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Excellence in Science

Mass Spectrometry – A Powerful tool for metabolomics

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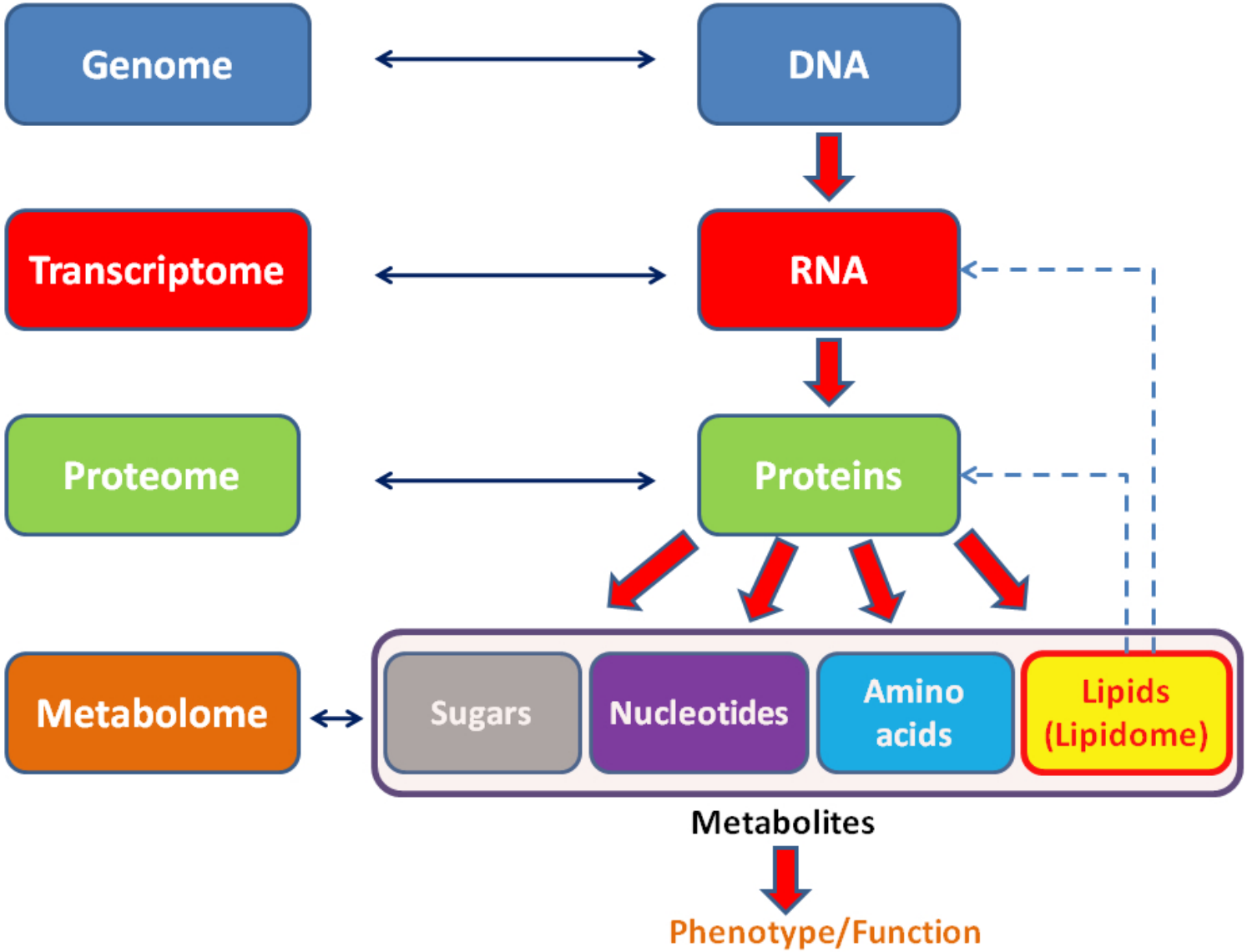
Manager, MS & Chromato
Shimadzu [Asia-Pacific] Pte Ltd


Genome
DNA
Transcriptome
RNA
Proteome
Proteins
Metabolome
Sugars
Nucleotides
Amino acids
Lipids (Lipidome)
Metabolites
Phenotype/Function

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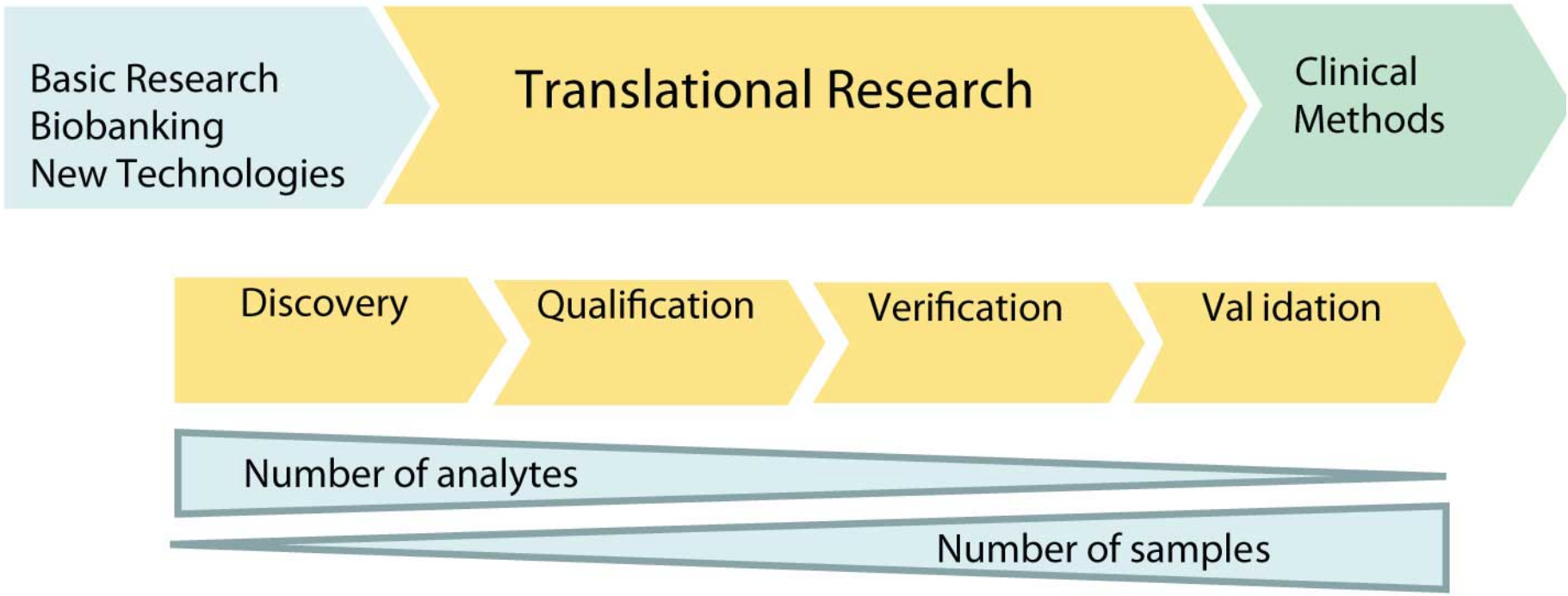
Biomarkers Identification and Validation



Mass Spectrometry –For Metabolomics

GCMS-QP2010 Ultra

GCMS-TQ 8040

LCMS-8060

Nexera UC- Online SFE/SFC

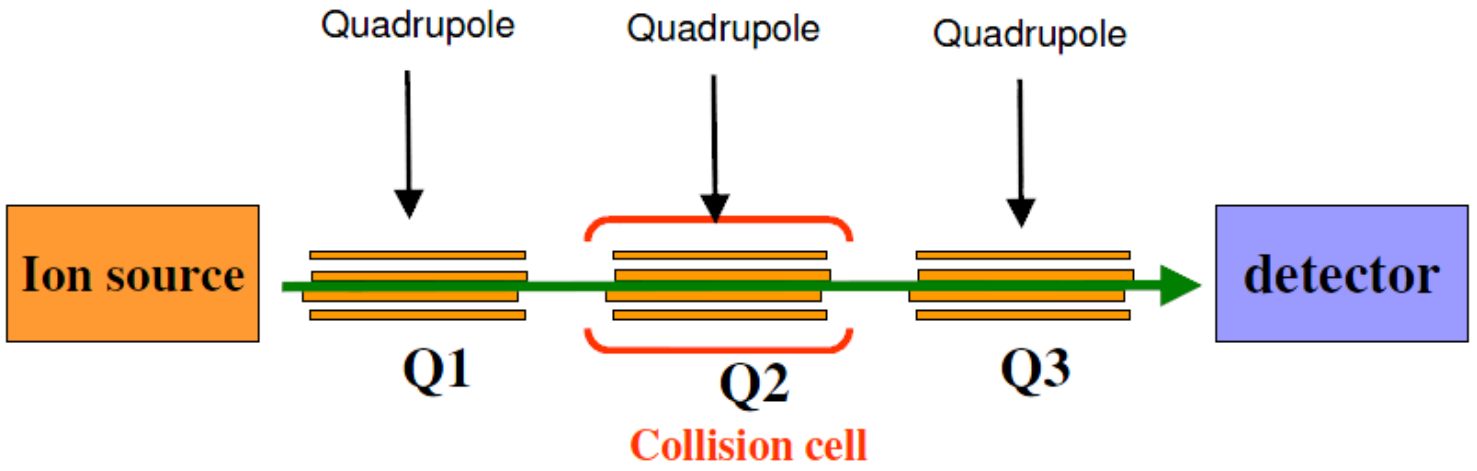
iDPlus-Performance

Bacterial Identification

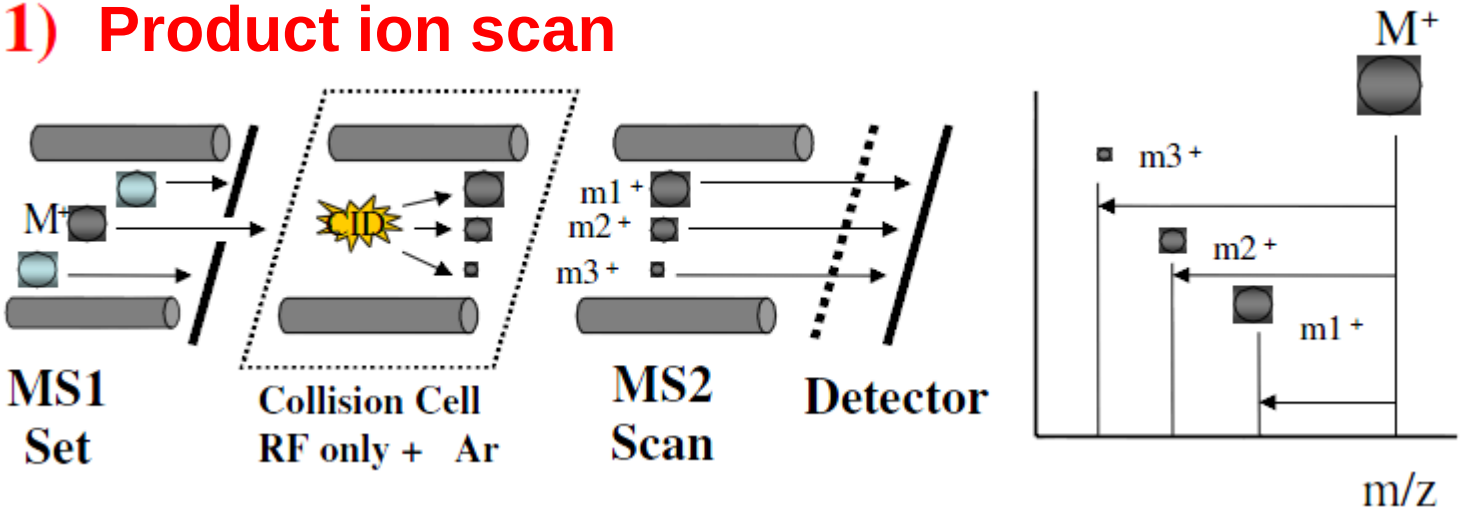
MALDI -7090 High Resolution TOF

iMScope-Trio –Mass imaging

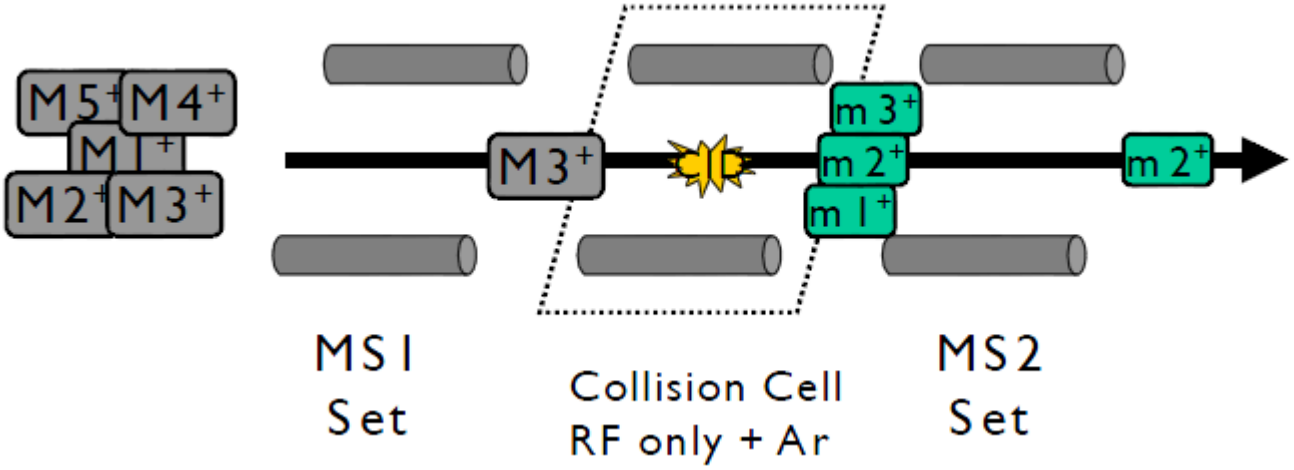
Triple Quadrupole MS/MS



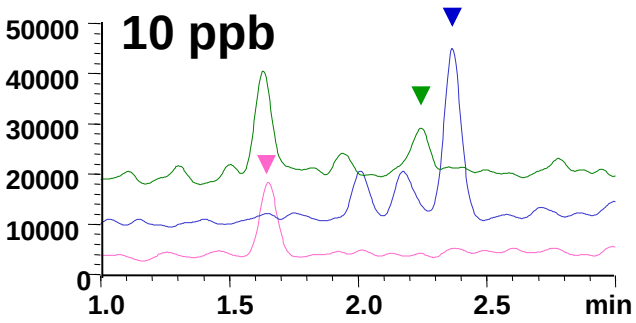
1) Product ion scan



MRM- Multi Reaction Monitoring

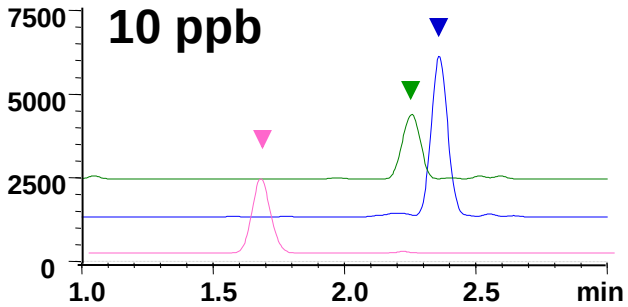


SIM (single analysis)



High sensitivity but high background

MRM (MS/MS analysis)



