Aug. 14, 2018
Lab Seminar
Mahidol University

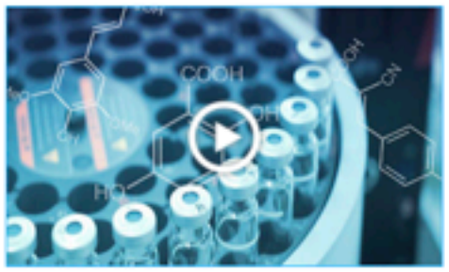
大阪大学
OSAKA UNIVERSITY

Seeing Is Believing
- Current trends in imaging mass spectrometry -

Shuichi Shimma, PhD

Associate Professor
Graduate School of Engineering,
Osaka University

https://www.edx.org/ **e-learning in English**



Metabolomics in Life Sciences

Learn about metabolomics principles and their applications in life sciences.

大阪大学
OSAKA UNIVERSITY

Archived
Future Dates To Be Announced

Enroll Now

I would like to receive email from Osaka University and learn about other offerings related to Metabolomics in Life Sciences.

About this course

2 Reviews 3.5 / 5

This course is an introduction to metabolomics principles and their applications in various fields of life sciences.

We will provide a summary of all steps in metabolomics research; from experimental design, sample preparation, analytical procedures, to data analysis. The course also provides case studies of various kinds of research samples to attract students that are not familiar with metabolomics, providing them enough explanation to utilize metabolomics technology for their respective research fields.

Several examples of metabolomics applications will be introduced throughout the lectures. These include examples within food science and technology, metabolic engineering, basic biology, introduction to imaging mass spectrometry, and application in medical science.

No previous knowledge on metabolomics is needed but we recommend that students have an undergraduate-level understanding of Biochemistry, Analytical Chemistry, and Biostatistics, and that they learn about basic principles of multivariable analysis prior to taking this course.

What you'll learn

- The basic principles of metabolomics
- Workflow of metabolomics research from design of experiment to data interpretation
- Applications of metabolomics in various fields of life sciences

View Course Syllabus

Meet the instructors

Eiichiro Fukusaki
Professor
Osaka University

Shuichi Shimma
Associate Professor
Osaka University

Sastia Prama Putri
Assistant Professor
Osaka University

Length:	4 weeks
Effort:	2-3 hours per week
Price:	FREE Verified Certificate option closed
Institution:	OsakaUx
Subject:	Biology & Life Sciences
Level:	Introductory
Languages:	English
Video Transcripts:	English

Share this course with a friend

Prerequisites

- Undergraduate level in Biochemistry
- Undergraduate level in Analytical Chemistry
- Undergraduate level in Biostatistics

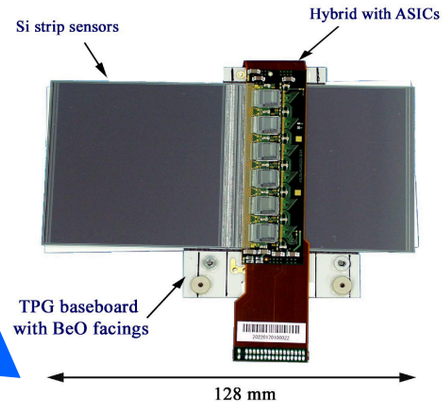
My research backgrounds

University (BC, MC, DC1)

Physics (High-energy physics)

(Univ Tsukuba, KEK, CERN)

2001~2004

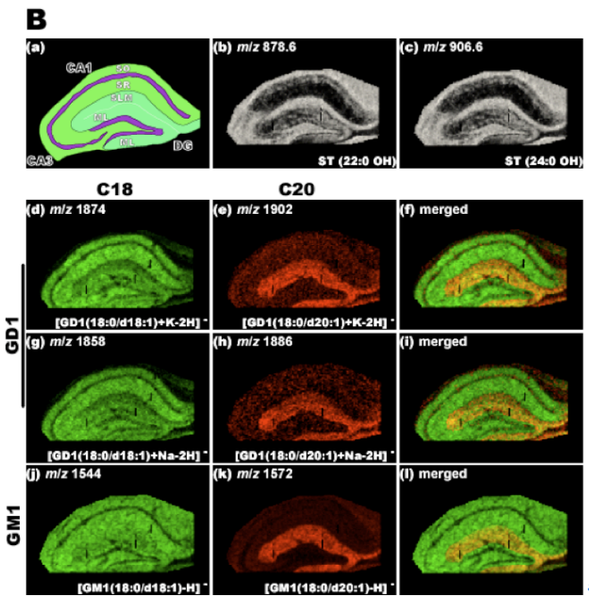


Basic physics, detector R&D, material physics, quality control

University (DC)

Biology (Physiology, mol. biol.)

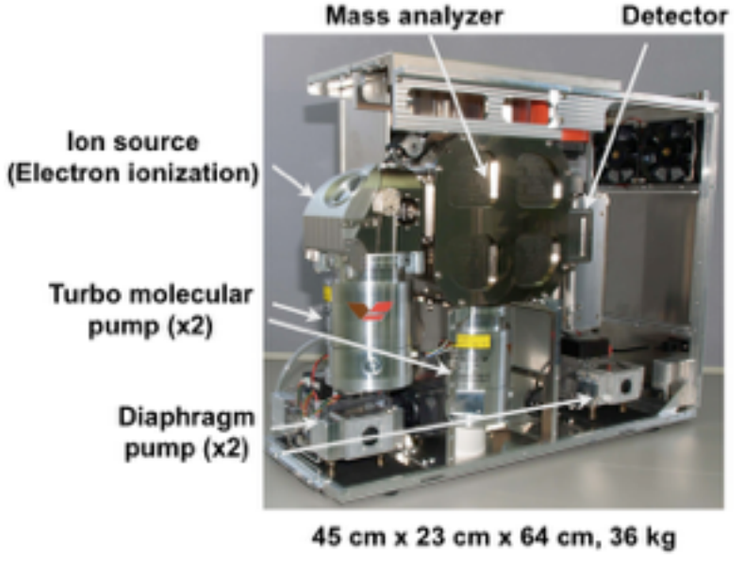
2004~2007



Post-Doc and Assistant Prof.

Engineering (Development of miniMS)

(Osaka Univerisity) 2007~2012



Research Scientist

Pharmacology (Clinical, imaging)

2012~2015

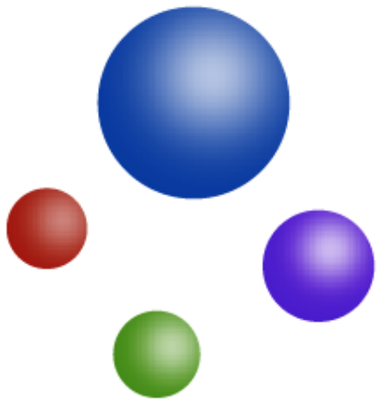
(NCC: National Cancer Center)

2015 April~ Osaka University

What is mass spectrometry (MS)?

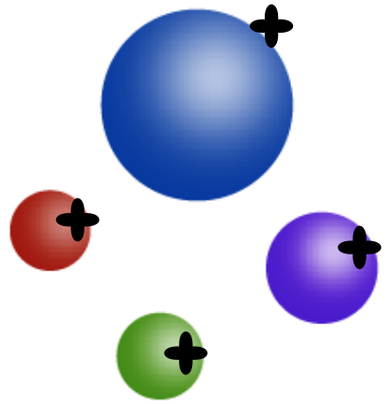
Mass spectrometry is analytical methodology to obtain mass of ions.

Molecules
(neutral charge)

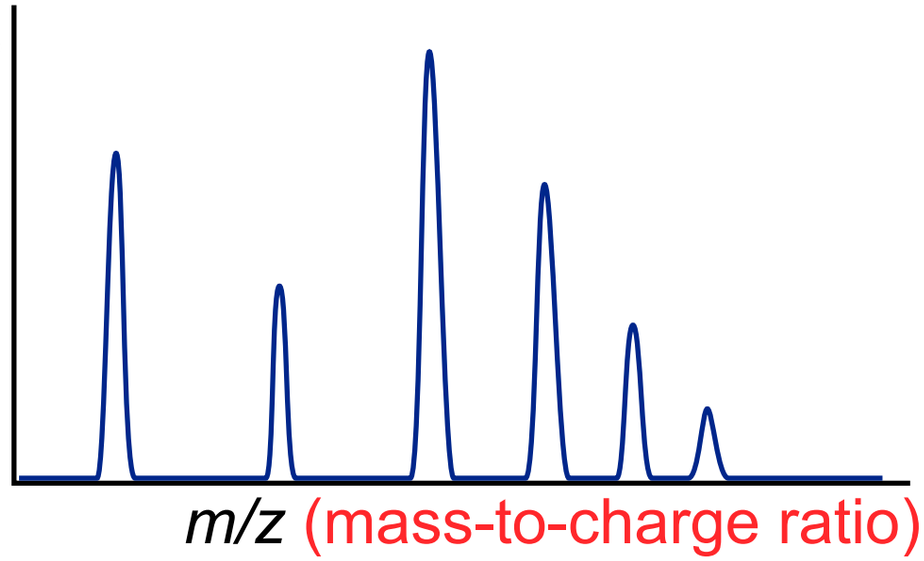


Ionization

Ions
(charged particles)



Mass spectra



All molecules normally exist as neutral charge.

To generate ions is the 1st step in MS. Generated ions can move inside electric and magnetic force.

Mass spectra are recorded by different motion of ions (velocity, curvature, oscillation) in different mass spectrometers.

Mass spectra include information of *m/z* in horizontal axis and ion intensity in vertical axis.

We can find “what kinds of molecules” and “how much molecules” in mass spectra.

Important facts in MS

Mass spectrometry is utilized to analyze components.

In MS,
the main purpose is to measure *m/z* and ion intensity.

Highly sensitive method

MS can detect
pmol (10^{-12} mol) or **fmol (10^{-15} mol)**
order concentrations.

Qualitative analysis

Structural analysis of molecules

Elemental composition analysis

Quantitative analysis

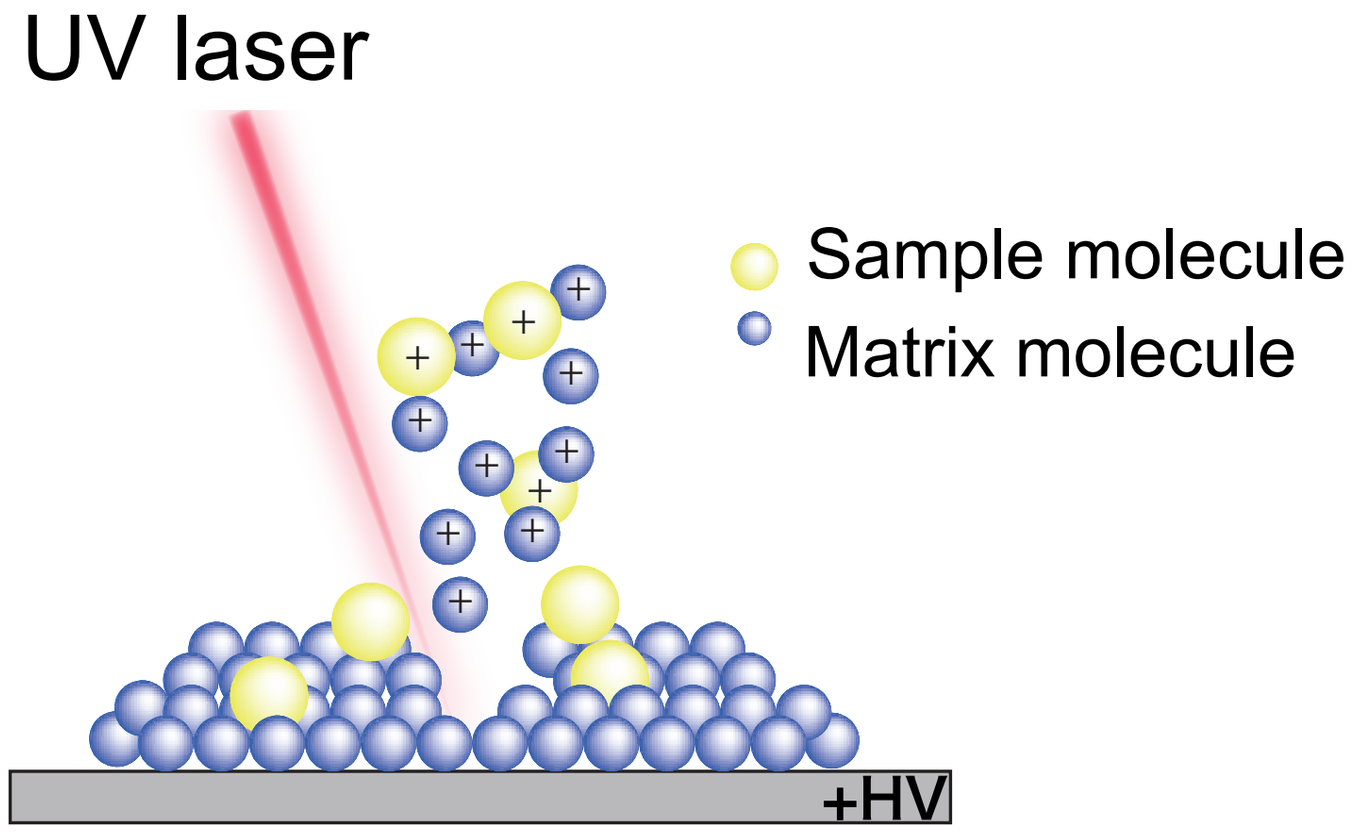
Determination of concentrations

Doping test/residual pesticides

Important ionization method

Matrix-assisted laser desorption ionization (MALDI)

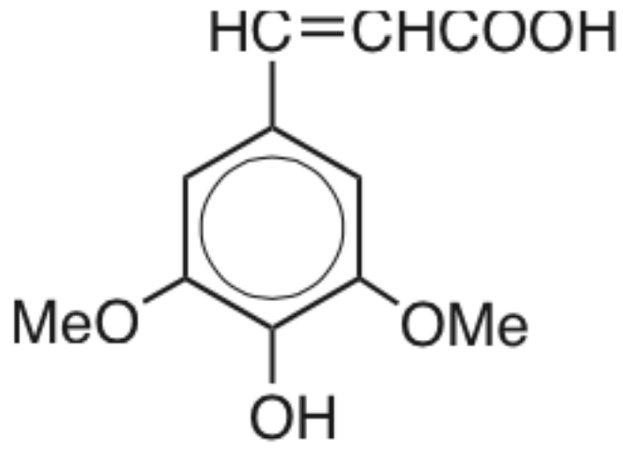
Ions are generated by irradiation of laser from matrix-analyte cocrystal.



