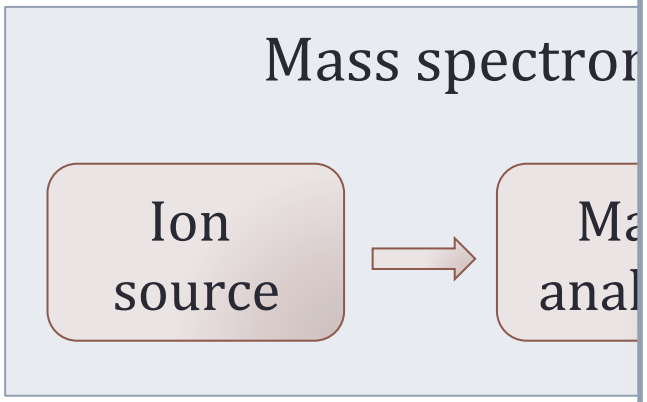
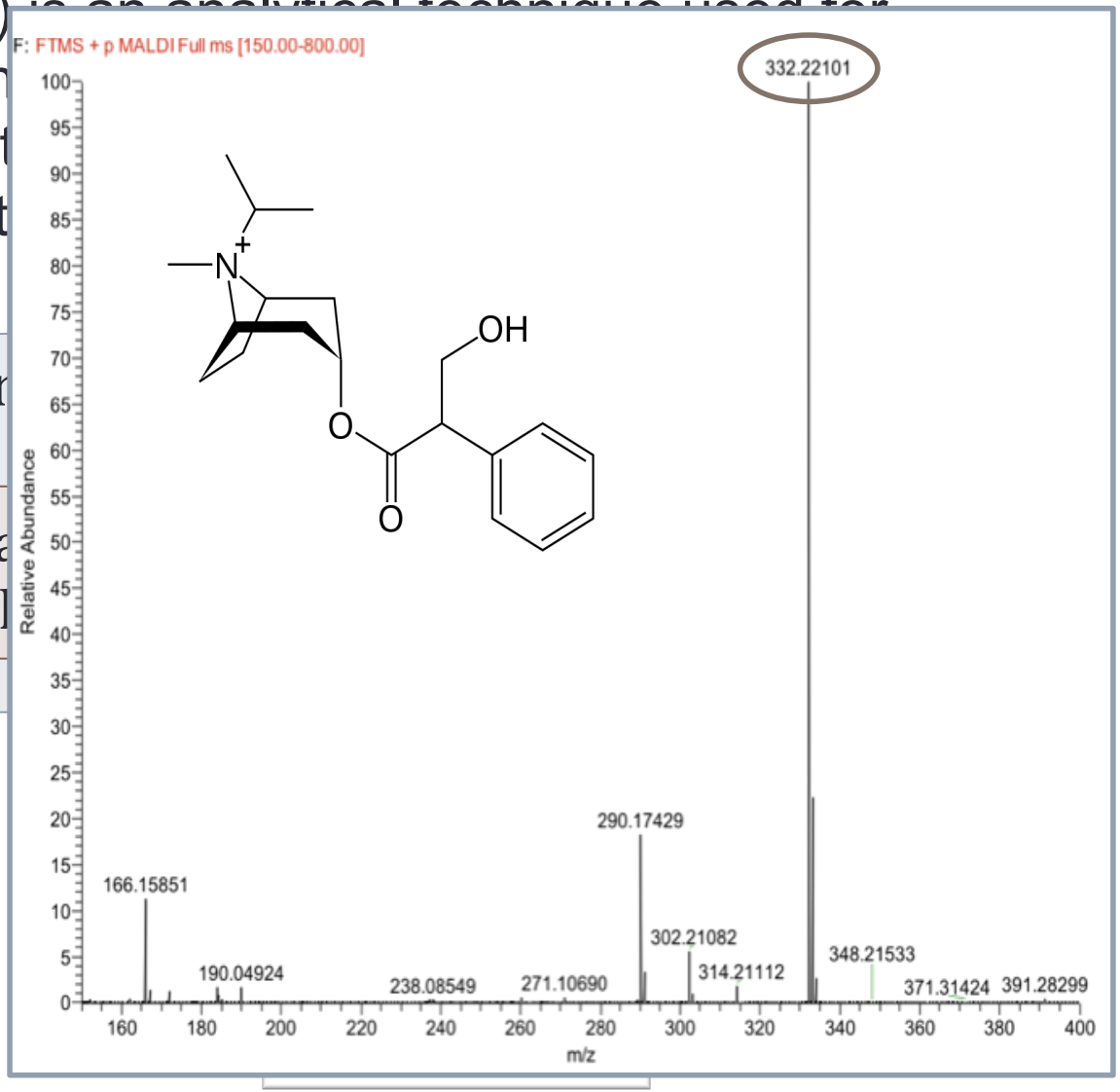
INTRODUCTION TO BIOLOGICAL MASS SPECTROMETRY II.

Mass spectrometry

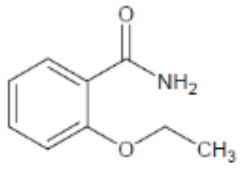
Mass spectrometry (MS) is an analytical technique used for identification of unknown by measuring the mass-t ions. It allows quantificat



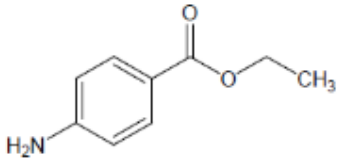


Is it enough to measure only mass?

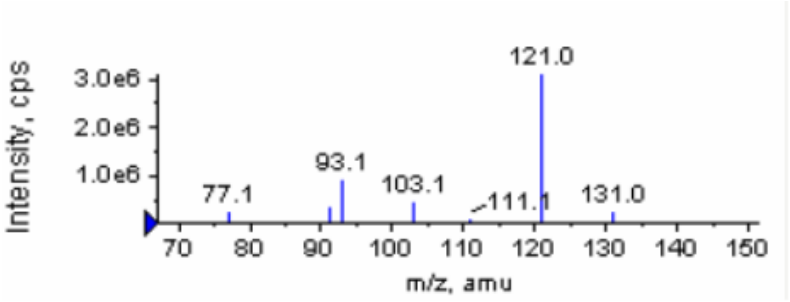
Ethenzamide

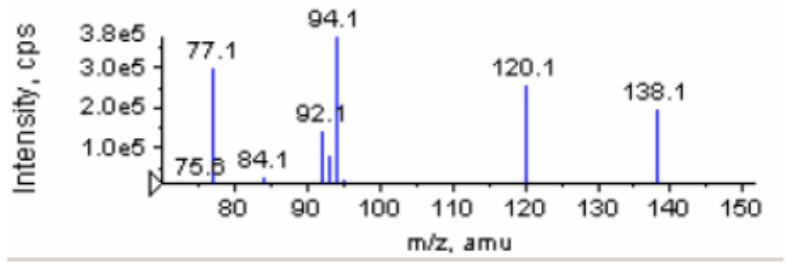


Benzocaine



Formula: $C_9H_{11}NO_2$
Molecular mass: 165.189 g/mol



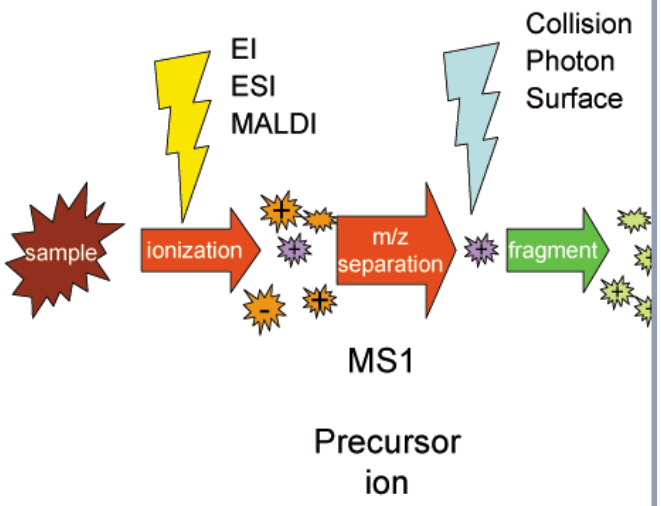


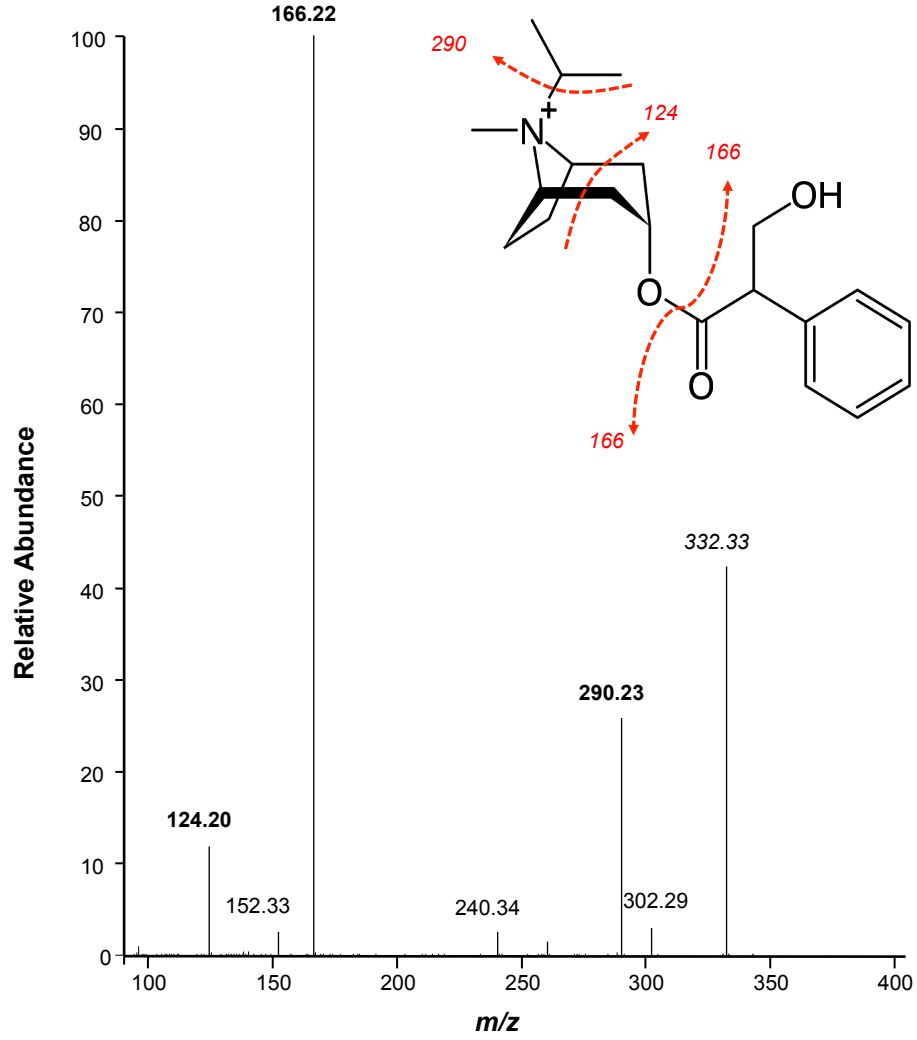
Tandem mass spectrometry *(MS/MS)*

A technique to **break down selected ions** (*precursor ions*) **into fragments** (*product ions*).
The fragments then reveal aspects of the chemical structure of the precursor ion.

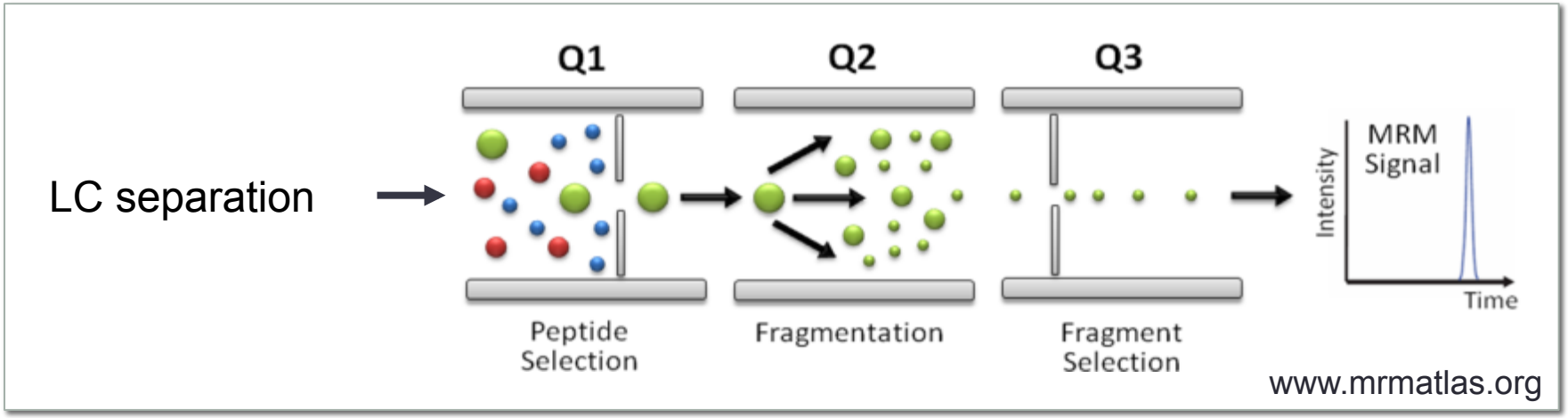
Tandem mass spectrometry (MS/MS)

