MALDI Imaging – Drug Imaging

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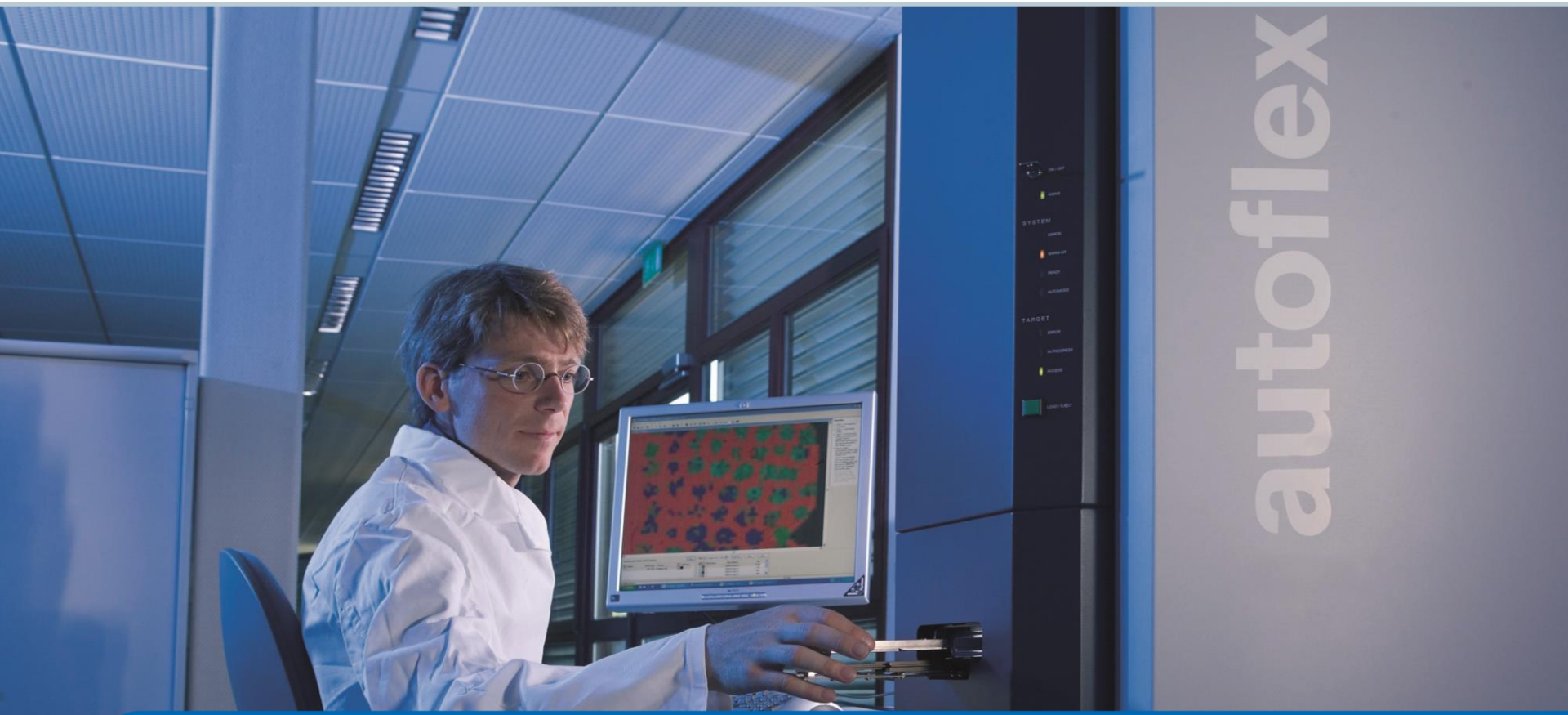
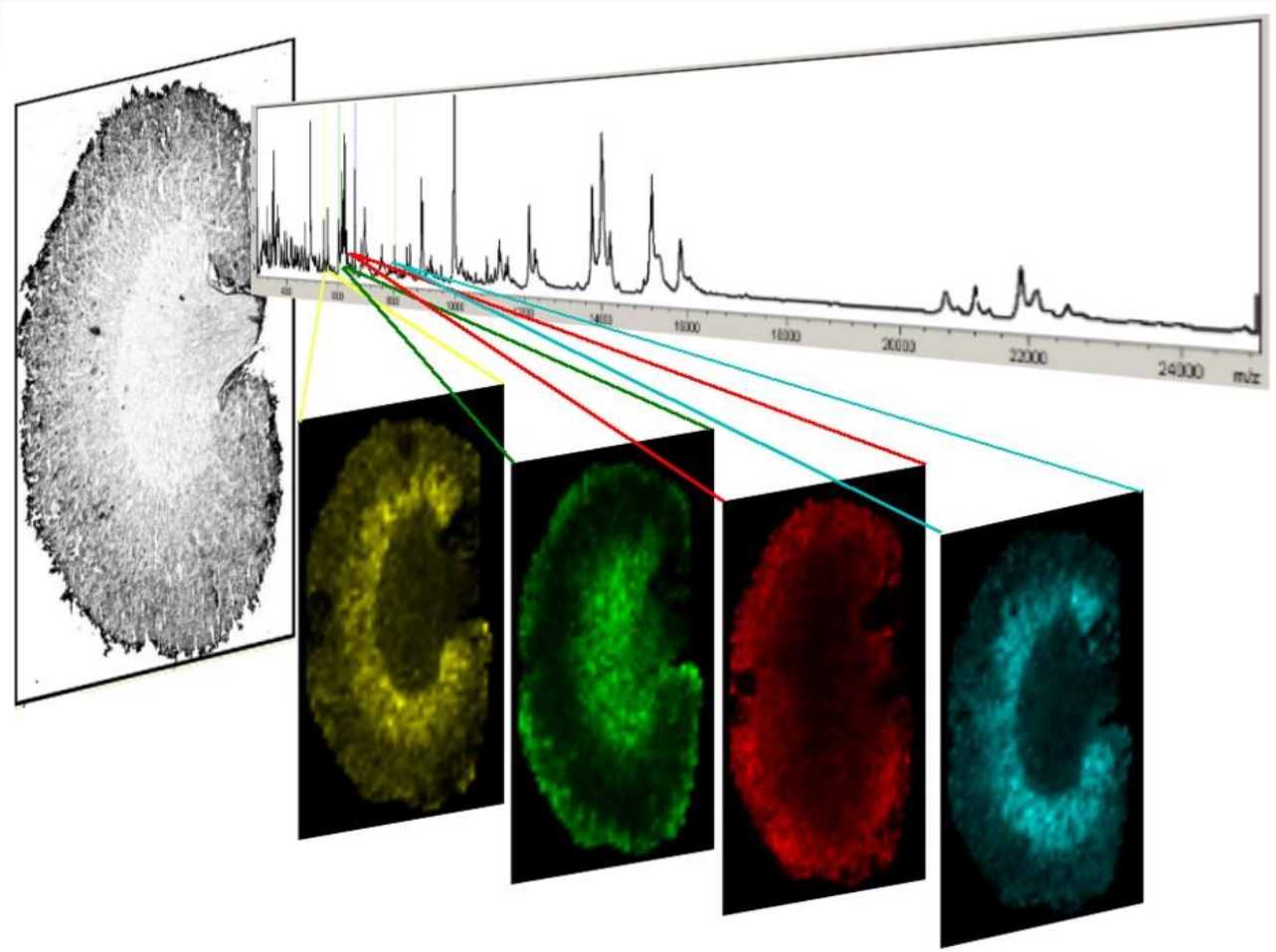
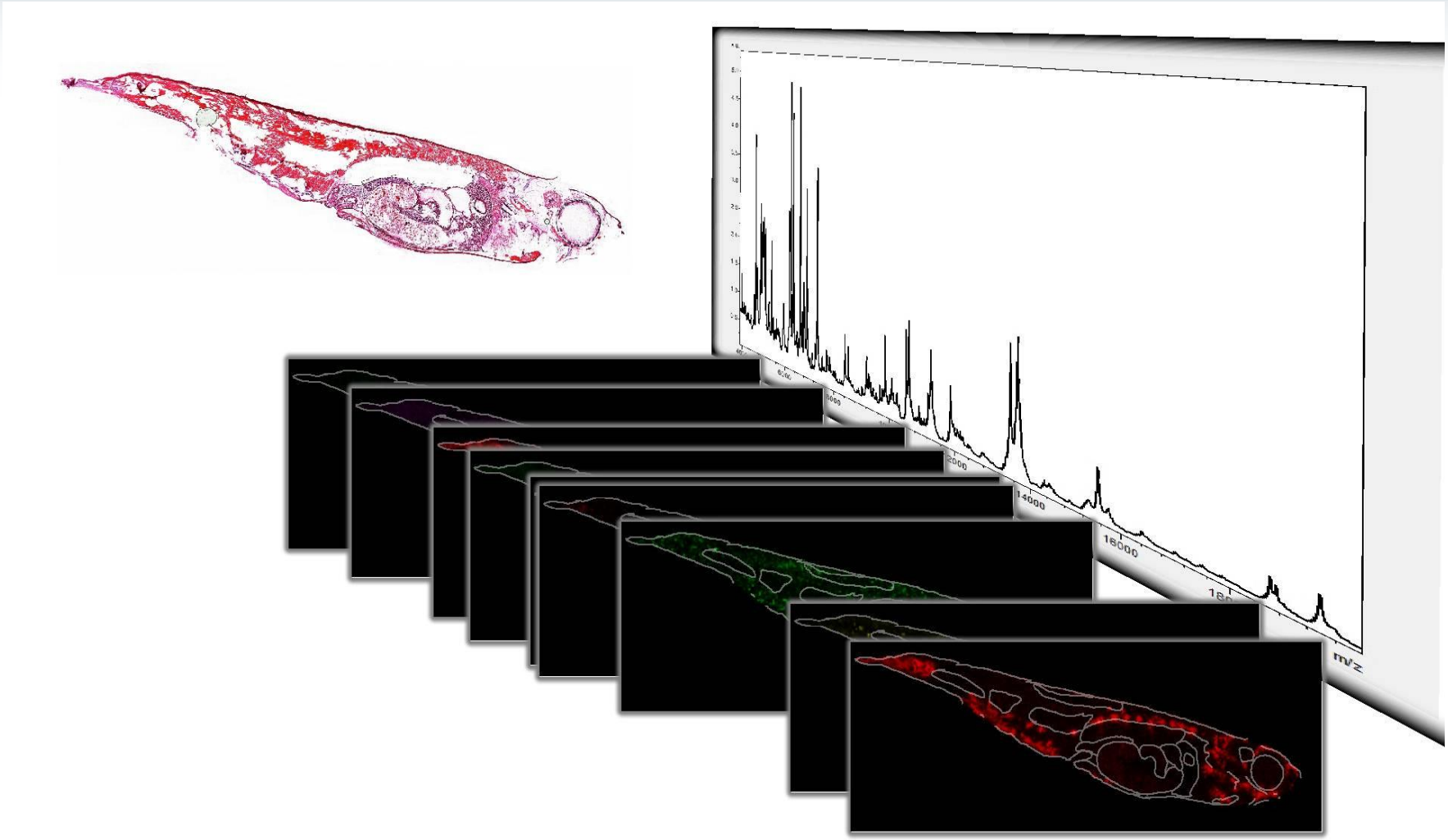


The principle of MALDI imaging



- Spatially resolved mass spectra are recorded
- Each mass signal represents a molecule (protein, peptide, drug, metabolite….)
- Molecular images of the distribution of those compounds are reconstructed