Matrix: ionization supportive compounds.

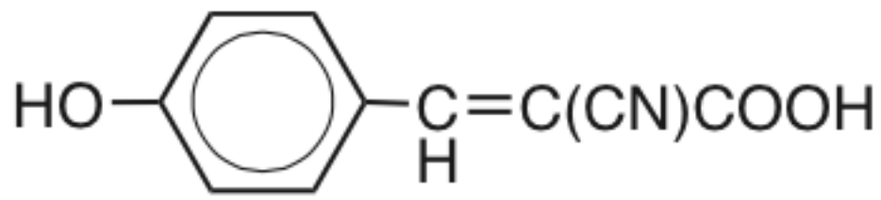
To absorb laser energy, matrices have an aromatic ring.

Common matrices in MALDI



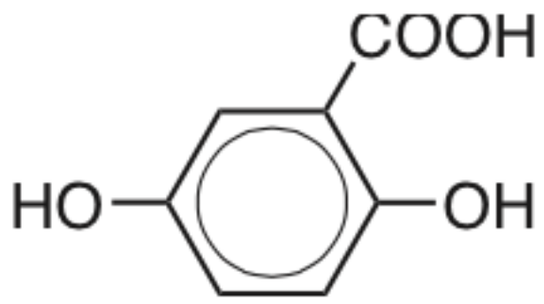
Sinapinic Acid （SA）

Proteins and peptides



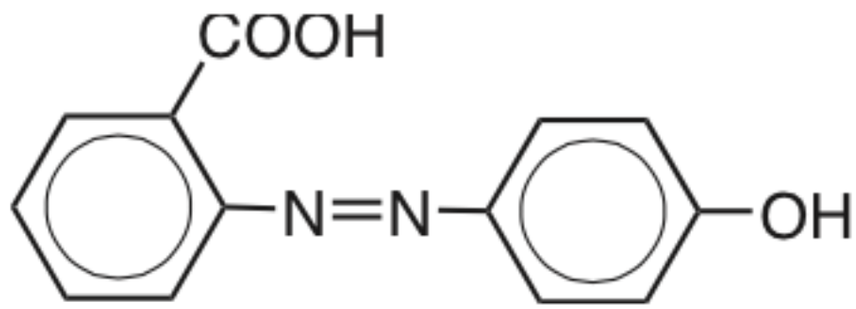
α-Cyano-4-hydroxycinnamic Acid
（CHCA）

Peptides and small molecules



2,5-Dihydroxybenzoic Acid
（DHB）

Sugars, peptides and synthetic polymers



2-(4-Hydroxyphenylazo)benzoic Acid
（HABA）

Synthetic polymers

Today’s topic

Molecular imaging using mass spectrometry

Molecular imaging using mass spectrometry

Imaging mass spectrometry

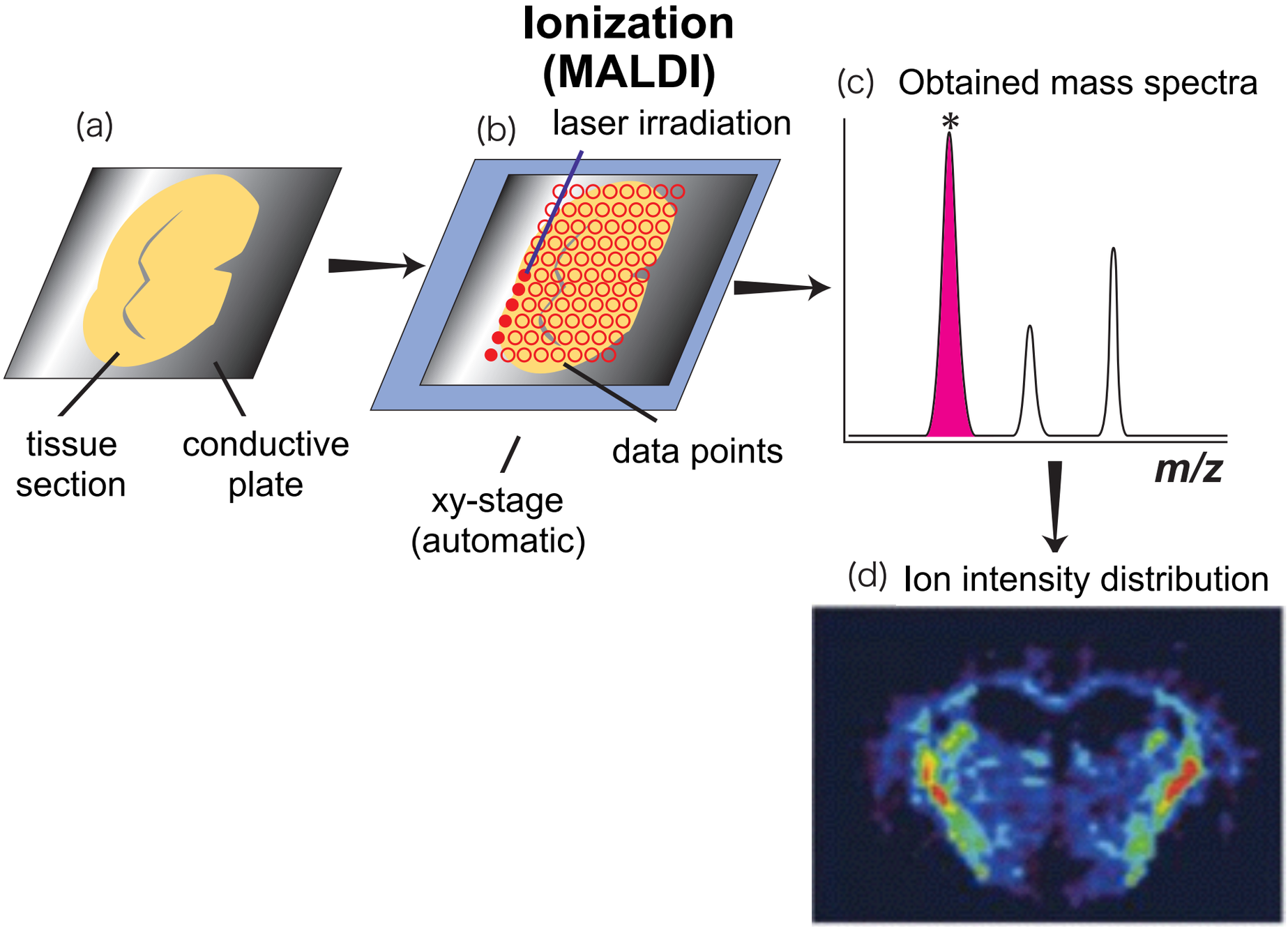
Important keyword

Important keyword

Seeing is Believing

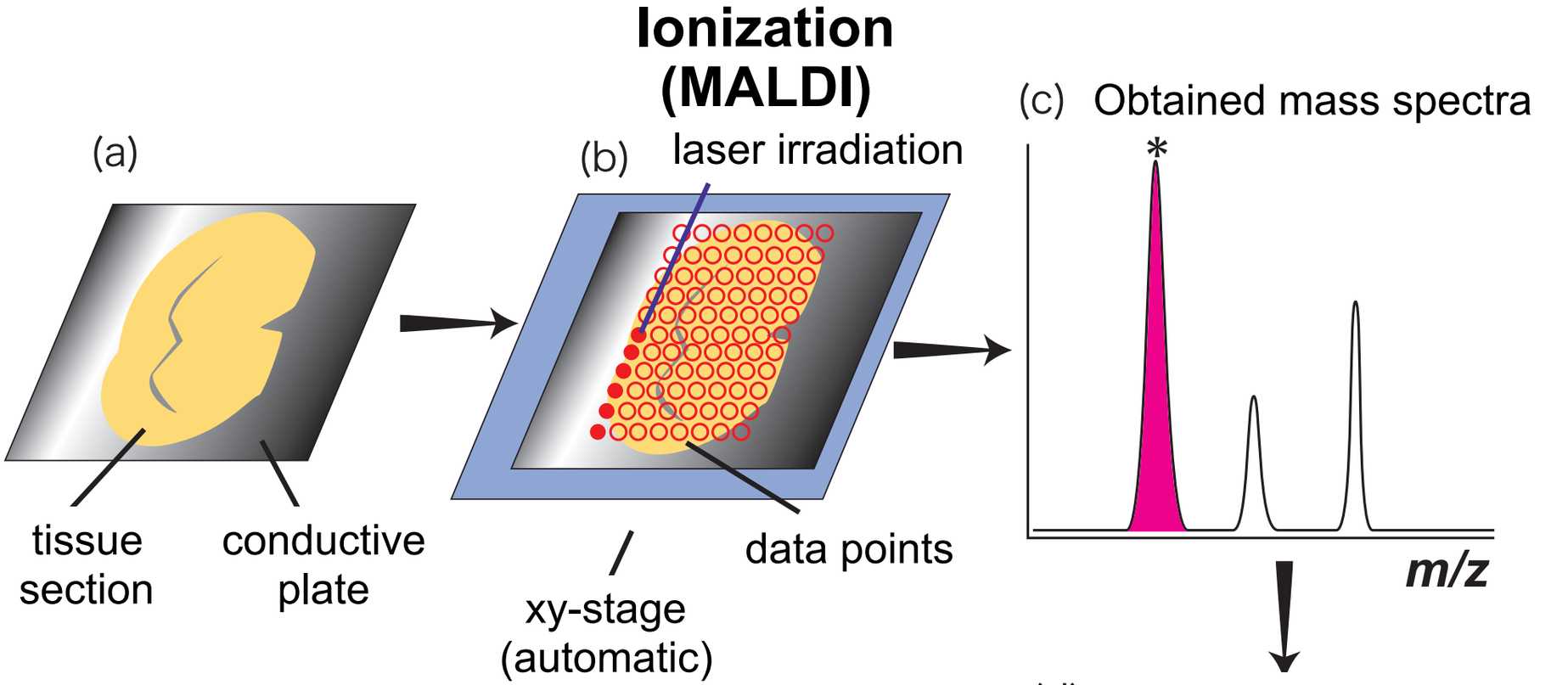
Imaging mass spectrometry (IMS)

-Direct mass spectrometry on the tissue surface-



Imaging mass spectrometry (IMS)

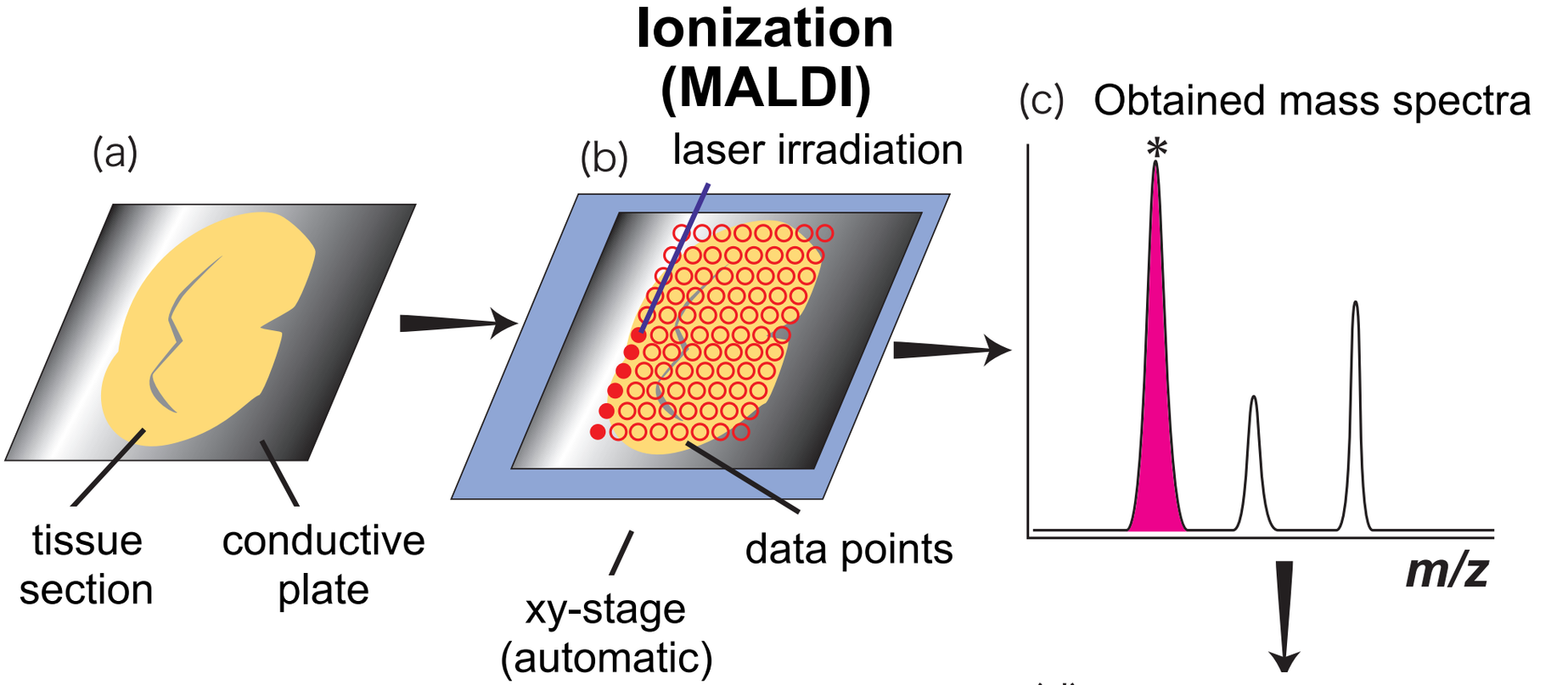
-Direct mass spectrometry on the tissue surface-



Generate ion intensity map using obtained mass spectra.