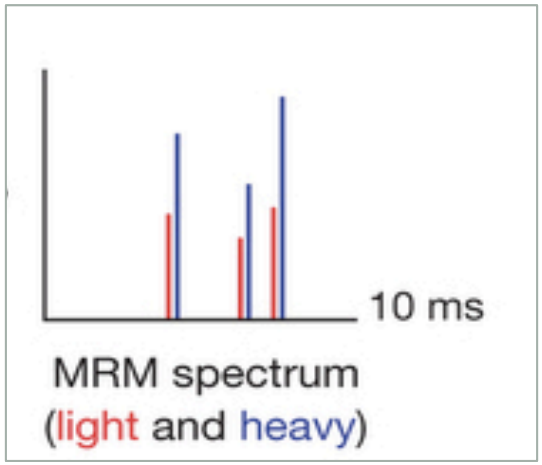
In a tandem mass spectrometer
the first mass analyzer (MS1). F
fragment ions (product ions) are
resulting ions are then separate
Examples: QqQ, QTOF, Qtrap,

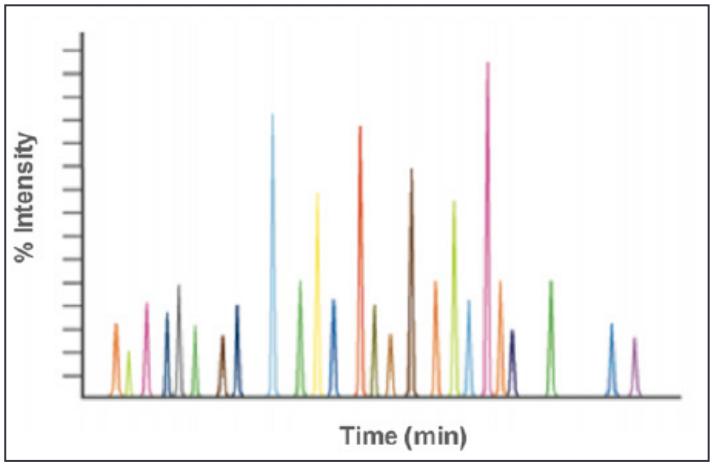




Selective reaction monitoring (SRM)







Multiple reaction monitoring (MRM)

Sample complexity in biological samples – the challenge

- Body fluids (urine, blood, cerebrospinal fluid (CSF))
- Tissue samples (biopsy, tumor, etc.)
- Other complex mixtures of various samples

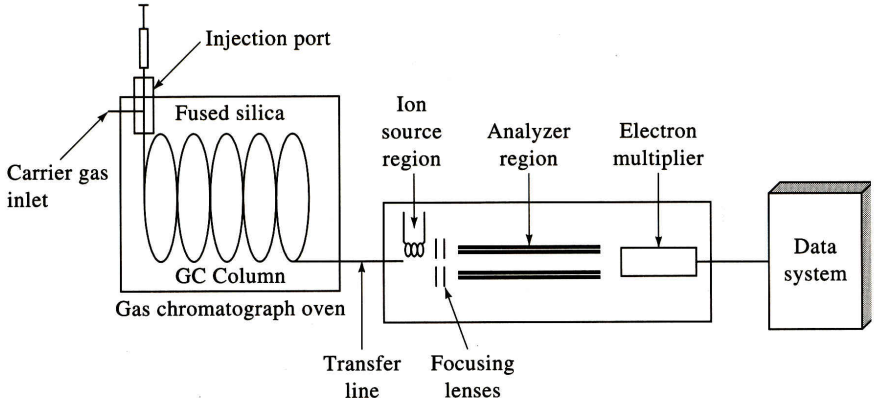
The solution is **separation**.

Off-line or on-line coupled separation.

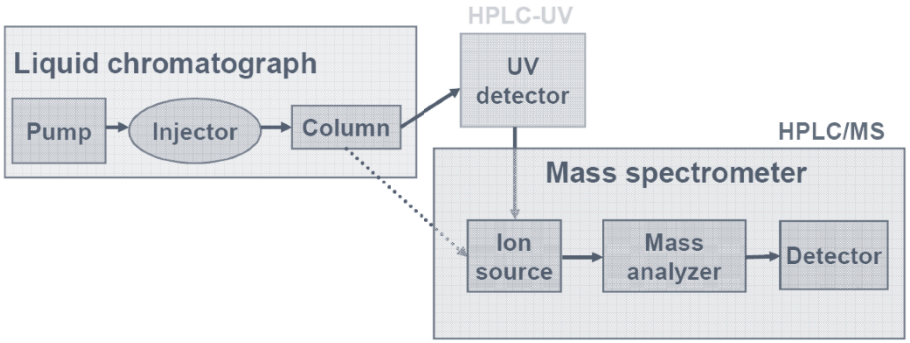
Chromatography	- gas chromatography - liquid chromatography (size-exclusion, ion-exchange, reversed-phase, hydrophobic interaction, etc.)
Electrophoresis	- gel electrophoresis (1D/2D-PAGE, DIGE) - capillary electrophoresis

Coupled systems

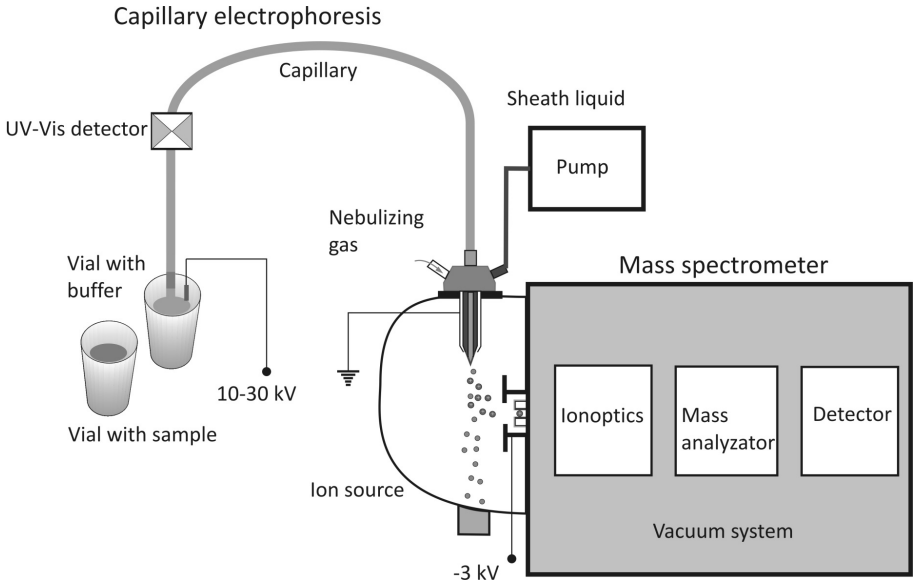
GC-MS system



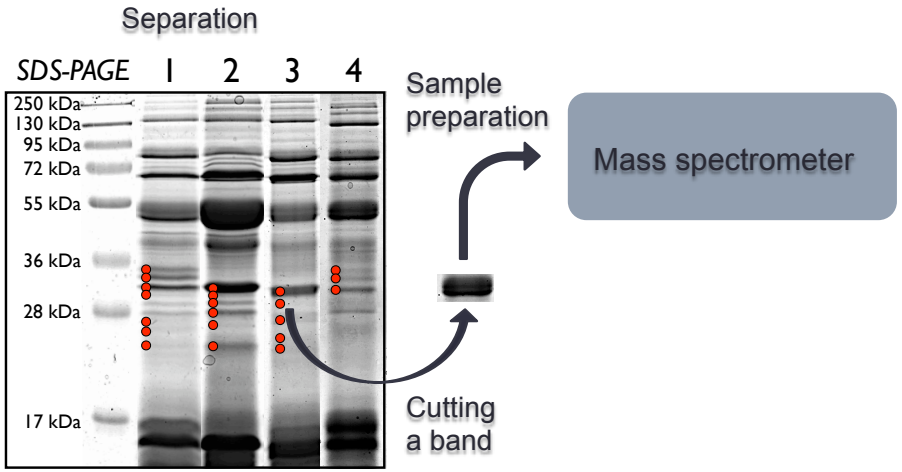
LC-MS system



CE-MS system



Off-line separation



PHARMACEUTICAL APPLICATIONS

- Drug synthesis, purity check and screening

-to determine the chemical structure of drugs and to detect/quantify impurities

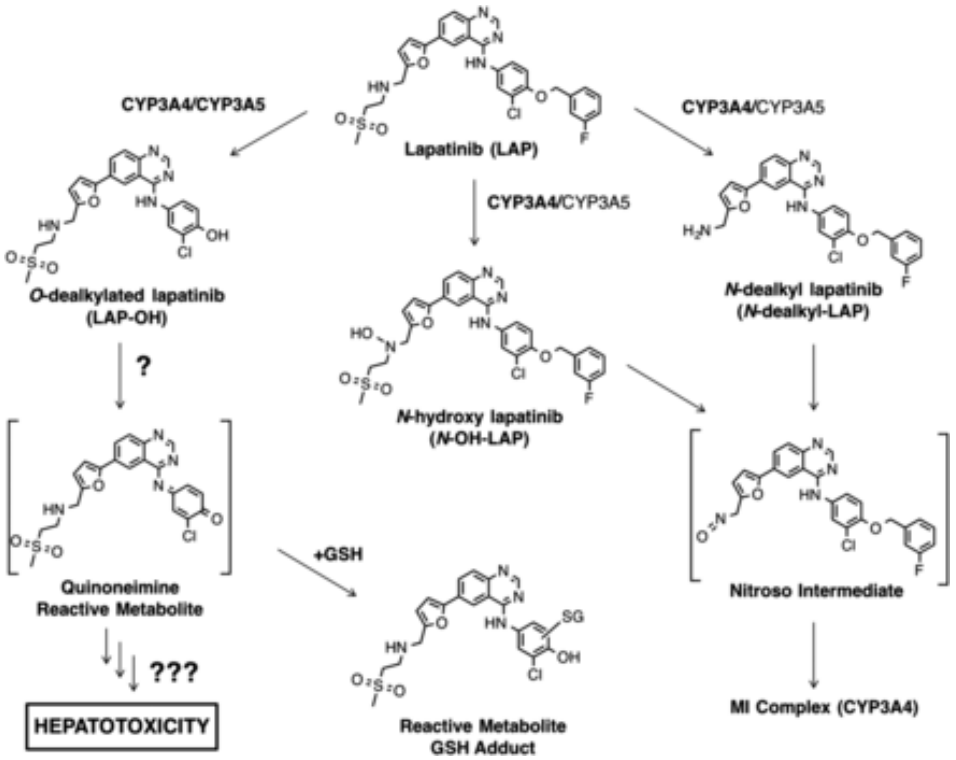
- Drug metabolism & pharmacokinetics (DMPK)

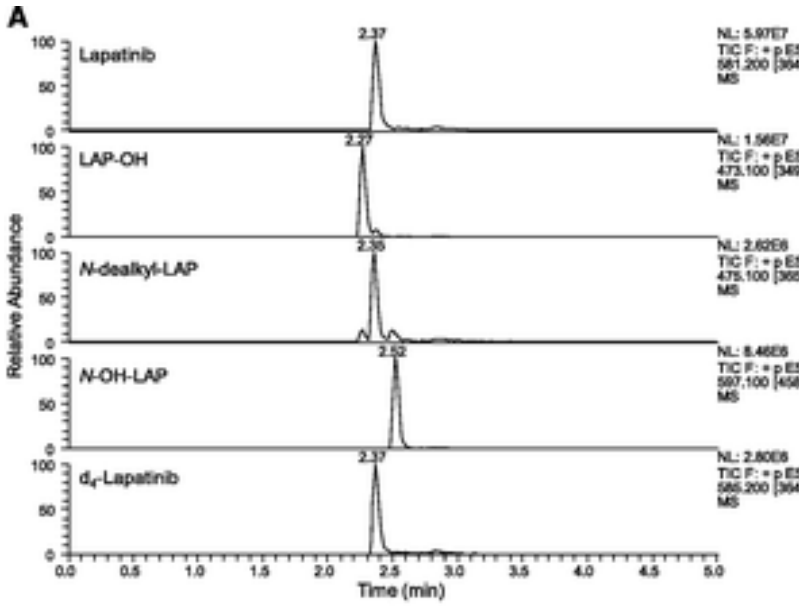
-to detect drugs and their metabolites in biological fluids and tissues

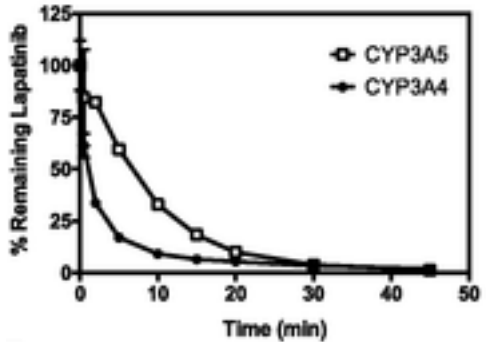
Commonly used techniques:

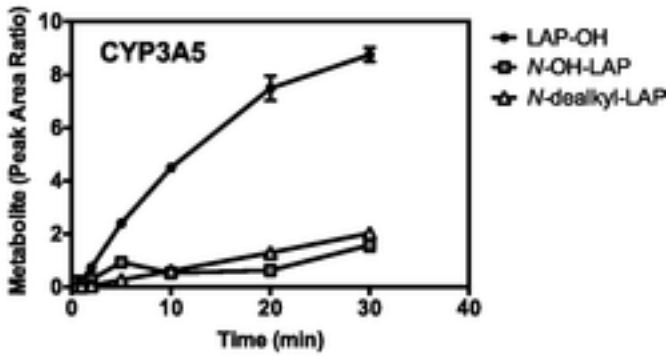
- GC-MS
- LC-MS
- CE-MS
- MALDI-MS imaging