Eliminates background for trace-level quantitation with high S/N

GCMS- Gold Standard for Metabolomics

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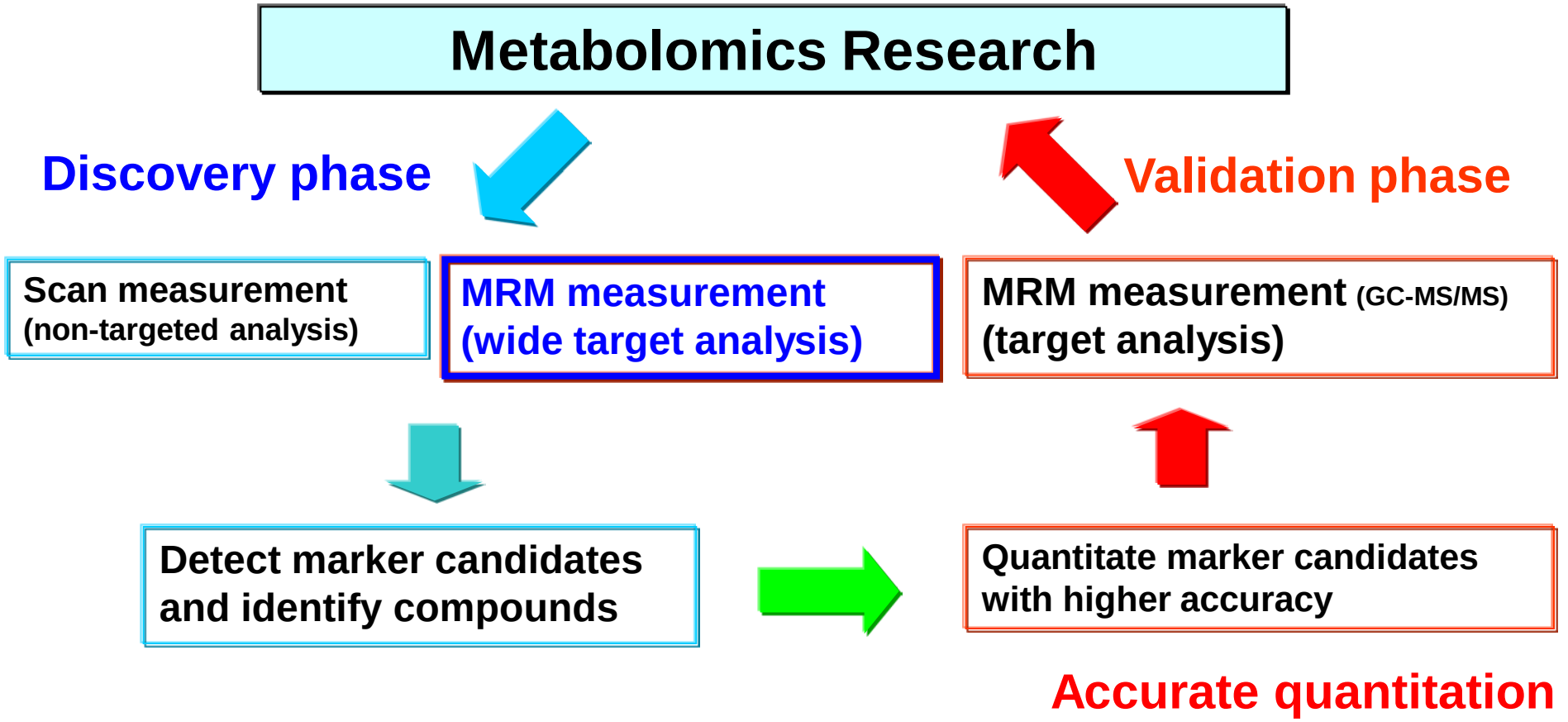
Metabolomics Research Using GC-MS/MS



GC/MS Metabolite Database

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C146-E277

Smart Metabolites Database

Database Software for
GC/MS and GC-MS/MS Analysis
of Metabolites

Easy Work Flow

AART function for Automatic Adjustment of Retention Indices with just one injection

Select components for measurement from the database.

Smart MRM database

Method is created Automatically

Start acquisition.

GCMS-TQ8040

1. Smart Productivity

- Analysis of 400 pesticides that used to require 2 or 3 methods, can now be accomplished in a single acquisition method by the new firmware protocol.

2. Smart Operation

- **Smart MRM** technology creates optimal MRM methods automatically. The “MRM Optimization Tool“ automates best MRM transitions for new compounds.

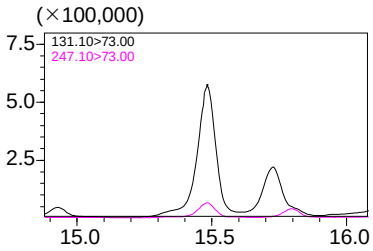
3. Smart Performance

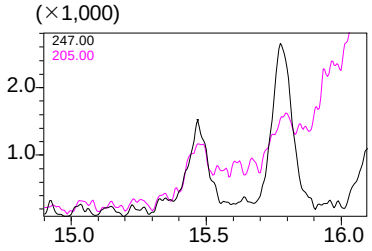
- ASSP achieves high sensitivity at scan speeds of 20,000 u/second. Fastest MRM 800trans/sec. Single GC/MS mode with the maximum possible sensitivity and repeatability.

Advantages of GCMSMS

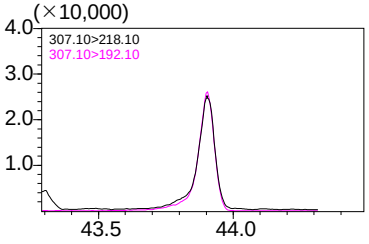
Comparison of Reproducibility for Measuring Metabolites in Human Blood Serum Using MRM and Scan Modes

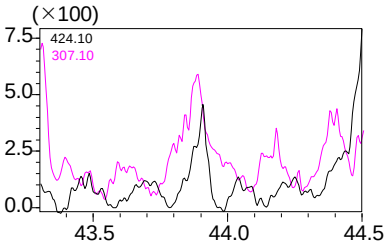
3-Hydroxyisovaleric acid-2TMS





Kynurenine-3TMS





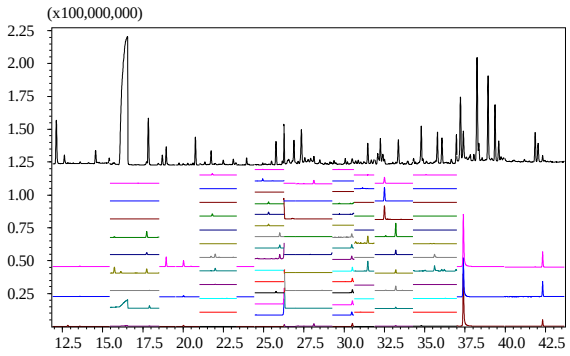
Compound name	%RSD (n=6) MRM	Scan
3-Hydroxyisovaleric acid-2TMS	3.99	14.0
Homocysteine-3TMS	5.04	23.4
Aconitic acid-3TMS	5.98	N/A
Kynurenine-3TMS	6.48	24.5

MRM is able to eliminate the effects of interfering substances so that it can measure trace components accurately.

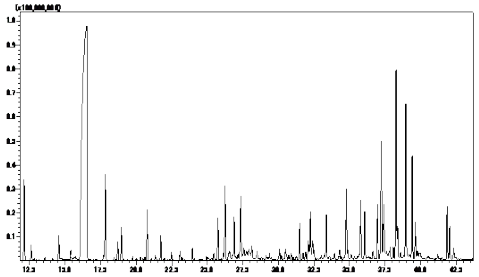
193 types of trimethylsilylated metabolites
50 types of fatty acid methyl esters (compatible with EI/PCI ionization)

Advantage of GCMSMS

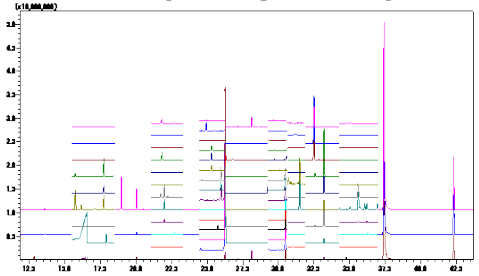
Scan/MRM data



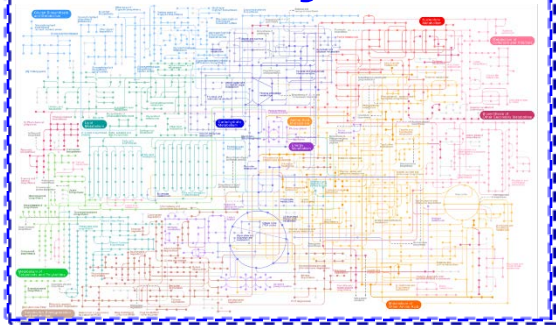
Scan (Non-targeted)



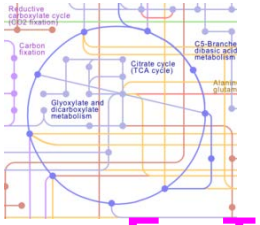
MRM (Targeted)



Discovery



Validation



Ex. TCA Cycle

Measurement of Scan/MRM mode (SmartMRM)

Scan section: Comprehensive search of metabolites

MRM section: Accurate quantitation of target compounds

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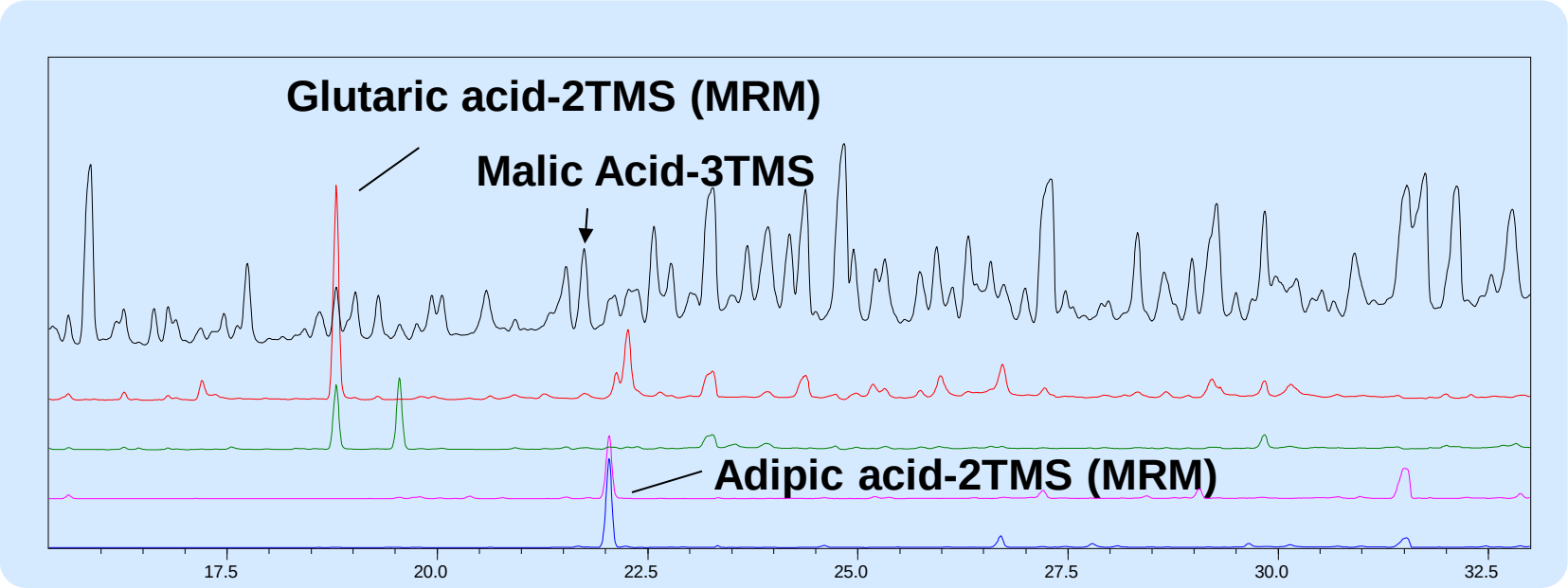
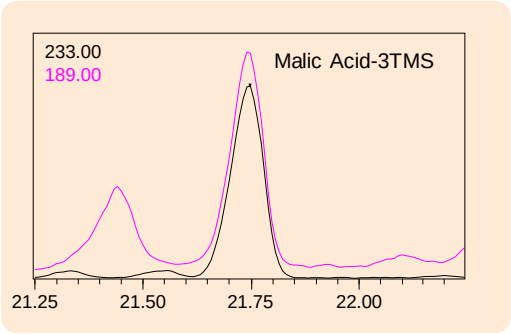
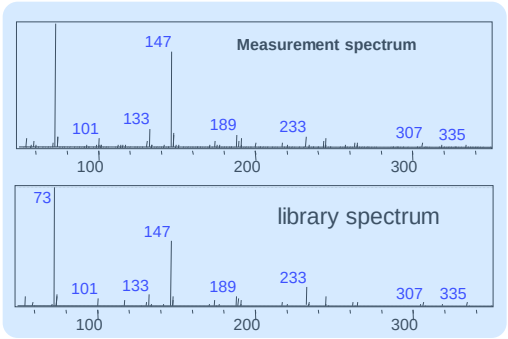
Metabolites in urine using Scan/MRM



Scan



Semi-quantitation



Measured

Library

Identification

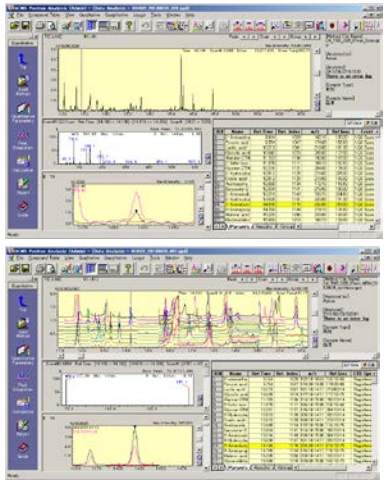
Metabolomics Solutions

– Measurements to Pathway Analyses

Measure by GC-MS(/MS)

Scan

MRM



GC/MS Metabolite Database Ver. 2

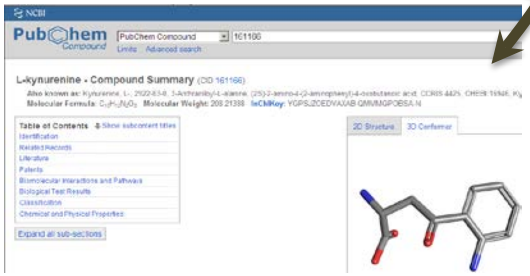
		Negative	Negative	Positive	Positive	Positive
ID	Name	Serum001	Serum002	Serum003	Serum004	Serum005
1	2-Aminoethanol-2T	1927822	1574800	1017918	1904832	1850236
2	Pyruvic acid-meto-	10448	5442. 353	9897. 21	7413. 618	8810. 286
3	Lactic acid-2TMS	147827	104531. 8	109895. 2	98769. 1	88488. 87
4	Glycolic acid-2TMS	216069	170218. 9	210625. 3	144679. 6	152893. 4
5	Alanine-2TMS	26855	20167. 9	22414	17969. 86	25549. 17
6	2-Keto-isovaleric ac	71265	65720. 31	63684. 02	59946. 26	51291. 02
7	Glycine-2TMS	14620	10804. 38	11295. 5	9904. 312	9789. 166
8	2-Hydroxybutyric ac	35699	27065. 86	27361. 46	20173. 2	30344. 84

