ImagePrep™ device.

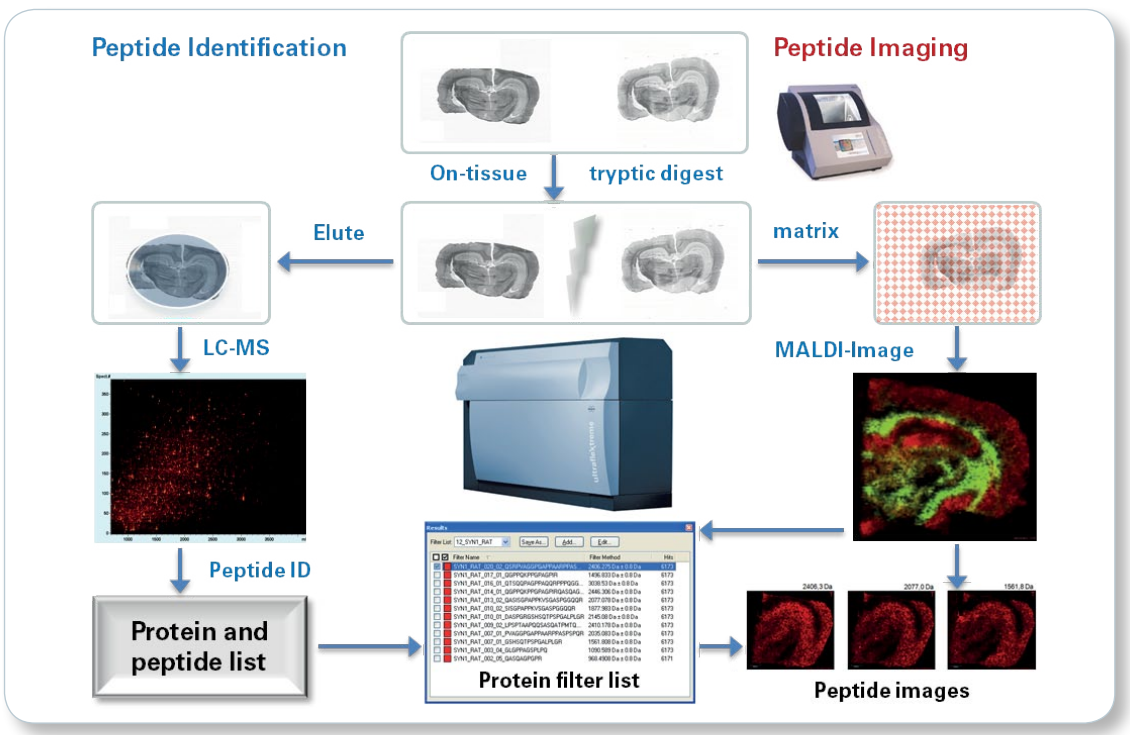
Highly abundant tryptic peptides can be identified by MS/MS directly off tissue, but the identification results will always be superior after an LC-separation.

Brukers imageID workflow combines direct MALDI Imaging with LC-MS identification. It allows the software assisted correlation of LC-MS and MALDI Imaging data for the comprehensive identification of tryptic peptides.



Left: H&E staining and MALDI Image of a Formalin fixed Paraffin embedded lung cancer section. Different tryptic peptides are displayed in the MALDI Images. They highlight distinct areas in agreement with the histomorphological examination.

Right: Kongo red staining and MALDI Image of a serum amyloid peptide in an Formalin fixed paraffin embedded amyloidosis sample. Areas with high amyloid density are marked on the stained section.



Overview of the imageID workflow for the comprehensive identification of tryptic peptides from tissue.

Sample Preparation

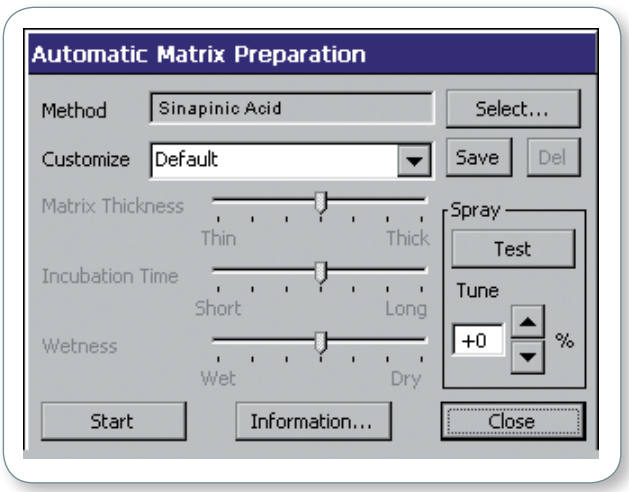
High-quality sample preparation is key to successful MALDI Imaging