Example: Proteins in adult medakafish



MALDI imaging applications

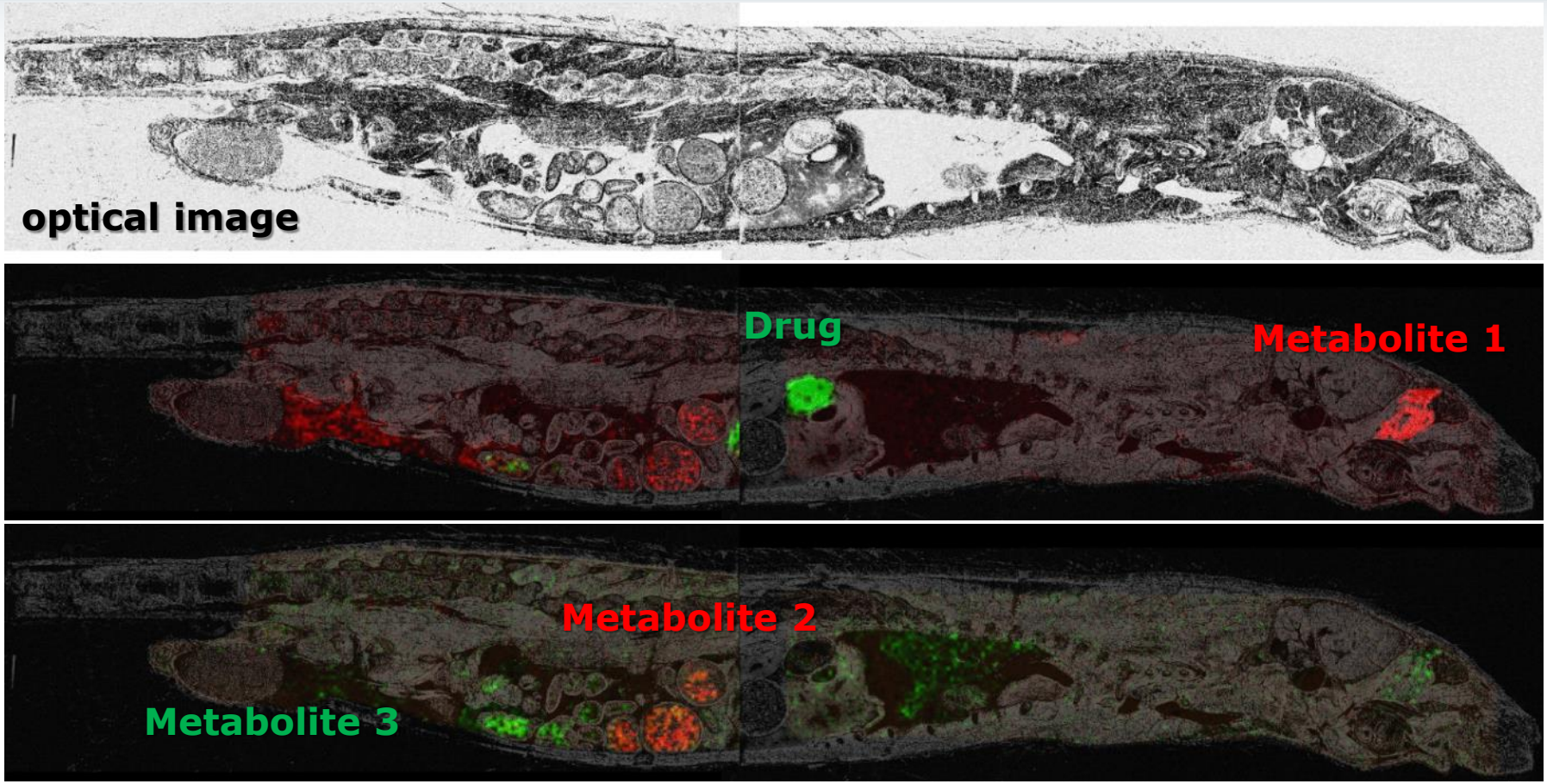
- **Drug development**: MALDI imaging allows label-free detection of drug and metabolite distribution in tissue
- **Cancer research**: MALDI imaging allows untargeted detection of tissue specific molecular phenotypes and comparison of patient cohorts
- **Lipid research** and **metabolomics**: Untargeted analysis of lipids and endogenous metabolites
- **Molecular histology**: Combination of virtual microscopy with MALDI imaging adds a molecular dimension to histology

MALDI imaging in DMPK – how it started

- MALDI imaging as a tool to complement whole body autoradiography (WBA)

	MALDI imaging	WBA
sensitivity	50-200 ng/g, drug dependent can be lower	high
cost	low	high
quantitation	requires extra effort	yes
unique advantages	No radioactivity Can differentiate drug and metabolites	

Examples: Drug in Whole Body Rat



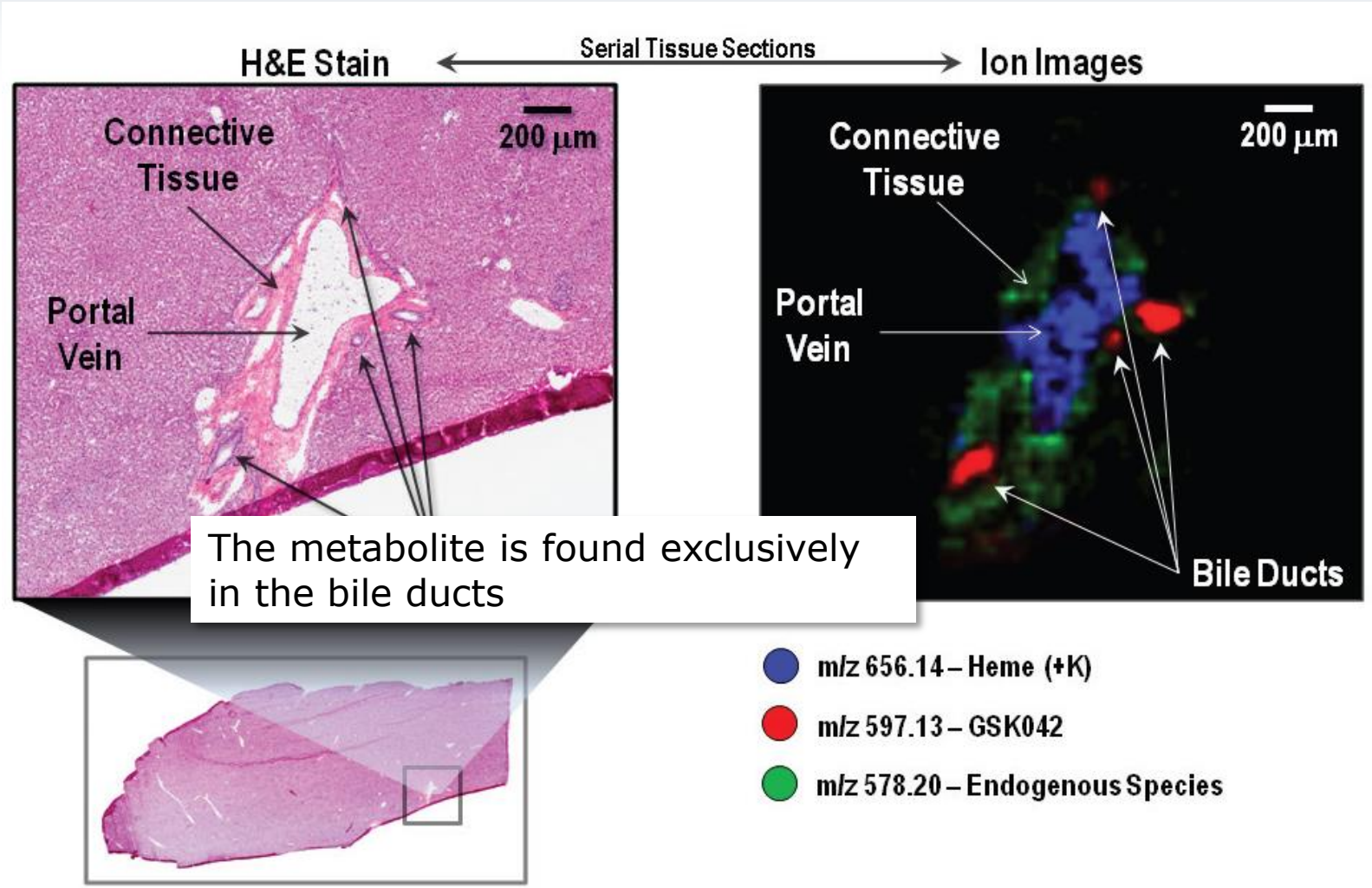
MALDI imaging requires no radioactive label for drug imaging and can differentiate drug and different metabolites

MALDI imaging in DMPK – state of the art

- **Integration** of **MALDI imaging** and **histology** allows a new understanding of drug distribution
- **Bridging biology and chemistry** in drug development

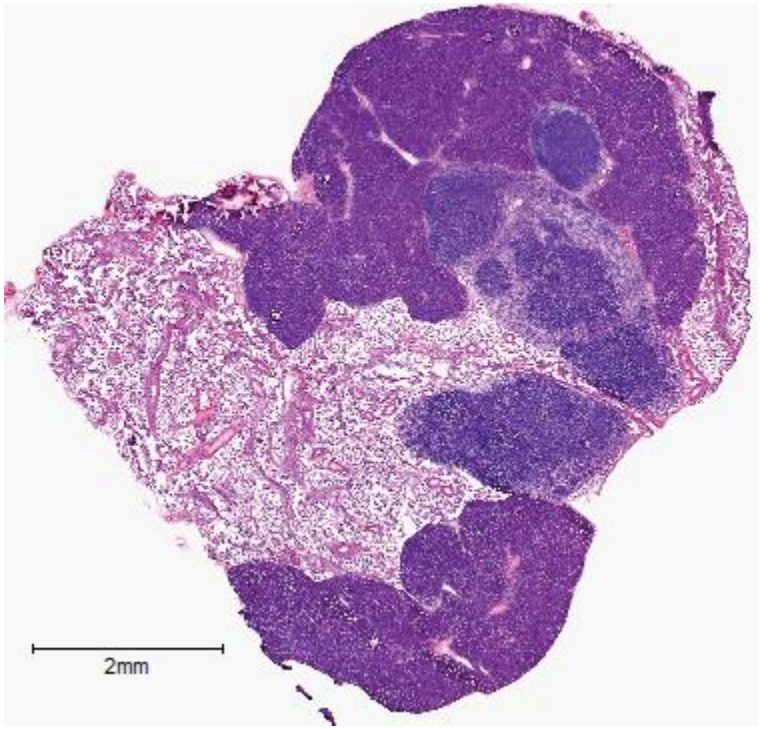
As described in:
Castellino et. al, (2011)
Bioanalysis , Vol. 3, No. 21, 2427

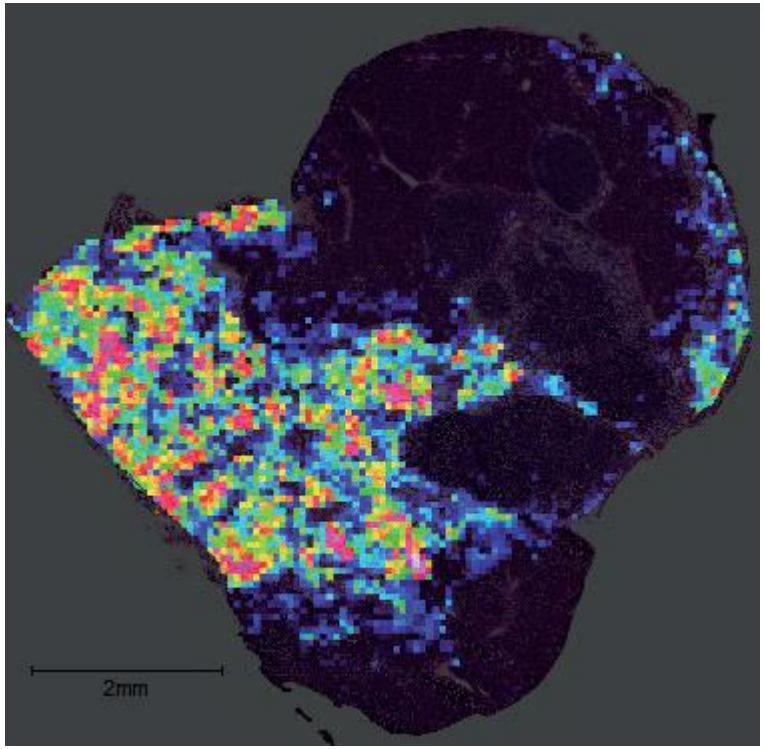
Drug imaging and histology



Castellino et al.(2011),Bioanalysis 3,2427

Drug imaging and histology





Example from a study on a mouse pancreas tumor: The comparison of the drug distribution (right) and the histology shows that the drug is contained in the fatty tissue and does not enter the targeted tumor tissue