Identify compounds

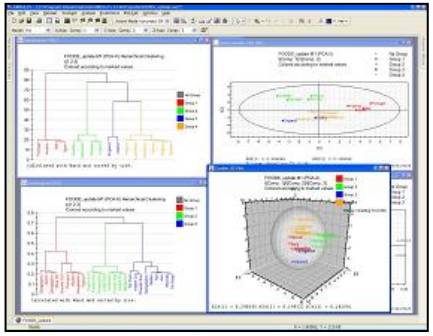
Output data

CID 161166

Enter PubChem ID



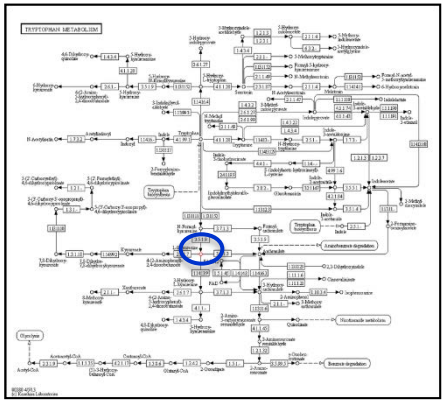
Handbook



Multivariate analysis

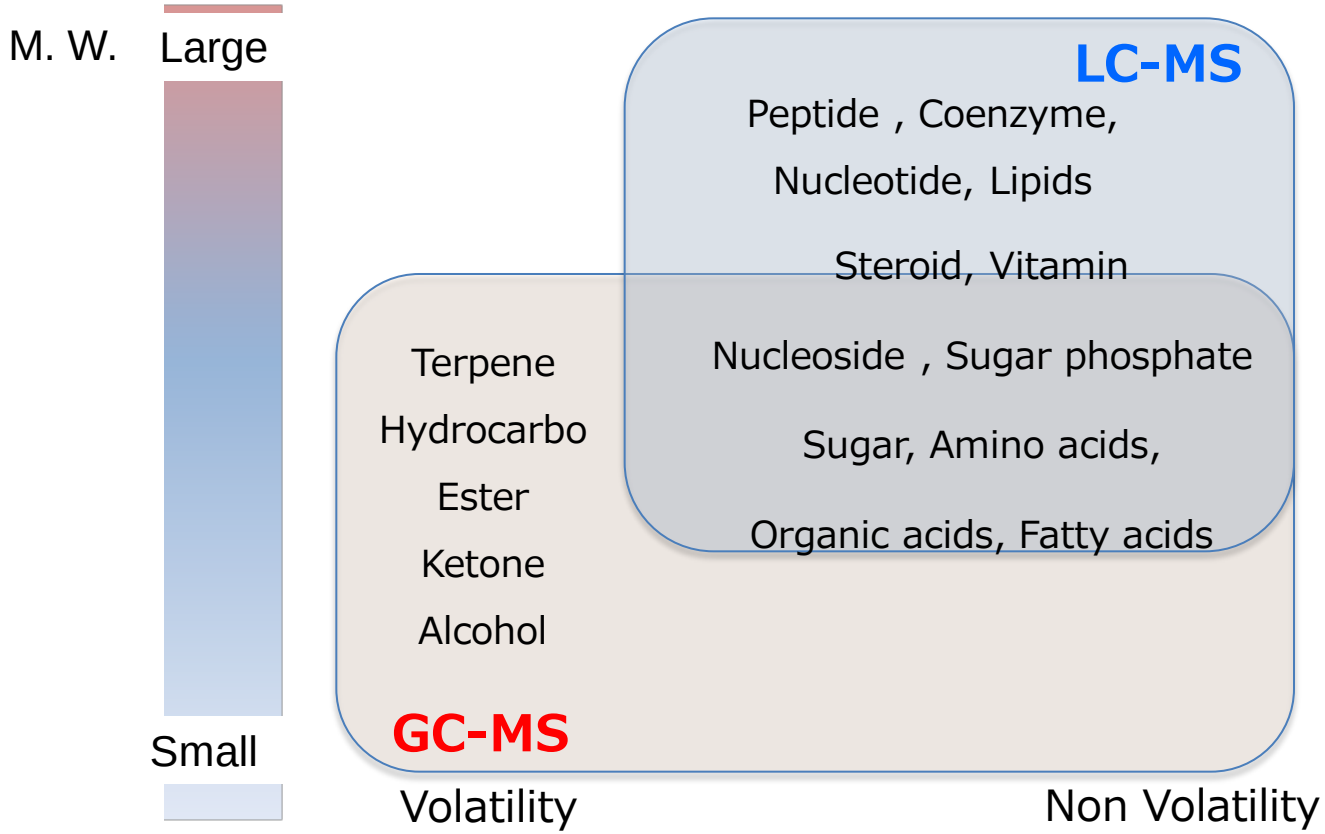
(Detect marker candidates)

From PubChem, link to KEGG



Analyze pathway data

Conventional Metabolomics



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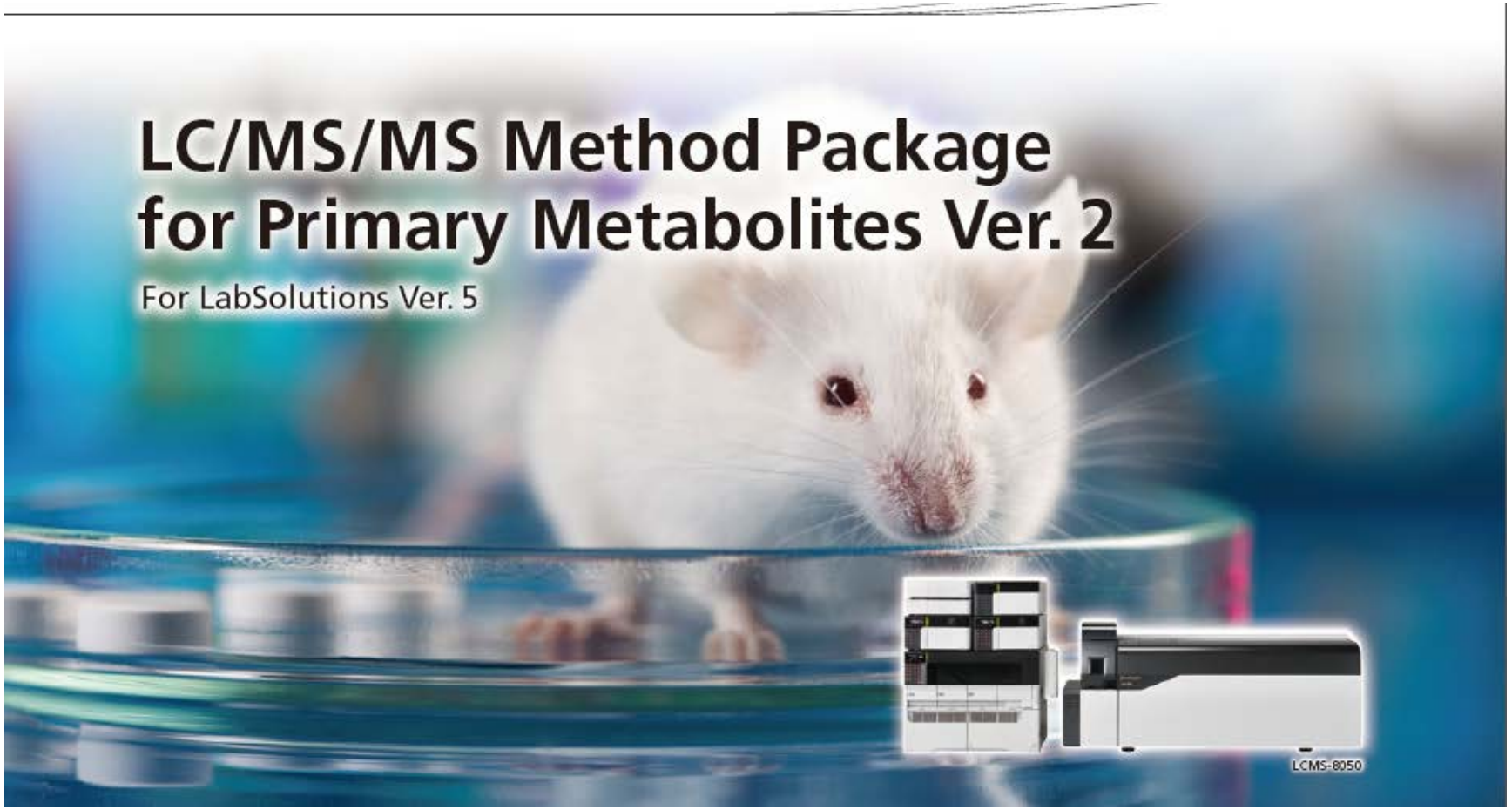
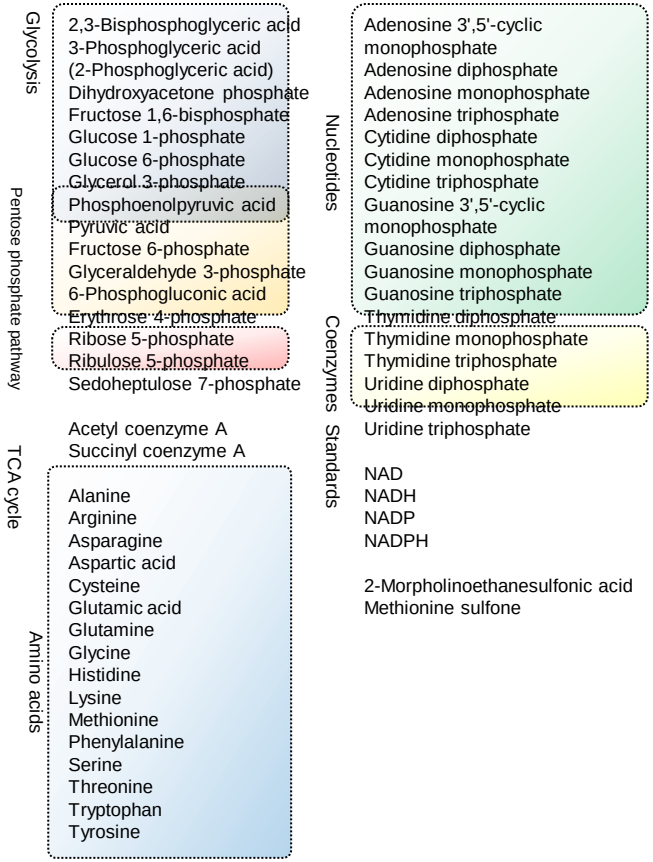
LCMSMS – Readymade Solutions

LCMSMS- Readymade Solutions

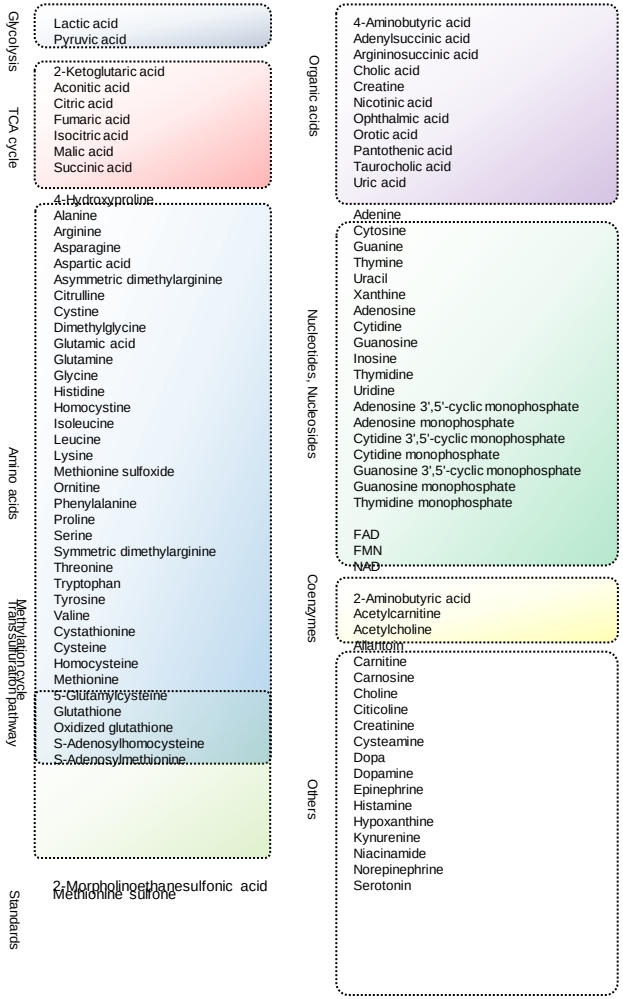


Metabolites for major pathways in single method

Ion pairing method (55 compounds)



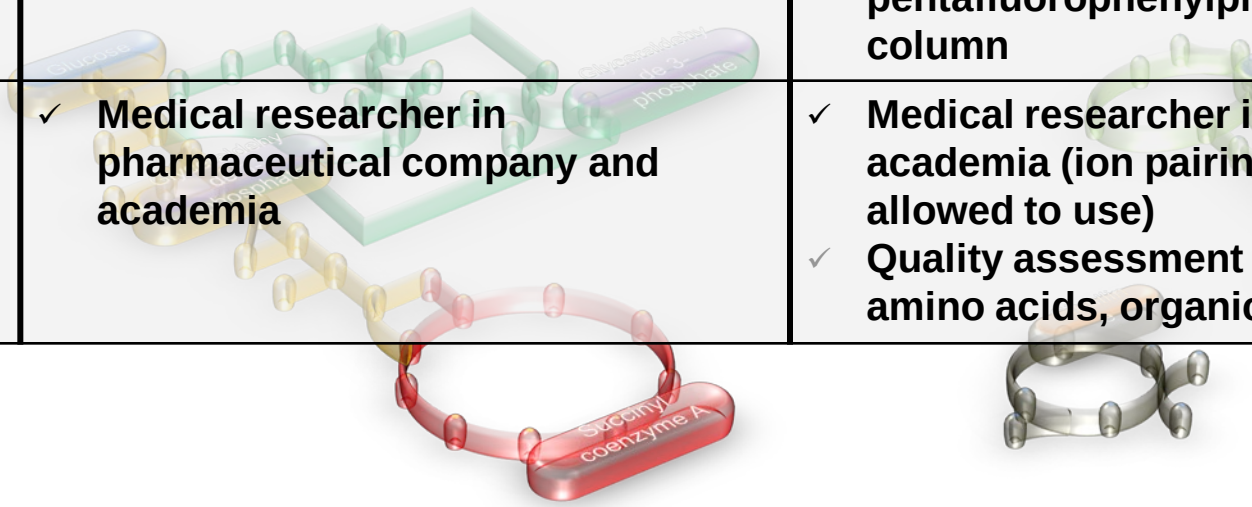
Non-ion pairing method (97 compounds)



✓ Ion pairing method contains metabolites in the central carbon metabolic pathway as target compounds and some amino acids and organic acids are added as target compounds in non-ion pairing method.

Method Package for Primary Metabolites

	Ion pairing method (55 compounds)	Non-ion pairing method (97 compounds)
Target compounds	✓ Glycolysis, TCA cycle (CoA), Pentose phosphate pathway, Amino acids, Nucleotides	Amino acids, TCA cycle (organic acid), Bases, Nucleosides, Nucleotides, Transsulfuration pathway, Methylation cycle
LC conditions	✓ Usage of ion pairing reagent ✓ Separation by ODS column	✓ Ion pairing reagent is not used ✓ Separation by pentafluorophenylpropyl (PFPP) column
Targeted Application	✓ Medical researcher in pharmaceutical company and academia	✓ Medical researcher in pharma and academia (ion pairing reagent is not allowed to use) ✓ Quality assessment by quantifying amino acids, organic acids etc.