Detection of lapatinib and its metabolites

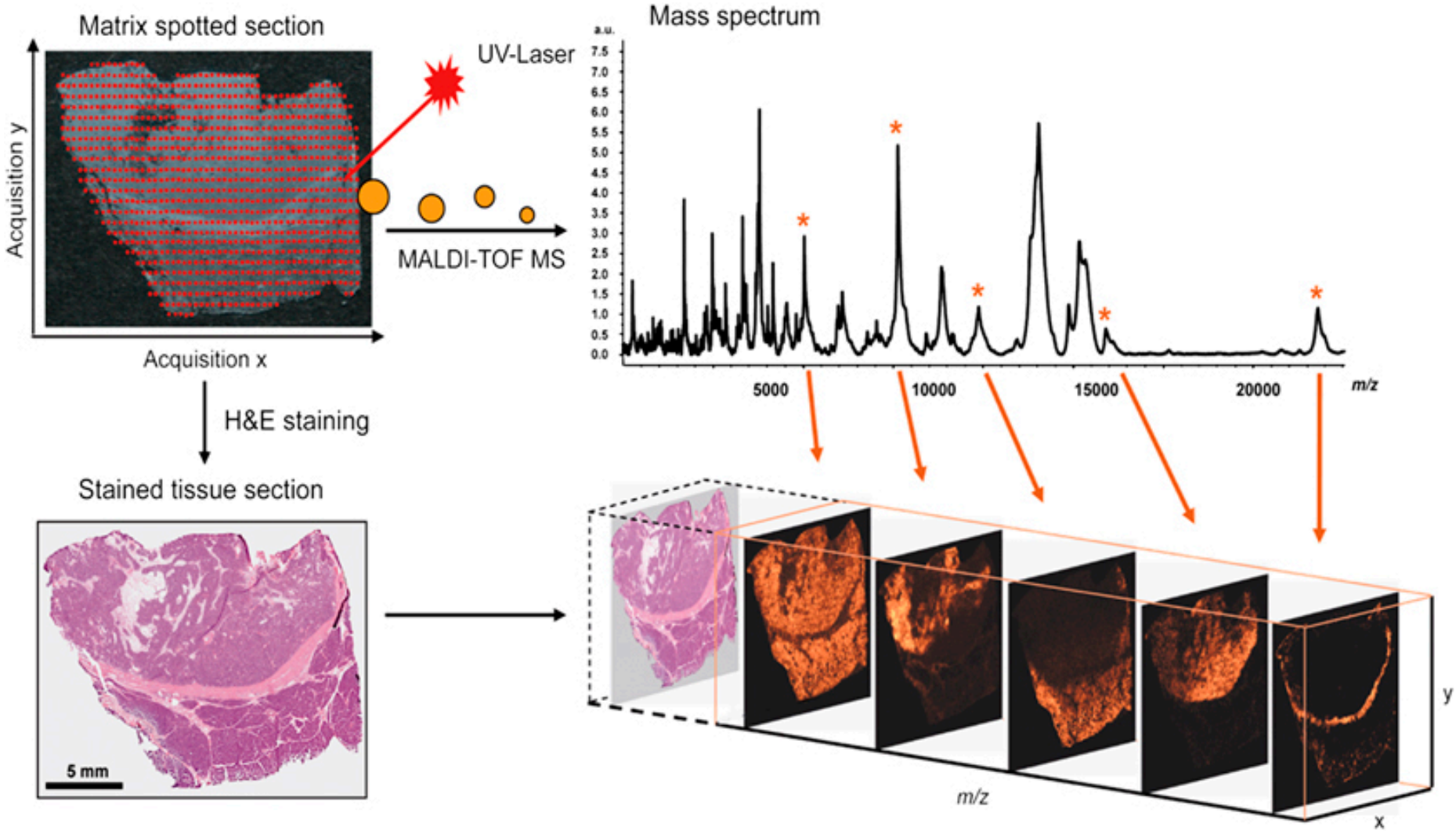




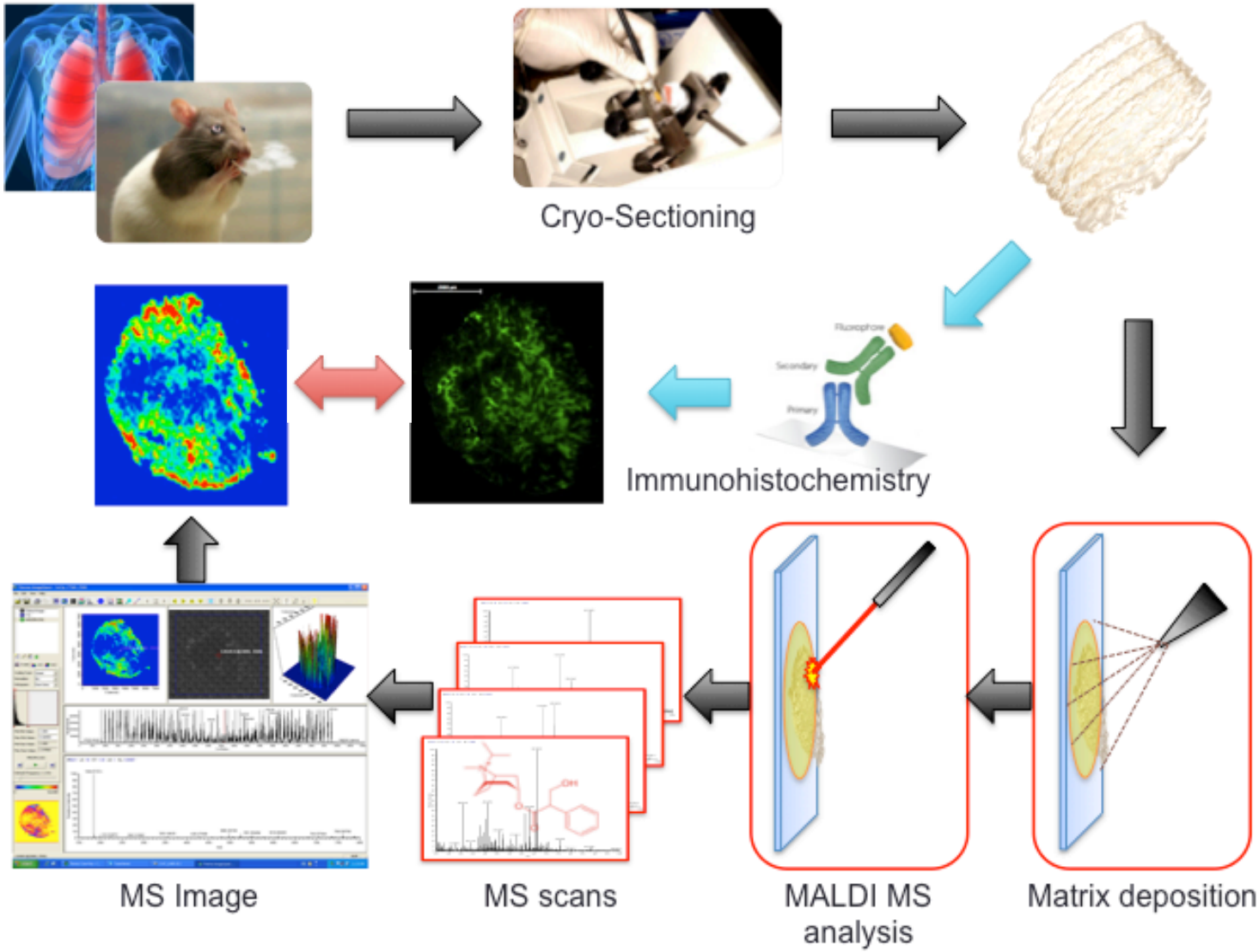




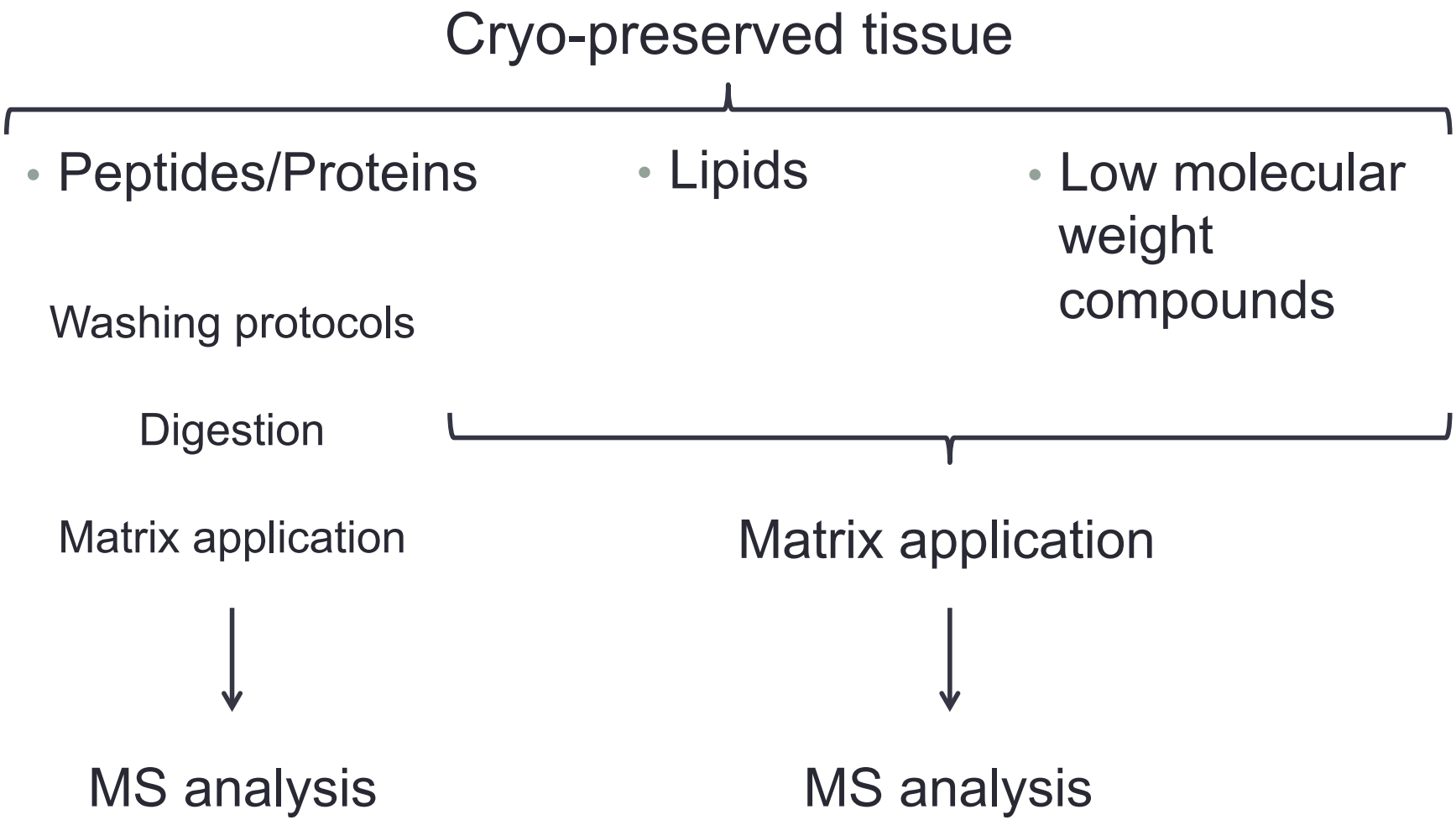
MALDI MS imaging



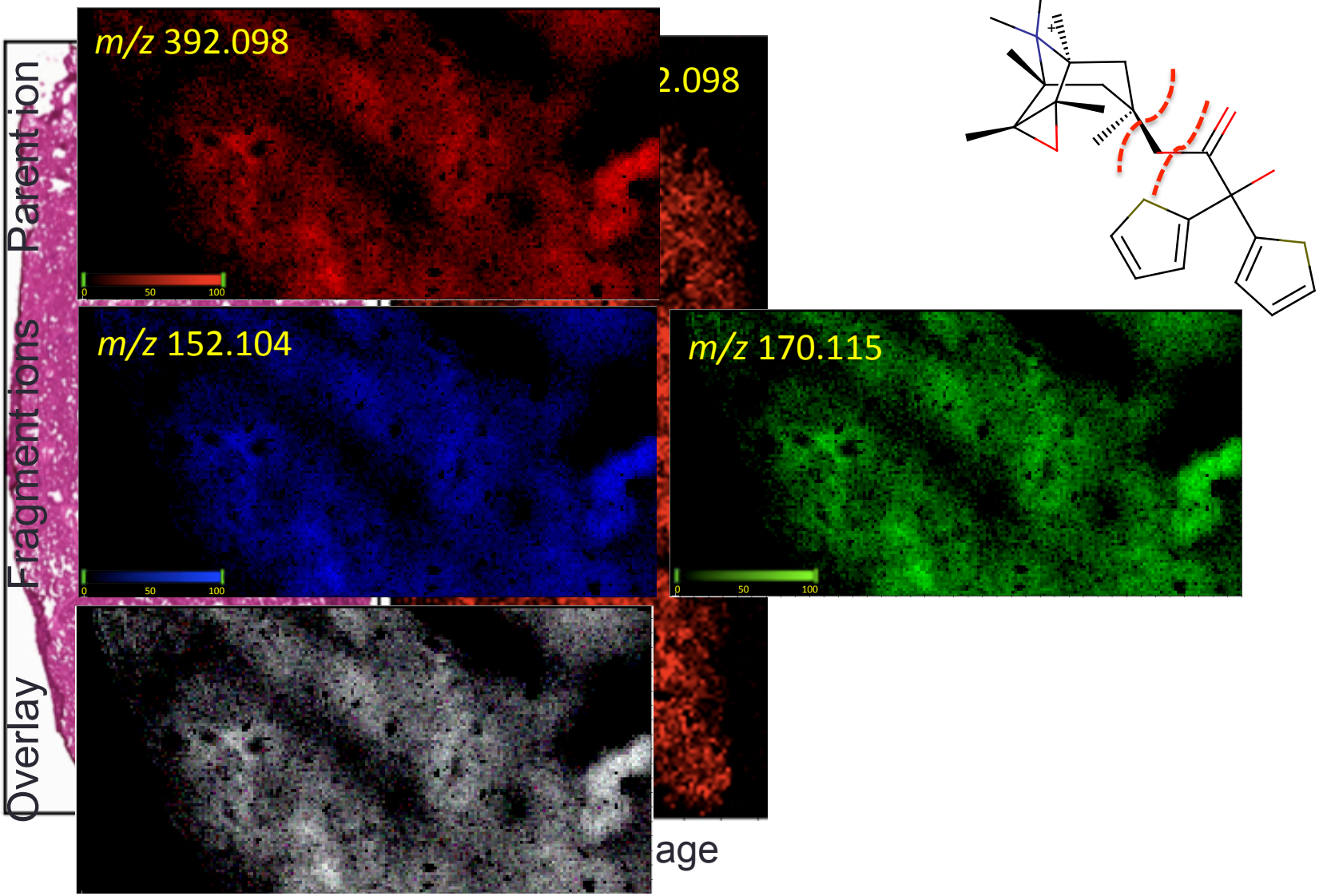
Workflow – human biopsies / animal models