Imaging mass spectrometry (IMS)

-Direct mass spectrometry on the tissue surface-



Generate ion intensity map using obtained mass spectra.

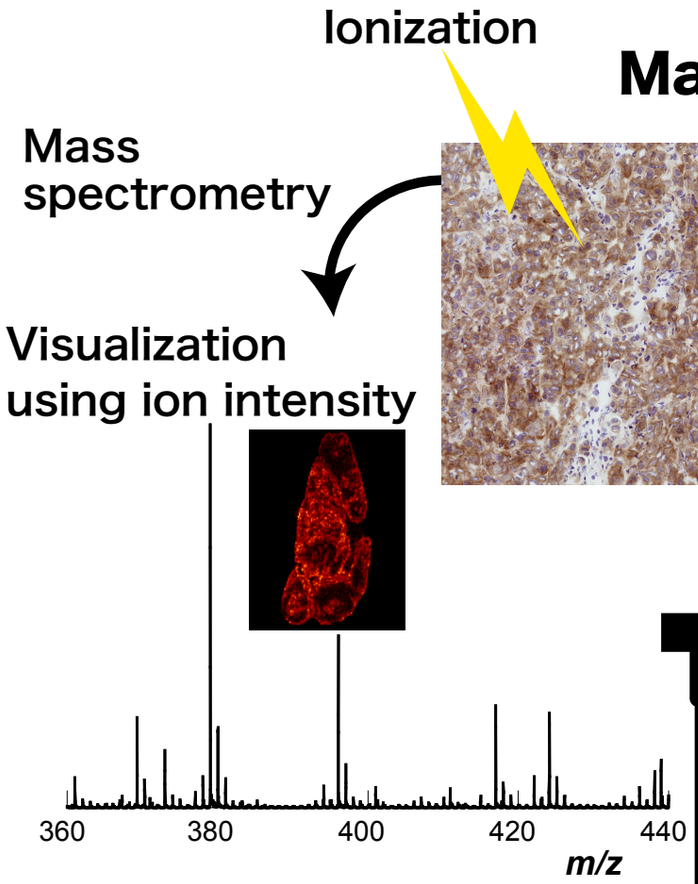
Non-labeled molecular imaging technique.
We can see “What, Where, How much”.

Mass Microscope

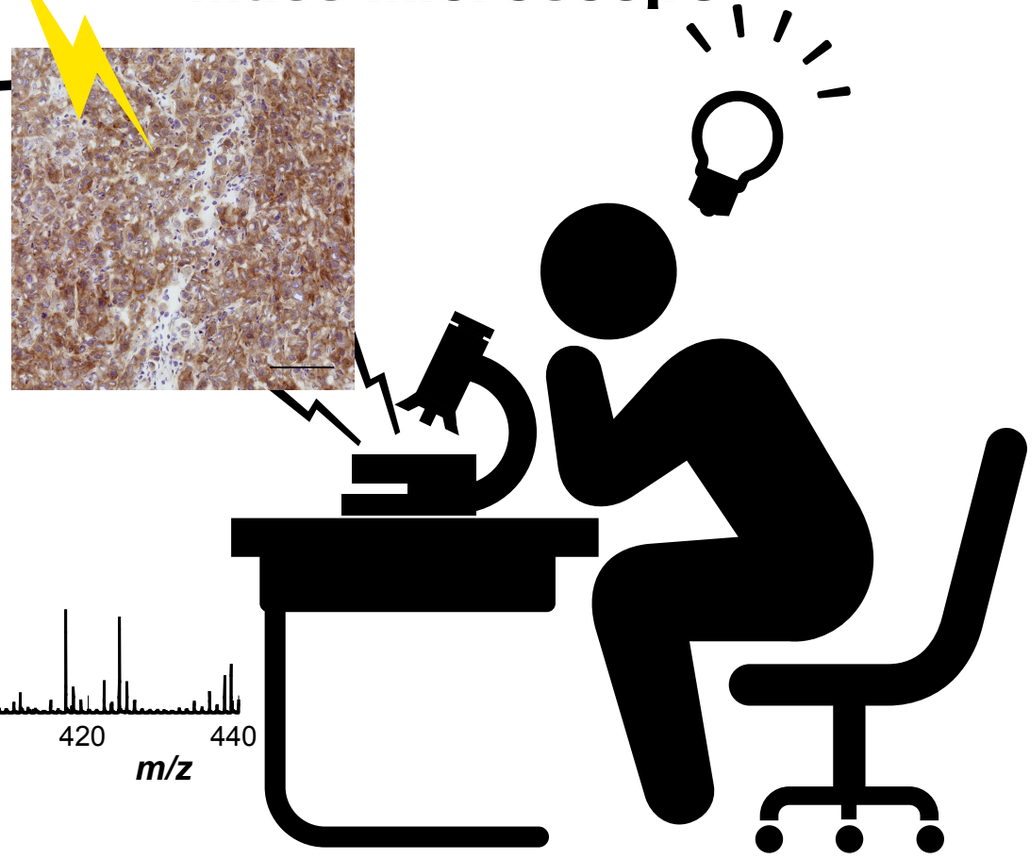
Mass Microscope is specially designed instrument for IMS.

Conventional microscope

You can see structure in ROIs.
But you cannot find components in ROIs.



Mass Microscope

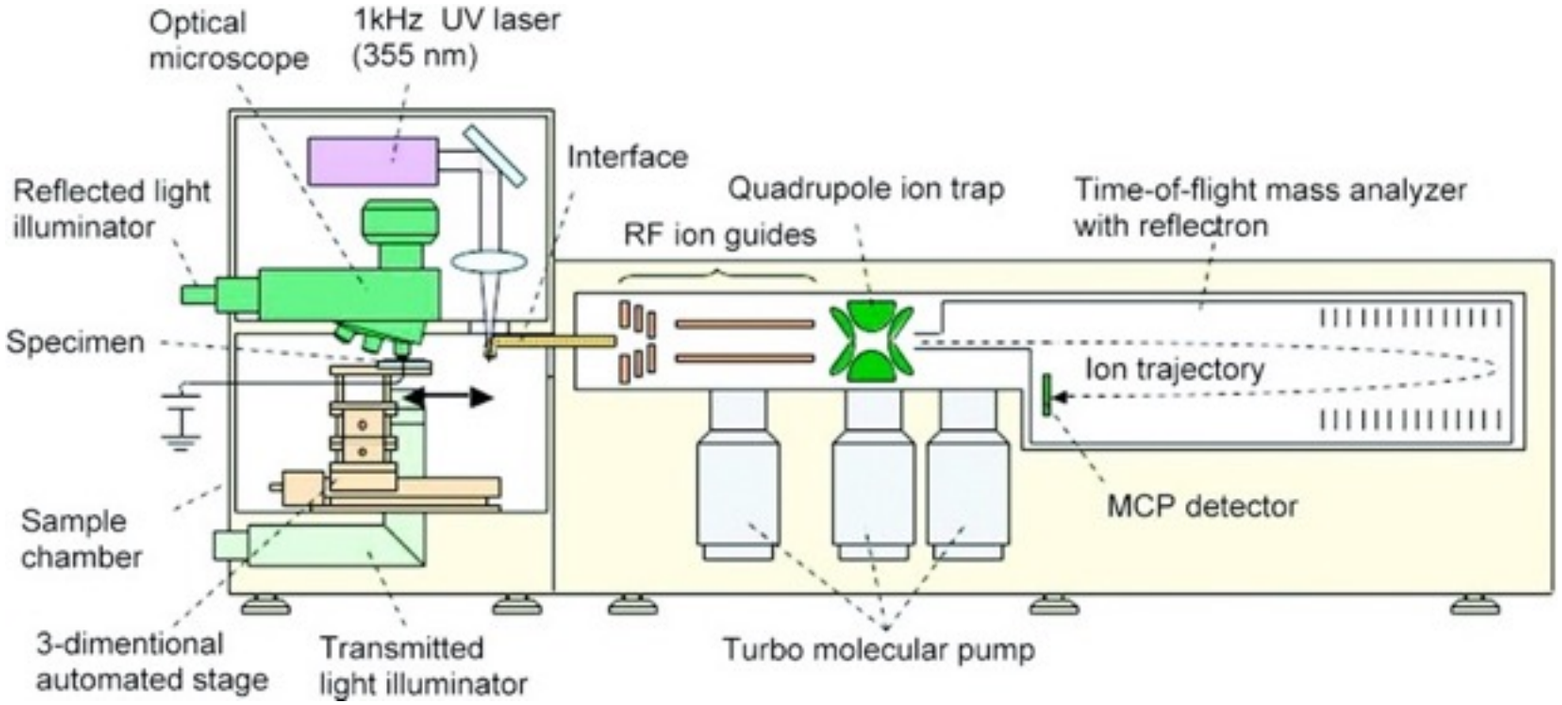


You can see many kinds of components in ROIs using mass microscope.

iMScope TRIO (Shimadzu, Kyoto, Japan)

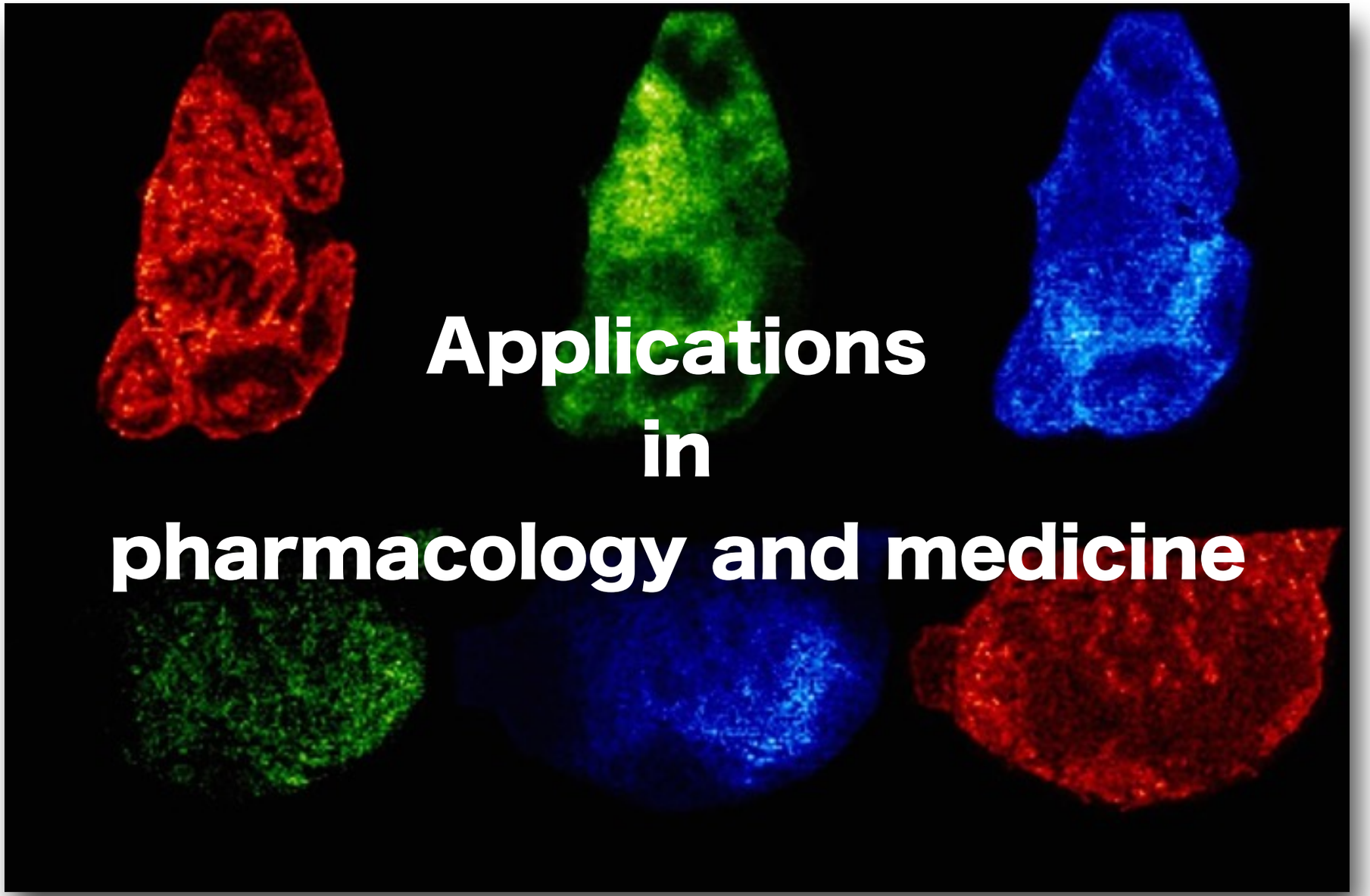
Picture of iMScope TRIO

Schematic drawing of instrument



- ✔ Optical microscope
- ✔ Ionization：Atmospheric pressure MALDI
- ✔ Laser for ionization｜Nd:YAG (355 nm, 1 kHz)
- ✔ Mass range｜*m/z* 50-3000
- ✔ Laser diameter｜variable (5 μm - 200 μm)