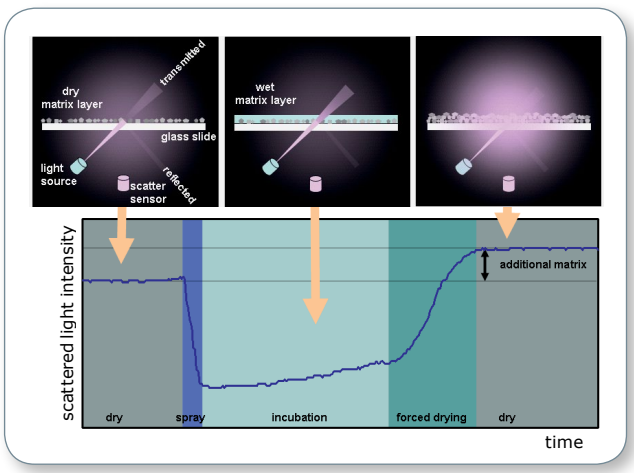
The Bruker ImagePrep provides automatic and reproducible sample preparation in an automated push-button procedure.

An intuitive user interface allows convenient adjustment of key parameters: matrix thickness, incubation time and wetness.

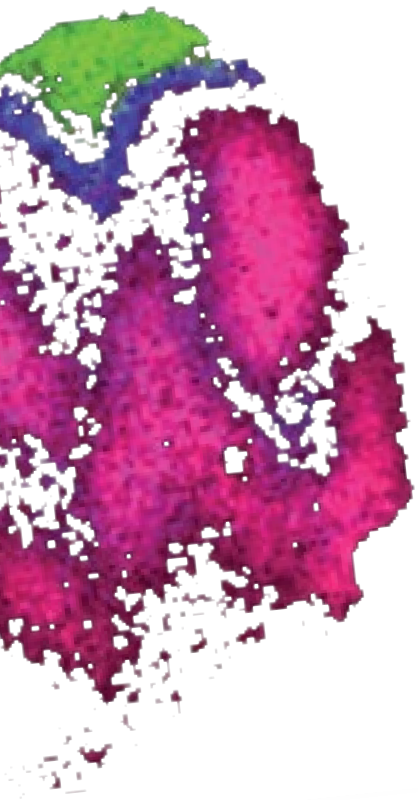
The matrix aerosol is generated by vibrational vaporization. Soft gravitational deposition of microdroplets delivers maximum spatial resolution.

ImagePrep can also be used to perform spatially resolved tryptic digests of tissue sections, which facilitates the study and identification of large proteins and FFPE tissue. See Application Note MT-98 for more details.





Patented optical sensor monitors wetness, drying rate and matrix build-up for maximum quality sample preparation.

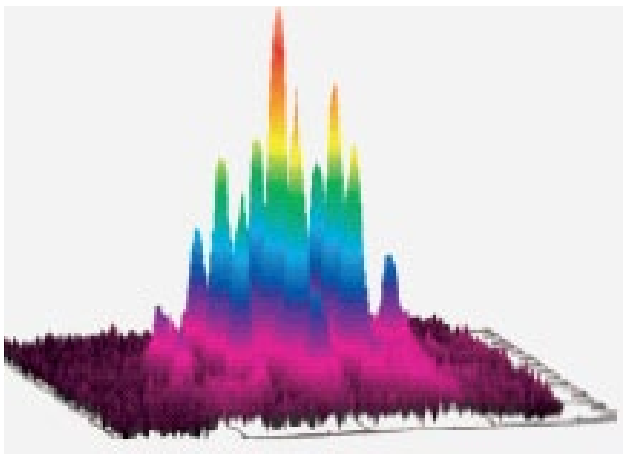


Peerless Ion Source Design

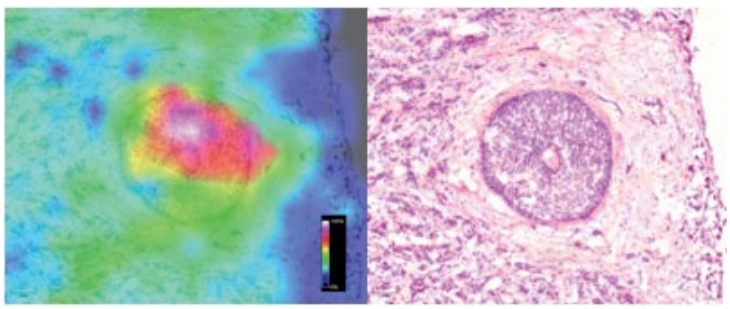
smartbeam™ II laser technology

The optimized energy profile of the proprietary smartbeam II laser ensures high quality spectra from a wide variety of matrices.