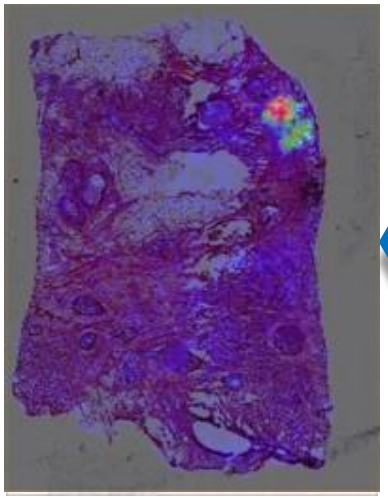
MALDI imaging workflow

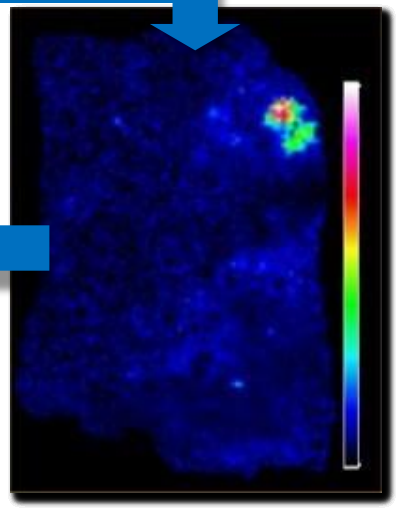
Tissue

Cryosection

Matrix coating

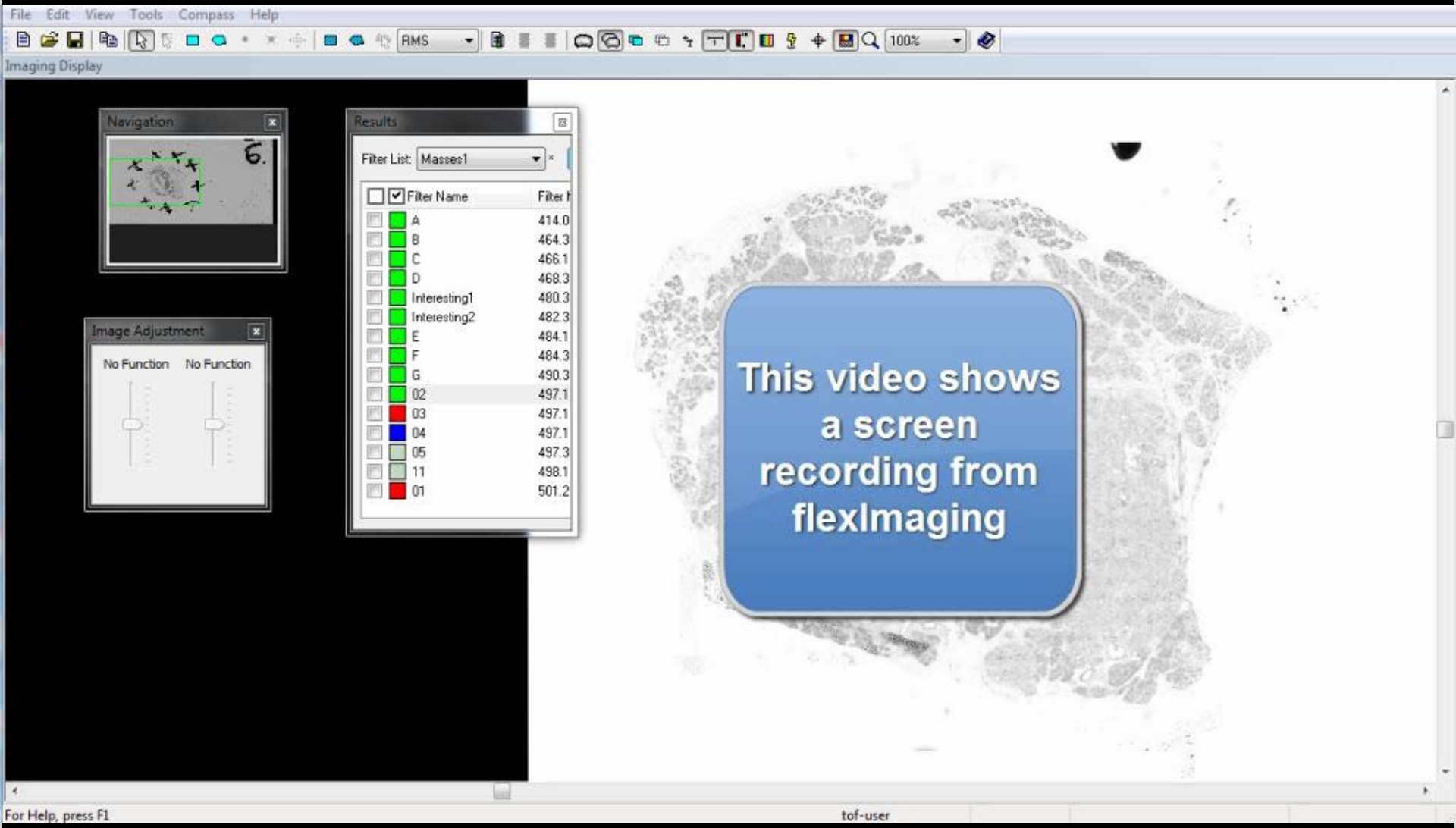
MALDI
Mass Spectrometry





MALDI imaging and virtual microscopy

- The video on the next slide gives an idea of the “look and feel” of the integration of histology and MALDI imaging
- The example is a mouse pancreatic tumor, measured at 50 µm resolution on a Solarix FTMS
- The histological image is a so-called digital slide that contains the full microscopic resolution.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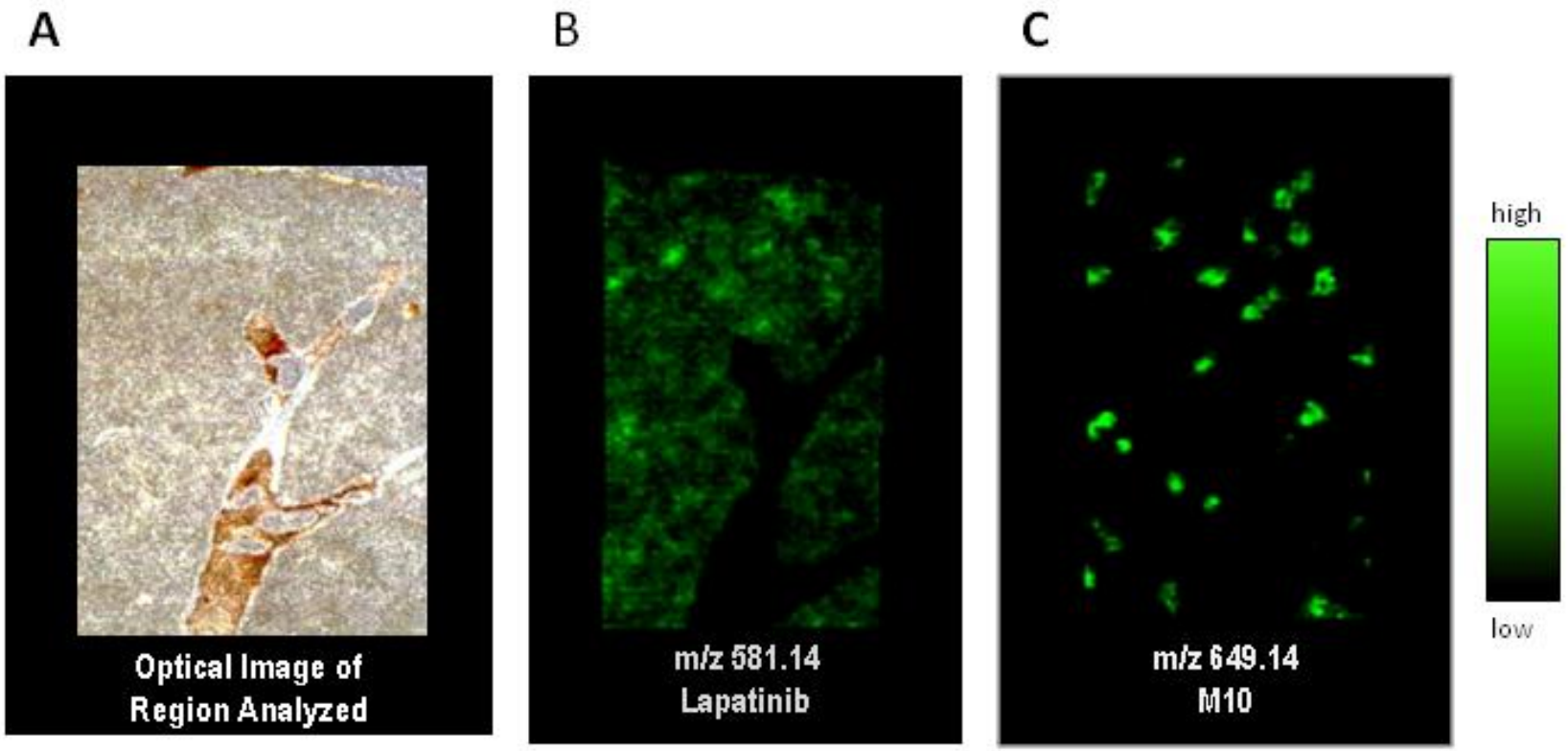
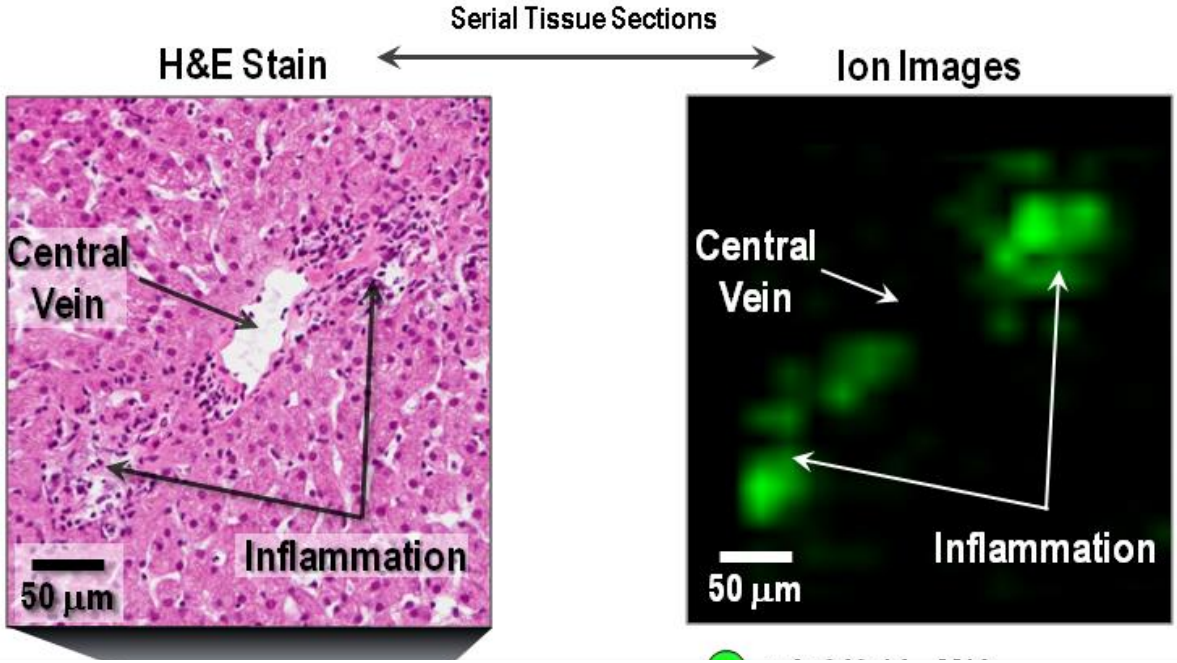
Drug imaging and histology



This example shows how Lapatinib and the metabolite M10 show a different distribution in a dog liver.

Castellino et al.(2011),Bioanalysis 3,2427



Comparison with histology shows that the metabolite M10 is found enriched in inflamed lesions.