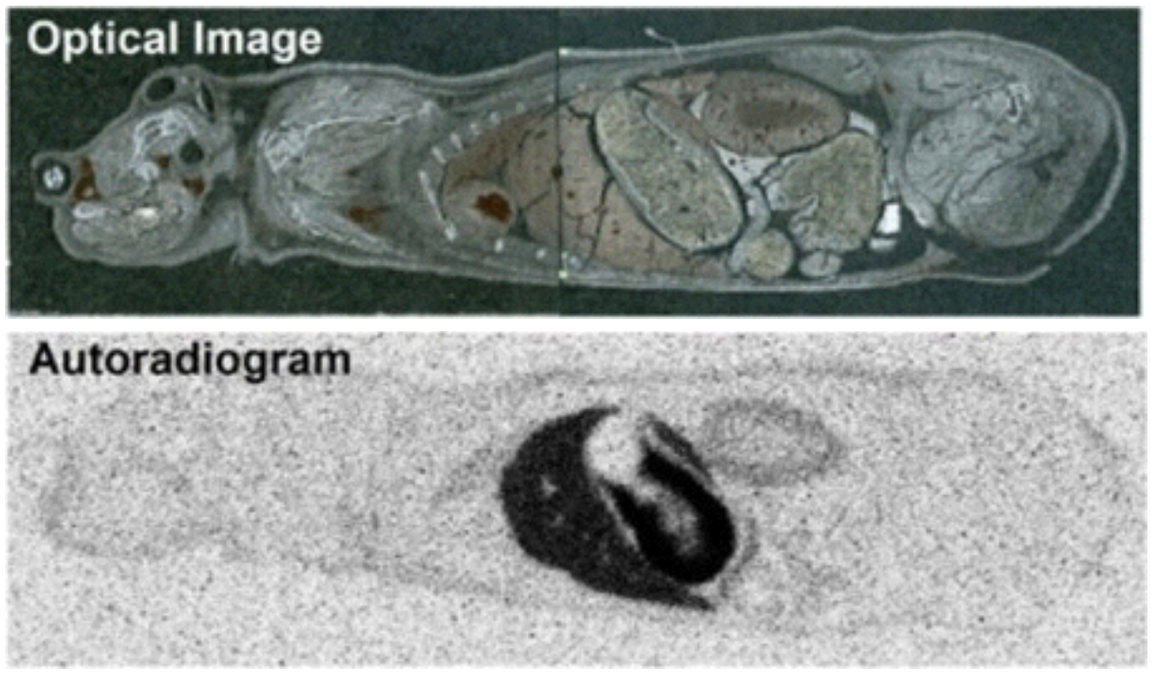
High spatial resolution imaging is available.


Applications
in
pharmacology and medicine

In-vivo pharmaco-imaging (conventional)

Autoradiography

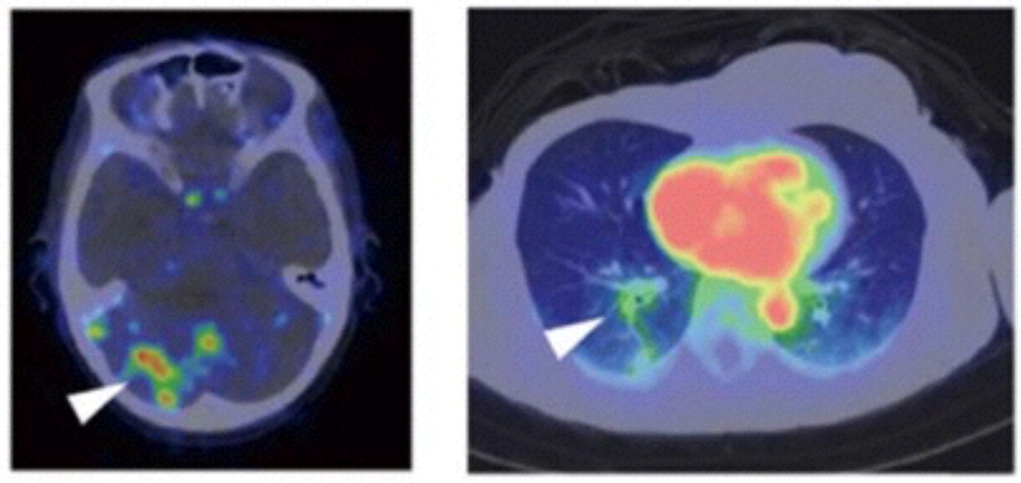
Detection of radiation



Takai et al. Rapid Commun Mass Spectrom, 26, 1549 (2012)

PET imaging

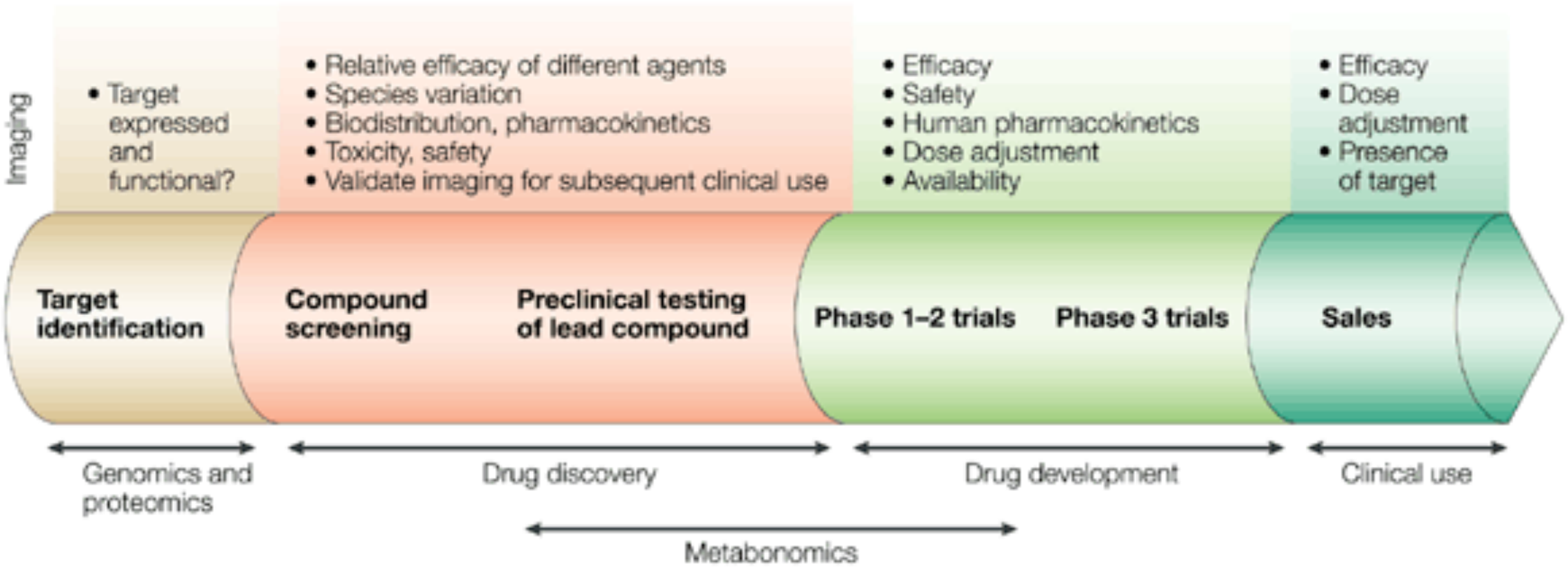
Detection of photon via annihilation



^{64}Cu-DOTA Trastsuzumab uptake into tumors (metastasis from breast cancer)

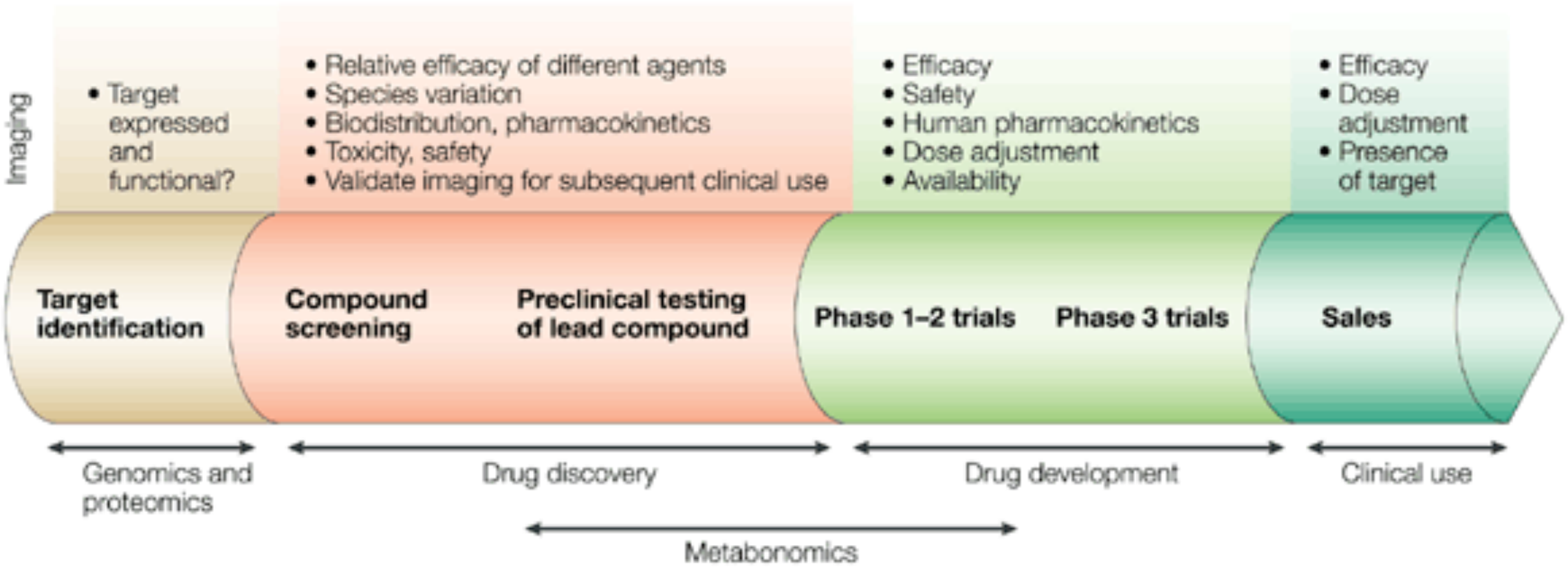
Tamura K et al. J Nuc Med 54, 1869 (2013)

Pipeline for drug development



Rudin M Nat Drug Discov. 2003

Pipeline for drug development



Rudin M Nat Drug Discov. 2003

In anti-cancer agents development,
cancer patients are recruited from phase I.

Expectations from clinicians and megapharma

Expectations from clinicians and megapharma

As developing biological studies,
almost therapies become **“targeted therapy”**.

Expectations from clinicians and megapharma

As developing biological studies,
almost therapies become **“targeted therapy”**.

Confirmation of **POM**

Expectations from clinicians and megapharma

As developing biological studies,
almost therapies become **“targeted therapy”**.

Confirmation of **POM**

Keyword 1: POM (proof-of-mechanism)

POM is one of the important keywords in drug development.

Expectations from clinicians and megapharma

As developing biological studies,
almost therapies become **“targeted therapy”**.

Confirmation of **POM**

Keyword 1: POM (proof-of-mechanism)

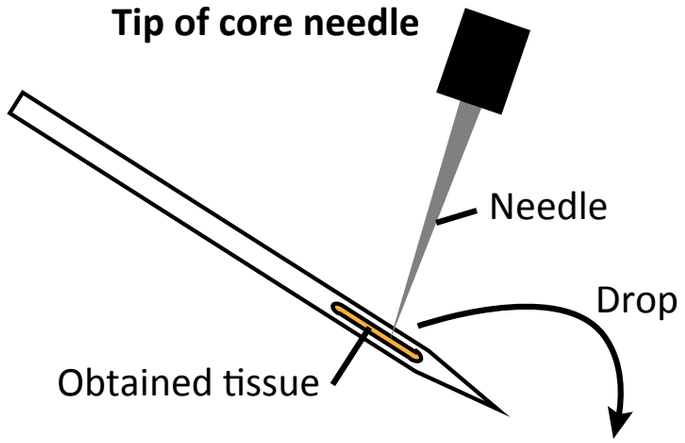
POM is one of the important keywords in drug development.

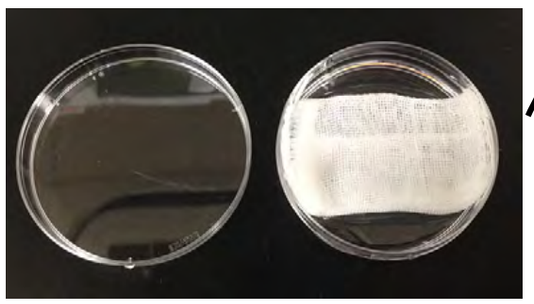
IMS can visualize POM of drugs.

This information is utilized for go/no go decision in drug development (especially in early phase).

Sampling and sectioning for core needle biopsy

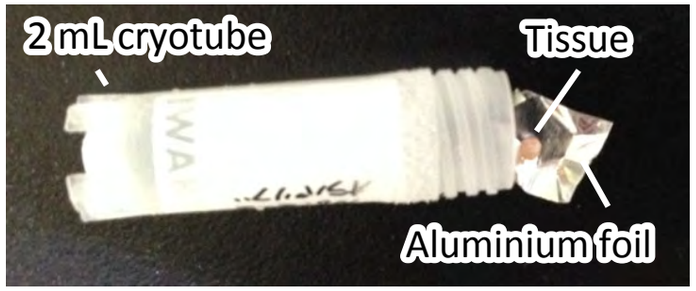
Sampling





Obtained tissue is dropped onto
a PBS dipped gauze in a 60 mm culture dish

The tissue is collected by needle
and put onto a piece of
an aluminium foil.