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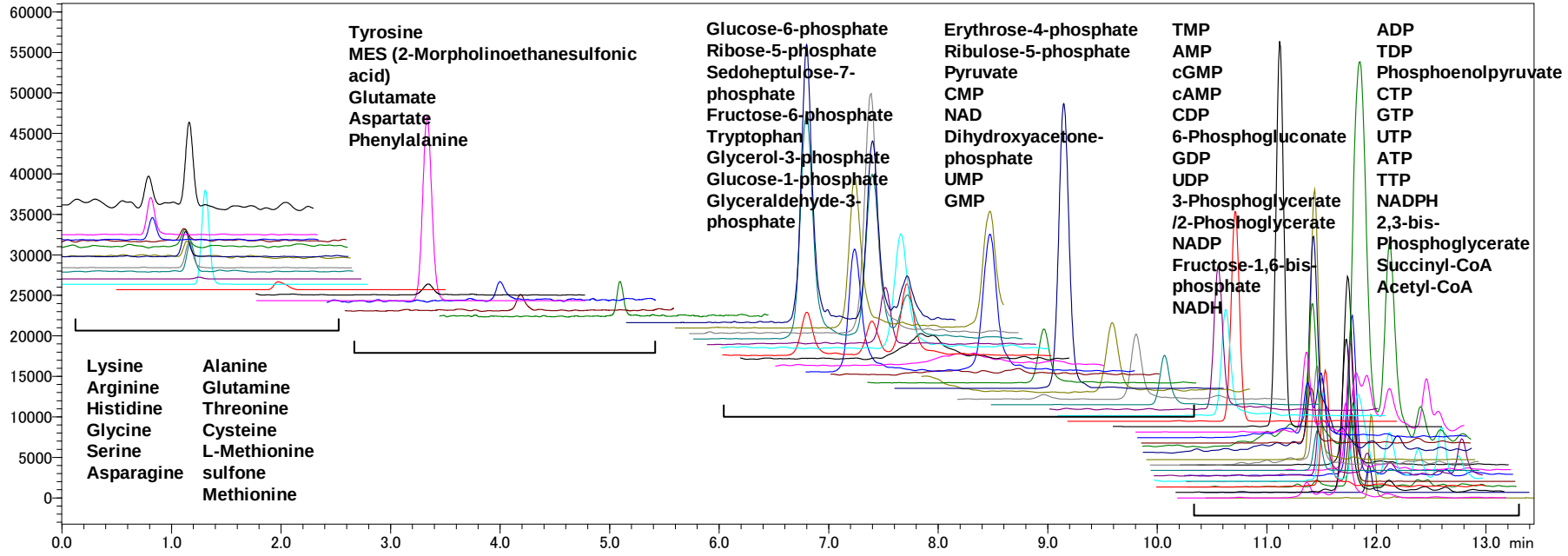
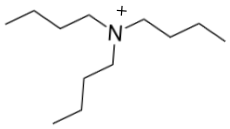
57 Standards Mix: Ion Pair Method

An aliquot of 57 STDs Mix was injected at a volume of 3 μL and separated using an ion-pairing chromatography.

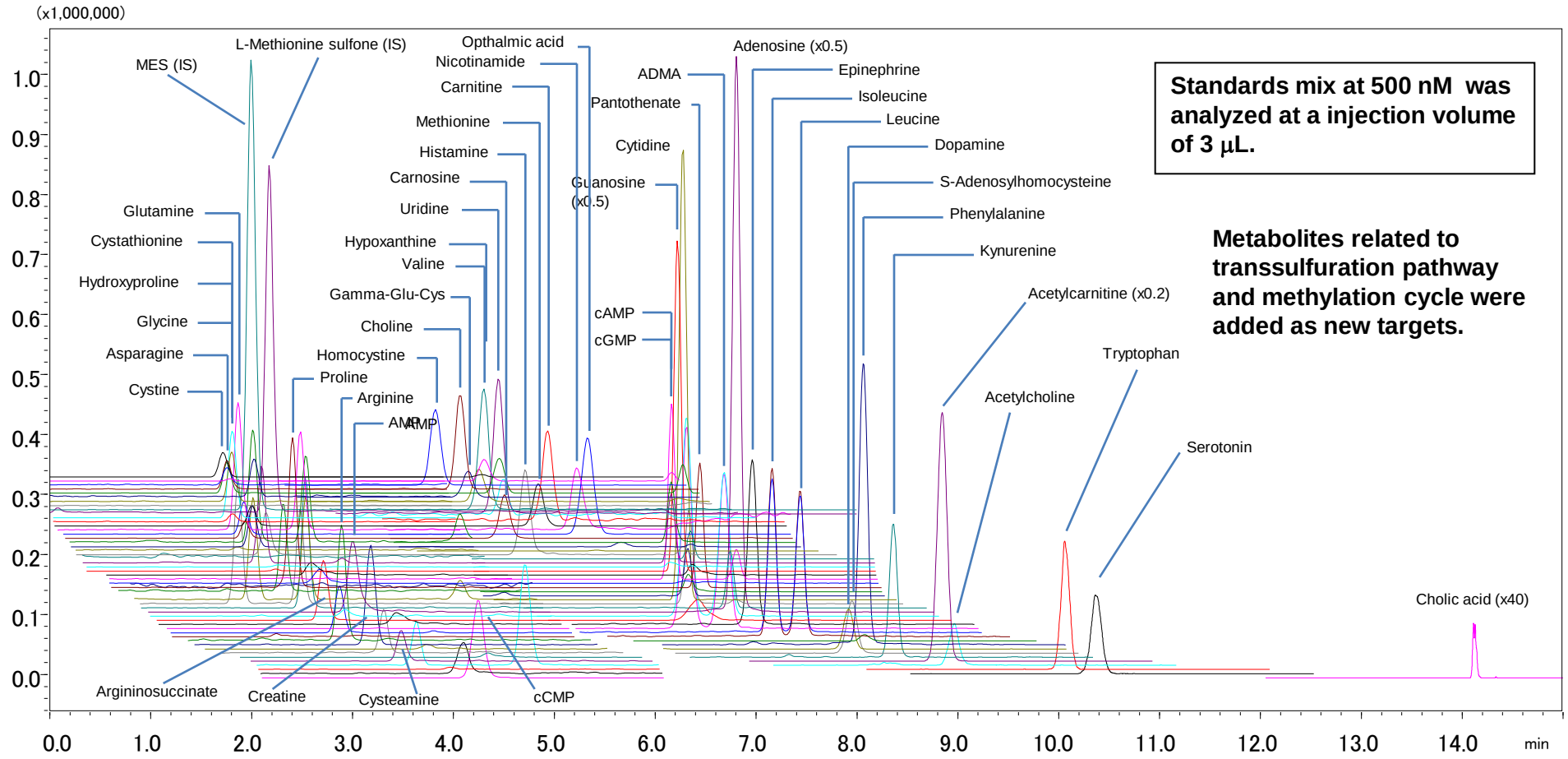


Column : Mastro C18 (2.0 X 150 mm, 3 mm)
Mobile phase: A : 15 mmol/L Acetate, 10 mmol/L Tributylamine - Water
B : Methanol
Injection vol. : 3 uL (100 nM 55 STD Mix)
Dwell Time : 10 ms
Ionization mode : ESI negative

Tributylamine



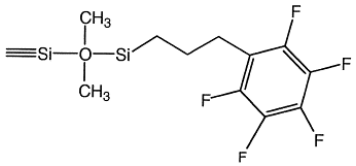
97 Standards Mix: Non-ion pair method



Standards mix at 500 nM was analyzed at a injection volume of 3 µL.

Metabolites related to transsulfuration pathway and methylation cycle were added as new targets.

Column : Discovery HS F5-3 (2.1 X 150 mm, 3 mm)
Mobile phase: A : 0.1% formic acid – Water, B : 0.1% formic acid – Acetonitrile
Injection vol. : 3 mL (1 mM 97 STD Mix)
Dwell Time : 5 ms, Ionization mode : ESI/positive/negative



Pentafluorophenylpropyl functional group

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Shimadzu series of LCMSMS

GLOBAL LAUNCH LCMS-8030

First mass spectrometry company to achieve a scan speed of 15,000u/sec and polarity switching time of 15msec

2010 AUGUST

LCMS-8040

Increased sensitivity by a factor of 5 compared to the LCMS-8030

2012 MAY

LCMS-8050

First mass spectrometry company to achieve 5msec polarity switching time and a scan time of 30,000u/sec. Increased sensitivity by 30 times compared to LCMS-8030

2013 AUGUST

LCMS-8060

A new vision in sensitivity.
It simply changes everything.
Increased sensitivity by 90 times compared to LCMS-8030

2015 ASMS 2015 MAY

SAP wins the 2015 MS Company of the Year

Frost & Sullivan, which is one of the worlds leading business intelligence giants, has highlighted in its prestigious white paper on best industry practises that:

•Shimadzu has established a strong presence in the APAC mass spectrometry market leveraging on its strengths in product innovation, high value-added and quality mass spectrometers, and excellent promotional activities.

•Shimadzu is working towards increasing its production capacity, thus ensuring the stable and reliable supply of products for years to come.

•Shimadzu's outstanding cost structure will further fortify its position as a leading participant in the MS market.

•Frost & Sullivan considered 2 key factors while deciding on the Company of the Year Award: Shimadzu's Visionary Innovation and Performance (This included criteria like Addressing Unmet Needs, Visionary Scenarios Through Mega Trends, Implementation Best Practices, Blue Ocean Strategy and Financial Performance) and Customer Impact (This included criteria like Price/Performance Value, Customer Purchase Experience, Customer Ownership Experience, Customer Service Experience and Brand Equity).

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LCMS-8060: Changes Everything

1. **Highest Sensitivity**
2. **Ultra Fast Technologies**
3. **Unsurpassed Robustness**

Increased ion production

Changes to the desolvation line capillary have increased ion production by a factor of >3

UF Qarray

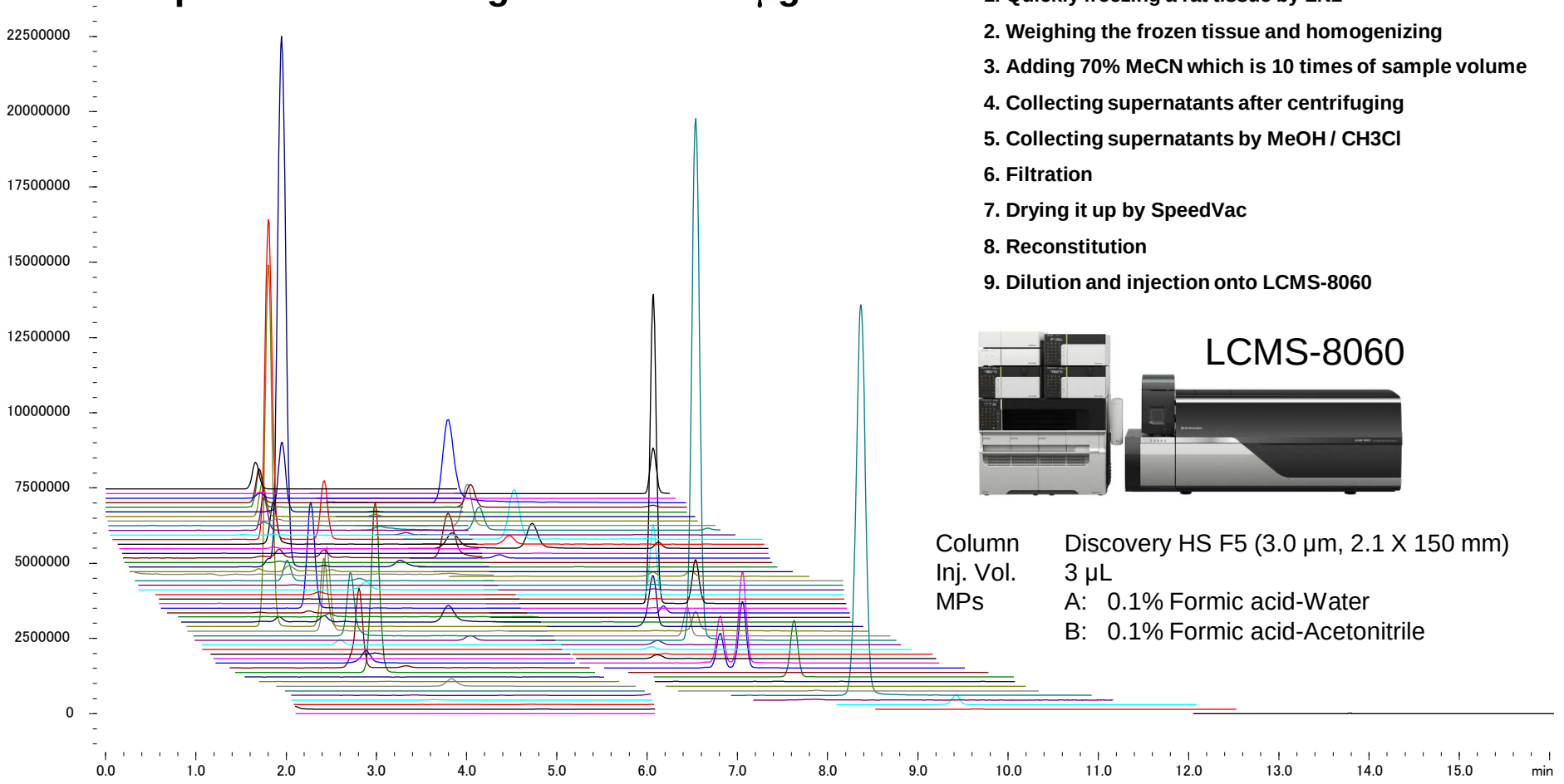
Redesigned to deliver a meaningful impact on ion focusing capability and higher sensitivity

Enhanced vacuum

New vacuum designed to increase ion transmission

MRM chromatograms of rat's kidney

- **Extraction corresponds to 5 mg of kidney tissue followed by 100 times dilution**
- **Sample amounts being measured: 3 μg of tissue**



1. Quickly freezing a rat tissue by LN2
2. Weighing the frozen tissue and homogenizing
3. Adding 70% MeCN which is 10 times of sample volume
4. Collecting supernatants after centrifuging
5. Collecting supernatants by MeOH / CH3Cl
6. Filtration
7. Drying it up by SpeedVac
8. Reconstitution
9. Dilution and injection onto LCMS-8060

LCMS-8060

Column	Discovery HS F5 (3.0 μm, 2.1 X 150 mm)
Inj. Vol.	3 μL
MPs	A: 0.1% Formic acid-Water
	B: 0.1% Formic acid-Acetonitrile