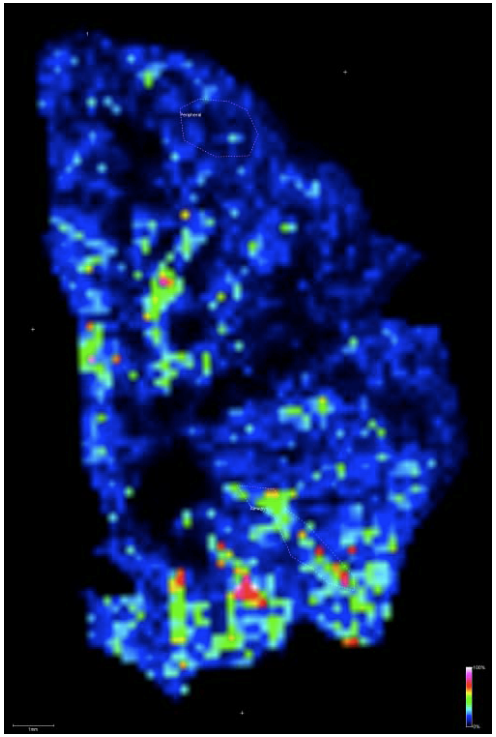
Analytes


Cryo-preserved tissue
• Peptides/Proteins
• Lipids
• Low molecular weight compounds
Washing protocols
Digestion
Matrix application
Matrix application
MS analysis
MS analysis

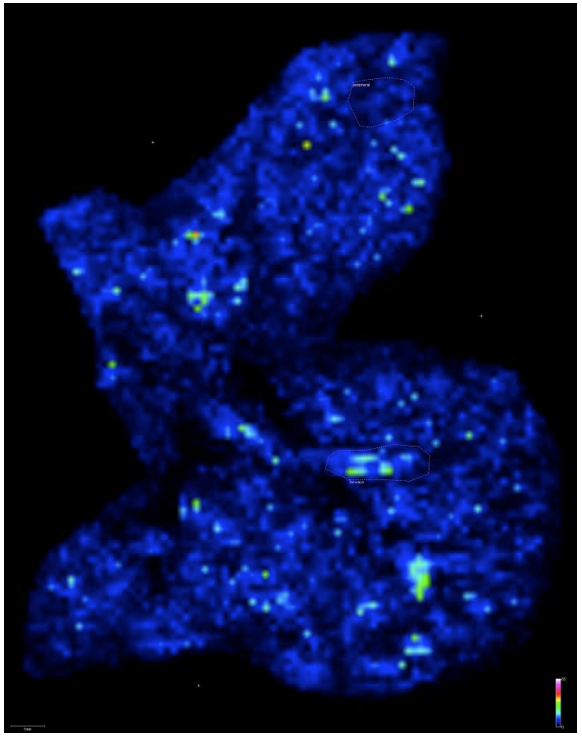
Drug distribution in rat lung



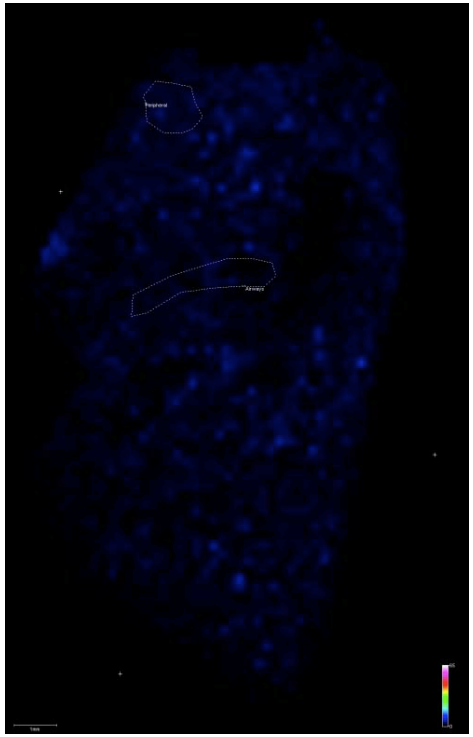
Temporal localization of drug in rat lung