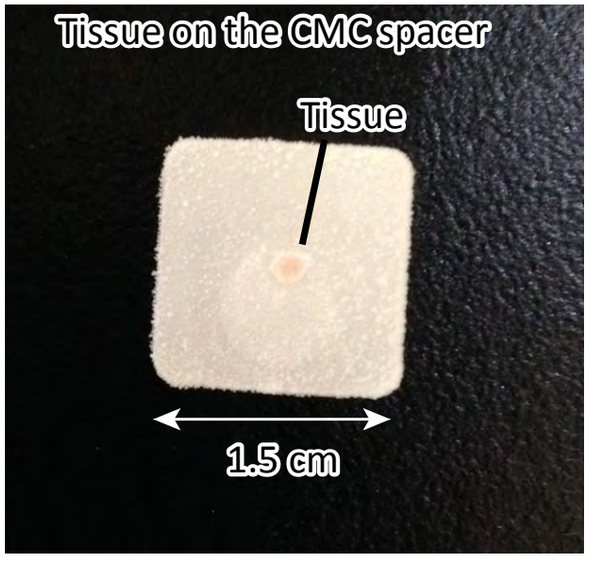
Freeze in liq. nitrogen

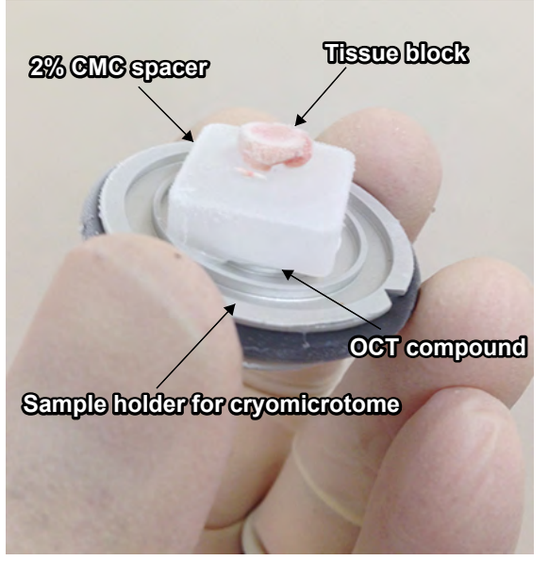
Store in a -80degC freezer

Tissue mounting for sectioning

CMC spacer

(1) 1.2 mL of 2% CMC solution is pipetted into disposable embedding dish.
(2) For biopsy samples, the dish is stored in the -80degC freezer for 9 min.
(3) After 9 min, the dish is put in room temperature for 1 min (slightly melt the surface after 1 min).
(4) Put the frozen tissue on the melting area with cold tweezer, and immediately stored in the -80degC freezer for 10 min.



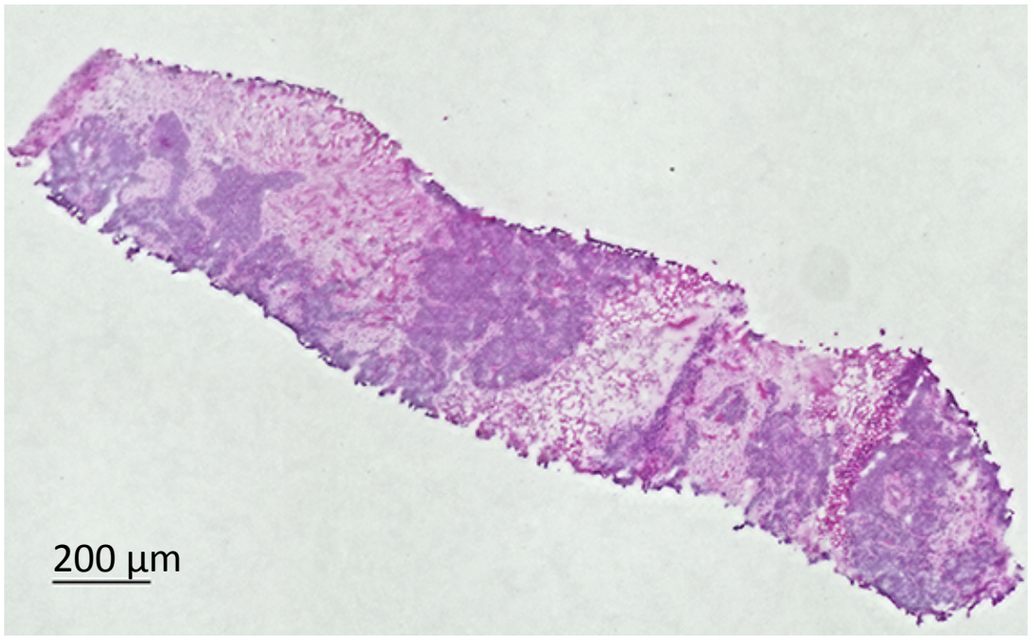


Quantitative-IMS of olaparib in human breast tumor tissues

MADRID 2014 ESMO congress

Shimoi T et al. ESMO2014

(A)

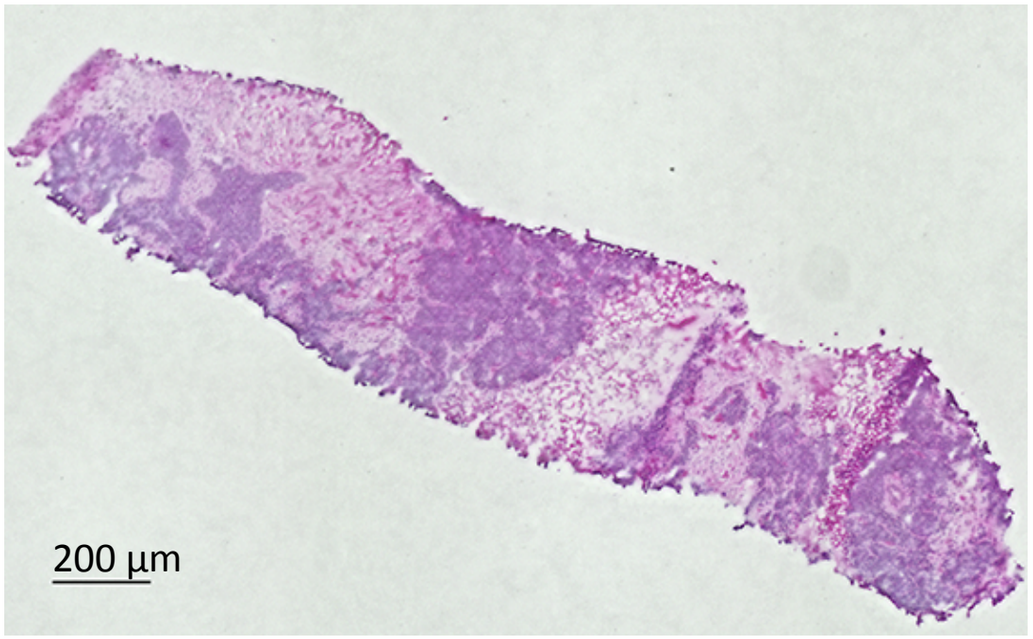


Quantitative-IMS of olaparib in human breast tumor tissues

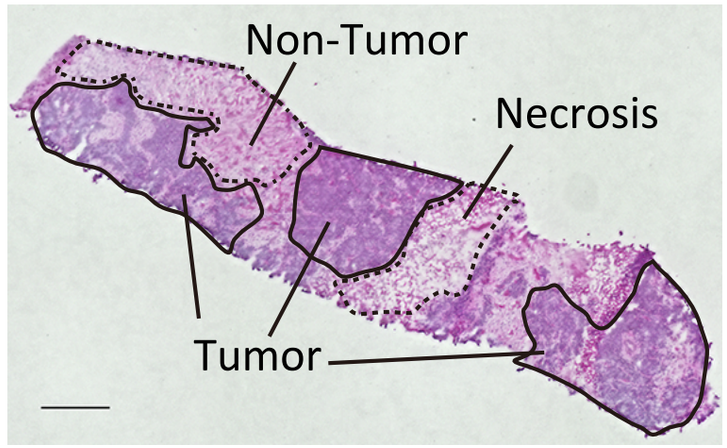
MADRID 2014 ESMO congress

Shimoi T et al. ESMO2014

(A)



(B)

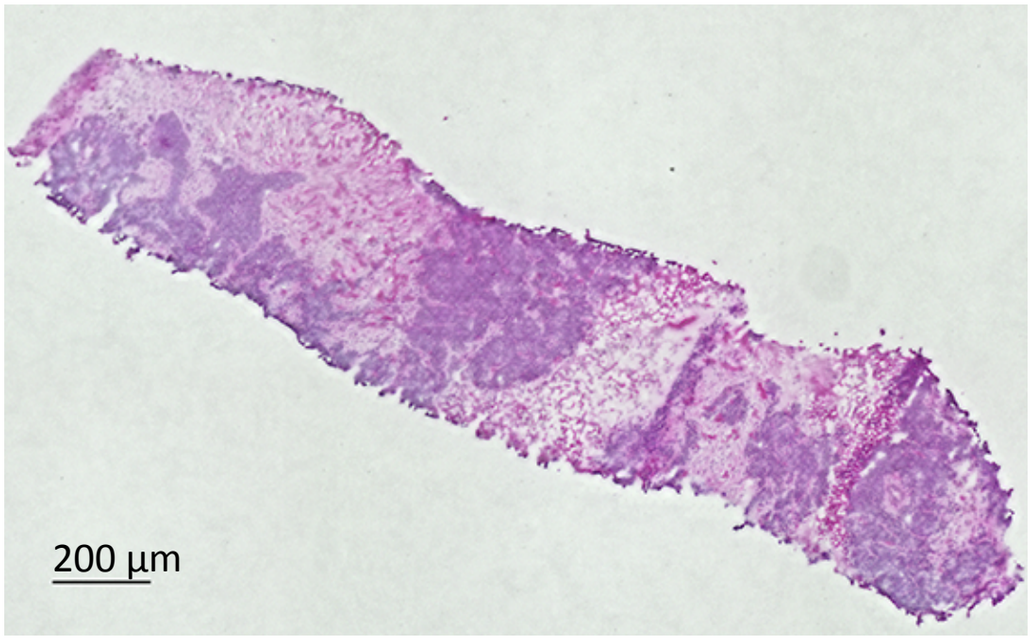


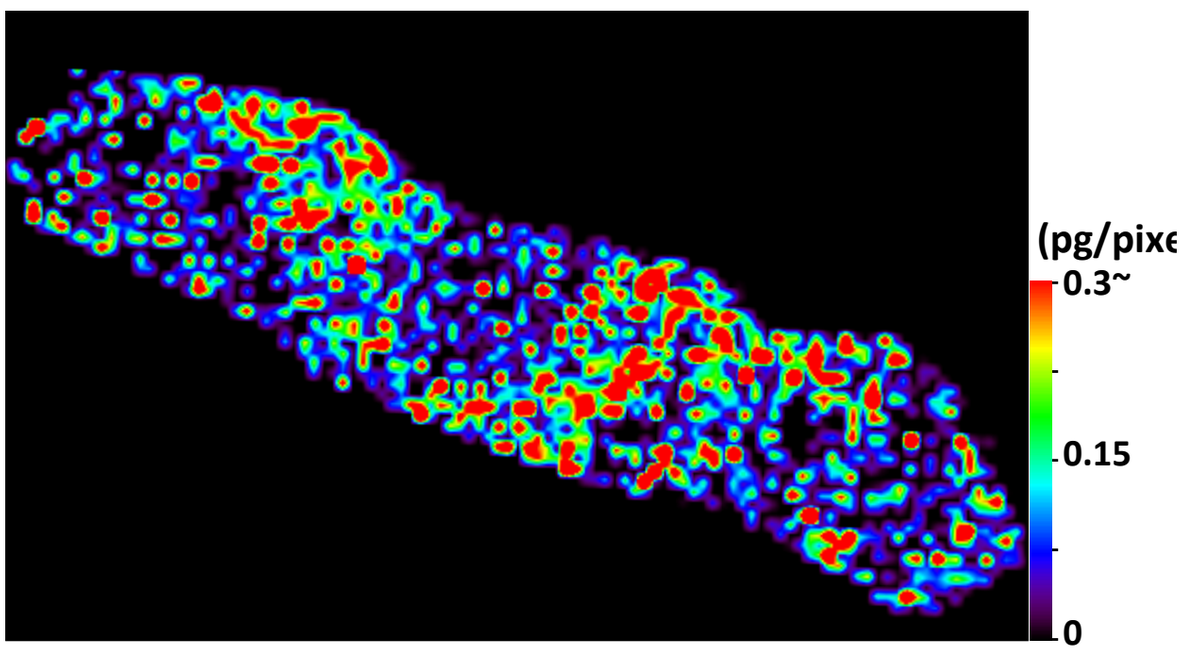
Quantitative-IMS of olaparib in human breast tumor tissues

MADRID 2014 ESMO congress

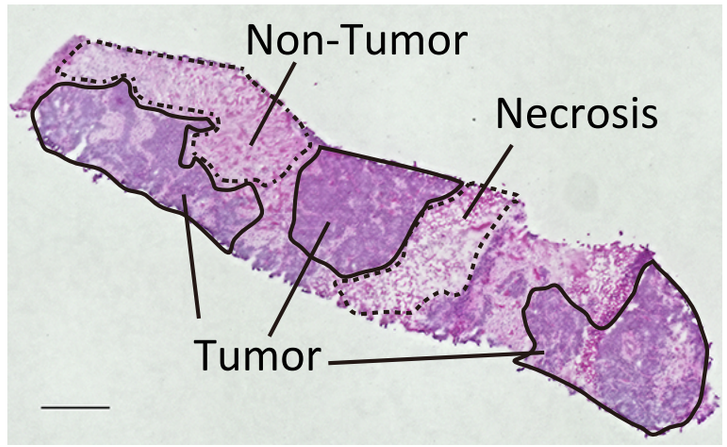
Shimoi T et al. ESMO2014

(A)





(B)