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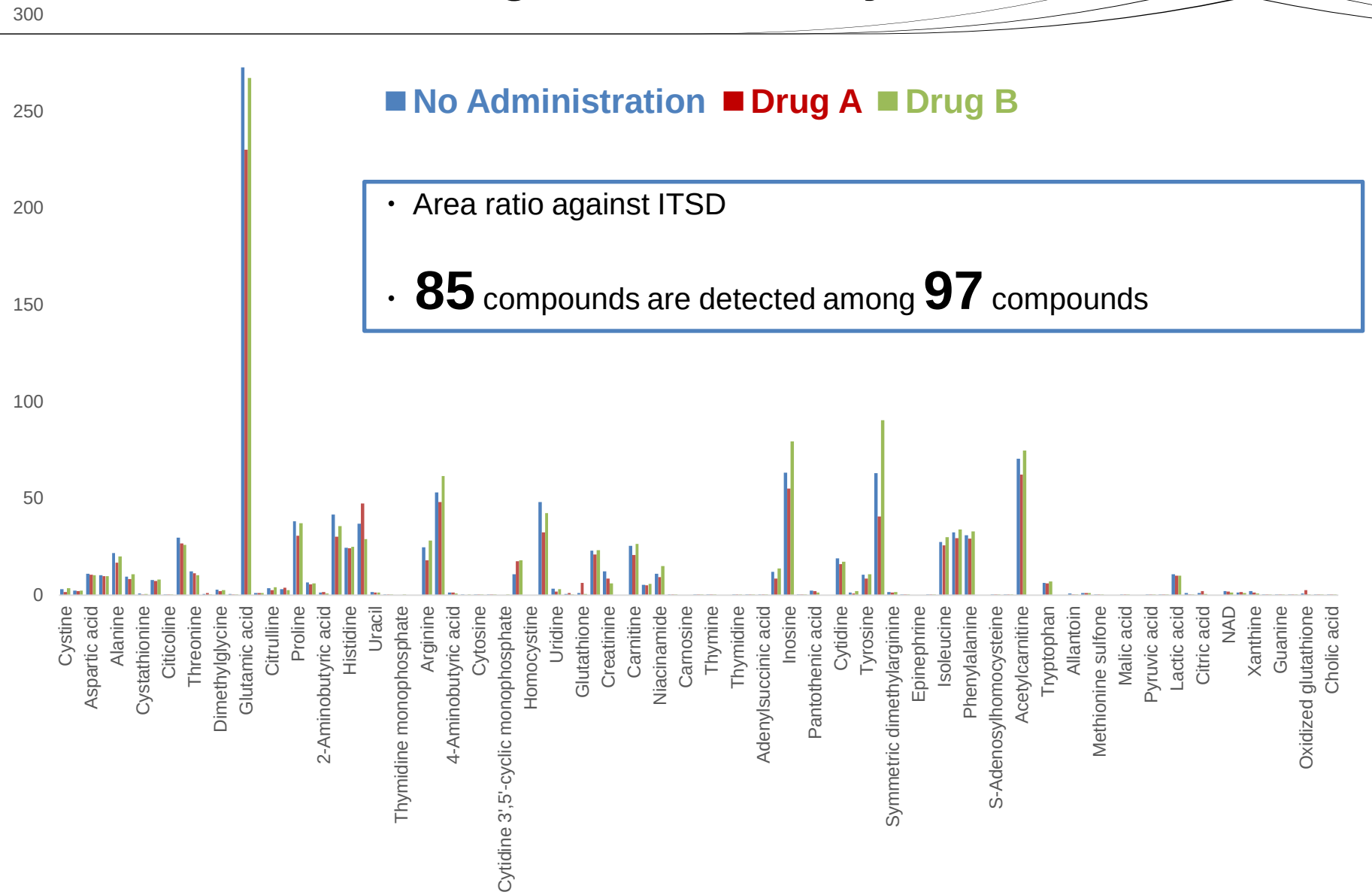
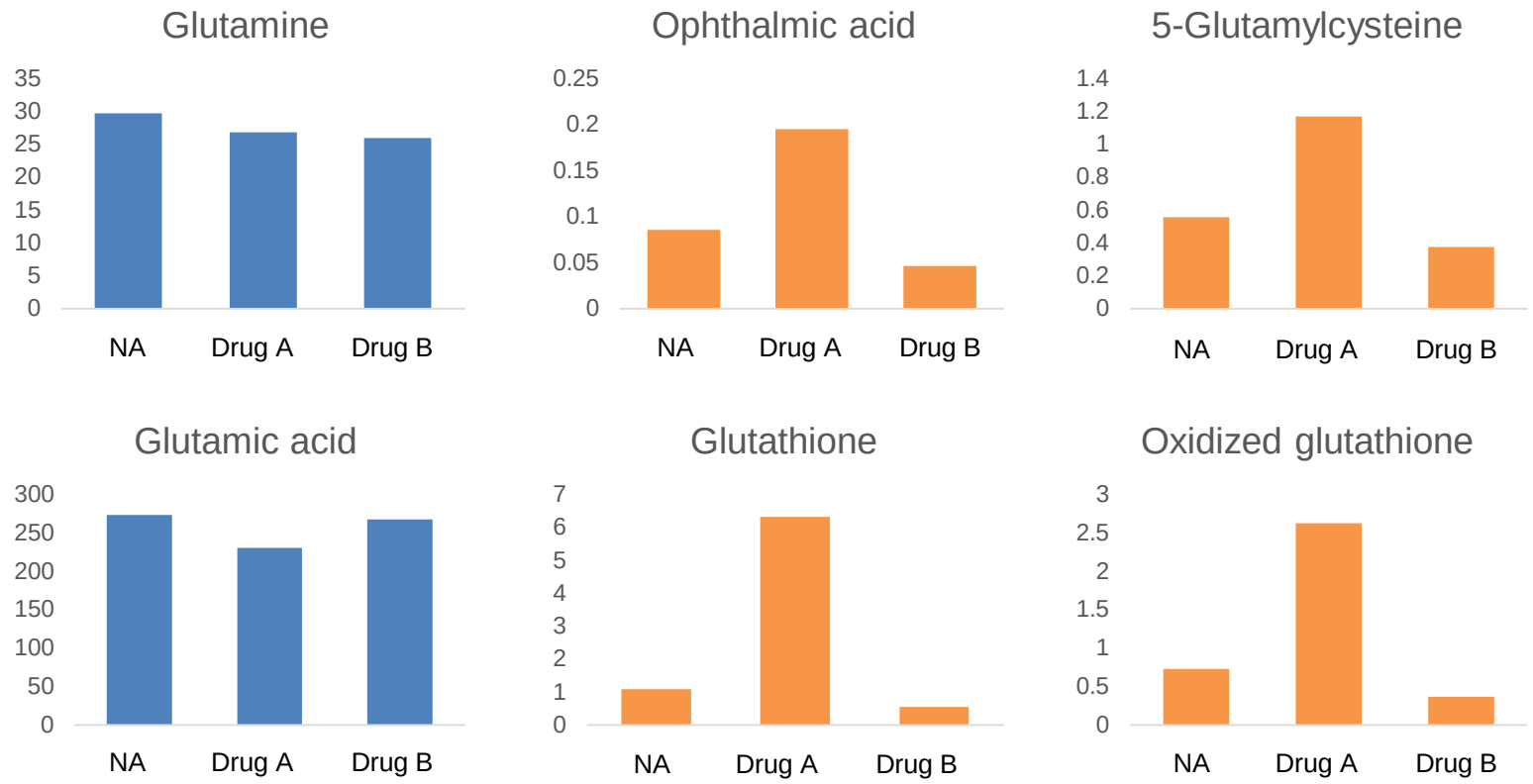
Metabolites Profiling in Rat Kidney



- Area ratio against ITSD
- **85** compounds are detected among **97** compounds

Metabolite Variation by Drug

- The level of ophthalmic acid, glutathione and 5-Glu-Cys were changed by the administration of drug A



Graph has shown average of area ratio (n=4)

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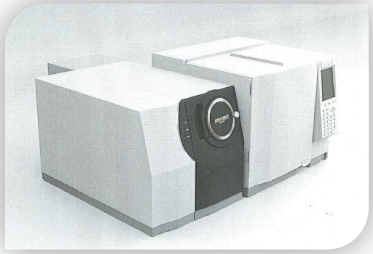
Full Line of Shimadzu
Ultra Fast Mass Spectrometers

UFMS ULTRA FAST MASS SPECTROMETRY *offers*

High sensitivity and Enhanced selectivity
High-Speed Performance

LCMS-8060



GCMS-TQ8040

GCMS-QP2010 Ultra

GCMS-QP2010 SE

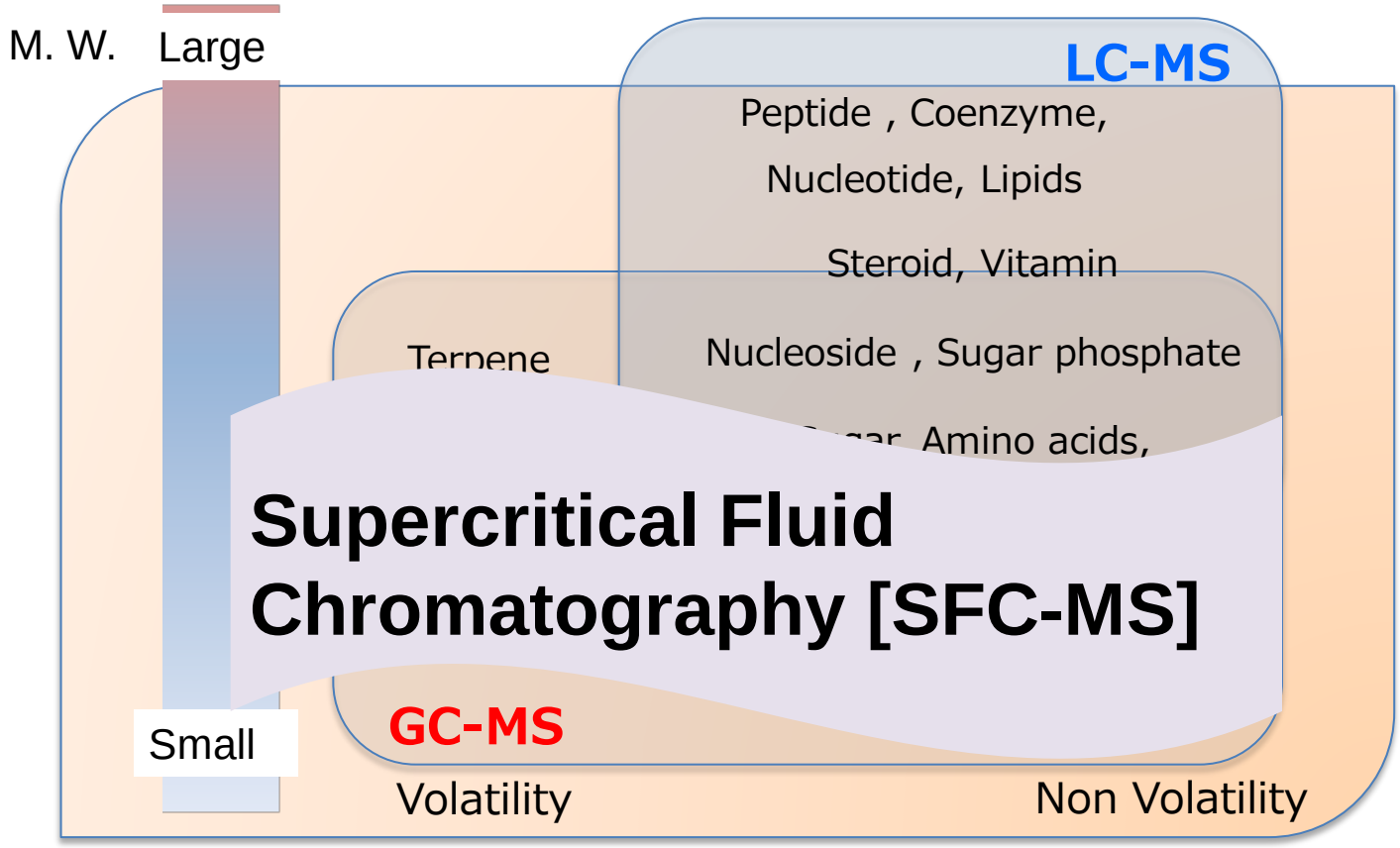
LCMS-8050

LCMS-8040

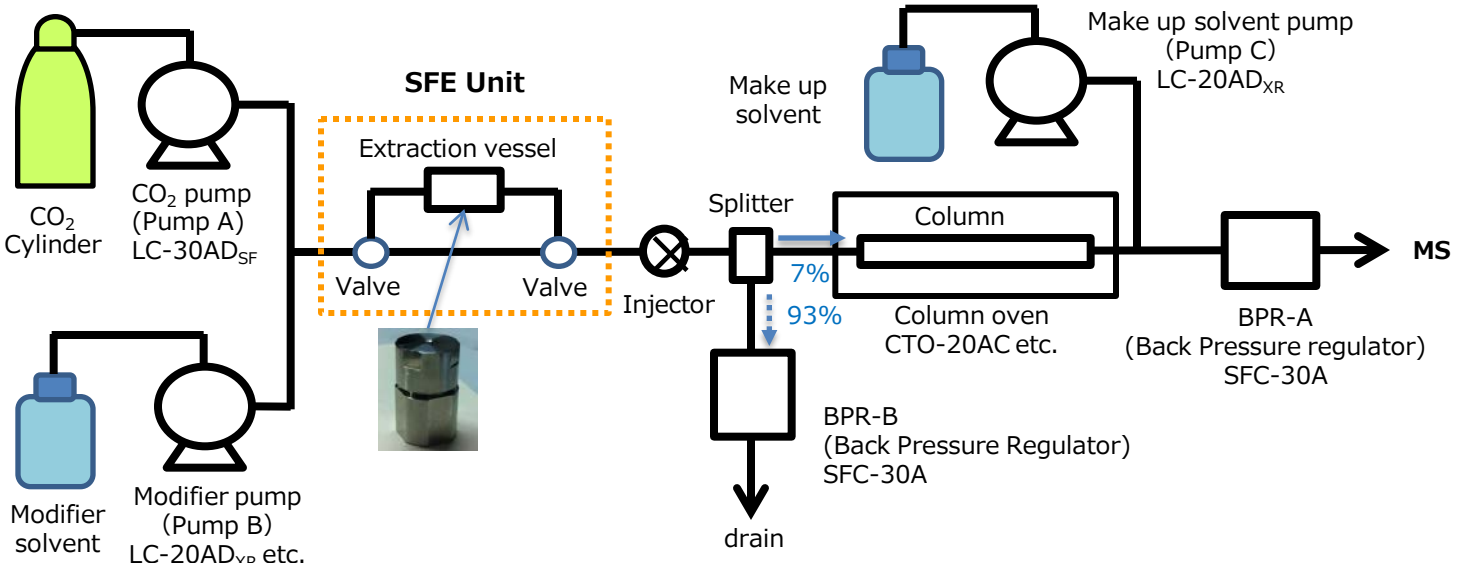
LCMS-8030

LCMS-2020

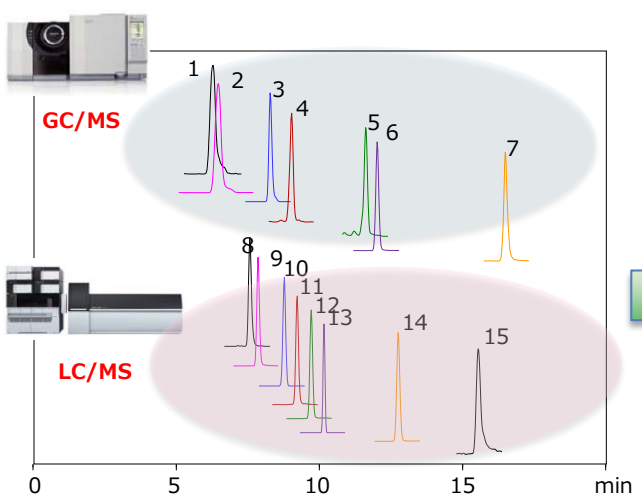
Conventional Metabolomics

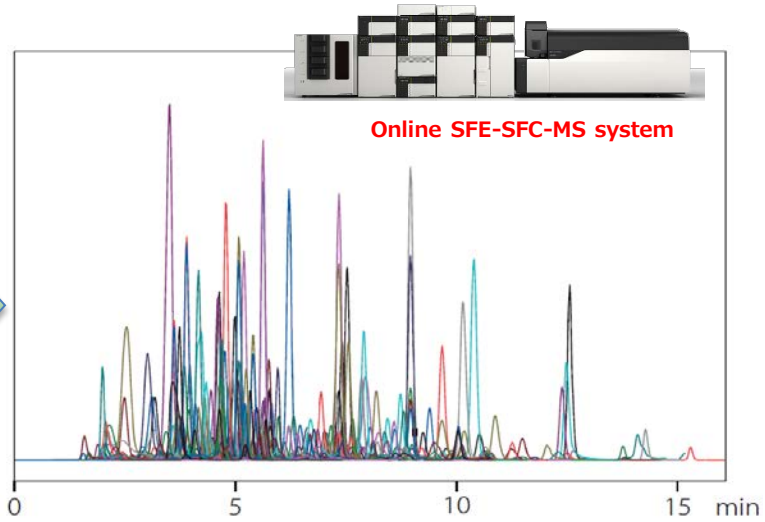


Online SFE/SFC/MS system



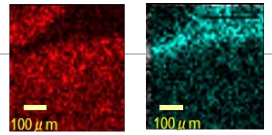
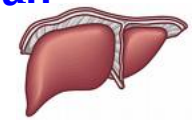
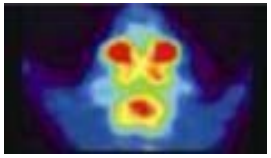
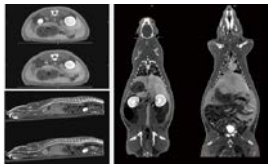
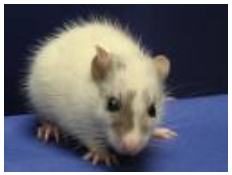
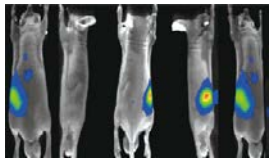
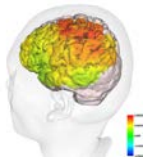
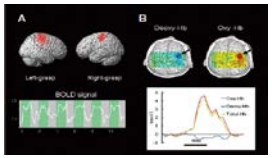
Advantage of SFC