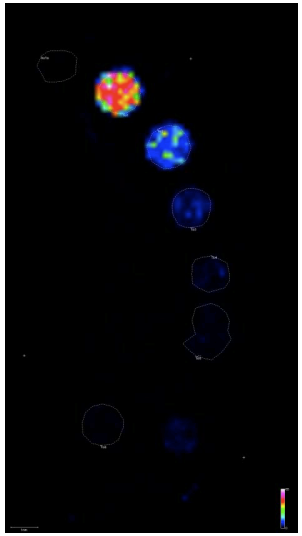
0 min



30 min

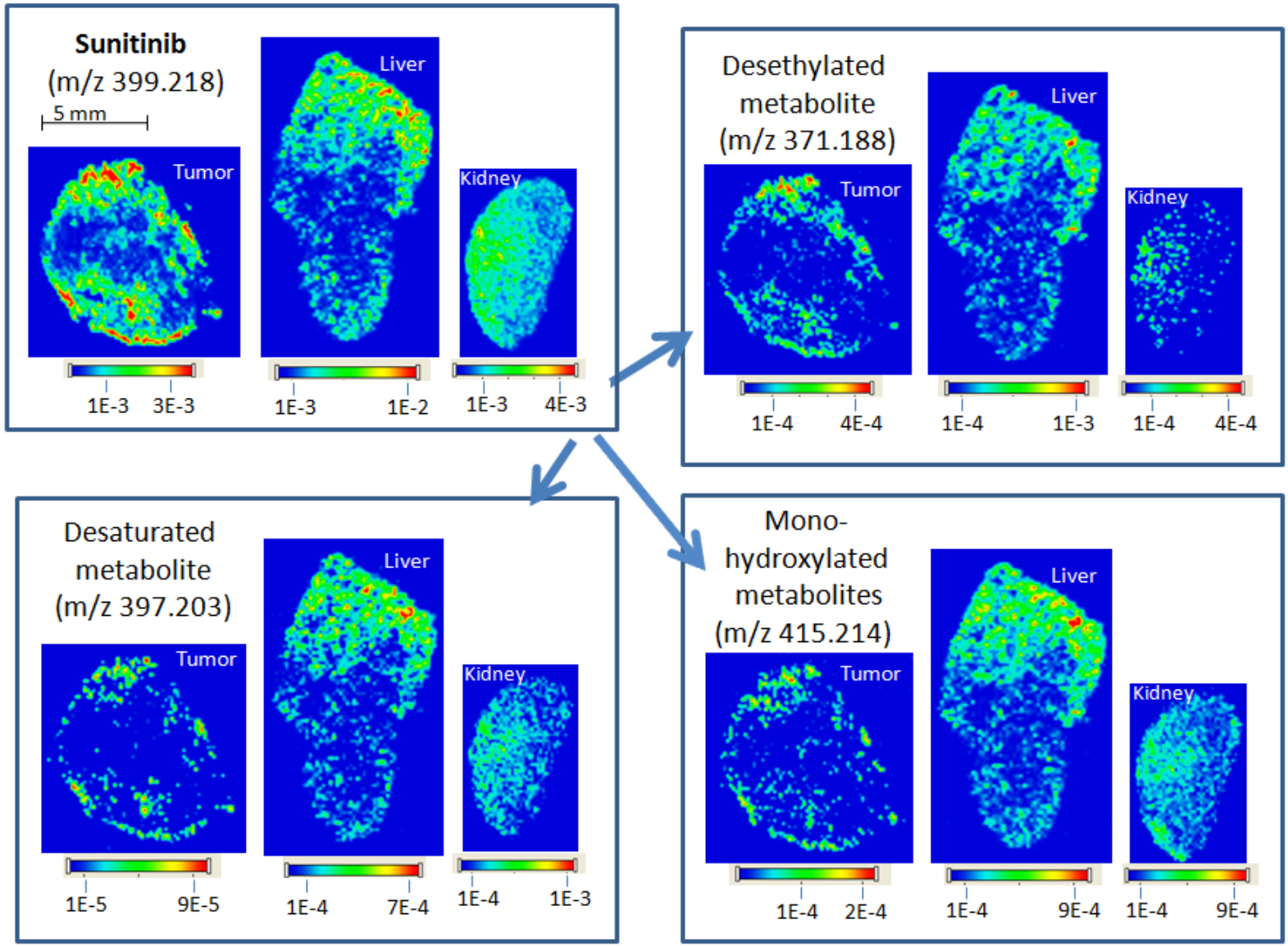


24 h

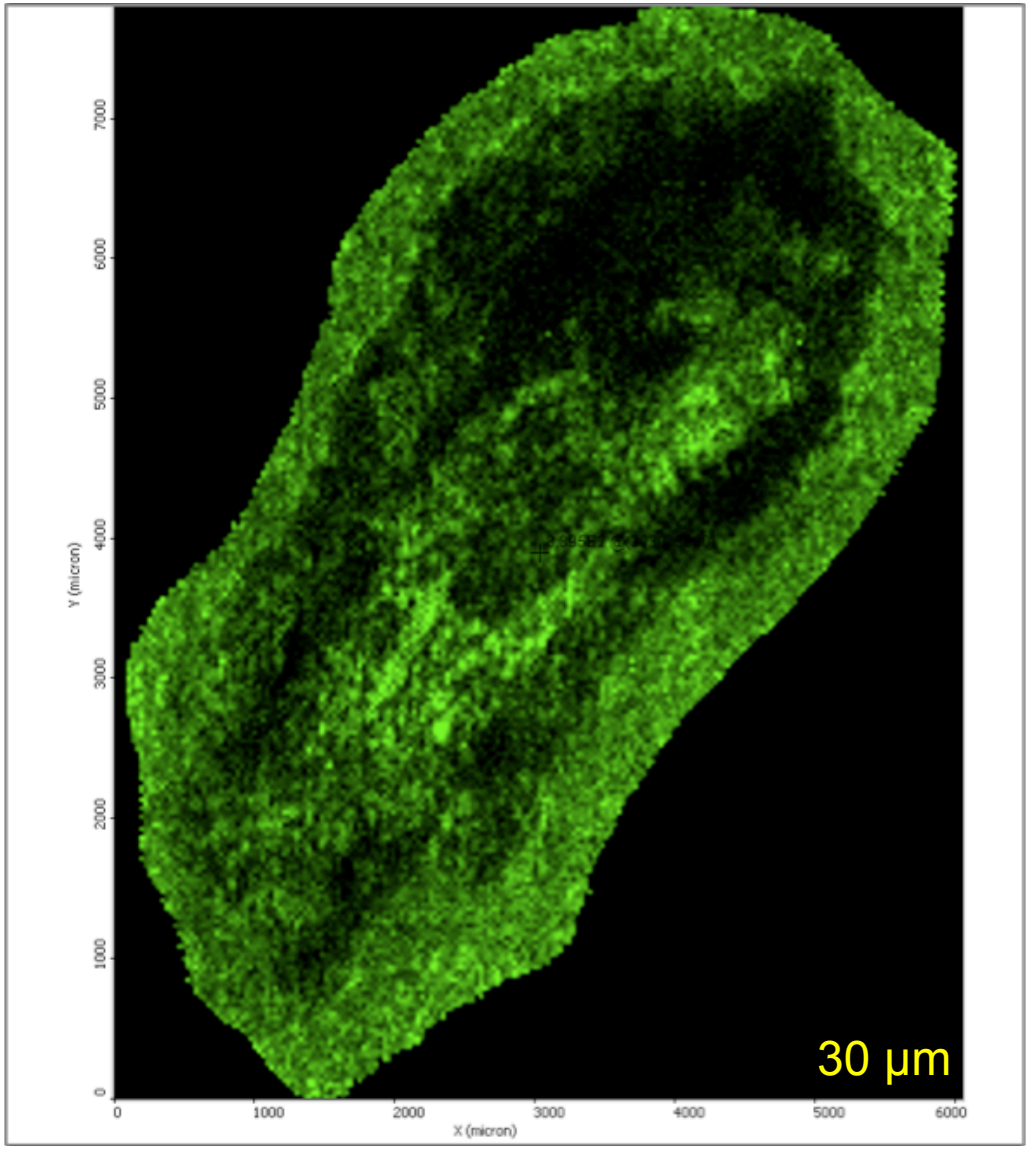


Calibration series

Distribution of a drug and it´s metabolites



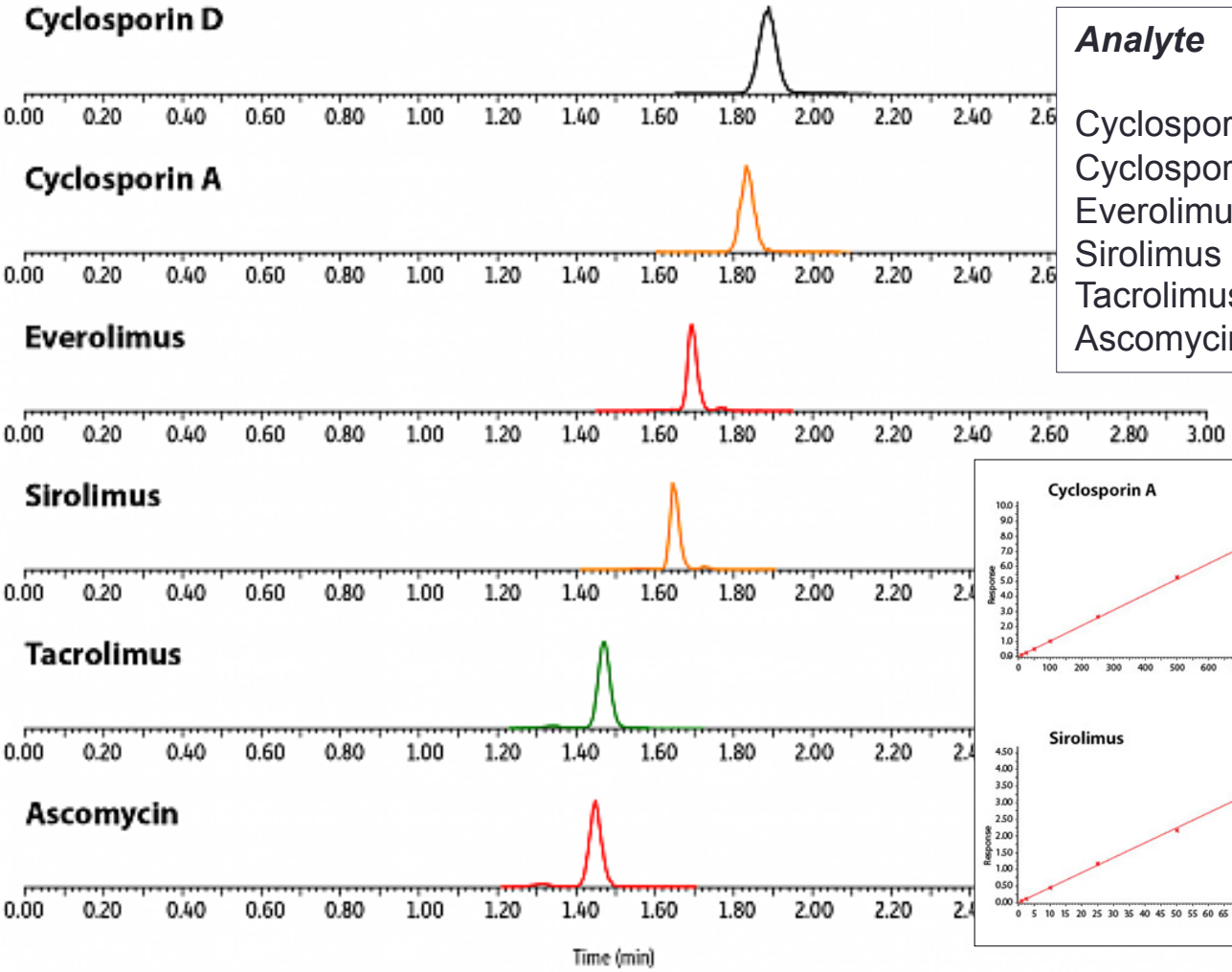
Importance of Sampling Resolution



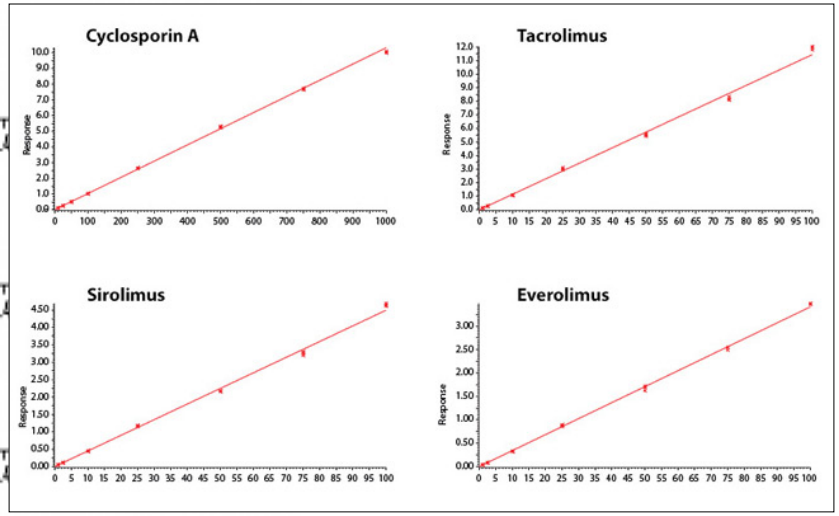
CLINICAL APPLICATIONS

- Therapeutic drug monitoring
- Screening for metabolic diseases
- Determination of hormones and other signaling components
- Toxicology
- Protein quantification and structure analysis
  - GC-MS, LC-MS, CE-MS
- Microbiological analysis
  - MALDI-TOF MS
- iKnife

Immunosuppressive Drugs in Whole Blood



Analyte	*Precursor Ion*	*Product Ion*
Cyclosporin D	1233.91	1216.88
Cyclosporin A	1219.83	1202.87
Everolimus	975.68	908.62
Sirolimus	931.63	864.57
Tacrolimus	821.61	768.51
Ascomycin	809.53	756.50



Newborn screening for metabolic diseases

Inborn errors of metabolism are disorders in which there is a block at some point in the normal metabolic pathway.

Disorders of

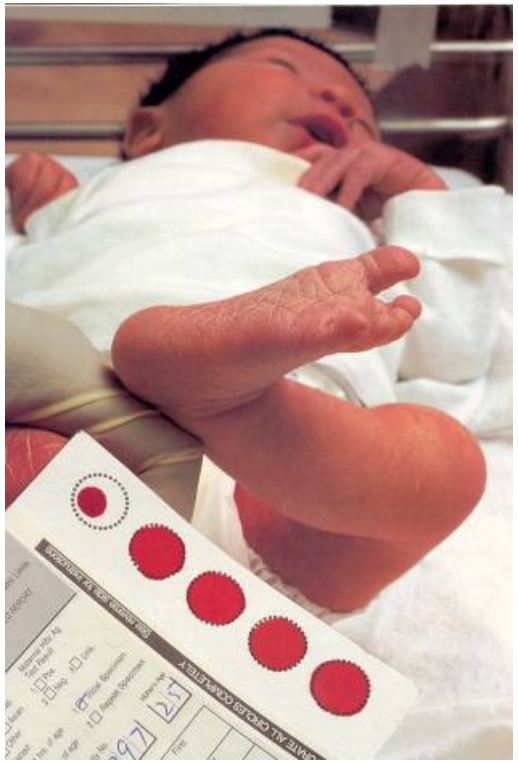
amino acid (*phenylketonuria, tyrosinosis, maple syrup disease, homocysteinuria, etc.*),

organic acid (*glutaric acidemia, propionic acidemia, etc.*)

and ***fatty acid metabolism*** (*carnitine palmitoyl transferase deficiency, short-, medium- and very long chain acyl-CoA dehydrogenase deficiency, etc.*)

Why tandem mass spectrometry (MS/MS)?

- One disease, one test is not cost-effective
- Many diseases, one test is cost-effective
- MS/MS allows for rapid, simultaneous analysis and detection of many metabolic disorders



Blood Sample on Guthrie Filter Paper Card

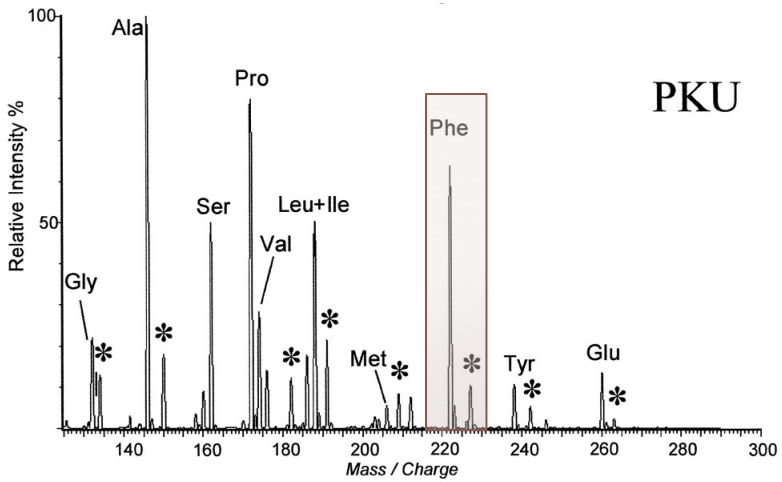
MS/MS methodology

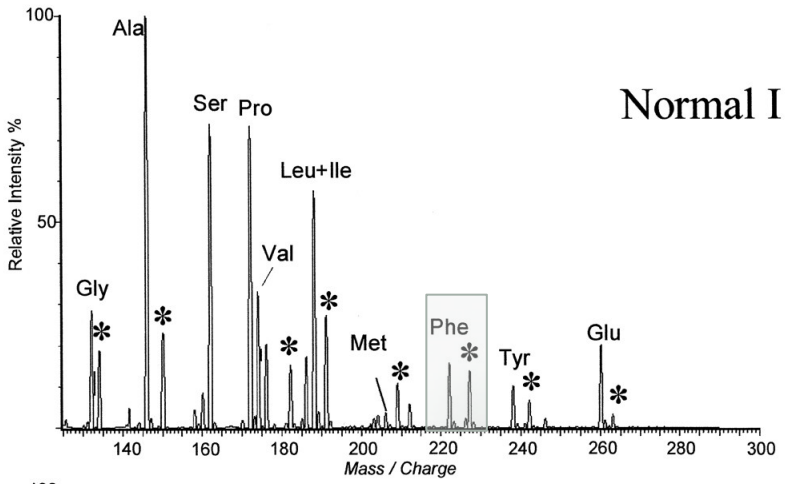
- Punched blood spots
- Added stable isotope internal standards
- Sample set up determines which masses and therefore which compounds are detected
- Short analysis time
- Automated data processing

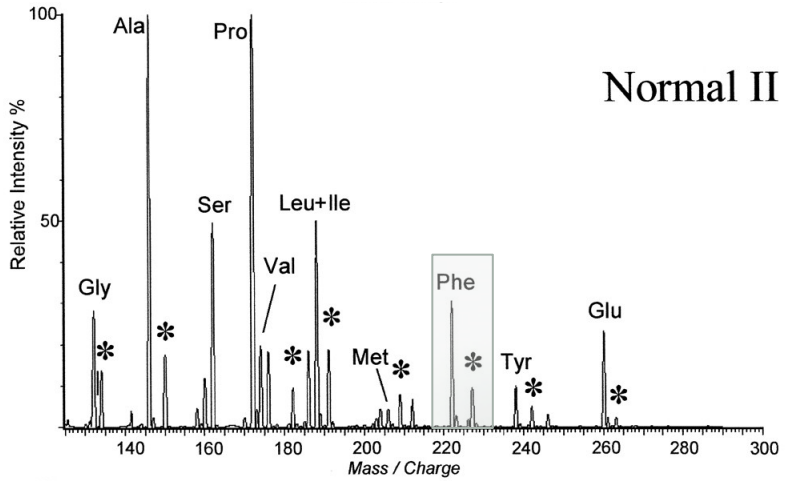
Compounds analyzed are amino acids and acylcarnitines

- Amino acids – to identify AA metabolic diseases
- Acylcarnitine –to identify organic acidurias and fatty acid oxidation disorders

Amino acid profile – phenylketonuria (PKU)