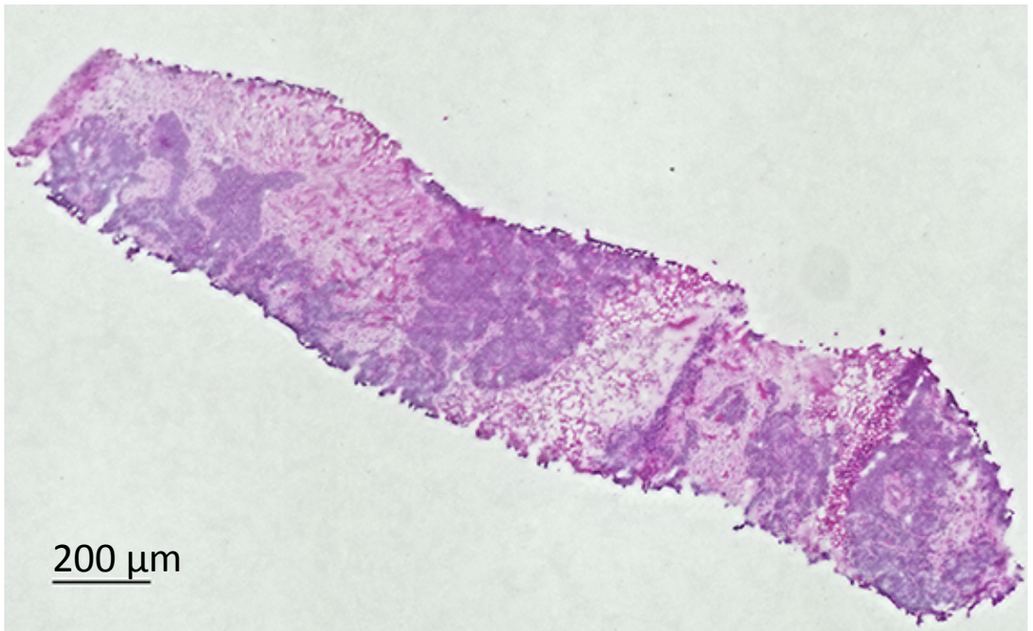


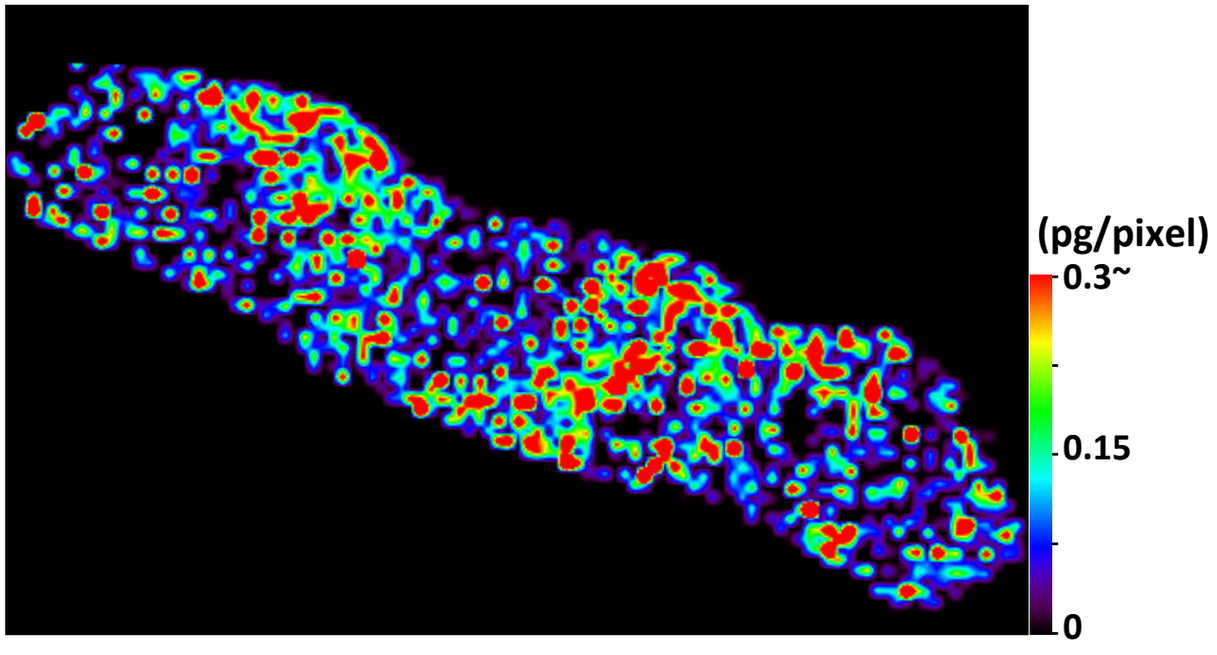
Quantitative-IMS of olaparib in human breast tumor tissues

MADRID 2014 ESMO congress

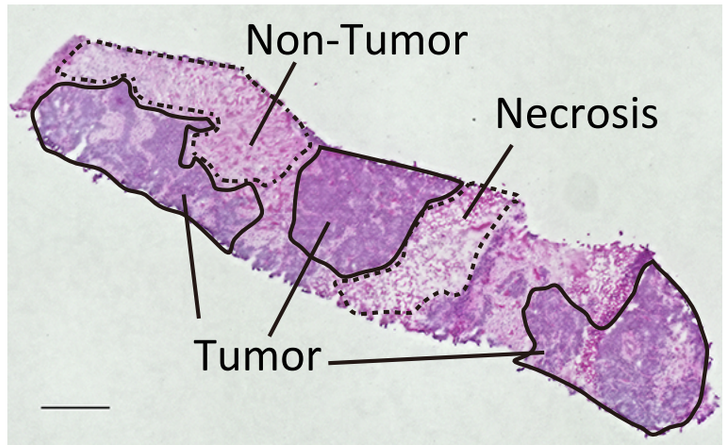
Shimoi T et al. ESMO2014

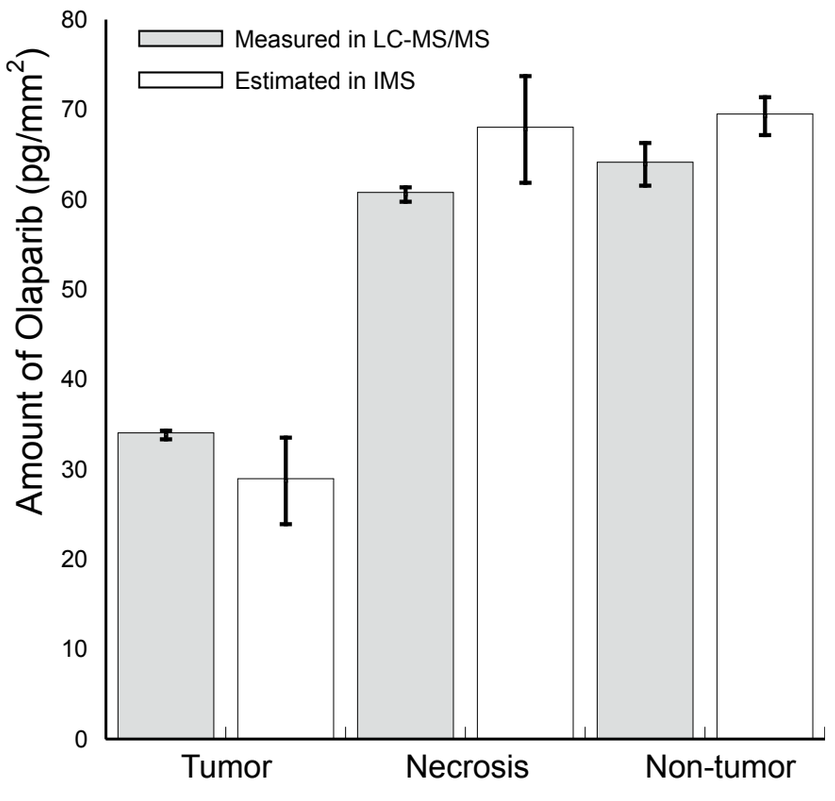
(A)





(B)



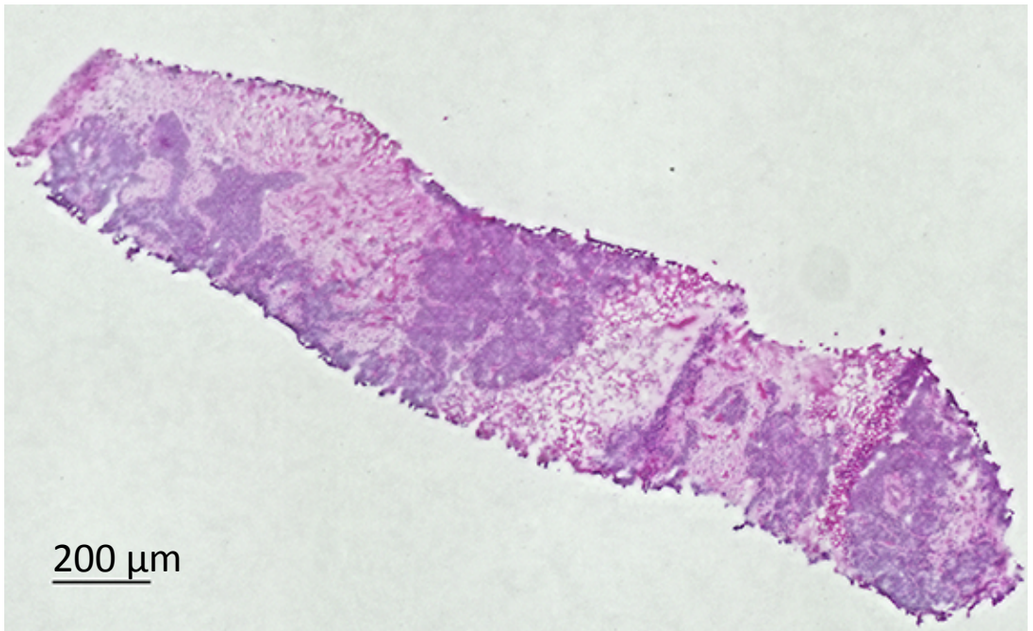


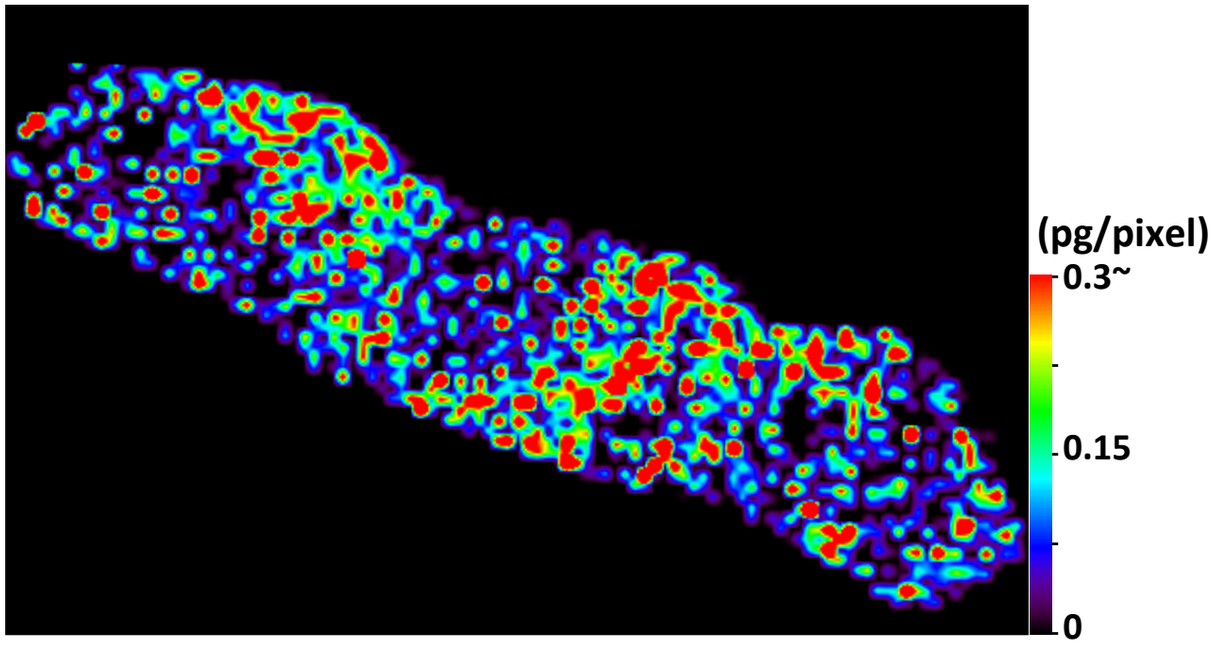
Quantitative-IMS of olaparib in human breast tumor tissues

MADRID 2014 ESMO congress

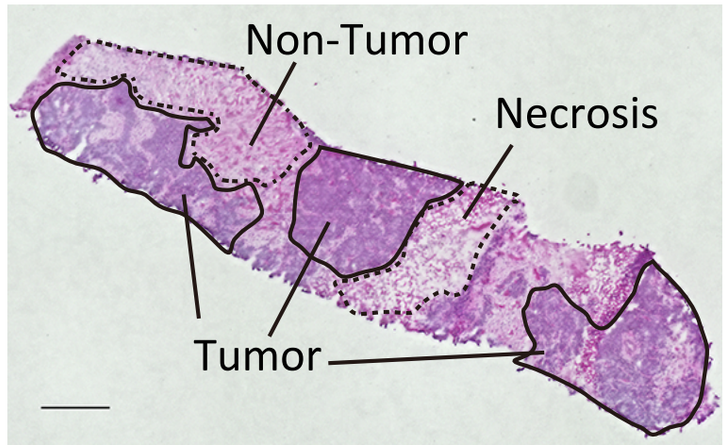
Shimoi T et al. ESMO2014

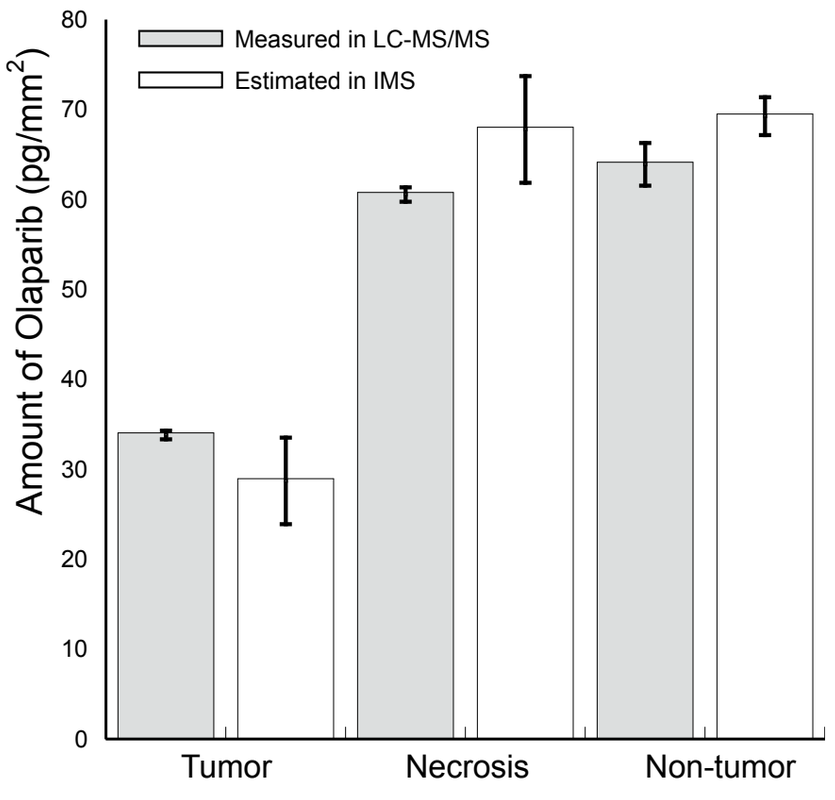
(A)