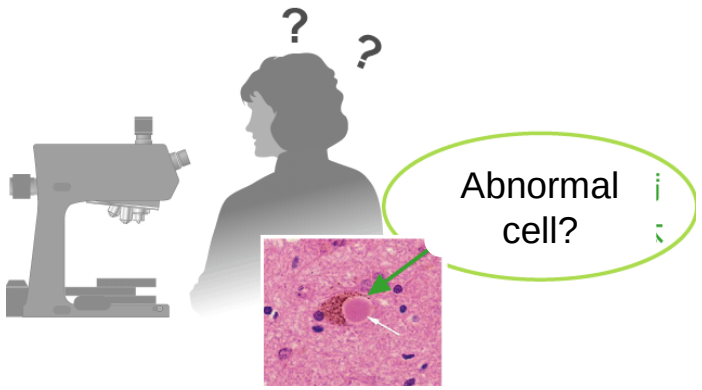
1. Diazinon
2. Metalaxyl
3. Tolclofos-methyl
4. Lenacil
5. Mepronil
6. Dioxathion
7. Cypermethrin
8. Aramite
9. Isouron
10. Acephate
11. Aminocarb
12. Cyazofamid
13. Diquat
14. Chromafenozide
15. Imidacloprid

- high sensitivity
- fast and high separation
- suitable for separation of hydrophobic compounds
- possible to change the polarity using modifier

	Target		Enabling Technology	
In vitro	**Cell** 		**MS Microscope**	**?**
	Tissue/Organ 		**MALDI-TOF MS Imaging**	**AXIMA & CHIP** 
In vivo	**Small animals** **Mouse** 		**PET**	**Clairvivo PET** 
			**CT**	**Clairvivo CT** 
	Rat 		**Optical Imaging**	**Clairvivo OPT** 
	Human **Brain** 		**fNIRS**	**LABNIRS** 
	Human **Whole body** 		**Clinical PET/CT**	**Eminence STARGATE** 

Imaging – Current challenges

Conventional optical microscope



iMScope- MS microscope

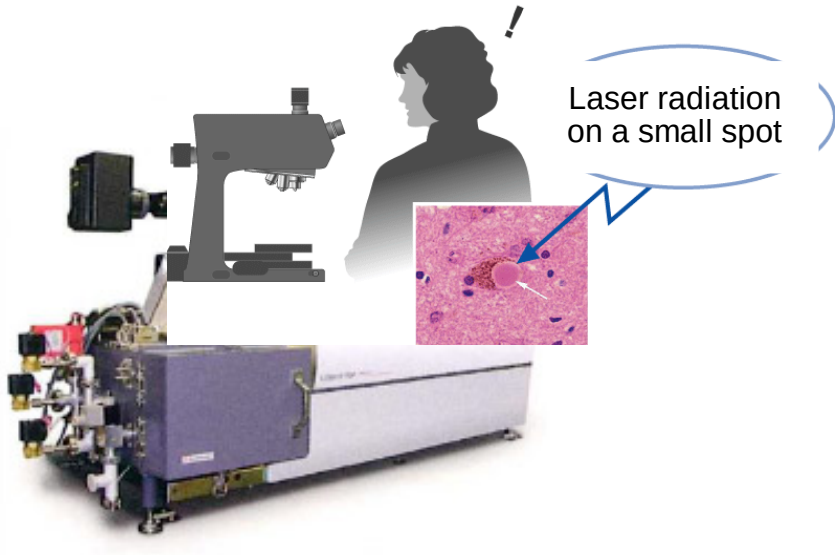
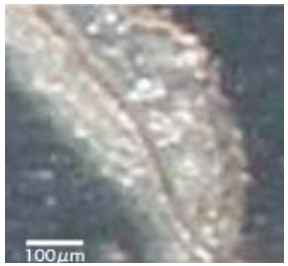


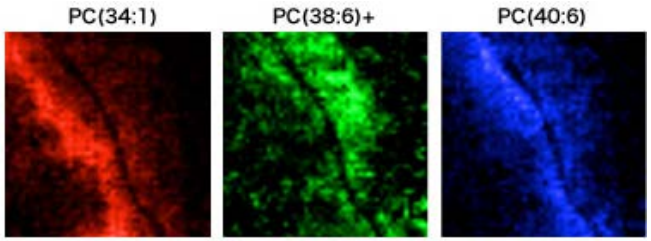
Image obtained with optical microscope

Simultaneous molecular identification by MS under microscope observation.

No information on compounds

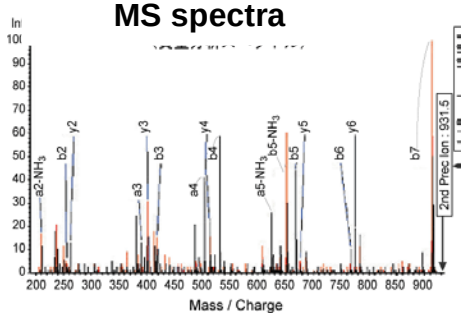


Images obtained with MS microscope



Identification of substances

MS spectra



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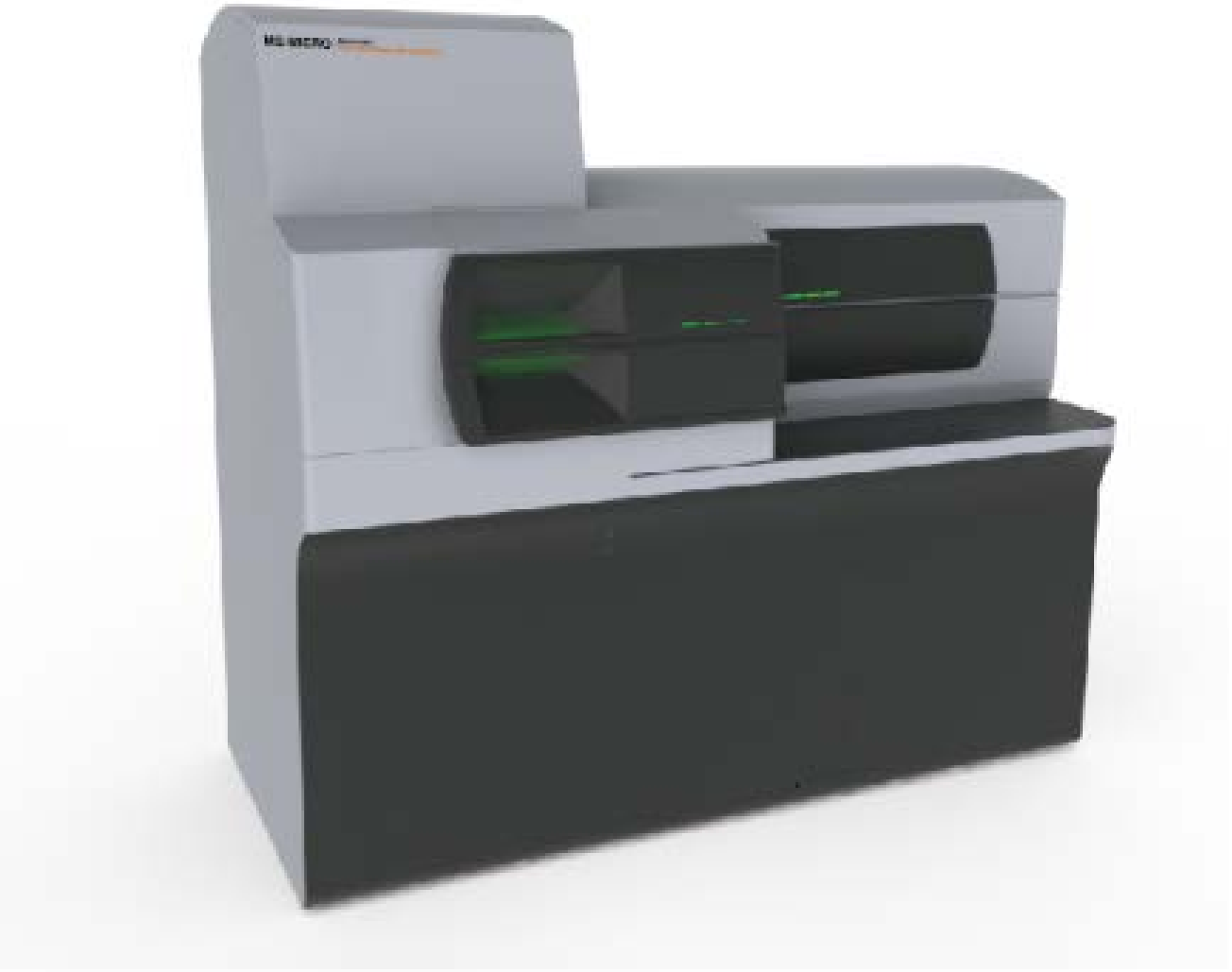
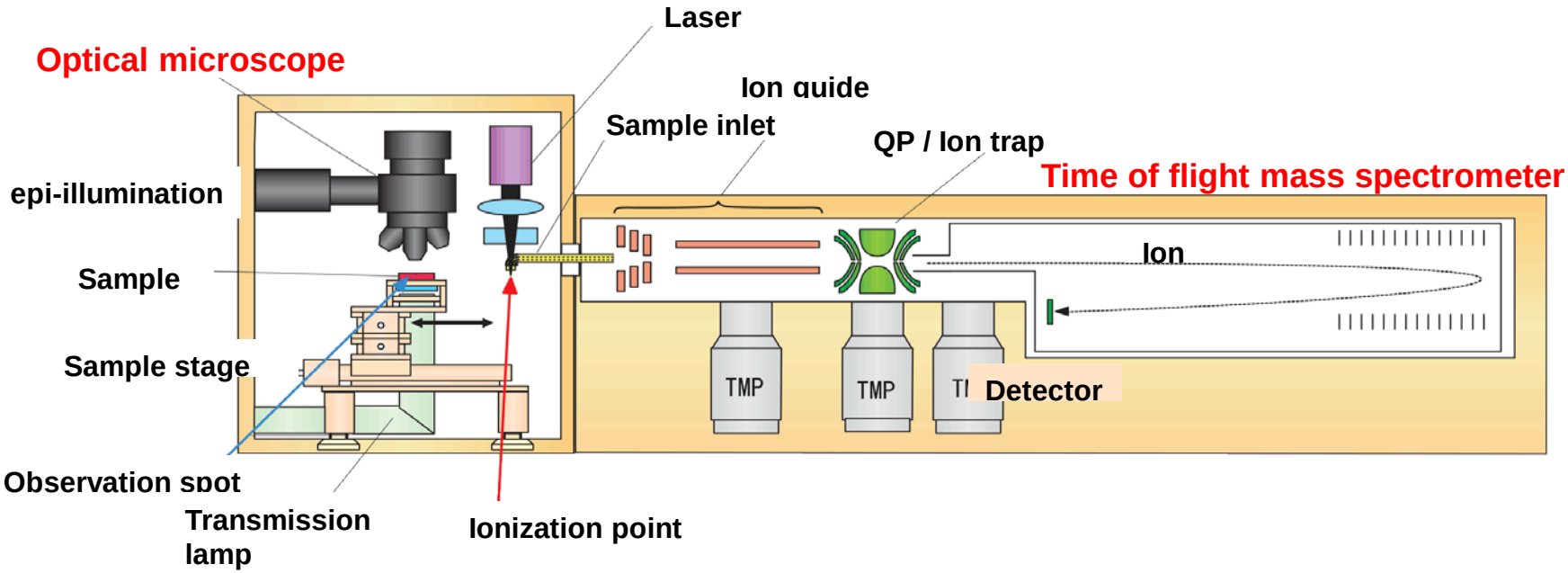
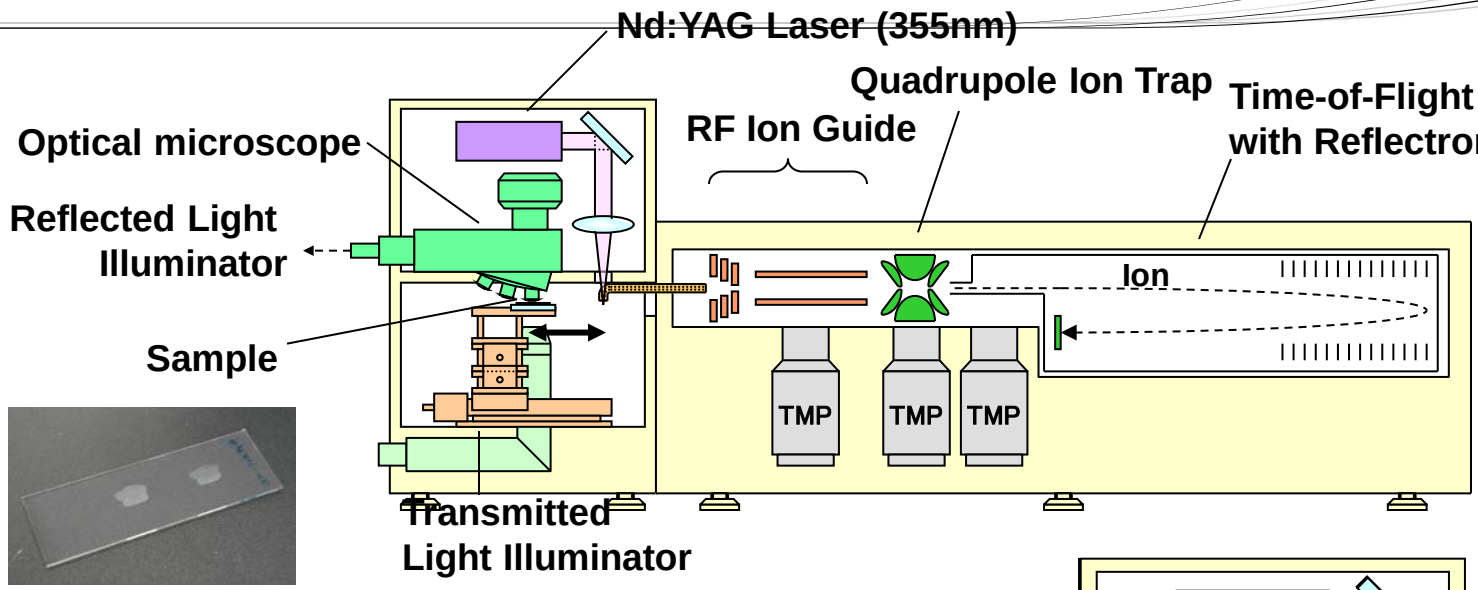
iMScope Trio– Imaging Mass Microscope