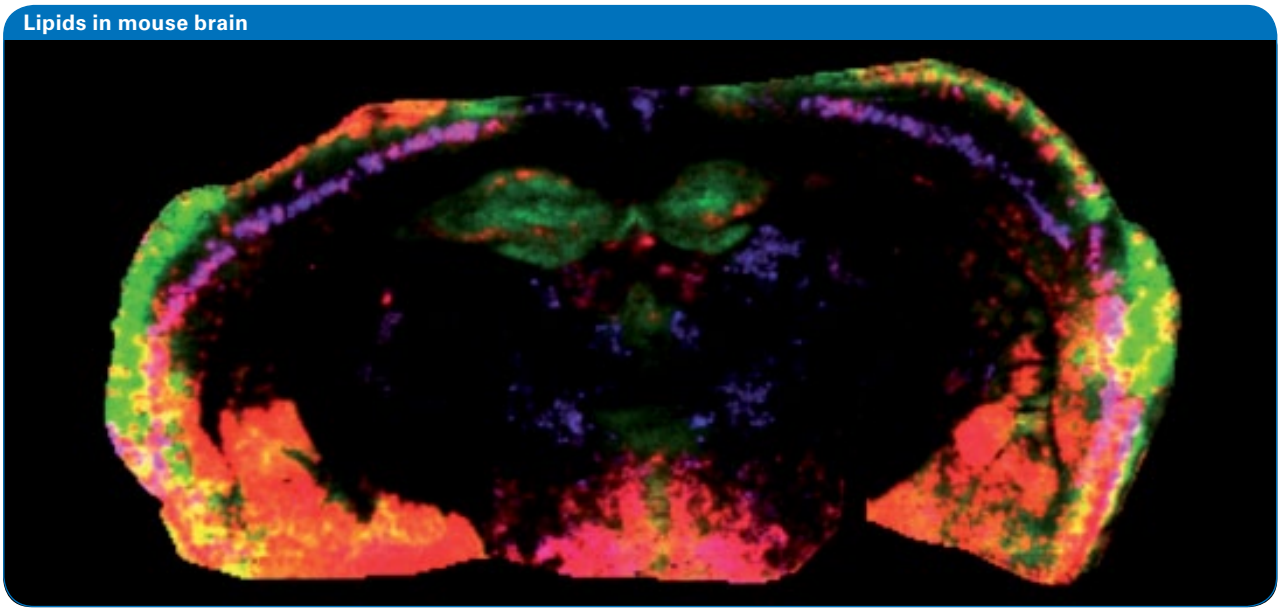
- Adjustable focus diameter for high spatial resolution
- Up to 2000 Hz repetition rate for fast acquisition
- 3.5 x 10^{10} shots lifetime



The unique soft ionization properties of the smartbeam laser leave tissue intact, enabling subsequent histological staining and correlation of molecular and histological features.