(B)

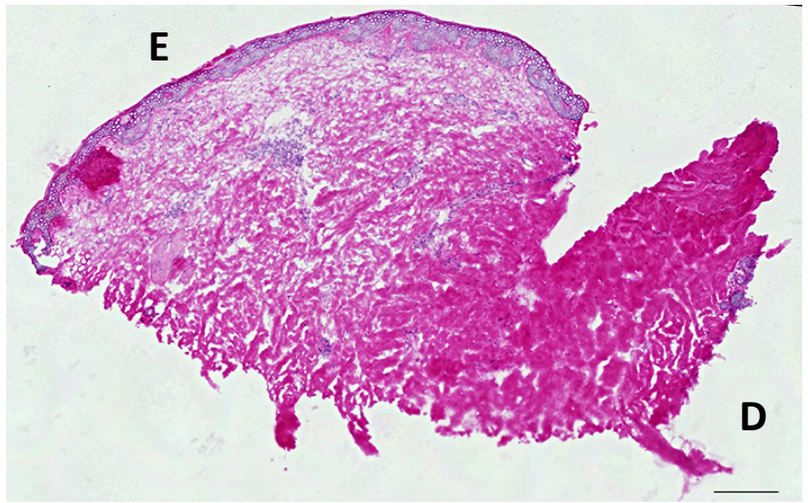




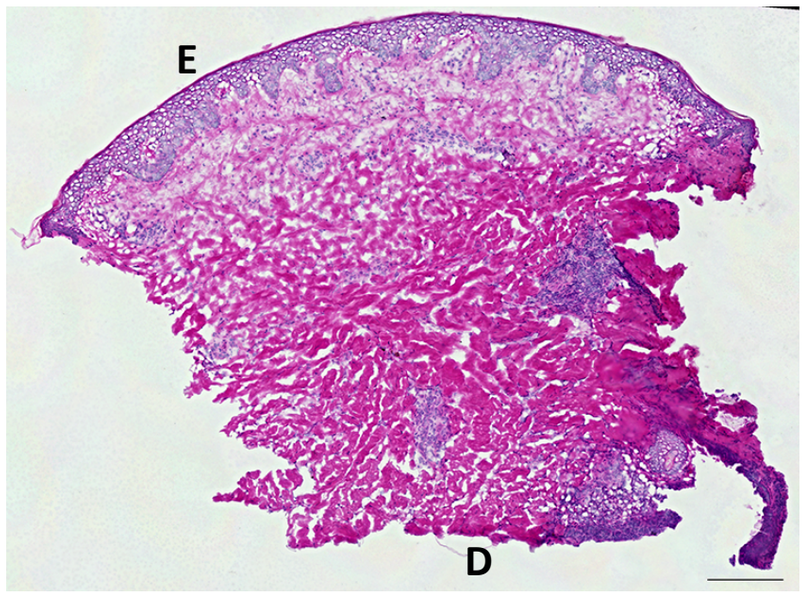
Olaparib was accumulated outside tumor regions.

Quantitative-IMS of Erlotinib for side effect study

Normal skin

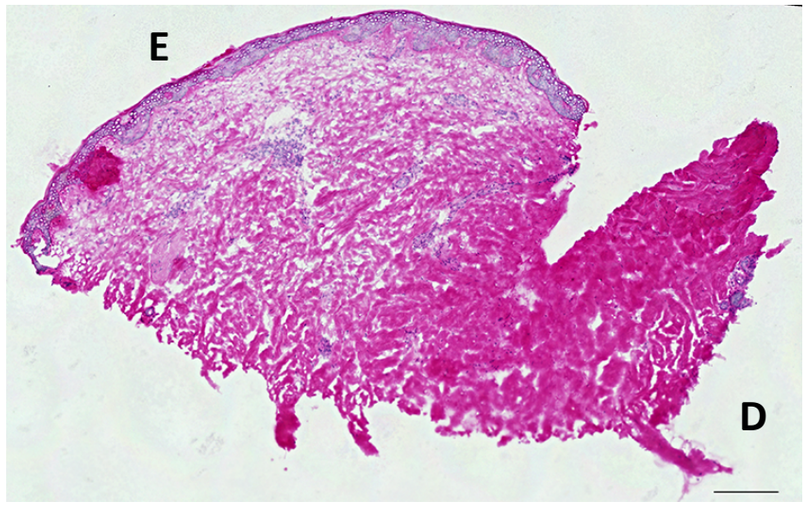


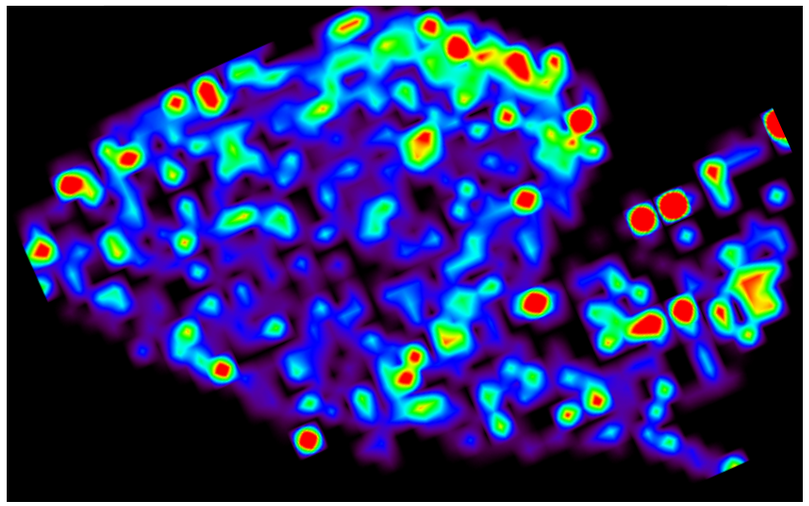
Rash skin



Quantitative-IMS of Erlotinib for side effect study

Normal skin

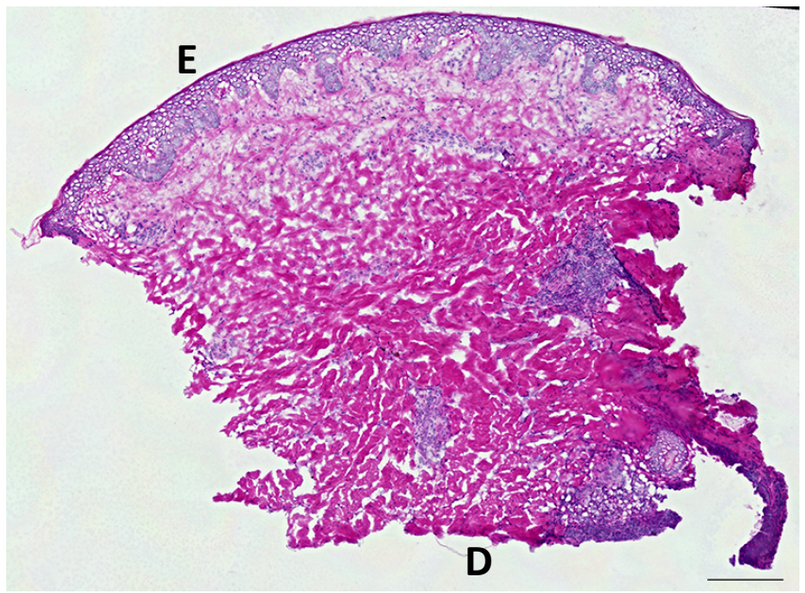


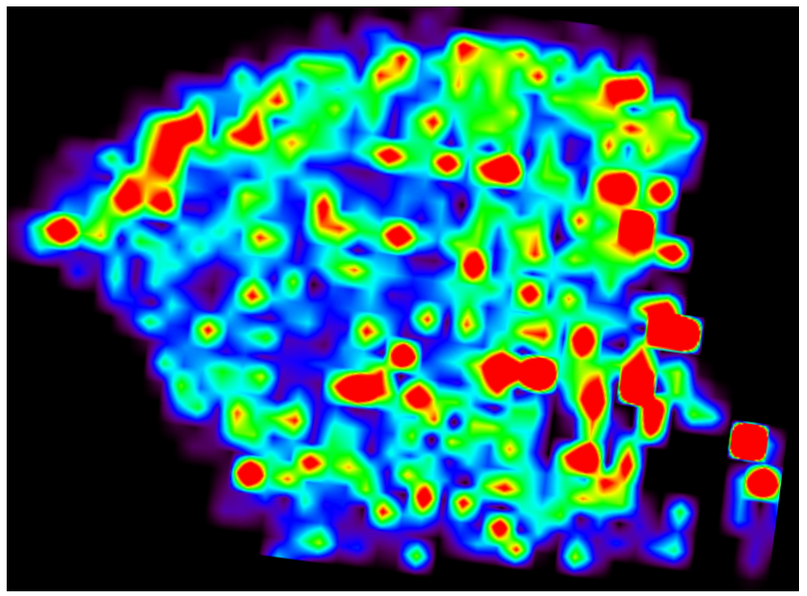


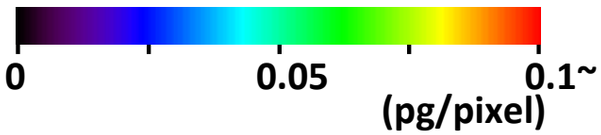
患者番号 4	切片 1 枚当たり薬物量	表層	真皮
rash	37.8† (1.92)‡	2.49	1.81
normal	21.7 (2.04)	1.48	0.90

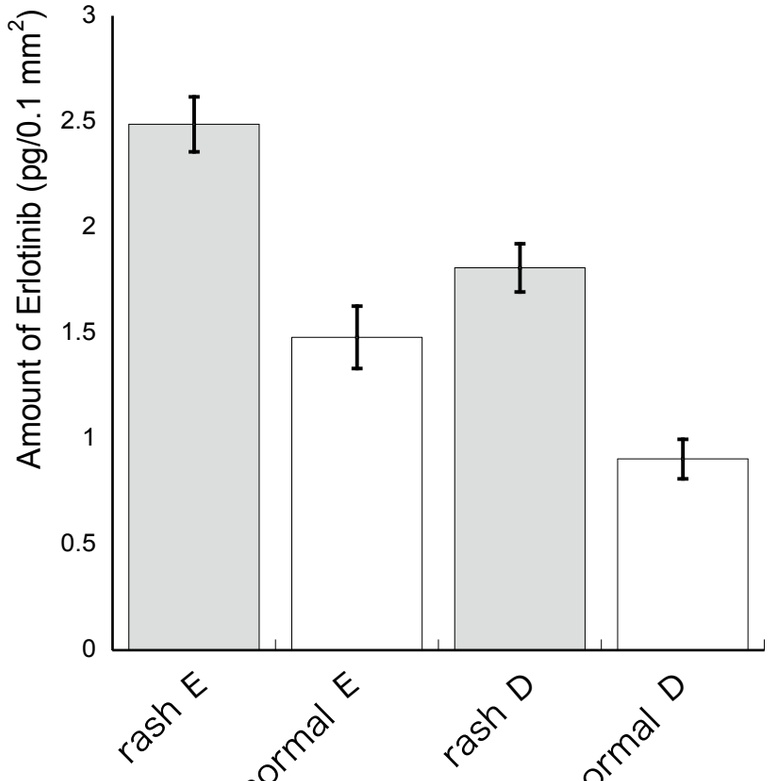
† 連続切片を採取し LC-MSMS を用いて測定した実験値　（単位：pg）
‡ 連続切片の面積．単位：mm^2
§ 皮疹部の真皮には炎症と見られる部位への蓄積があるが，そこを除いて薬物量を推定した．