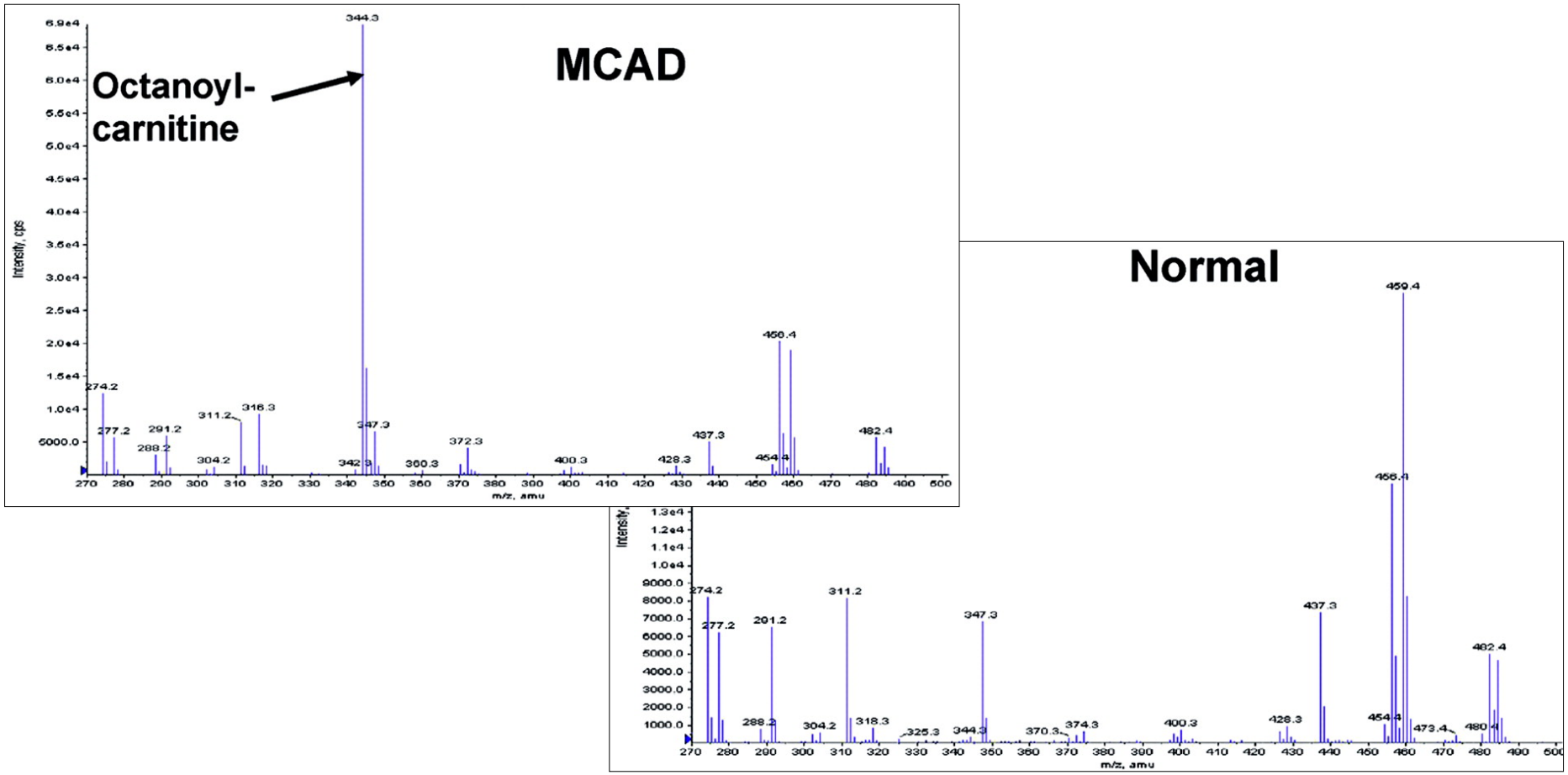






* internal standards

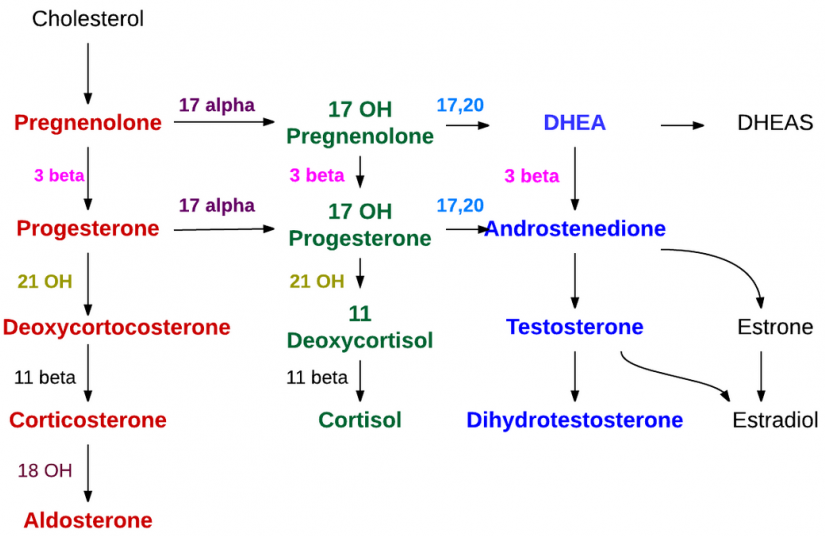
Acylcarnitine profile – MCAD deficiency



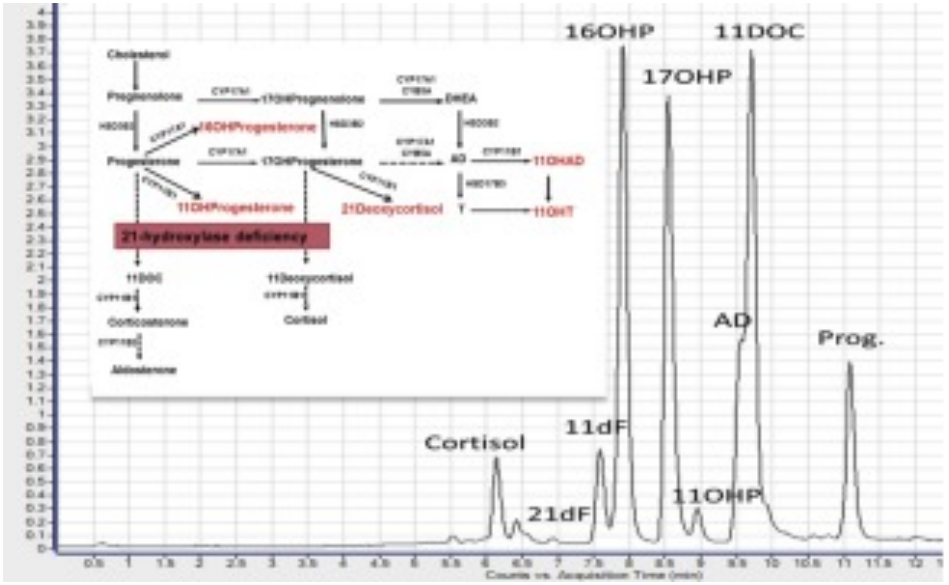
Typical acylcarnitine profile from a healthy individual and medium-chain acyl-CoA dehydrogenase (MCAD) deficiency patient. The abnormal elevation of octanoylcarnitine (C8) is characteristic of MCAD deficiency.

Analysis of the steroid metabolites

- congenital adrenal hyperplasia (CAH)
- polycystic ovarian syndrome (PCOS)
- Cushing's syndrome
- Addison's disease
- gonadal dysfunctions

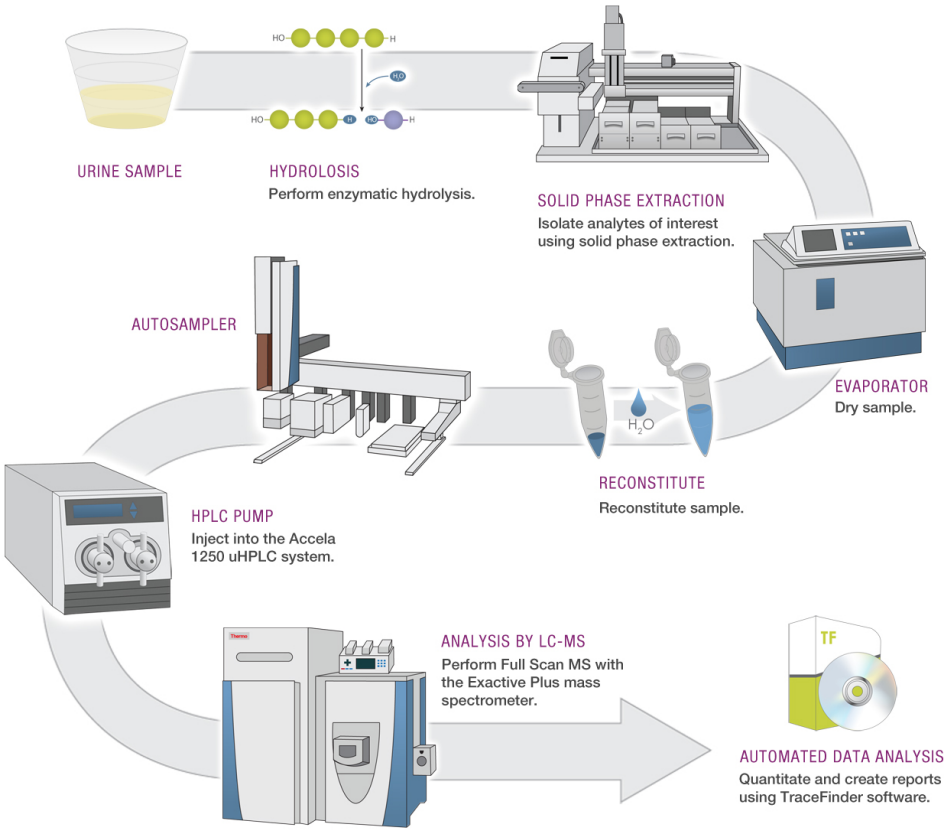


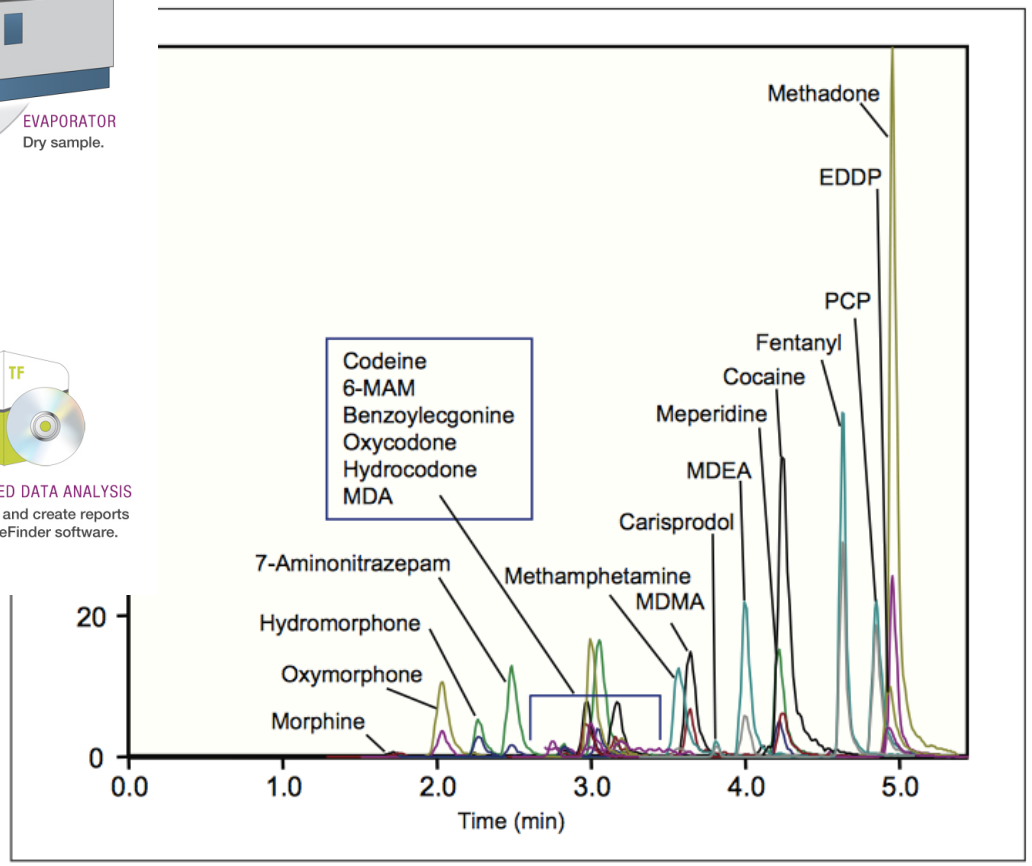
Congenital adrenal hyperplasia (CAH)



Diminished or absent activity of 21-hydroxylase. Increased levels of 17-hydroxyprogesterone (17-OHP), DHEA sulphate, androstenedione, and testosterone. Decrease levels of deoxycorticosterone, 11-deoxycortisol, corticosterone, cortisol, and aldosterone.