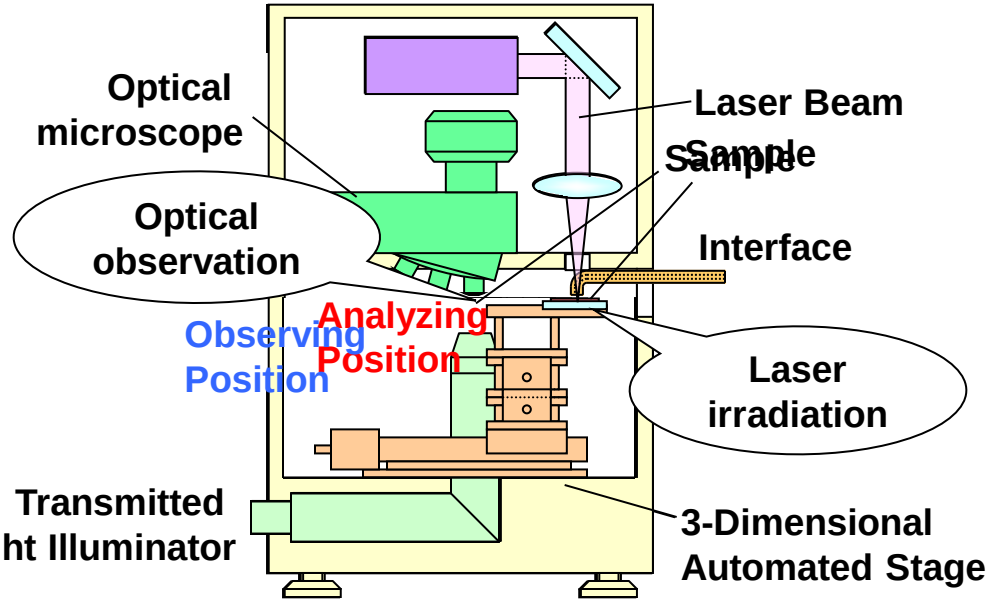


iMScope – Imaging Mass Microscope



iMScope- How it works

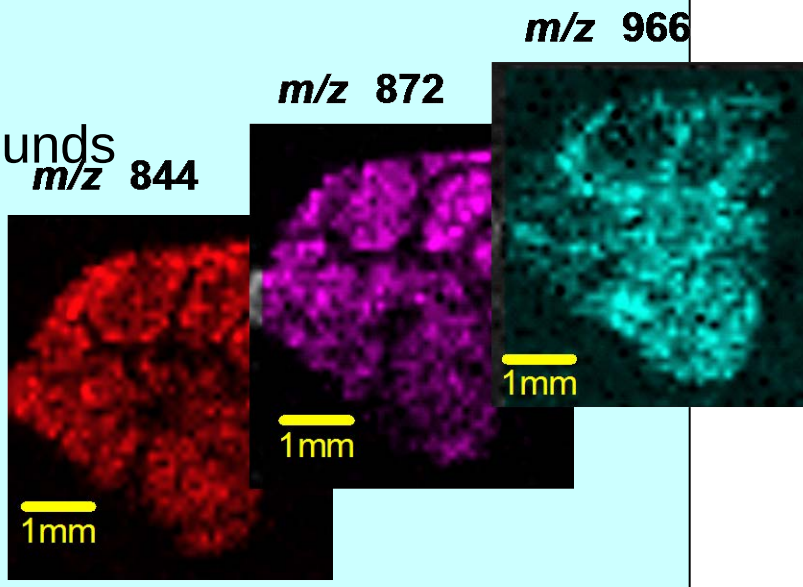




iMScope – Imaging Mass Microscope

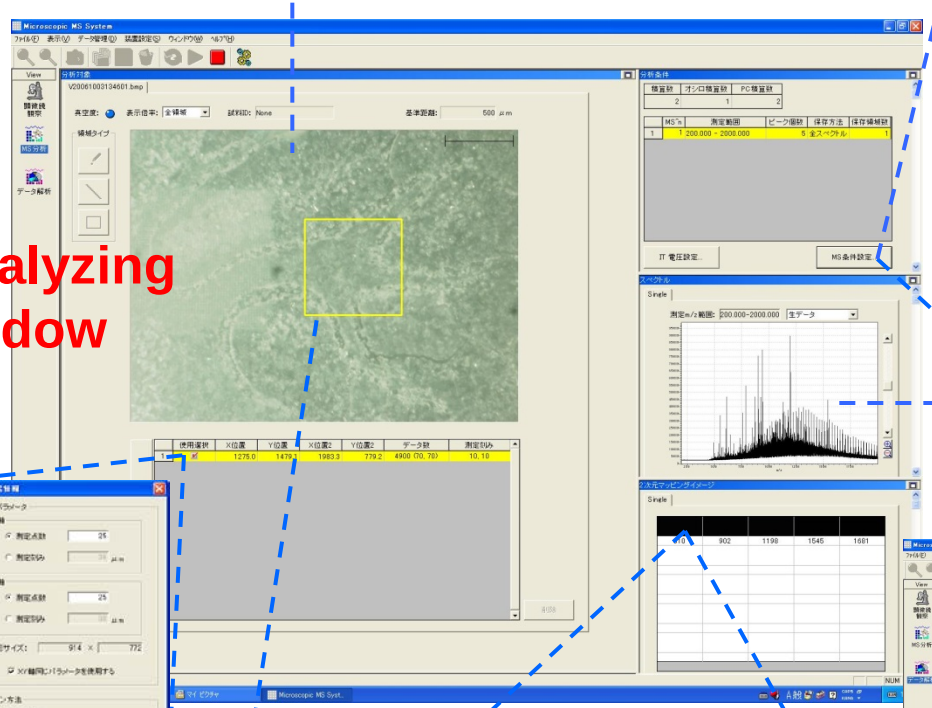
Features

- ✓Direct MS measurement of tissue slice
  - ➢ visualize a local distribution of compounds
- ✓High spatial resolution (5um)
  - ➢ observe compounds in a single cell
- ✓Atmospheric pressure ionization
  - ➢ Suitable for living cell
- ✓MS^n measurement
  - ➢ realize structural analysis of unknown compounds