„…in addition to parent and M10, ion images for 21 metabolites were also generated in the liver sections (data not shown). "

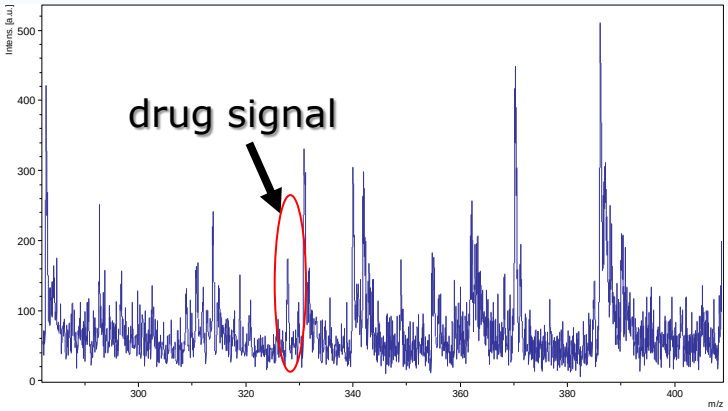
Castellino et al.(2011),Bioanalysis 3,2427

Drug imaging – technical considerations

- In MALDI imaging thousands of compounds are measured at the same time
- It is important to be specific and to ensure that the detected signal really corresponds to the drug
- This can be achieved in two ways, either by an MS/MS experiment (SRM)…
- …or by high mass resolution and accuracy

Drug imaging in MALDI-TOF

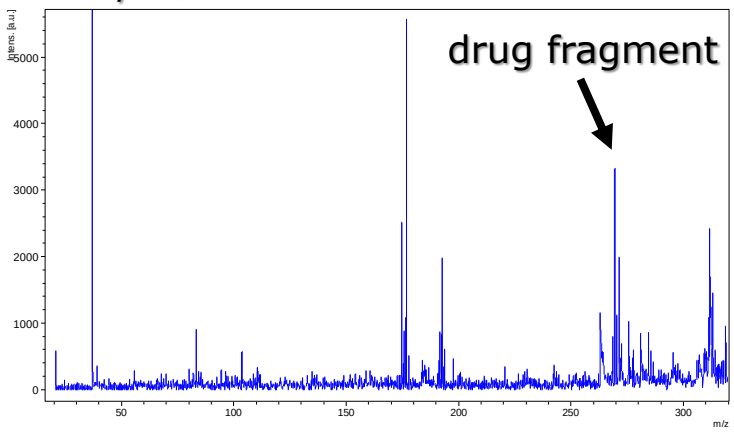
MS from tissue



Small molecule measurements: Interference of background signals in MS mode!

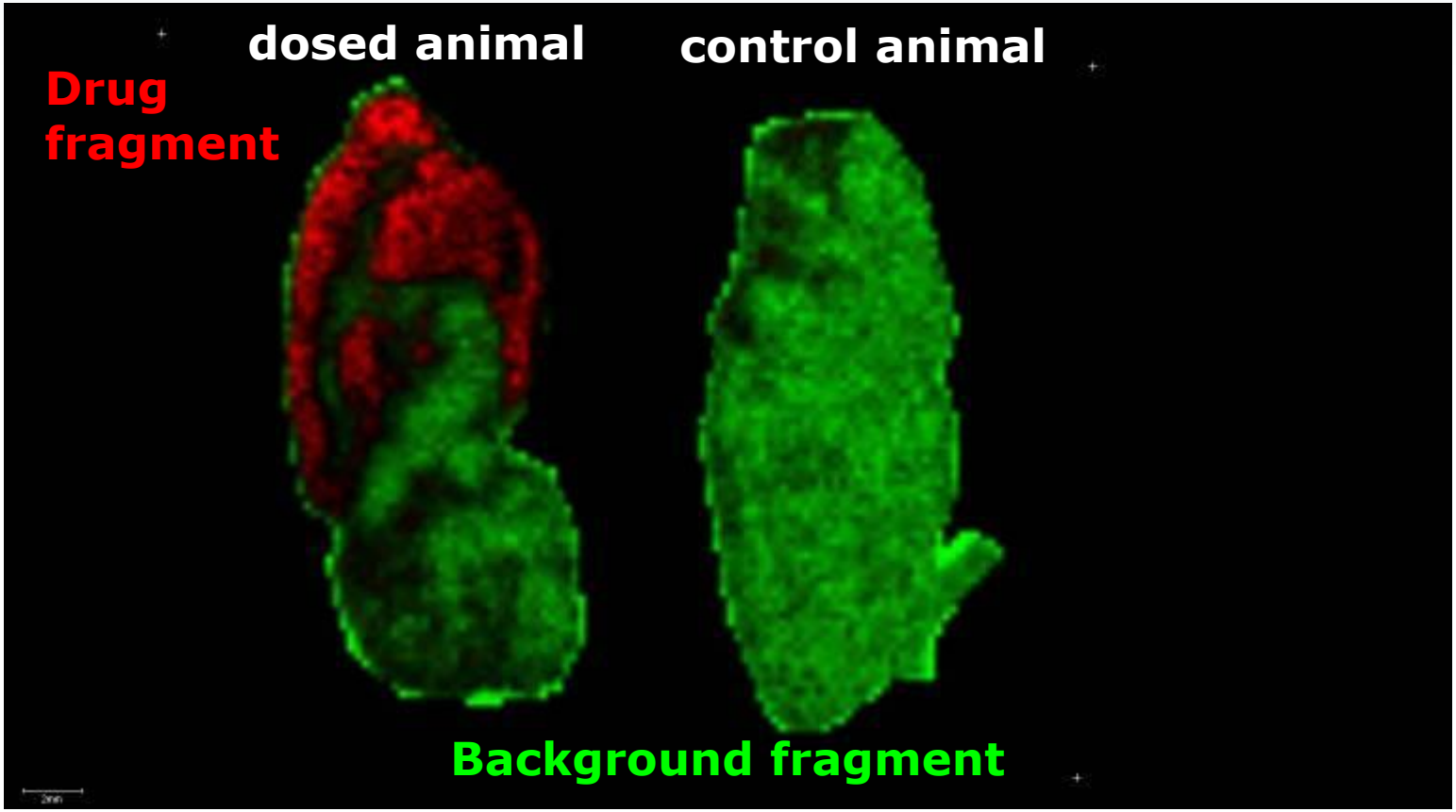
MS/MS

MS/MS from tissue



Fragmentation increases specificity of drug imaging!

Example of MALDI-TOF/TOF imaging

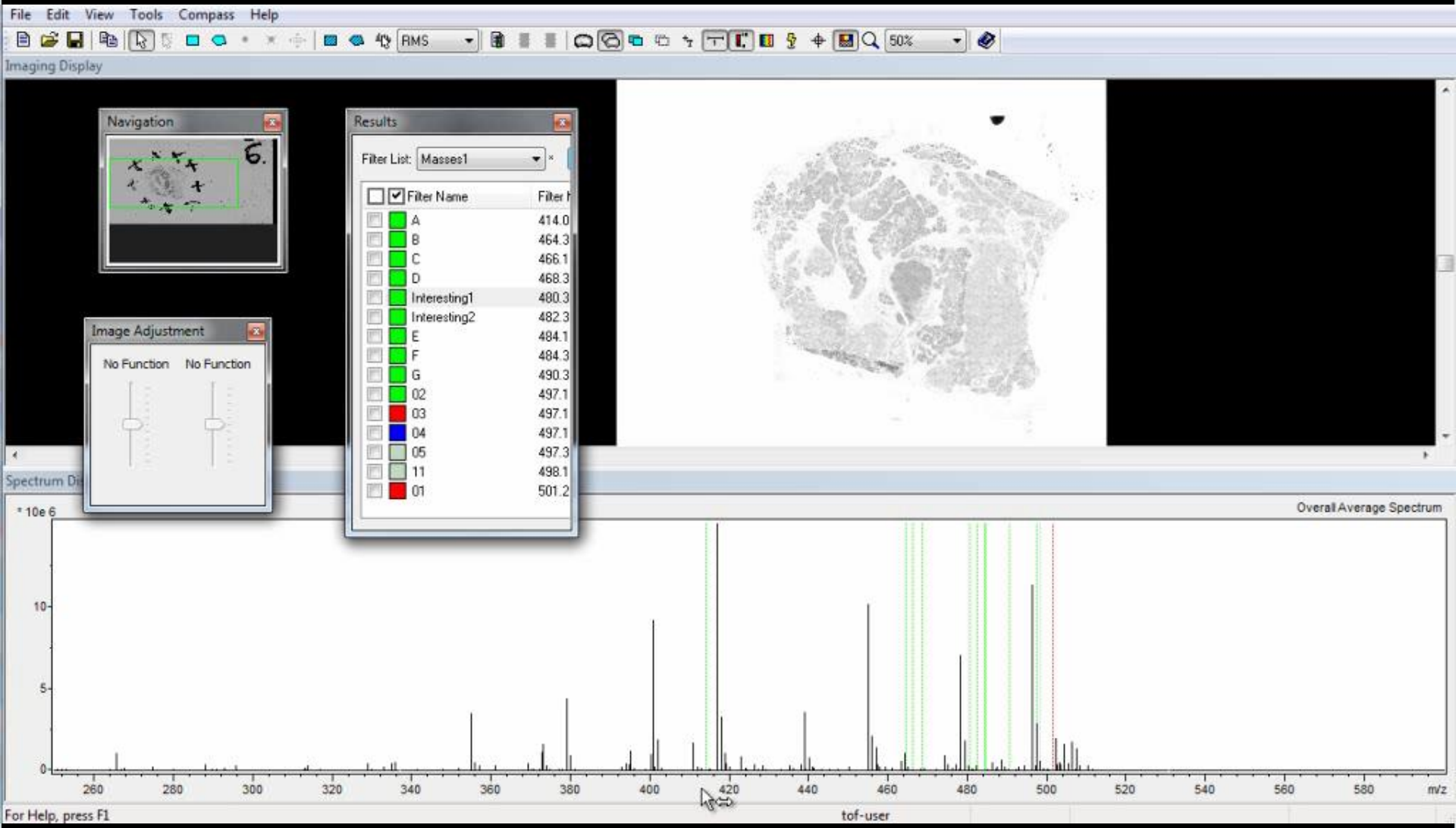


Courtesy of: Klaus Rumpel, Dale Shepherd, Pfizer Ltd.

High mass resolution imaging: FTMS

- On a **Solarix** very **high mass resolution** and **accuracy** can be achieved.
- **Advantages** for MALDI imaging:
- **Drugs** and **metabolites** can be **detected in the same measurement**
- Allows to **find** potentially **unexpected metabolites**
- Higher **flexibility**

The video in the next slide shows the „look and feel" of working with FTMS imaging data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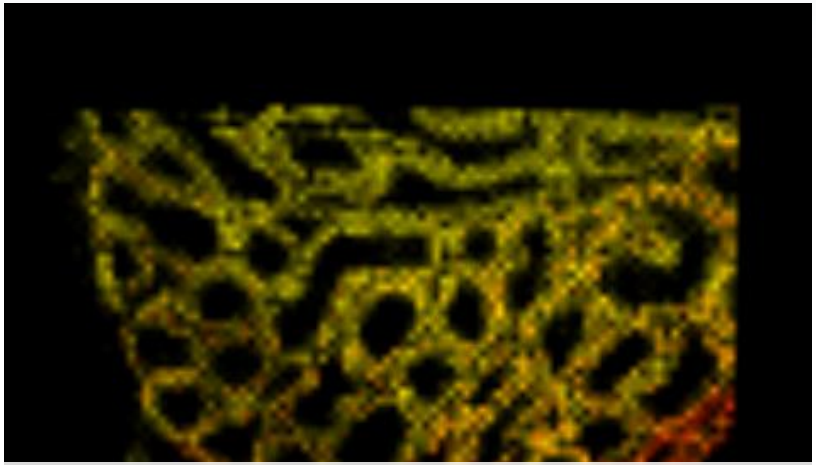
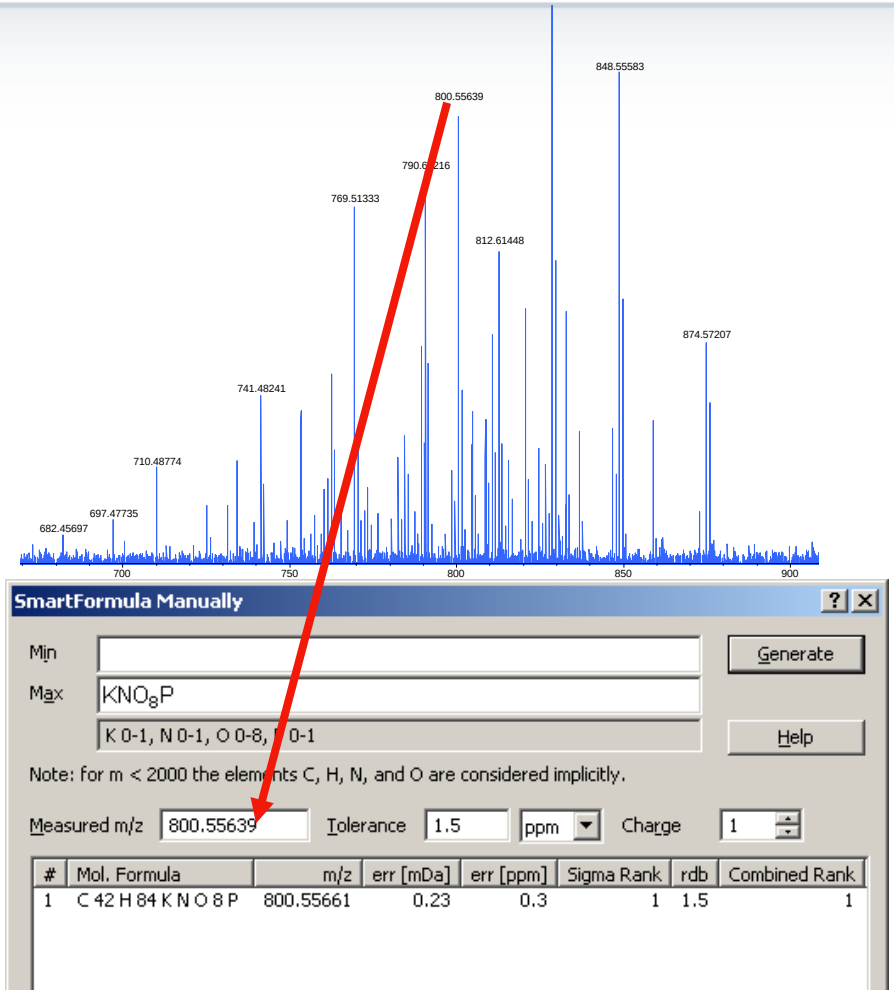
Solarix: Identification of compounds