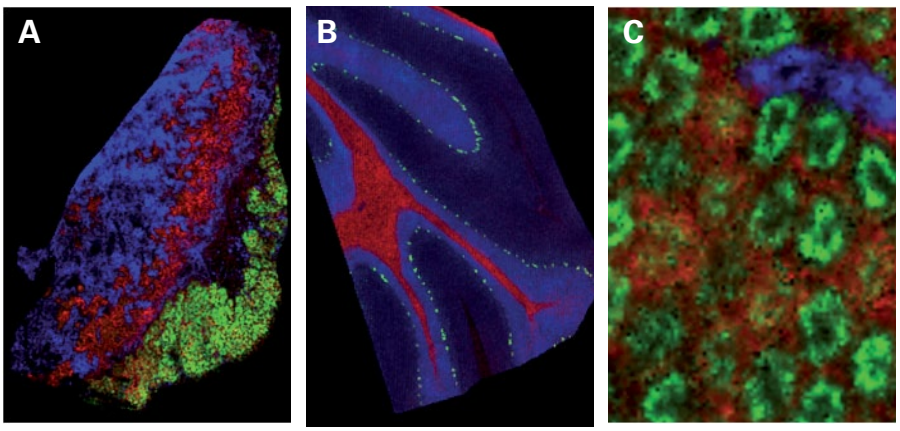


Lipids in mouse brain

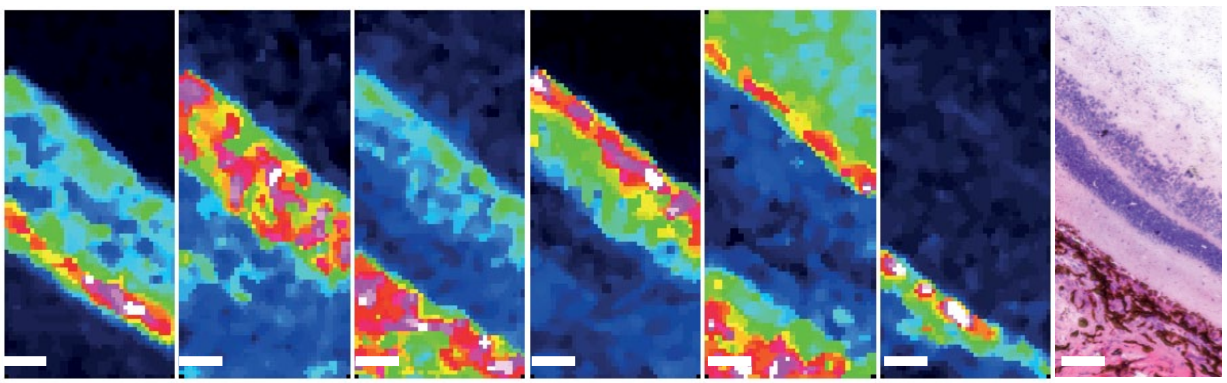


autoflex speed and ultrafleXtreme

- High-end MALDI-TOF and TOF/TOF instruments
- Fast smartbeam II laser for fast high-quality MALDI Imaging (2 kHz with ultrafleXtreme, 1 kHz with autoflex speed)
- Linear mode for detection of intact proteins
- Reflectron mode for the detection of peptides, lipids and small molecules
- FAST-SRM mode for convenient targeted analysis of small molecules and drugs
- Perpetual Ion Source for automated source cleaning



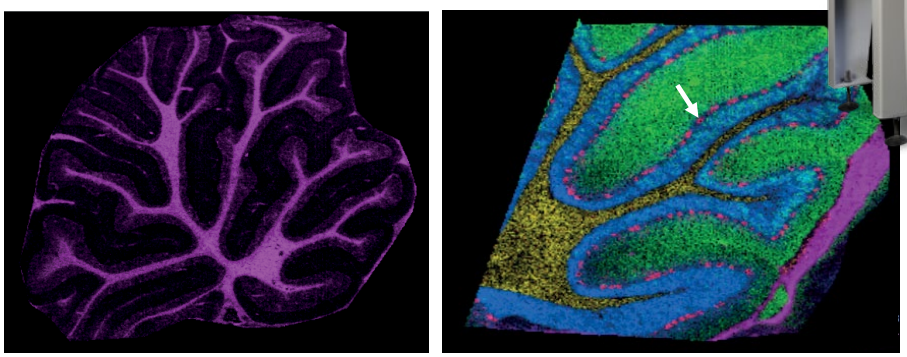
A: Proteins in bladder cancer section; B: Lipids in rat cerebellum at 10 µm pixel size. Individual Purkinje cells are resolved (green channel); C: Proteins in rat testis