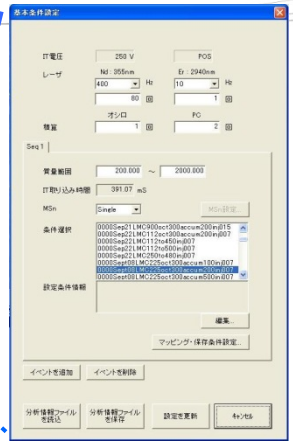


Powerful Software

(1) Optical Microscope Image



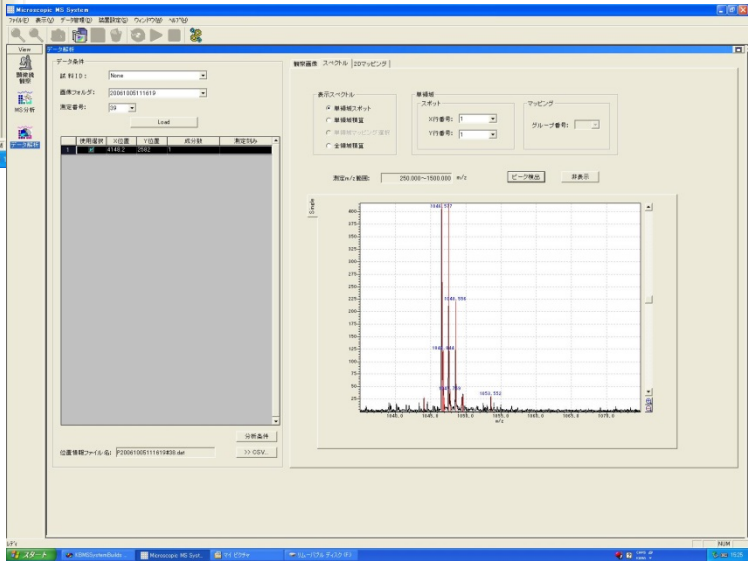
MS Analyzing Window



(3) Setting of mass analyses parameters

(4)-1 Real time mass spectrum

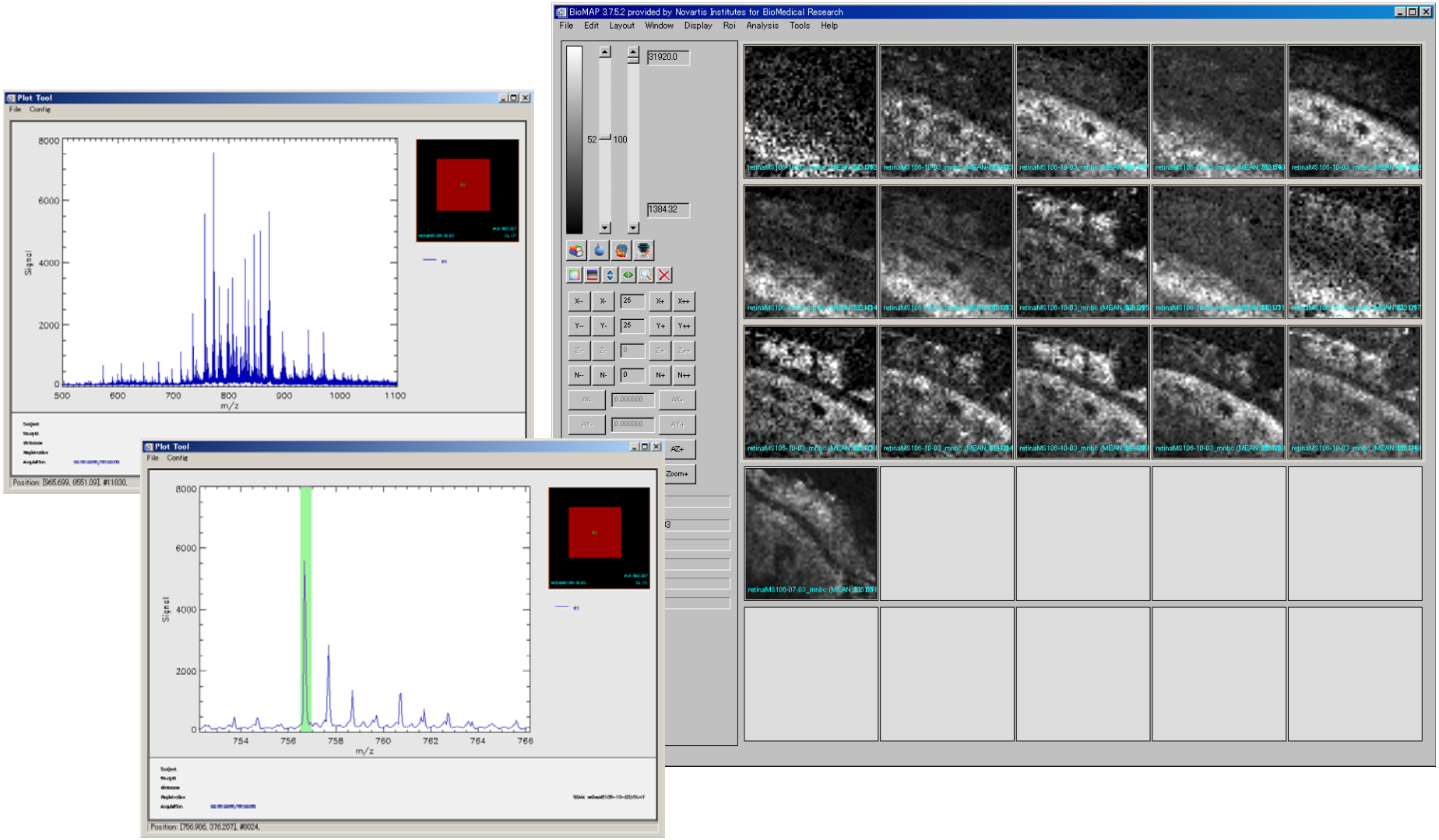
Data Analyzing Window



(2) Assignment of analyzing spot / area

(4)-2 MS Image

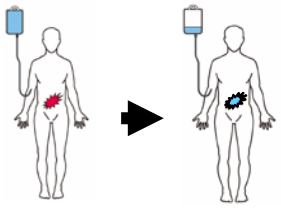
Imaging Viewer software



iMScope – Drug Delivery System

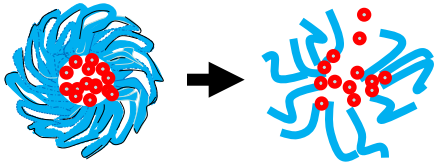
DDS

Targeting of drug to tumor



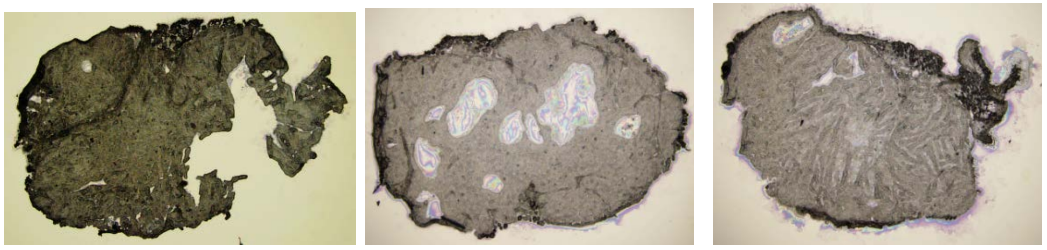
Targeting of drug to cancer cells

Controlled release of drug

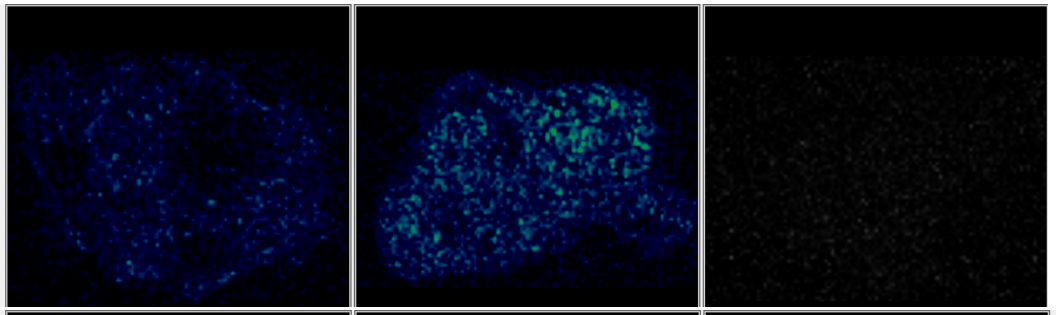


15min 1hr 24hrs

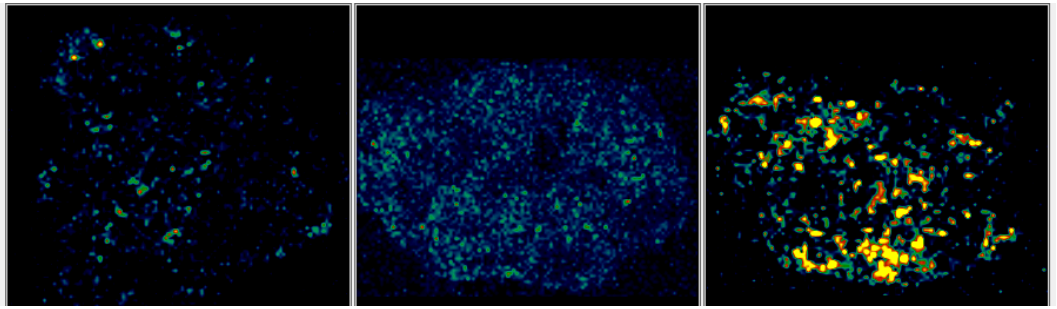
Tumor



Taxol in tumor



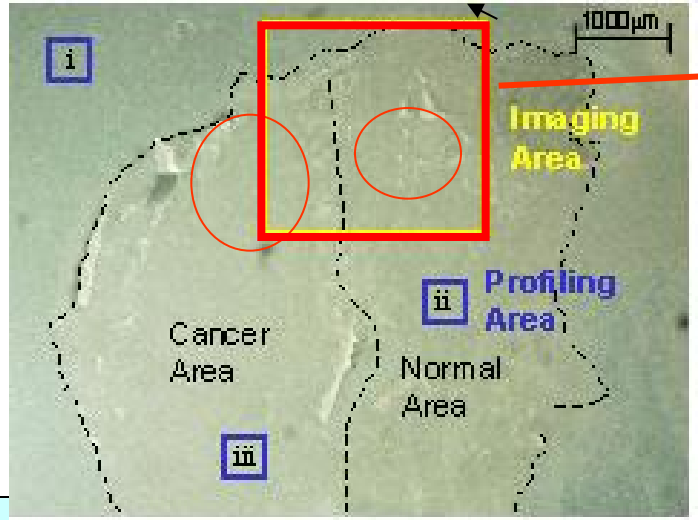
Taxol-Micelle in tumor



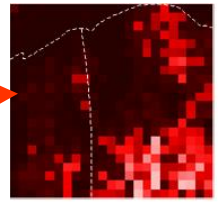
100%

0%

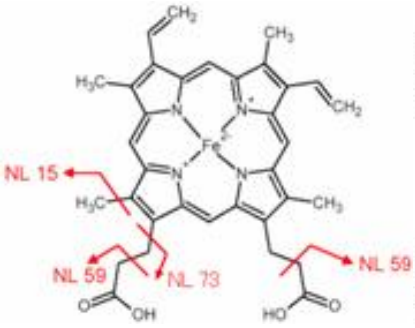
iMScope – Cancer Diagnostics



Comparison between normal and cancer area in human cancer tissue

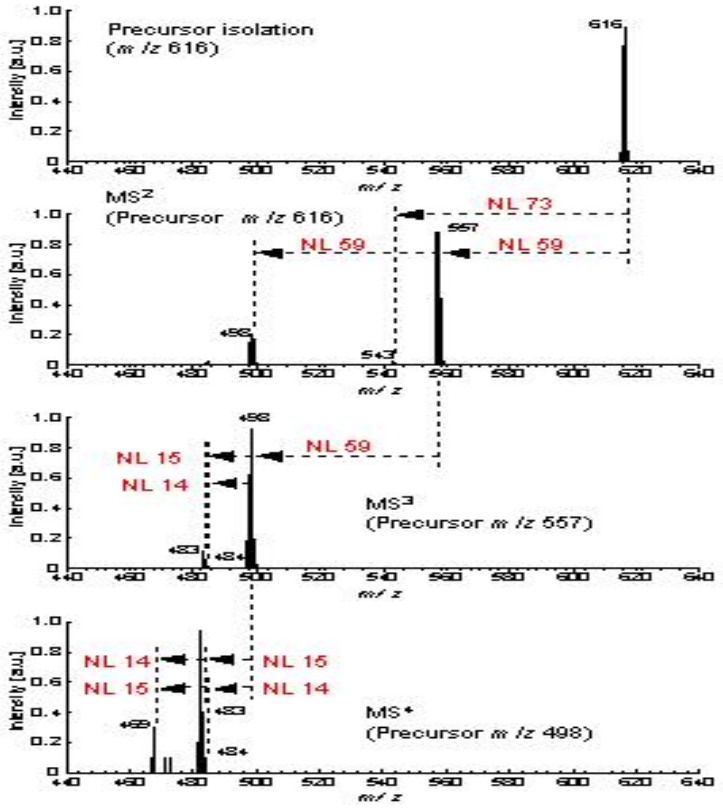


Heme b distribution MS imaging with m/z 616

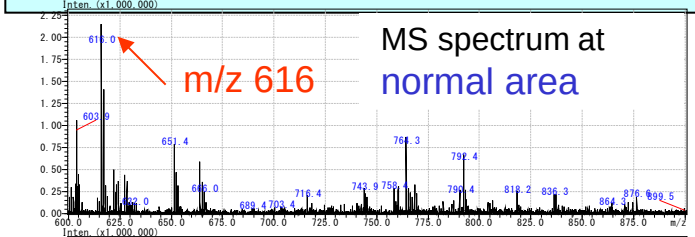


Heme b

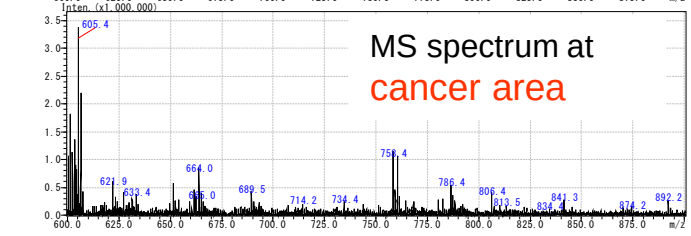
MS^n analysis of peak m/z 616



The peak m/z 616 can be identified as Heme b based on MS^n analysis



MS spectrum at normal area

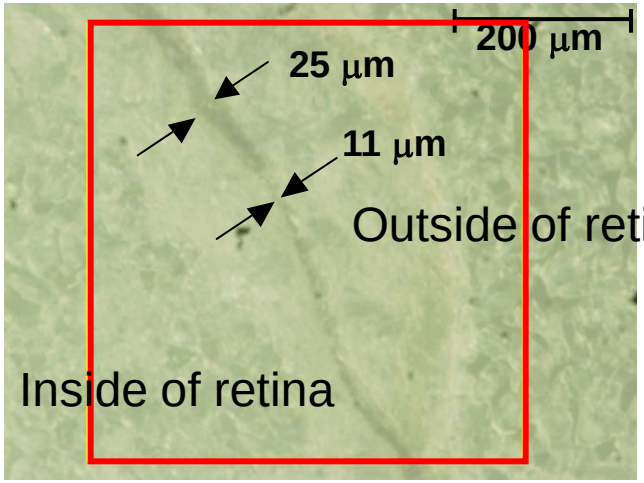


MS spectrum at cancer area

Heme b concentration is found to be low due to ischemic conditions at cancer area.

iMScope – Biological Tissue Slice

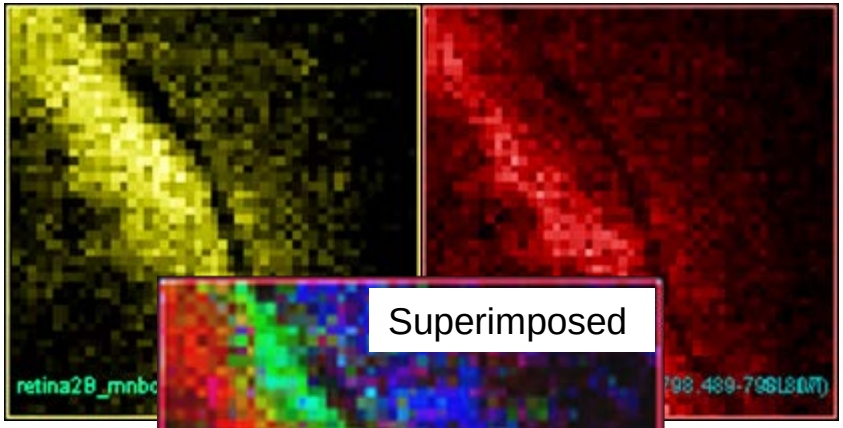
Optical microscope



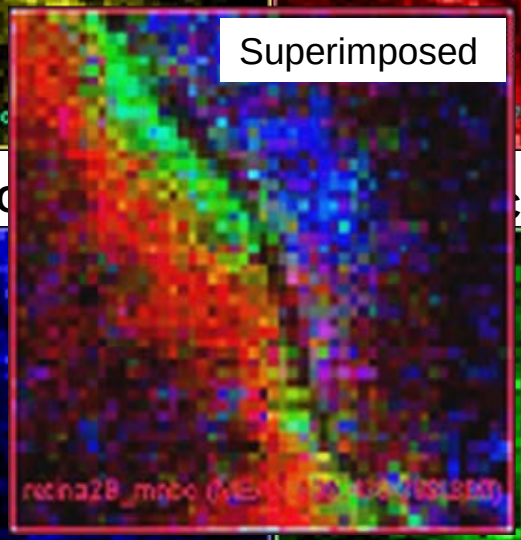
Visualizing the distribution of lipid isomers in the mouse retina to understand the structure of the lipid layers.

m/z772.5 PC(16:0 16:0)+K

m/z798.5 PC(16:0 18:1)+K

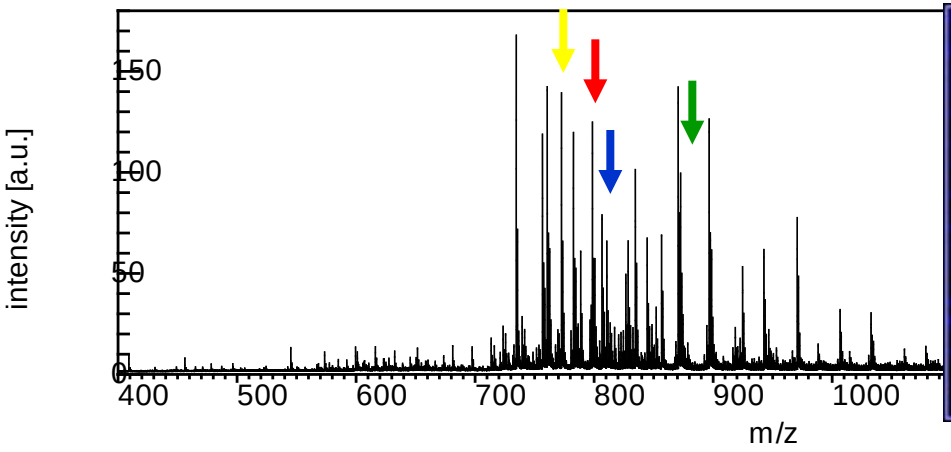


Superimposed



m/z806.5 PC

(18:0 22:6)+K



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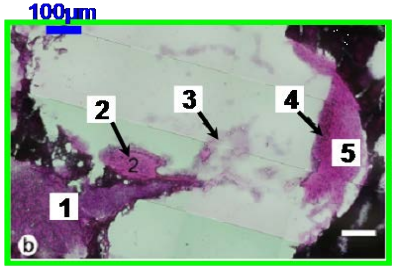
iMScope – Potential Applications

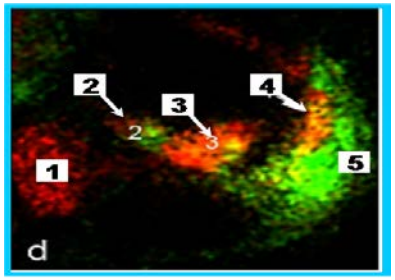
Medical diagnostics

Distribution of compounds in lesion area

Optical imaging

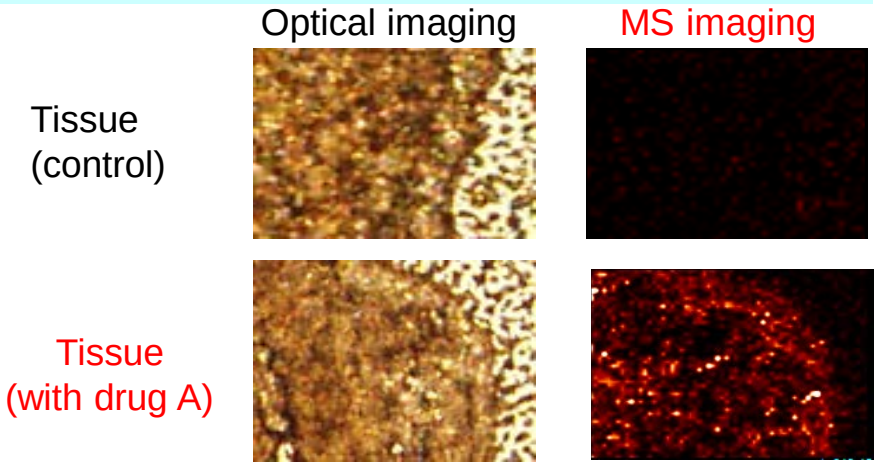


MS imaging



Drug metabolism

Distribution of drug A (m/z 313) in tissue



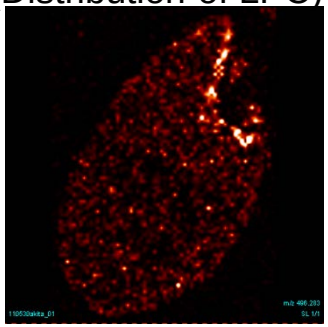
Agriculture

Distribution of active ingredient in rice

Optical imaging

germ

MS imaging (Distribution of LPC)



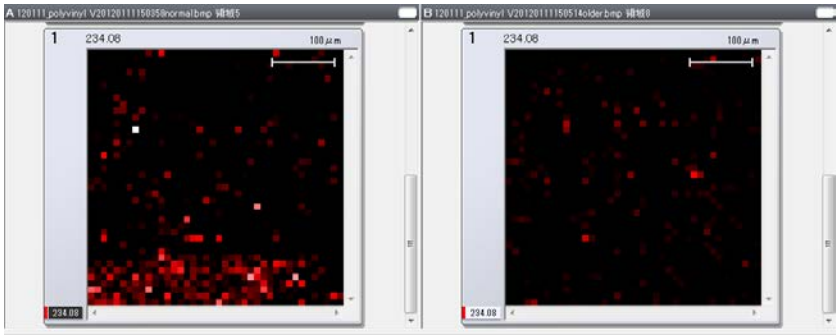
Chemical material

Distribution of compound B in rubber sheet

New rubber

MS imaging

Old rubber



Summary

- Shimadzu offers wide range of analytical instruments from Research to routine analysis
- In addition to analytical platforms Shimadzu offers readymade solutions to enhance your workflows
- iMScope can be extremely useful in various fields especially in Clinical Metabolomics due to its high spatial resolution.
- Imaging is powerful tool to study the localization of compounds within the cells/Tissues
- Shimadzu is keen to collaborate for your research with its advance instrumentation to ramp up your research