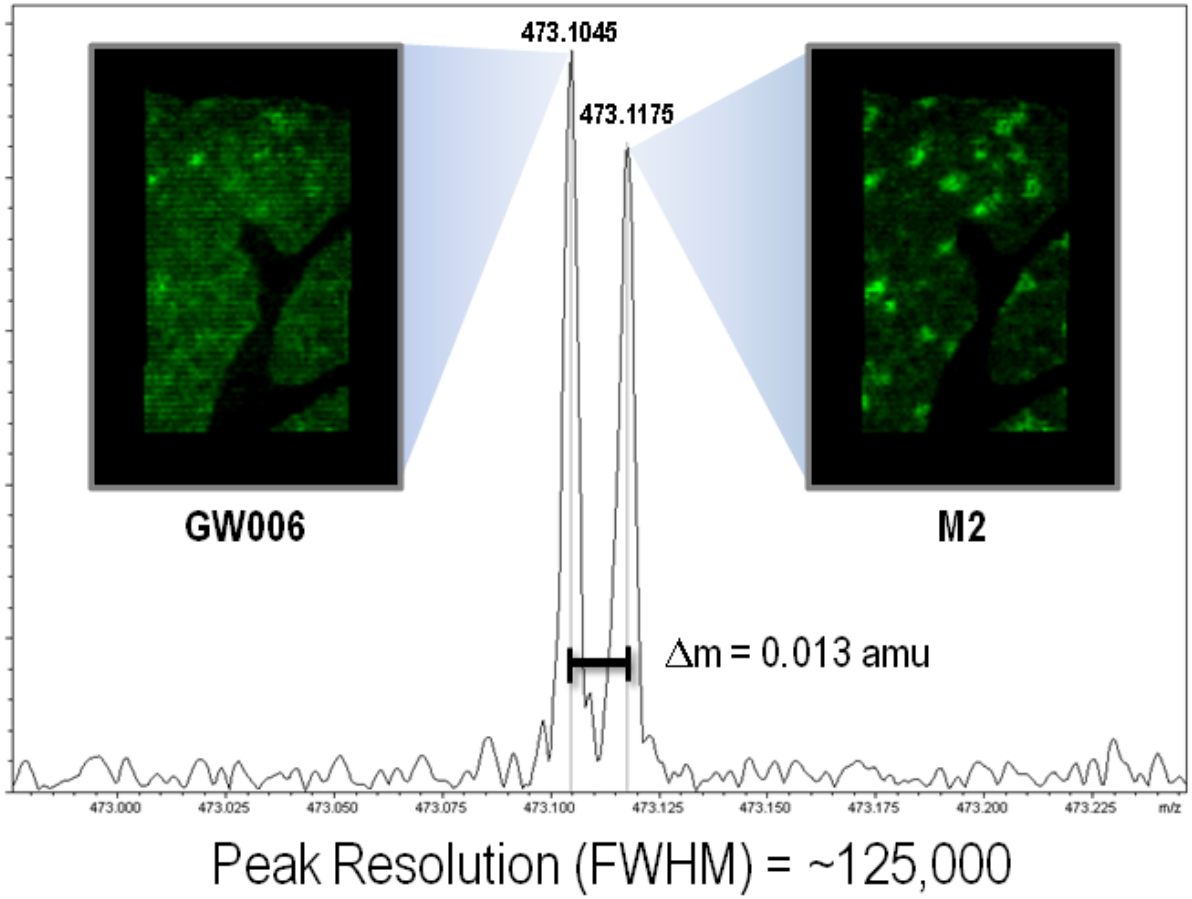
FTMS allows the identification of unknown compounds based on

- exact mass
- isotope fine structure
- advanced MS/MS options

Maximum confidence in drug imaging results



Drug imaging on Solarix FTMS

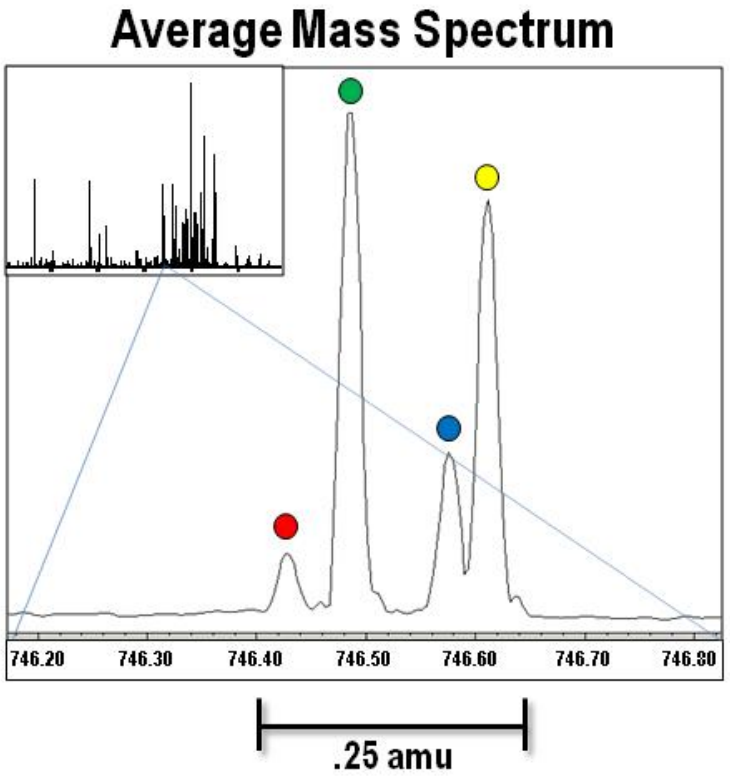


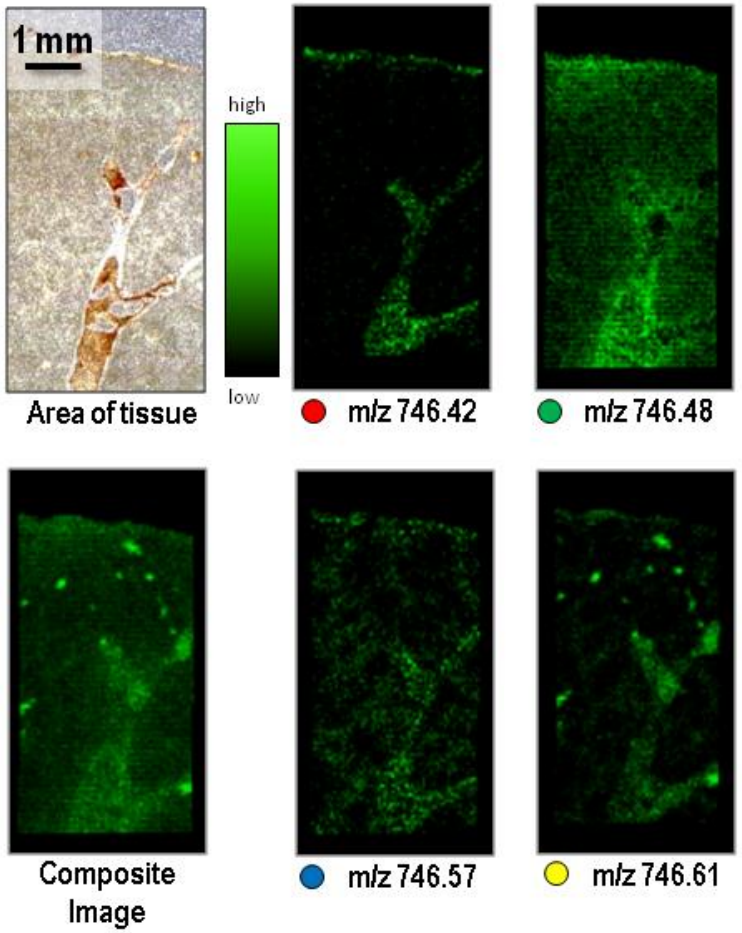
These two lapatinib metabolites show a different distribution in the dog liver and have a mass difference of only 0.013 Da

MALDI imaging on Solarix allows unambiguous assignment of the metabolites

Castellino et al.(2011),Bioanalysis 3,2427

High mass resolution imaging

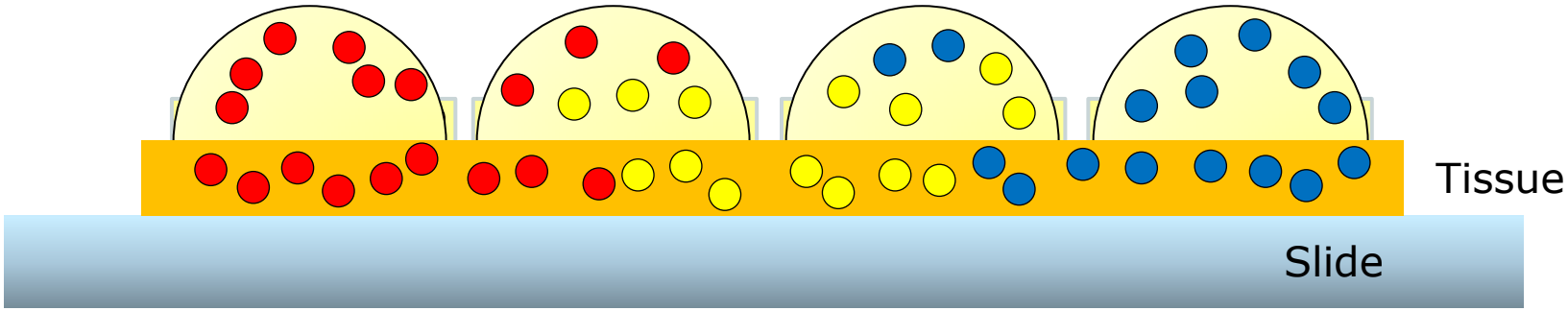




Sample Preparation

1. The analytes are in the tissue
2. Liquid matrix solution is applied
3. Analytes are extracted from tissue
4. Solvent evaporates, analytes are embedded in matrix

This is completely automated by the Imageprep device



All examples have been prepared with the imagePrep:
High resolution and spectral quality

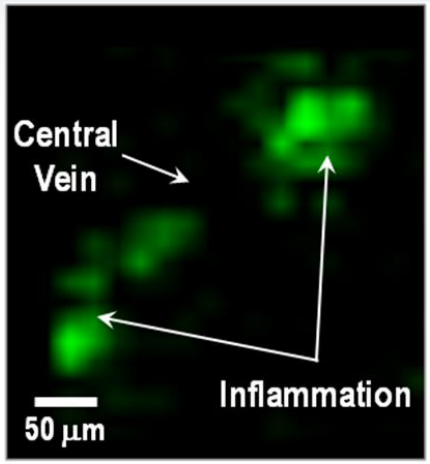
solarix Next-Generation FTMS

The **solariX** is a high-performing, research grade FTMS that is designed to address some of today's most challenging analytical problems:

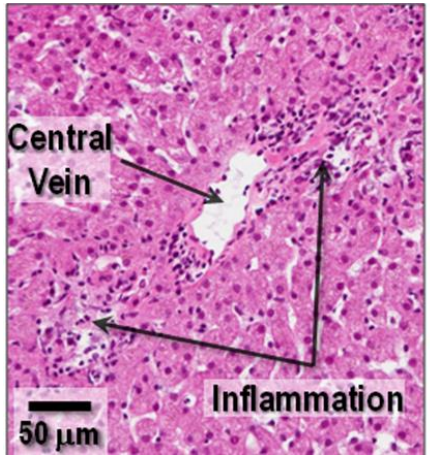
- High-end life science studies
- Molecular Imaging of Tissue
- Complex Environmental sample analysis
- Metabolomics Research
- Petroleum Product Analysis