Quantitation of drugs of abuse in body fluids

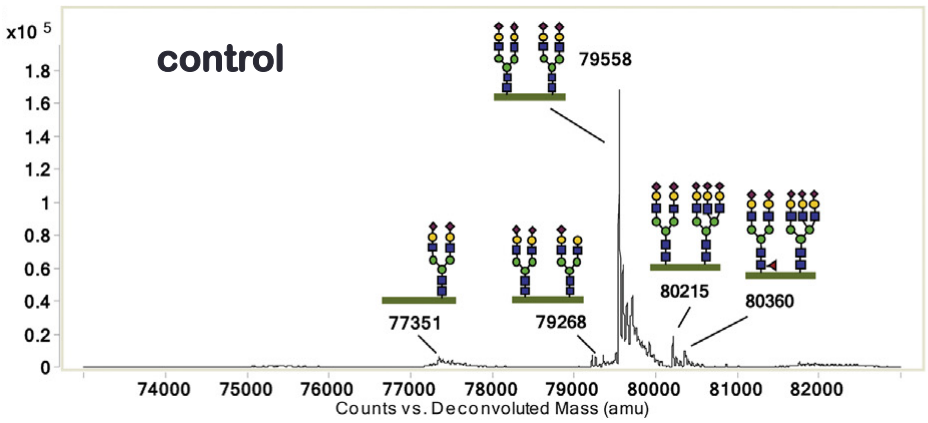


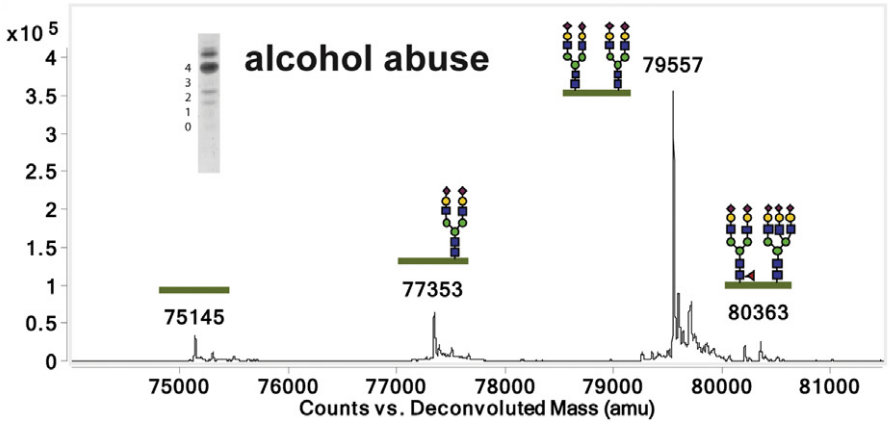


The workflow described here uses a simple dilute-and-shoot, solid-phase extraction (SPE) or liquid-liquid extraction (LLE) for sample preparation.

Carbohydrate deficient transferrin – chronic alcohol abuse

Carbohydrate-deficient transferrin (CDT) is currently the most specific laboratory marker of chronic alcohol abuse.
Transferrin is a glycoprotein, the most common form is tetrasialotransferrin, with 4 sialic acid chains. In persons who consume significant quantities of alcohol, the proportion of transferrin with 0, 1 or 2 sialic acid chains is increased.





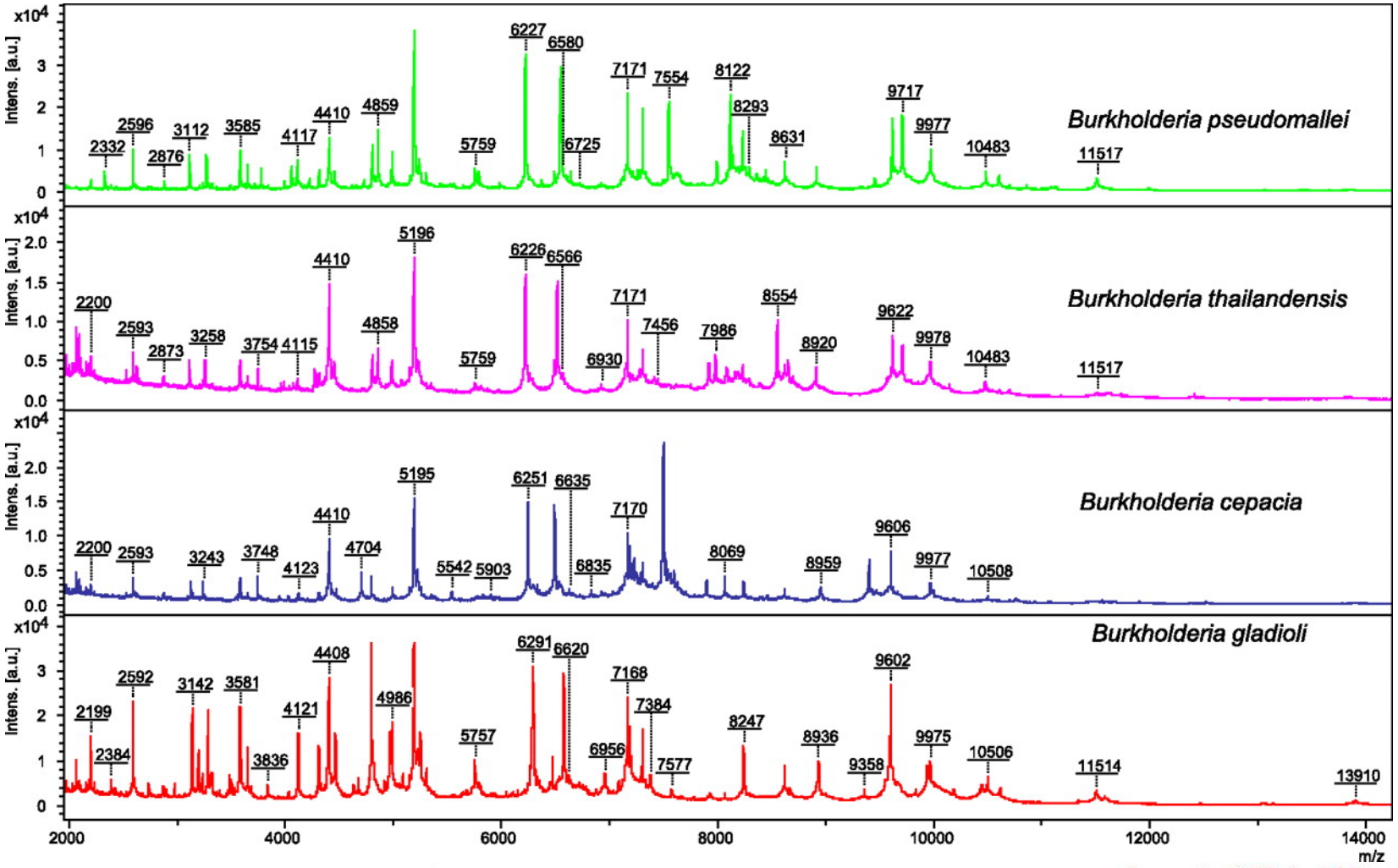
Translational Research 2015;166:639–649

Identification of microorganisms by MALDI-TOF MS

The identification of microorganisms by MALDI-TOF MS is based on the detection of mass signals from protein biomarkers that are specific for genus, species or sub-group.
Protein biomarkers are the most characteristic and accessible molecules in the analysis of whole cells (they provide good signals without extraction, separation or amplification).
A spectrum from whole bacterial cells typically contains 10–30 peaks with masses ranging from 2 to about 20 kDa (intact proteins).

Prerequisites for correct identification of microorganisms with MALDI-TOF are the use of a database consisting of spectra of known samples. The quality of the database is crucial.
At present commercially available libraries permit identification of more than 90% of clinically relevant bacteria.

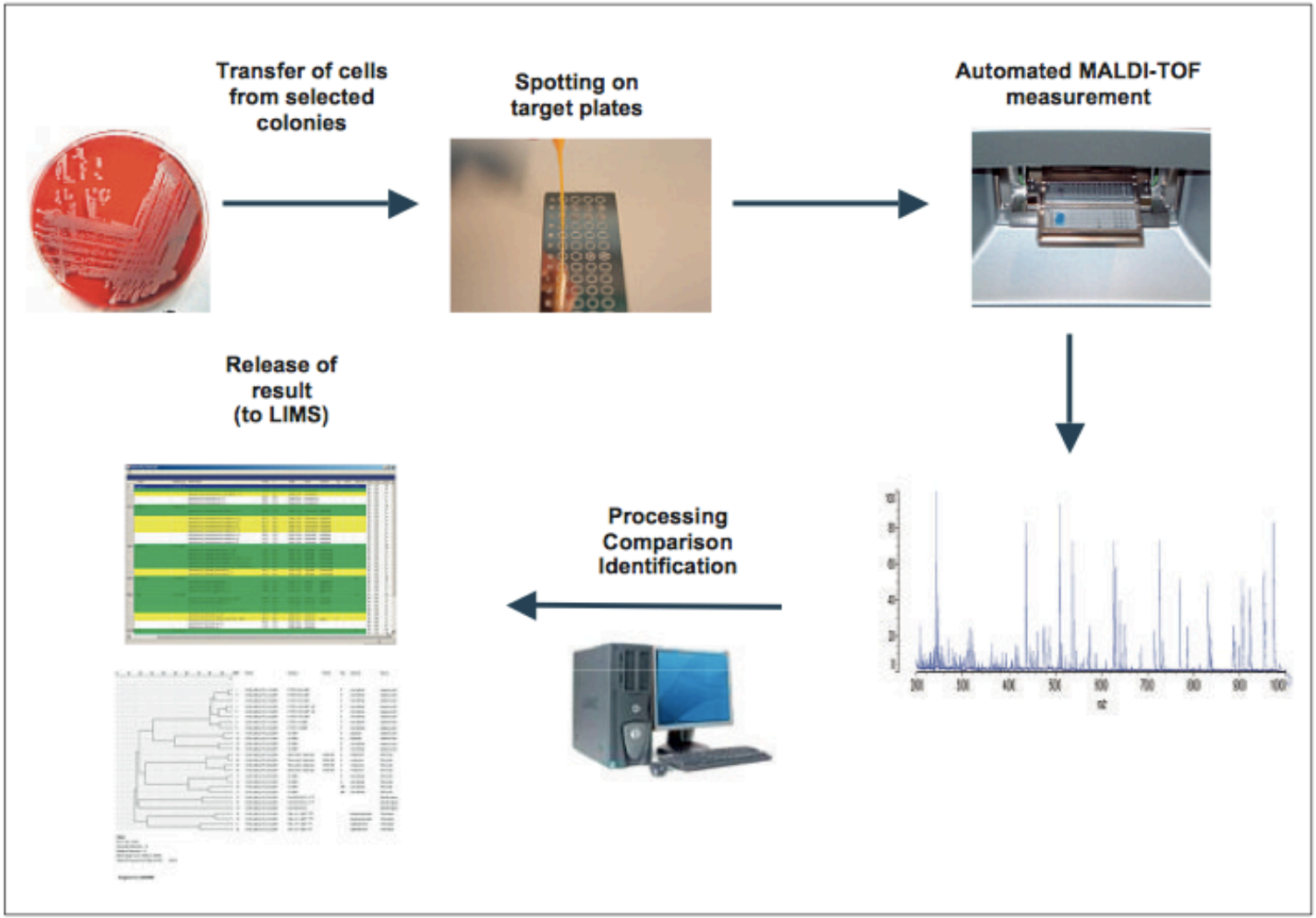
MALDI-TOF MS spectra of B. pseudomallei compared to those of other species



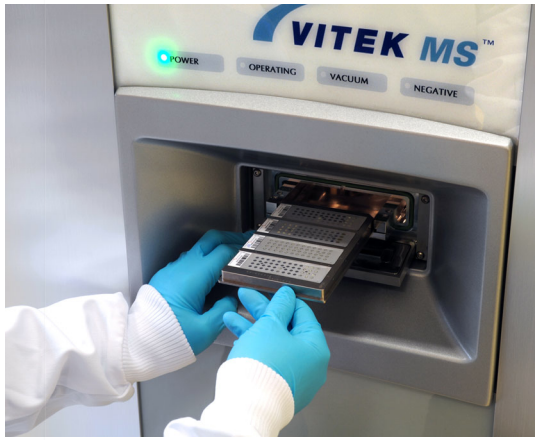
Susanna K. P. Lau et al. J. Clin. Microbiol. 2012;50:3142-3143

Journal of Clinical Microbiology

General workflow for microorganism identification/classification



Typical MALDI-TOF instrumentation



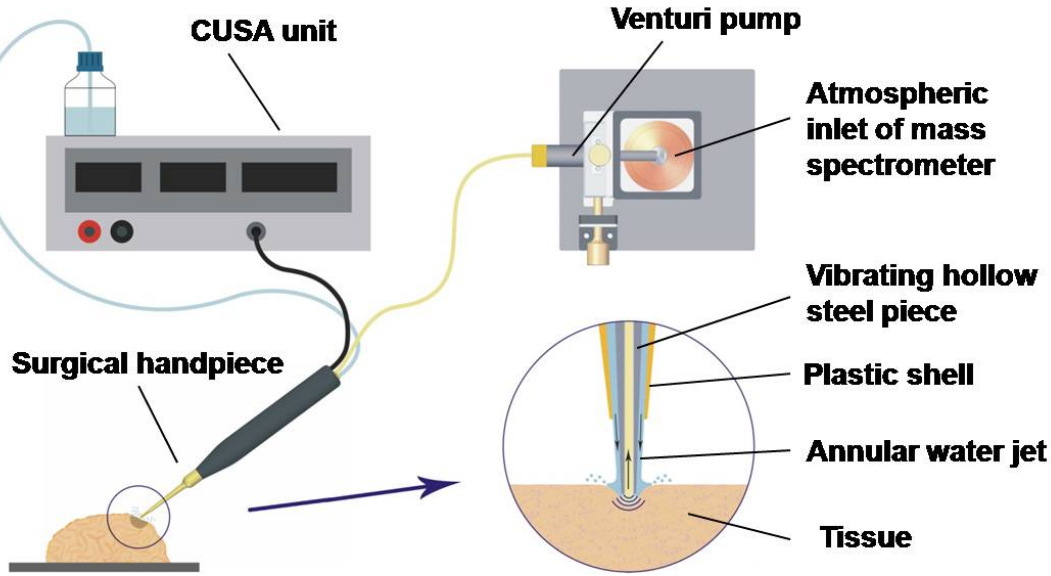
http://www.biomerieux-diagnostics.com

REIMS technology – intelligent surgical knife

Rapid evaporative ionization mass spectrometry (REIMS) is a technique that allows near–real-time characterization of human tissue in vivo by analysis of the aerosol (“smoke”) released during electrosurgical dissection.
The ***coupling of REIMS technology with electrosurgery*** for tissue diagnostics is known as the **intelligent knife (iKnife)**.