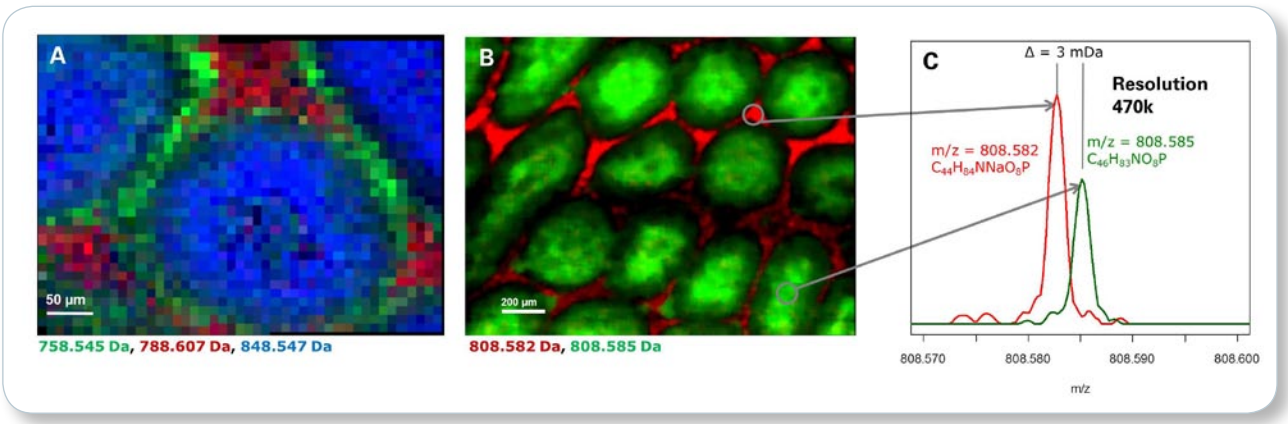
Lipids in pig retina measured at 10 µm pixel size show the different layers (right: H&E stain). Scale bar 100 µm.

solariX XR

- Extreme mass resolution in excess of 10 million
- Smartbeam II laser for high spatial resolution
- Unparalleled performance for small molecule measurements
- Perfectly suited for metabolomics and drug applications
- CASI™ mode: Continuous accumulation of selected ions for maximum sensitivity
- Isotopic fine structure allows direct readout of molecular formulas of unknown compunds
- Dual source for MALDI and electrospray



Lipids in rat cerebellum at 15µm pixel size (left) and 10µm pixel size (right). Individual purkinje cells cann be seen at 10µm pixel size (purple channel, one Purkinje cell is indicated by the arrow).



In this rat testis dataset, the two displayed lipid signals have a mass difference of only 3 mDa. The signal shown in green is found in the seminiferous tubules, the one in red is seen in the interstitial space. At a resolution of 470k the signals are clearly resolved.

MALDI Imaging Instrumentation and Software

Mass spectrometry and sample preparation systems

- ImagePrep provides high-quality, easy-to-use sample preparation and enables high-sensitivity imaging experiments.
- autoflex MALDI-TOF and ultrafleXtreme MALDI-TOF instruments are equipped with the smartbeam laser for high-quality mass spectra and images.
- solariX XR FTMS provides highest resolution MALDI Imaging of small molecules, peptides and lipids.

Integrated software solutions

- flexImaging dedicated molecular histology software controls data acquisition, data visualization and image generation from tissue samples.
- Sophisticated biostatistical software enables tissue-related biomarker candidate searches and tissue class profiling using pattern profiling algorithms.

"MALDI Imaging gives us for the first time the possibility to generate molecular images without the need for molecular probes. These molecular phenotypes can give us additional insight where the histological phenotype is not sufficient."

Dr. Axel Walch, Pathologist,Helmholtz-Centre Munich, Germany

"MALDI-MS Imaging fills an analytical void by permitting us to discretely examine the distribution of parent drug and metabolites in tissues. We have been able to achieve the spatial and spectral resolution required to examine sub-compartment tissue distributions and correlate them with histology in the preclinical setting. This ability to link chemistry and biology is permitting us to more closely examine the basis of drug toxicity and pharmacology as well as refine our understanding of pharmacokinetics and drug transport."

Stephen Castellino, PhD; Director US SID, US TKB, GlaxoSmithKline, USA

"In mass spectrometry, technology achievements can also be measured by the involvements of manufacturers. Imaging mass spectrometry is no exception. In this regard, in partnership with academia, Bruker Daltonics has been instrumental in proposing a complete, comprehensive and convivial imaging solution."

Dr. Pierre Chaurand, Associate Professor, Université de Montréal, Canada

"ImaBiotech purchased several Bruker instruments because their devices' high performance fit perfectly with our MALDI Imaging technology platform to provide superior services to the pharmaceutical and biomedical industries. Our extensive research made possible by integrating Bruker into our repertoire is proof of the unparalleled quality and robustness of Bruker technology."

Dr. Jonathan Stauber, CEO ImaBiotech, France

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