Conclusion

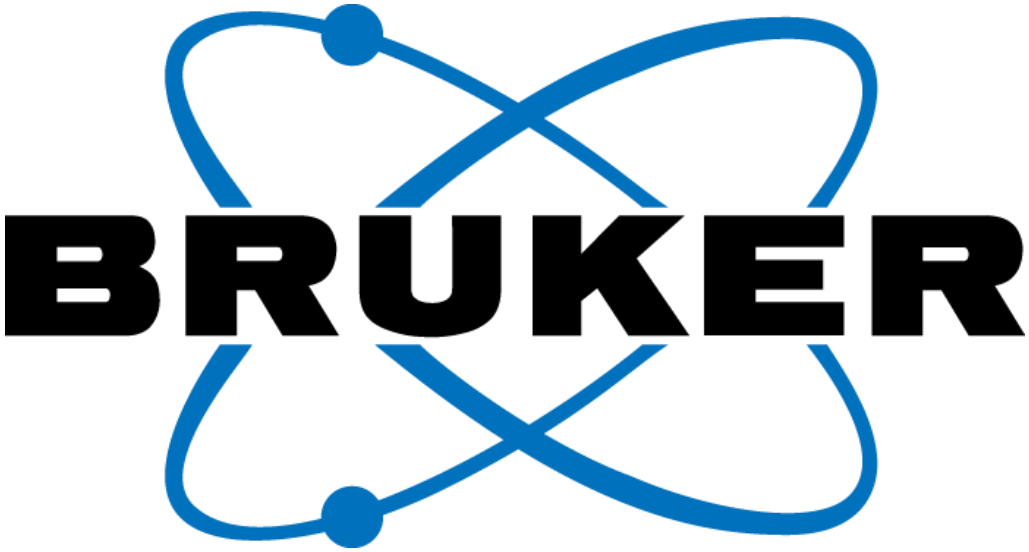
- MALDI Imaging is a powerful tool to investigate drug distribution
- Differentiate drug and metabolites
- Integration of histology and MALDI imaging allows new insight
- „Bridging chemistry and biology in drug development“



m/z 649.14 – M10



Castellino et al.(2011),Bioanalysis 3,2427



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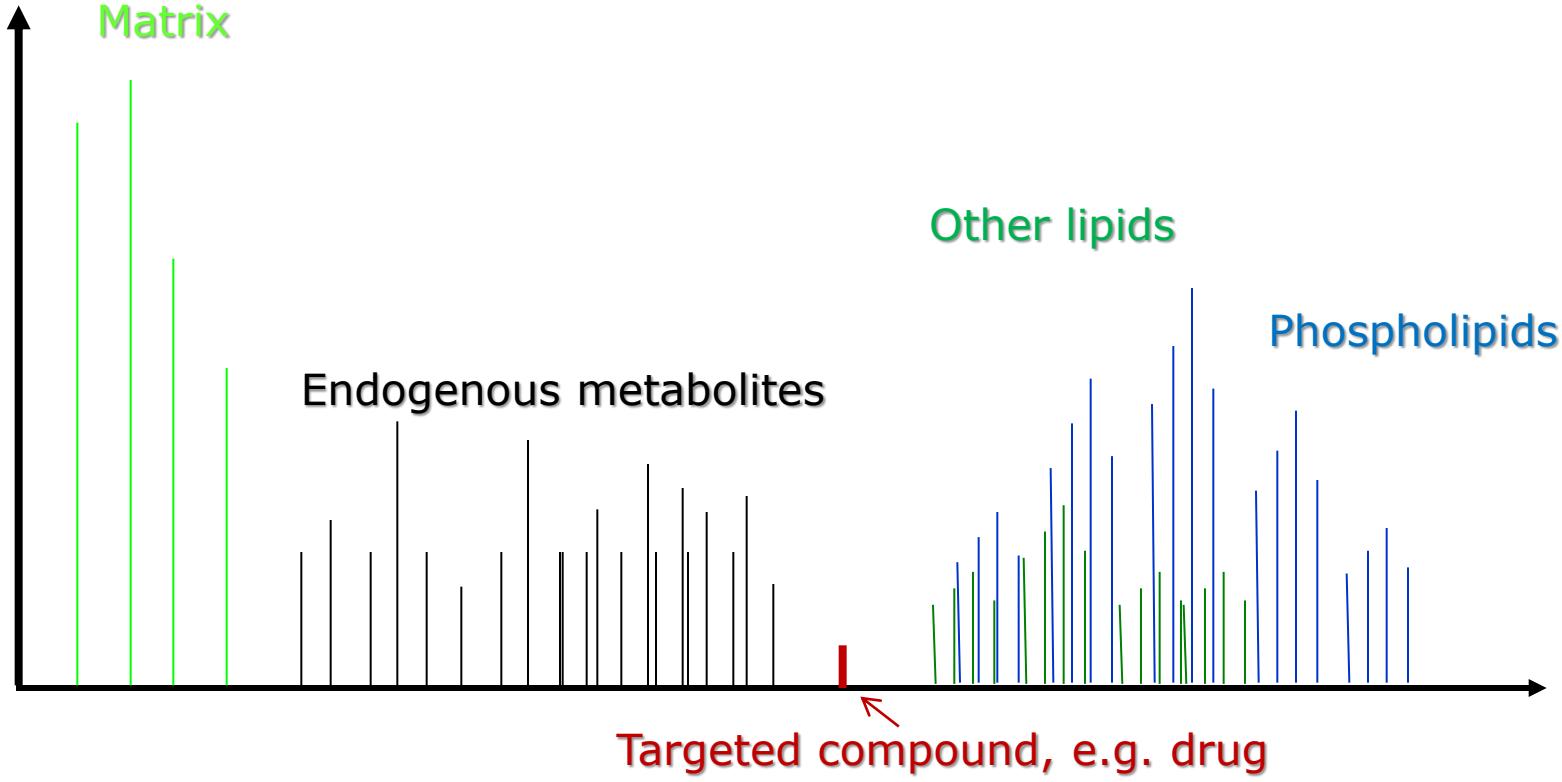
Dynamic range limitations in ion traps

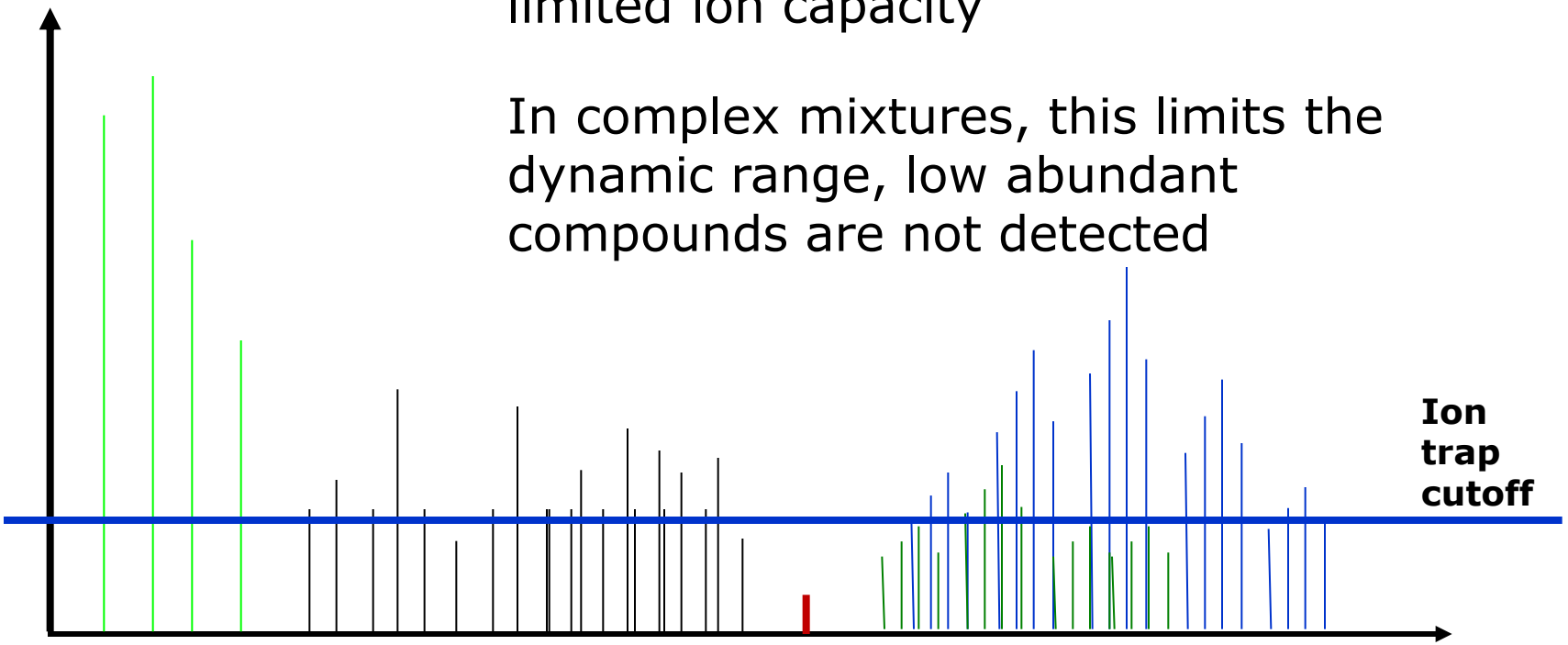
MALDI imaging spectra from spectra are extremely complex mixtures:



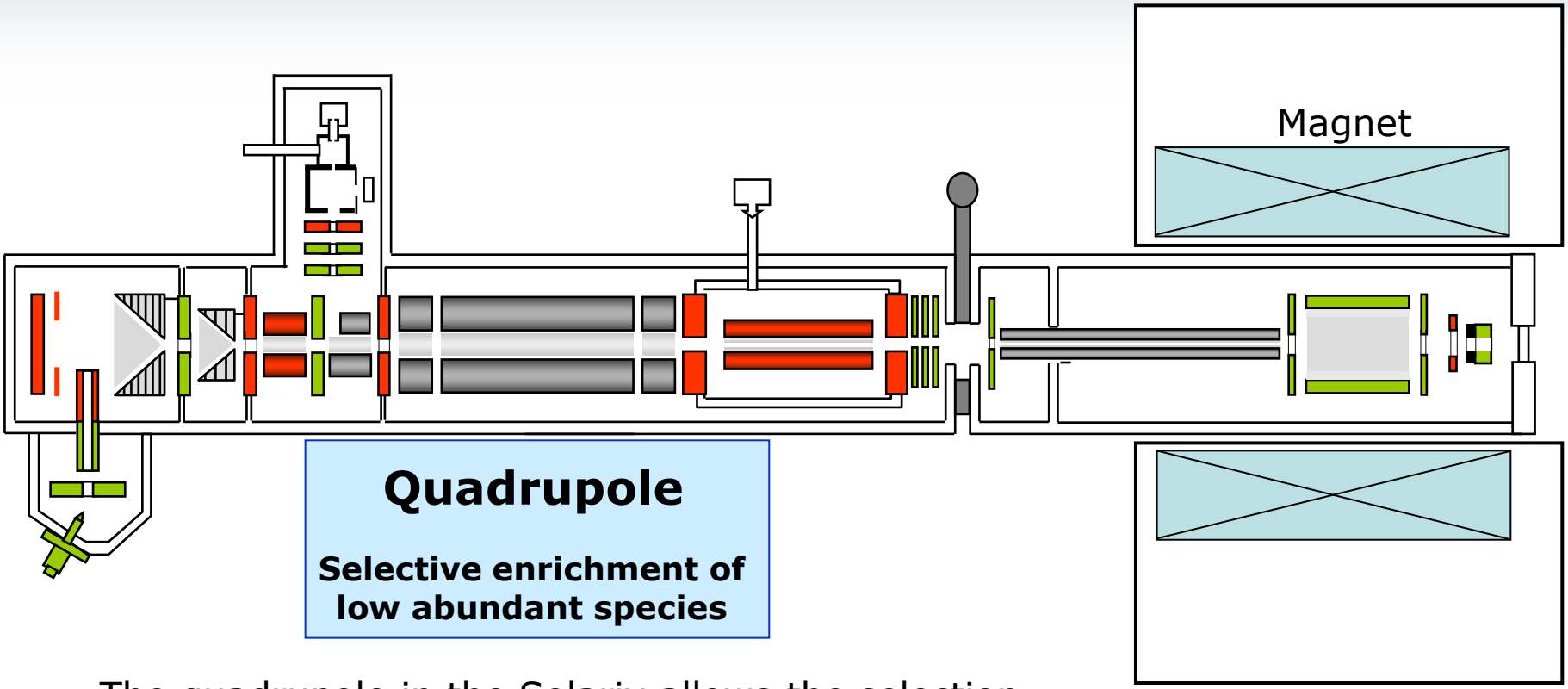
Dynamic range limitations in ion traps

Iontraps (that includes FTMS and orbitral trapping devices) have a limited ion capacity