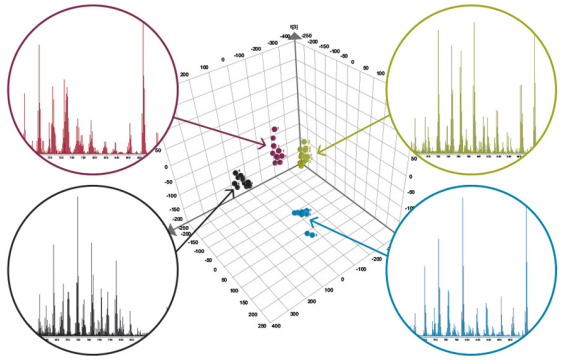
The REIMS-iKnife method is able to detect the tumor margin in a surgical environment in patients undergoing resection of brain, liver, lung, breast, or colorectal tumors.
It has the potential to influence “on-table” decision-making, it provides a valid alternative to frozen section histology and ultimately to improve oncological outcomes. It could also improve cosmetic and functional outcomes by minimizing surgical trauma and the unnecessary removal of healthy tissue.

Schema of REIMS instrumentation and data collection using Cavitron Ultrasonic Surgical Aspirator (CUSA).



The CUSA is an innovative tool for dissecting various tissues. It can potentially reduce intraoperative blood loss and perioperative morbidity. It is widely used in neurosurgery, gastrointestinal, hepatobiliary surgery, gynecology, and urology.

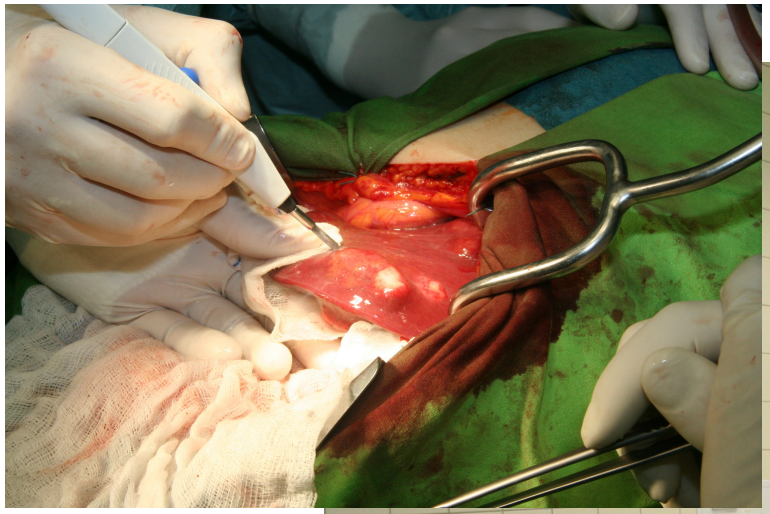
Before it´s use in the surgical suite, iKnife data is collected ex vivo from various benign and malignant tissue samples and, from these data, a histologically authentic spectral reference library is constructed. Database and multivariate statistical tissue identification algorithms are then tested during surgery.



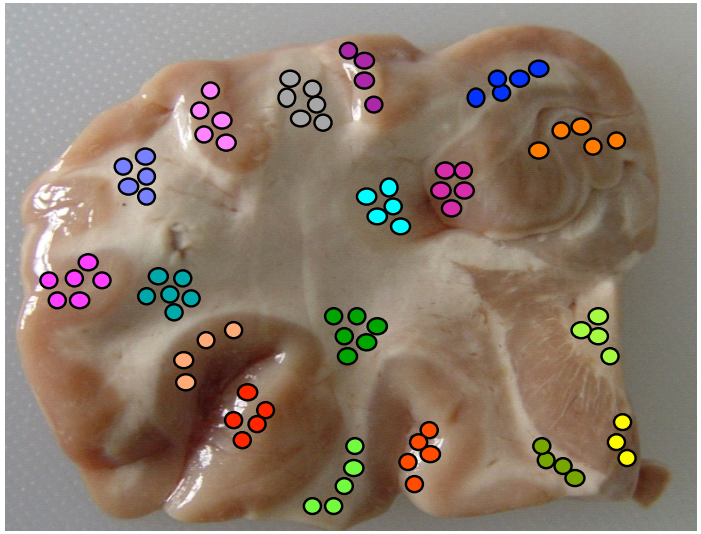
OPLS-DA Plot showing 4 distinct sample groups, with an example mass spectrum from each group.

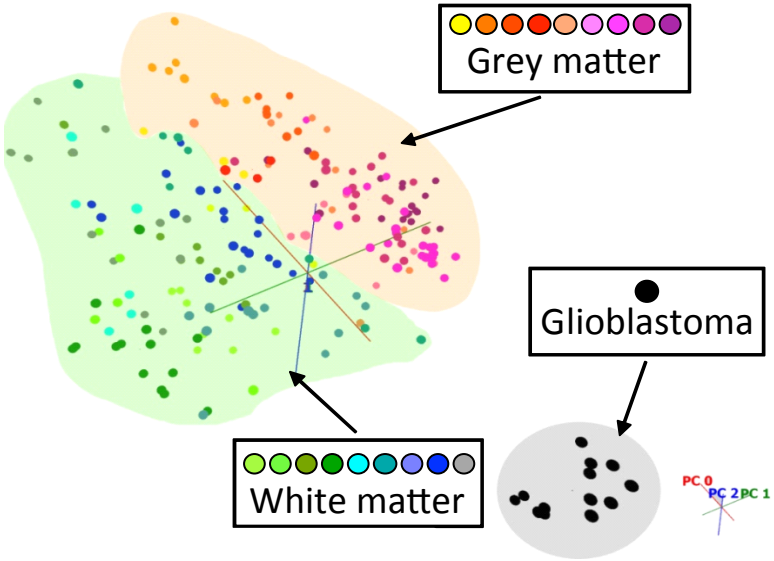
http://www.waters.com/waters/en_SE/REIMS-Research-System-with-iKnife-Sampling-Device

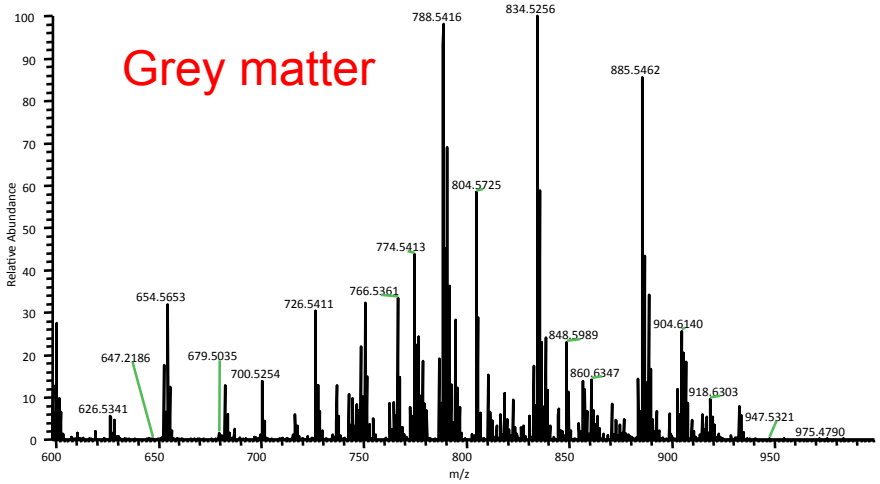
iKnife in the operating room



Human brain




Grey matter
Glioblastoma
White matter
PC 0
PC 2
PC 1


Grey matter
Relative Abundance
m/z
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885.5462
804.5725
774.5413
654.5653
726.5411
766.5361
848.5989
904.6140
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679.5035
700.5254
860.6347
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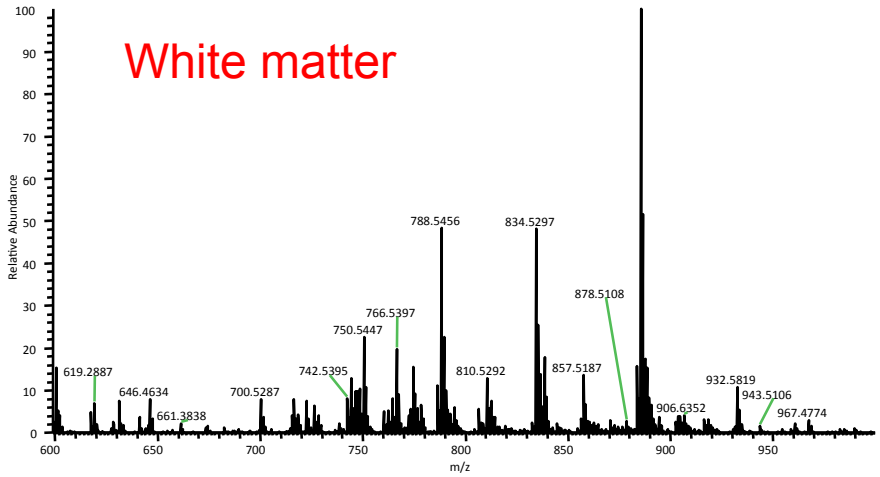
626.5341
947.5321
975.4790


White matter
Relative Abundance
m/z
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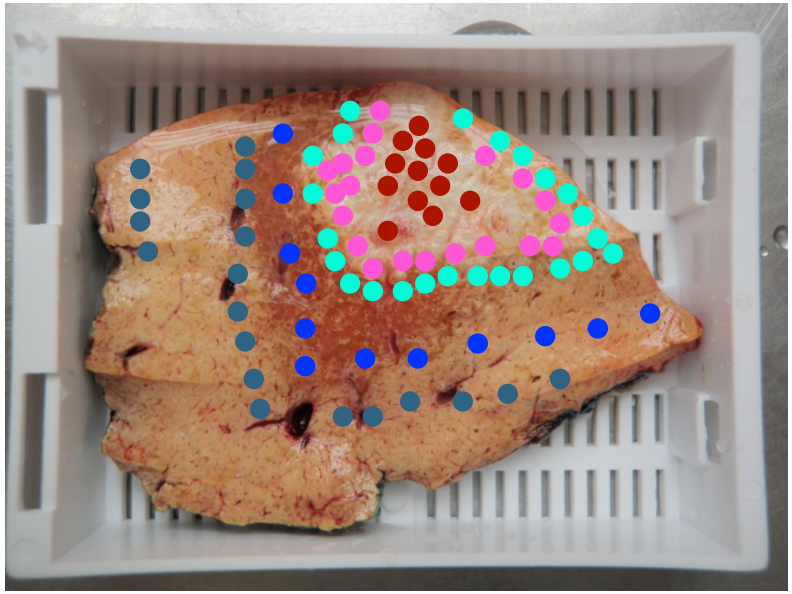
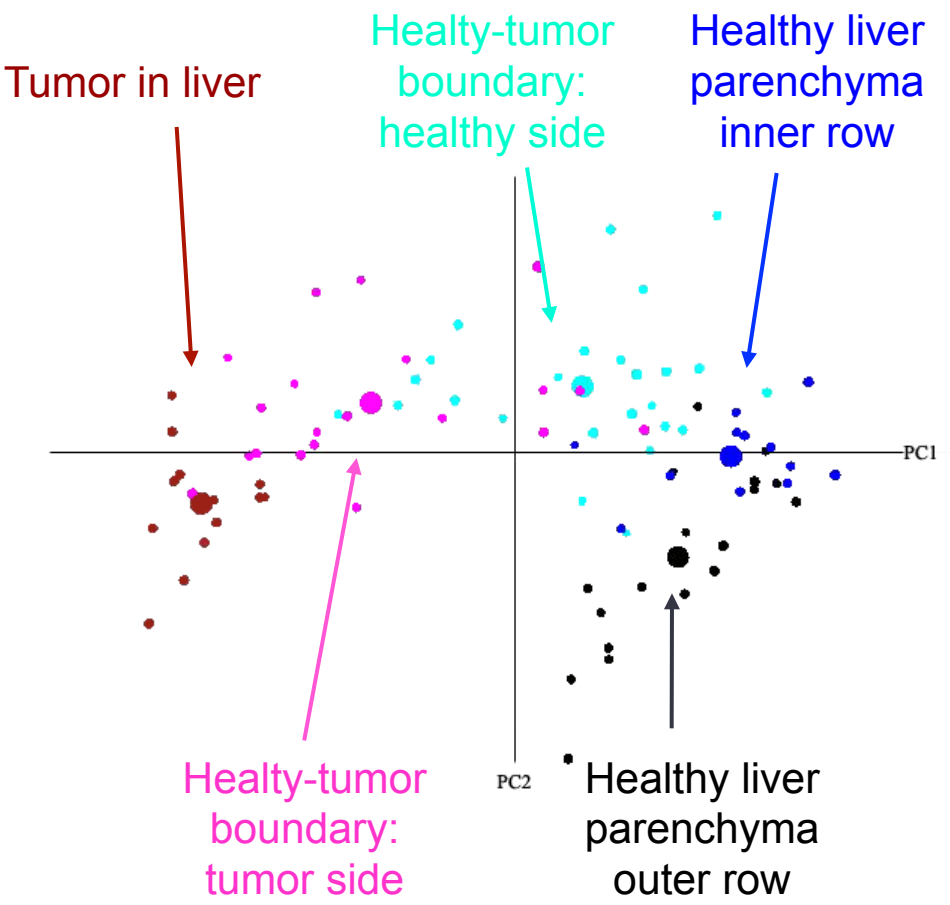
834.5297
878.5108
766.5397
750.5447
619.2887
742.5395
810.5292
857.5187
646.4634
700.5287
661.3838
932.5819
943.5106
906.6352
967.4774

Colon adenocarcinoma metastasis





Thank You!