In complex mixtures, this limits the dynamic range, low abundant compounds are not detected



CASI: Continuous accumulation of selected ions



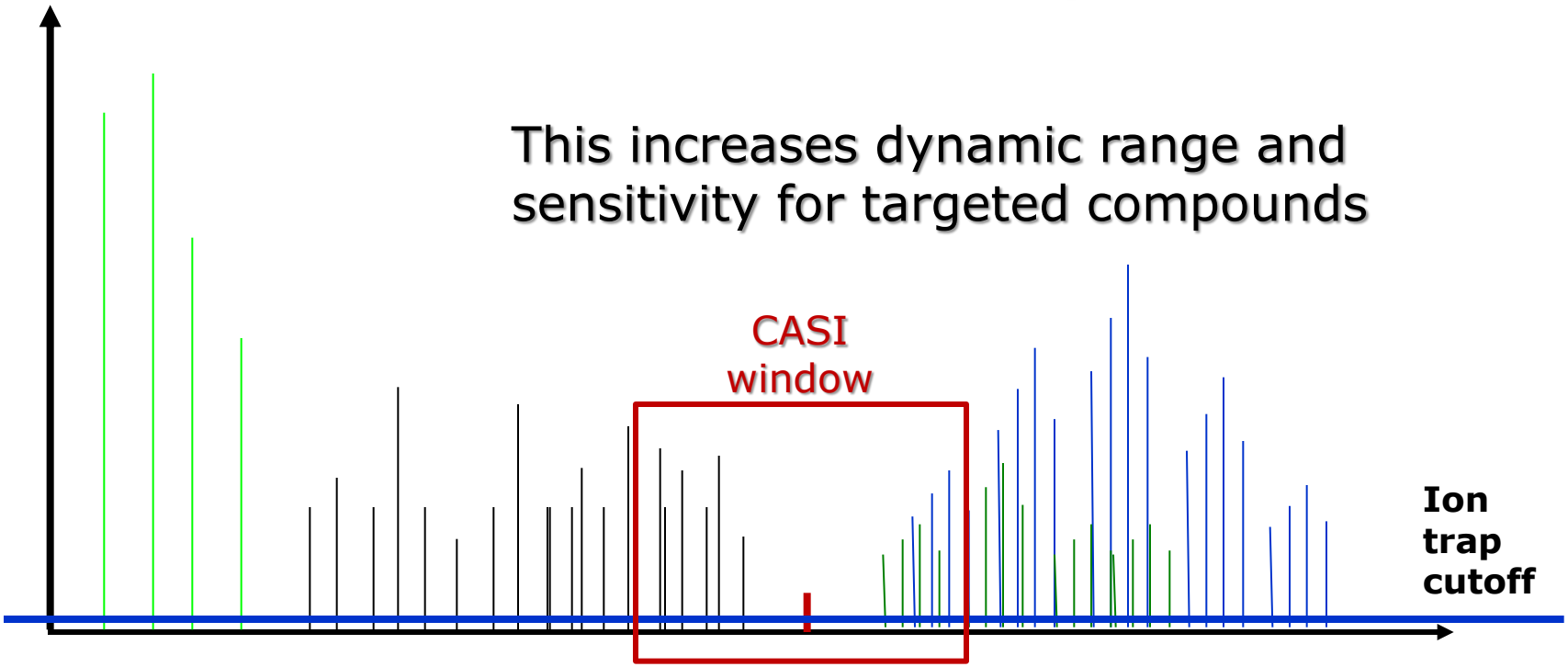
The quadrupole in the Solarix allows the selection of mass ranges

In drug imaging most phase 1 metabolites can be found in a mass window of 100 Da

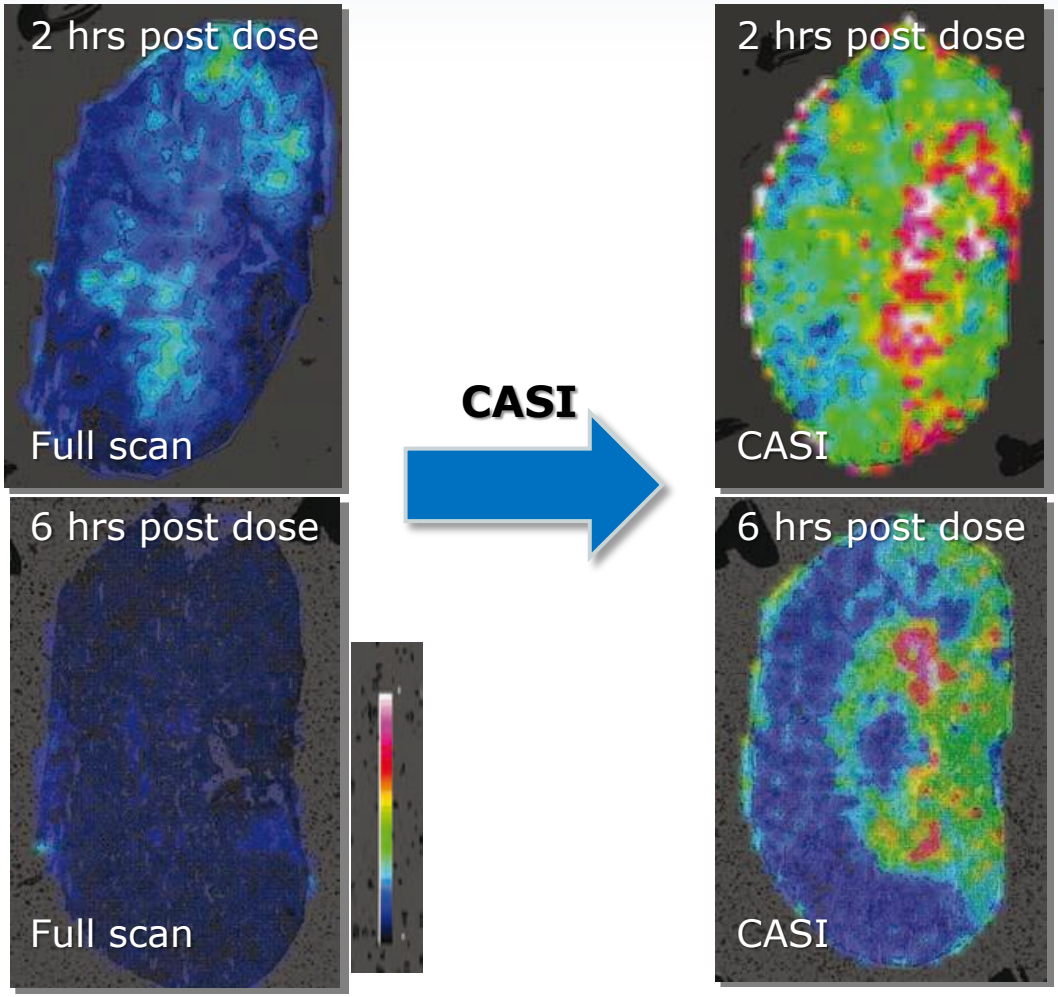
Dynamic range limitations in ion traps

The quadrupole in the Solarix can be used to selectively fill the trap with defined mass ranges.

This increases dynamic range and sensitivity for targeted compounds

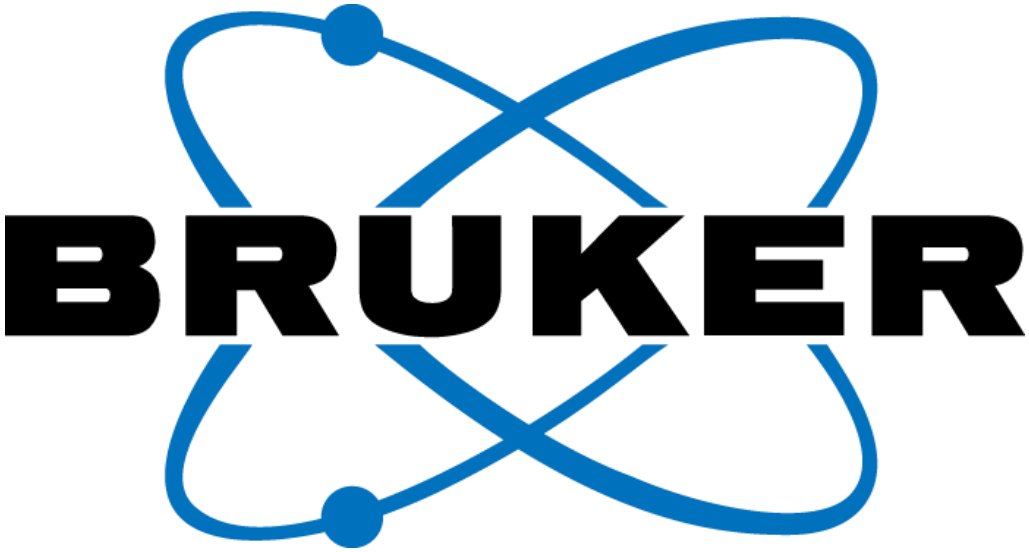


CASI: Continuous accumulation of selected ions



Example: Olanzapine in rat kidney

CASI boosts the dynamic range and allows confident detection of lower abundant compounds



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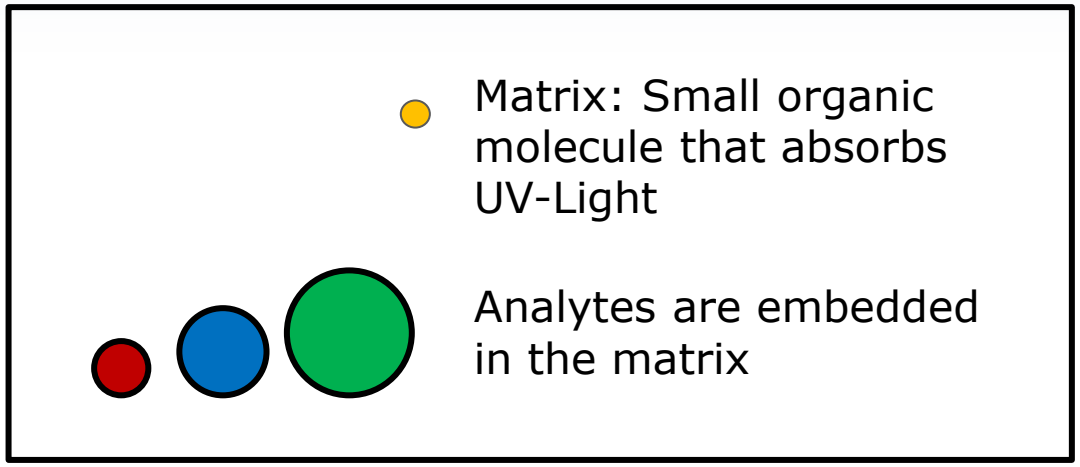
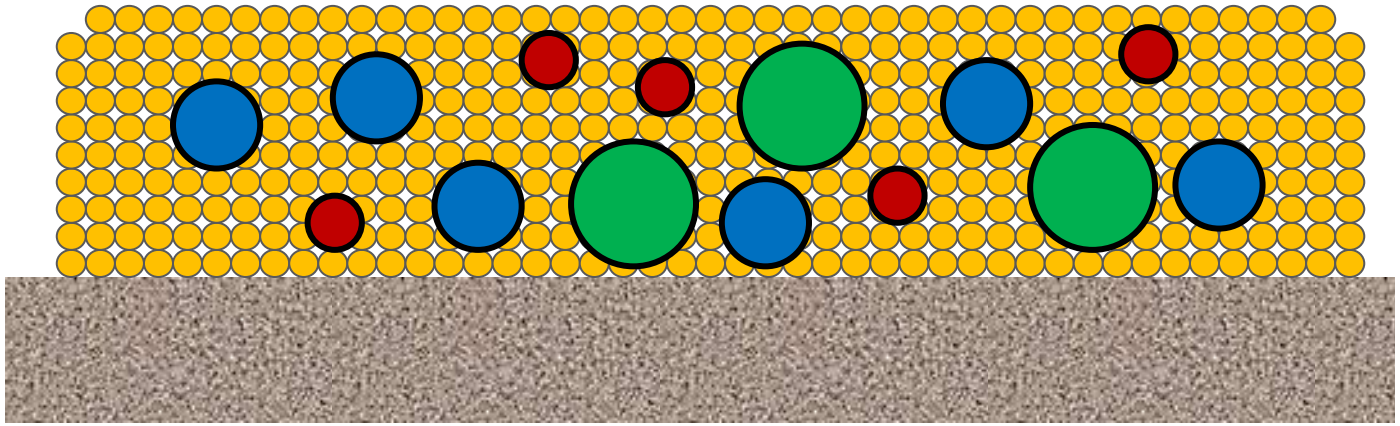
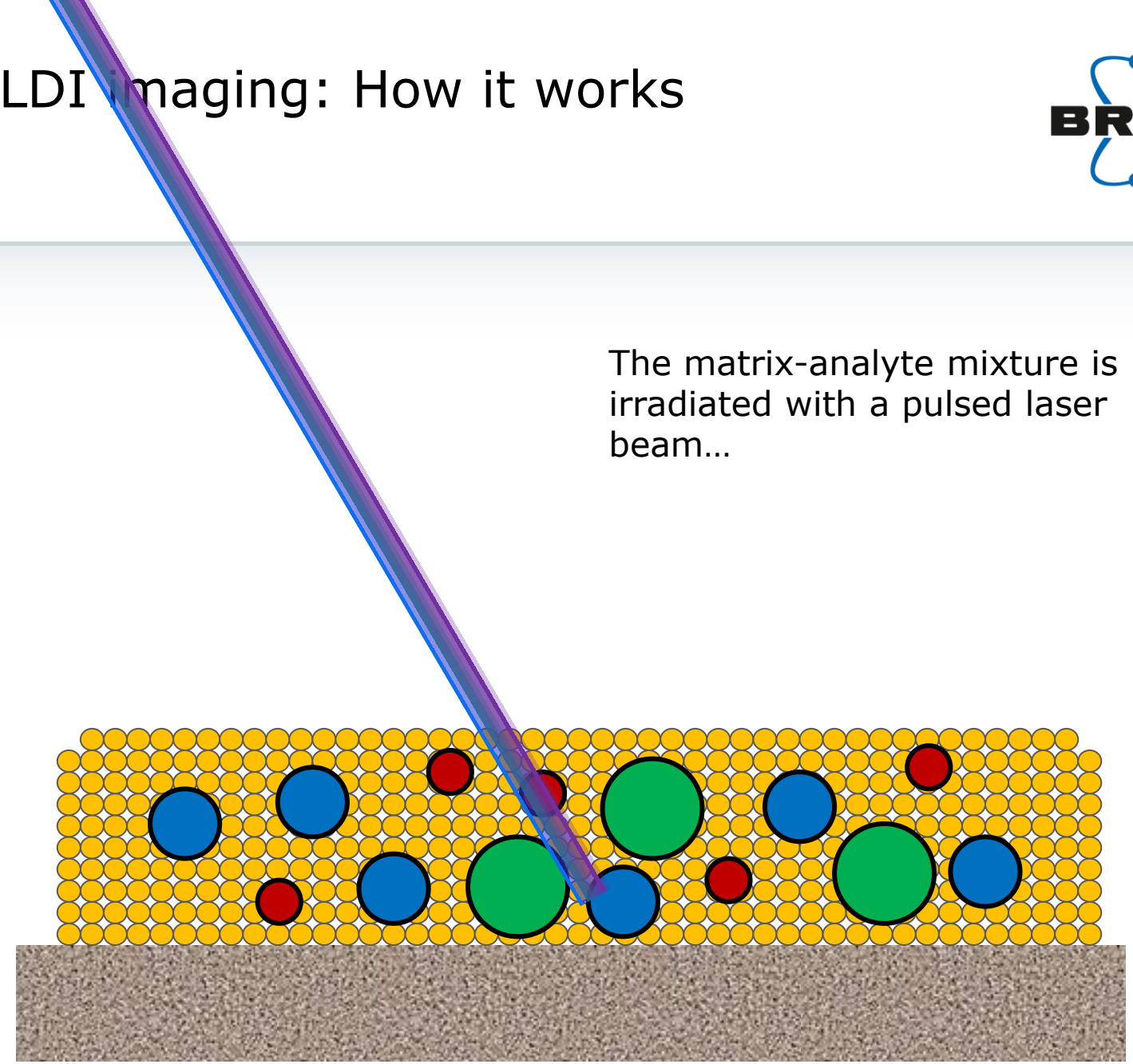
MALDI imaging: How it works



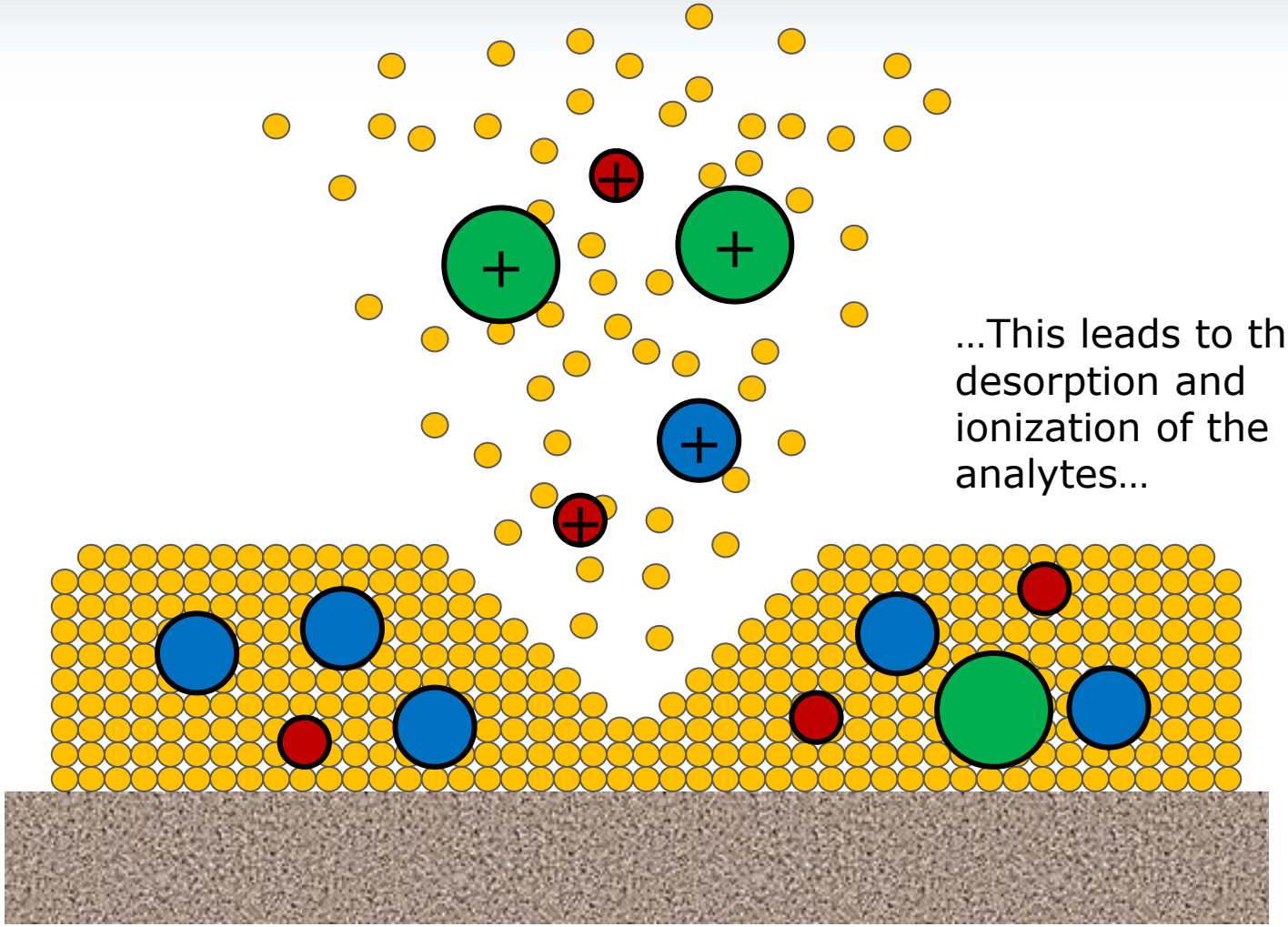


MALDI imaging: How it works

The matrix-analyte mixture is irradiated with a pulsed laser beam…



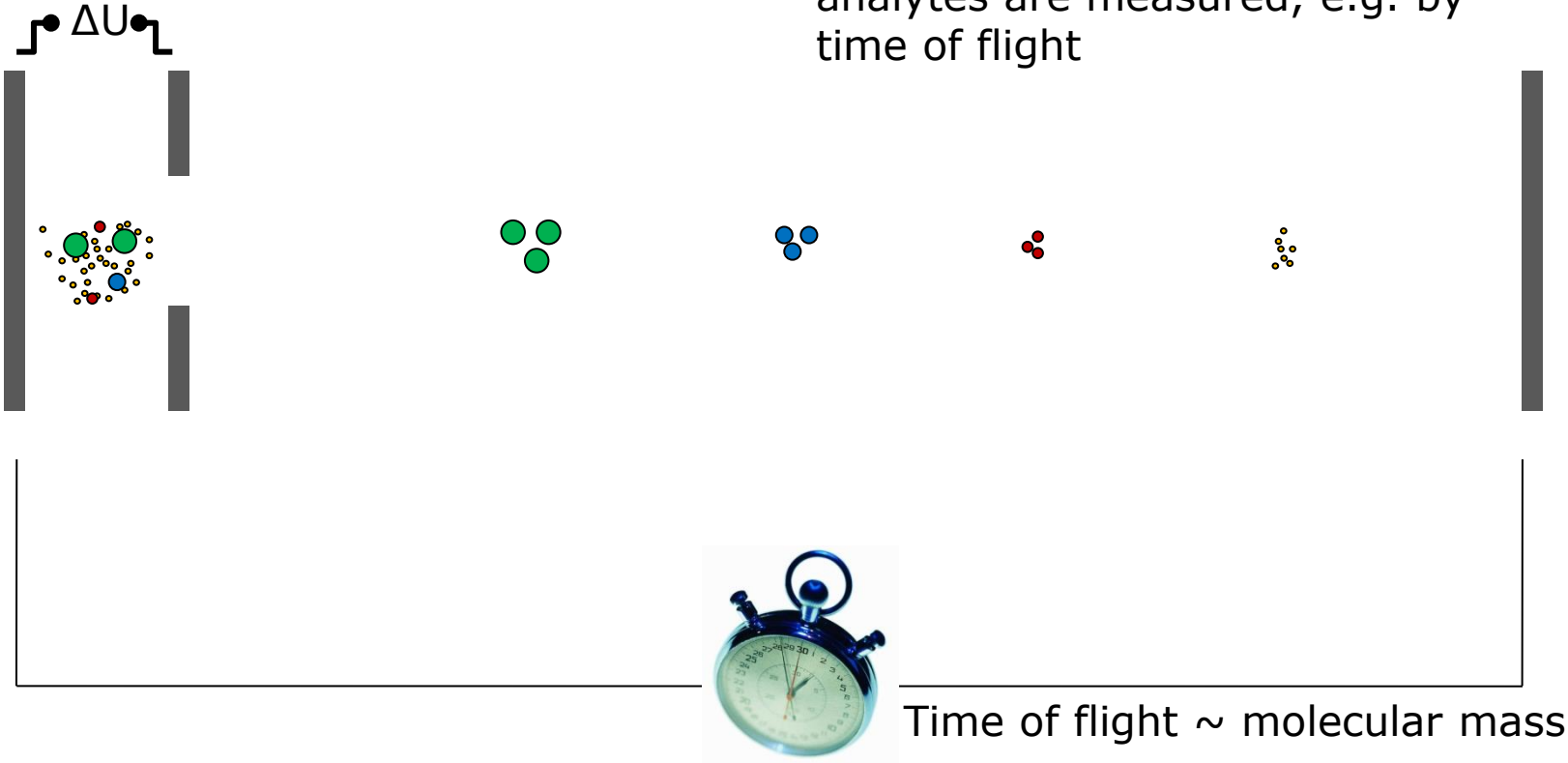
MALDI imaging: How it works



…This leads to the desorption and ionization of the analytes…

MALDI imaging: How it works

The molecular masses of the analytes are measured, e.g. by time of flight



Time of flight ~ molecular mass

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3. Analytes are extracted from tissue
4. Solvent evaporates, analytes are embedded in matrix