Rash skin





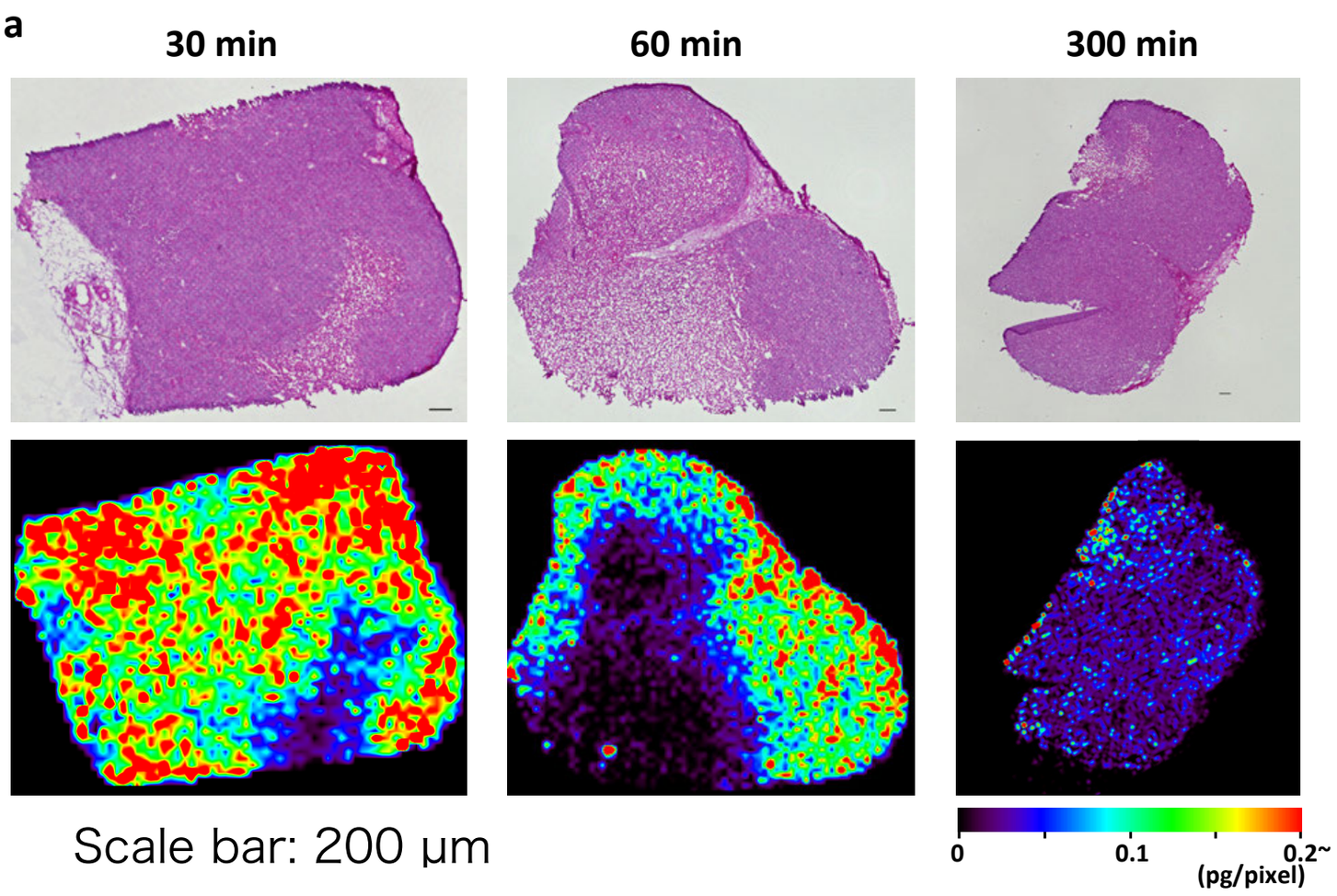


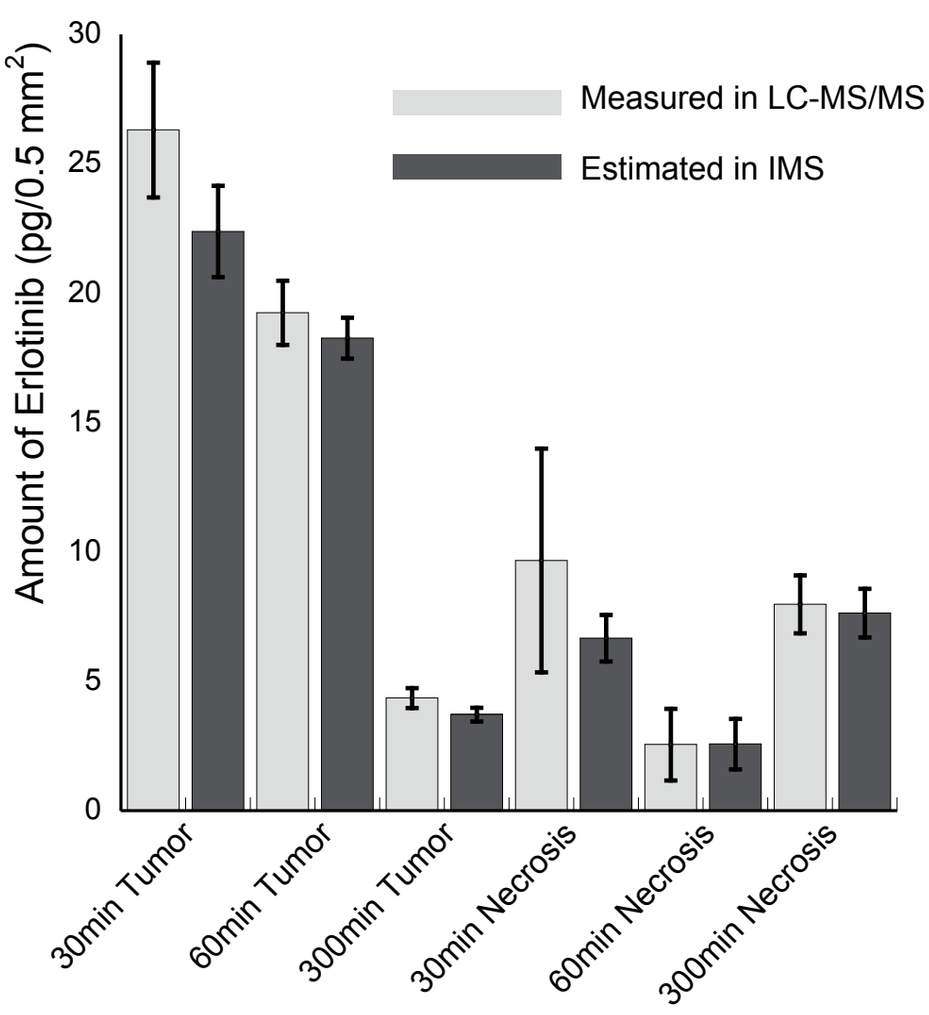


エラーバー：s.m
(皮疹，正常ともに 3 枚の切片をイメージングし，薬剤推定を表皮で 2 カ所，真皮で 2 カ所おこなった)

Quantitative Imaging in PK study

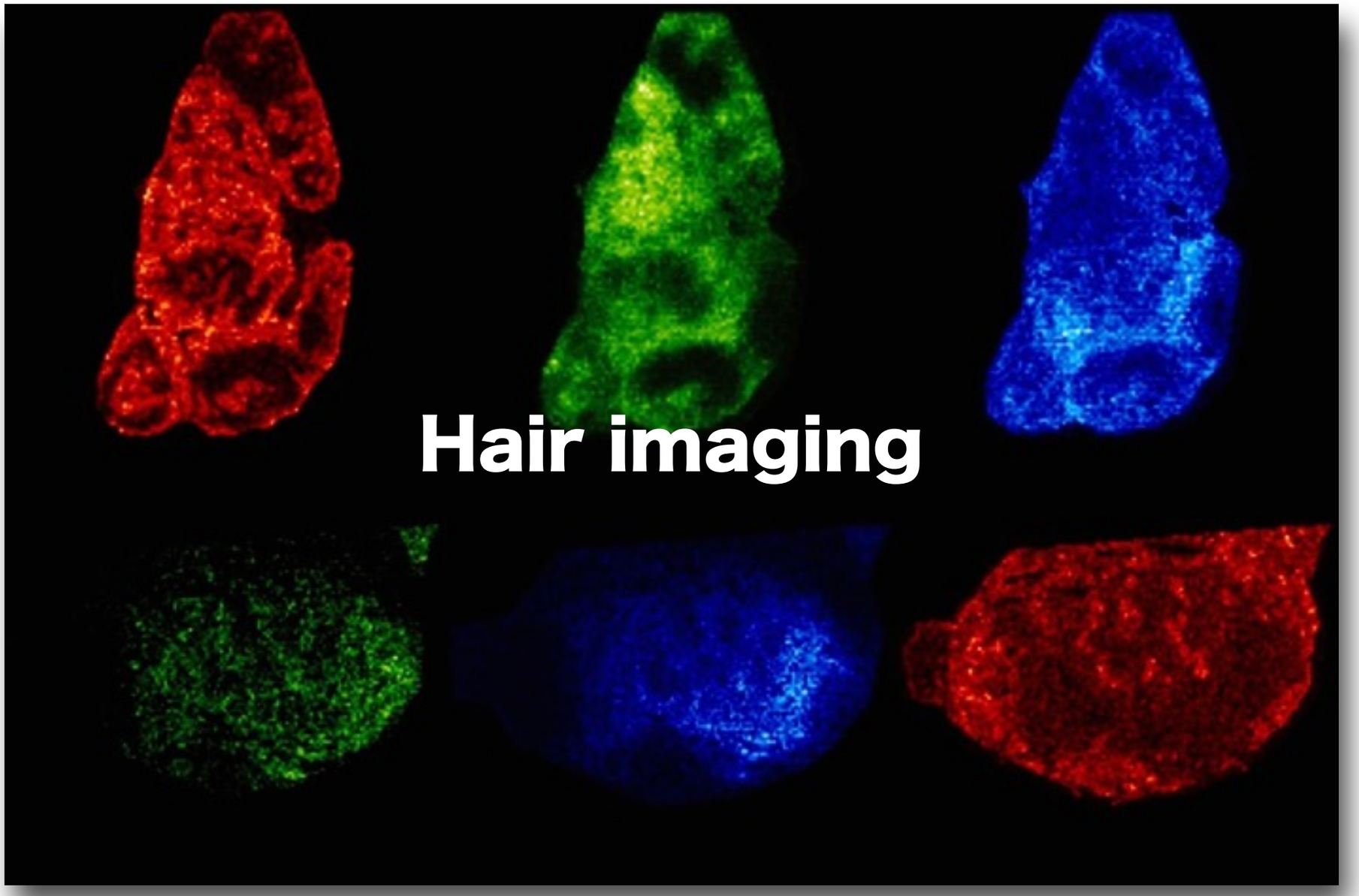
Quantitative pharmacokinetic-IMS



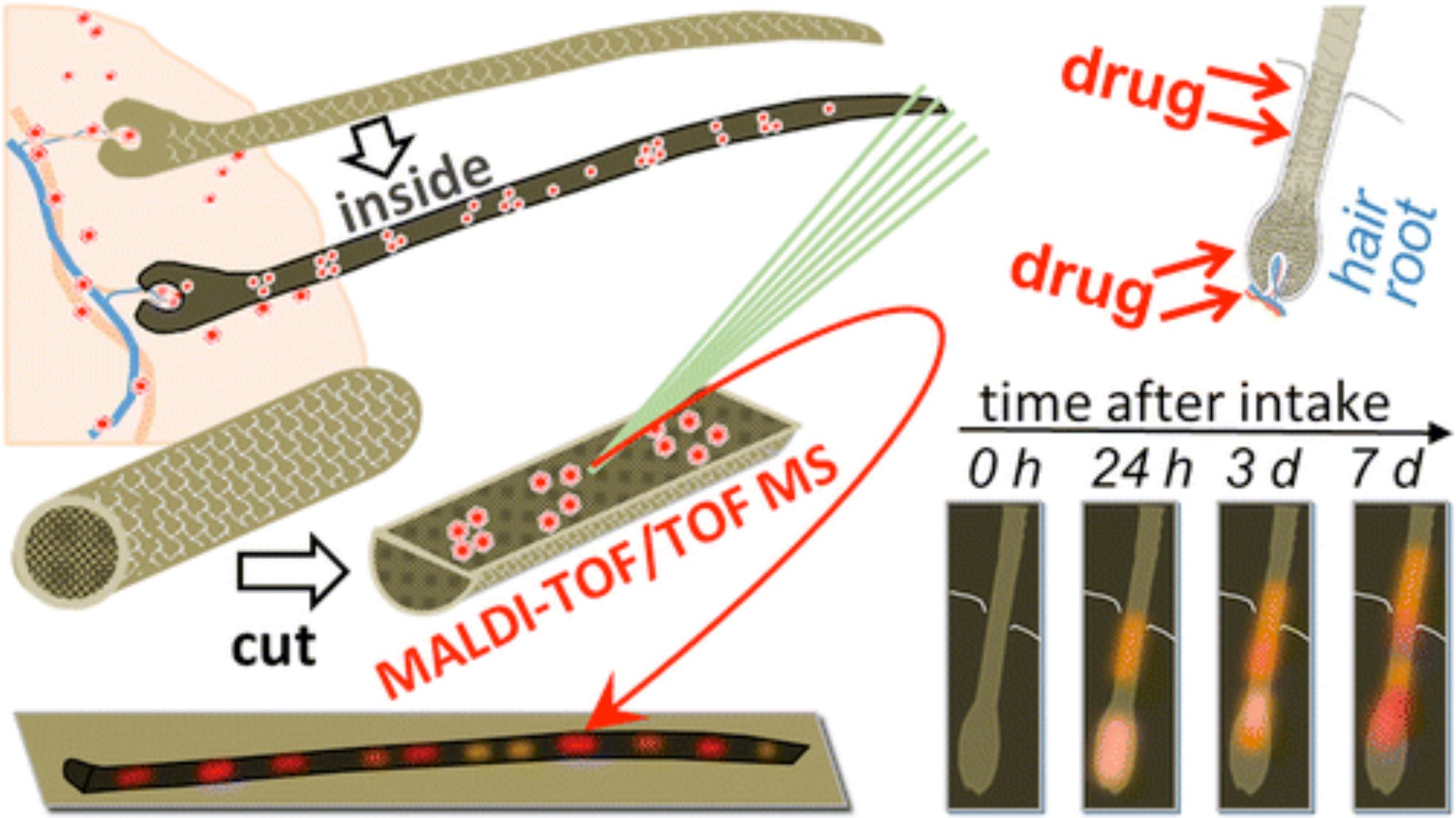


This procedure has been accepted in Surf Int Anal (Takeo E and Shimma S).

Estimated drug amounts using IMS results are equivalent to measured values using LC-MS/MS.

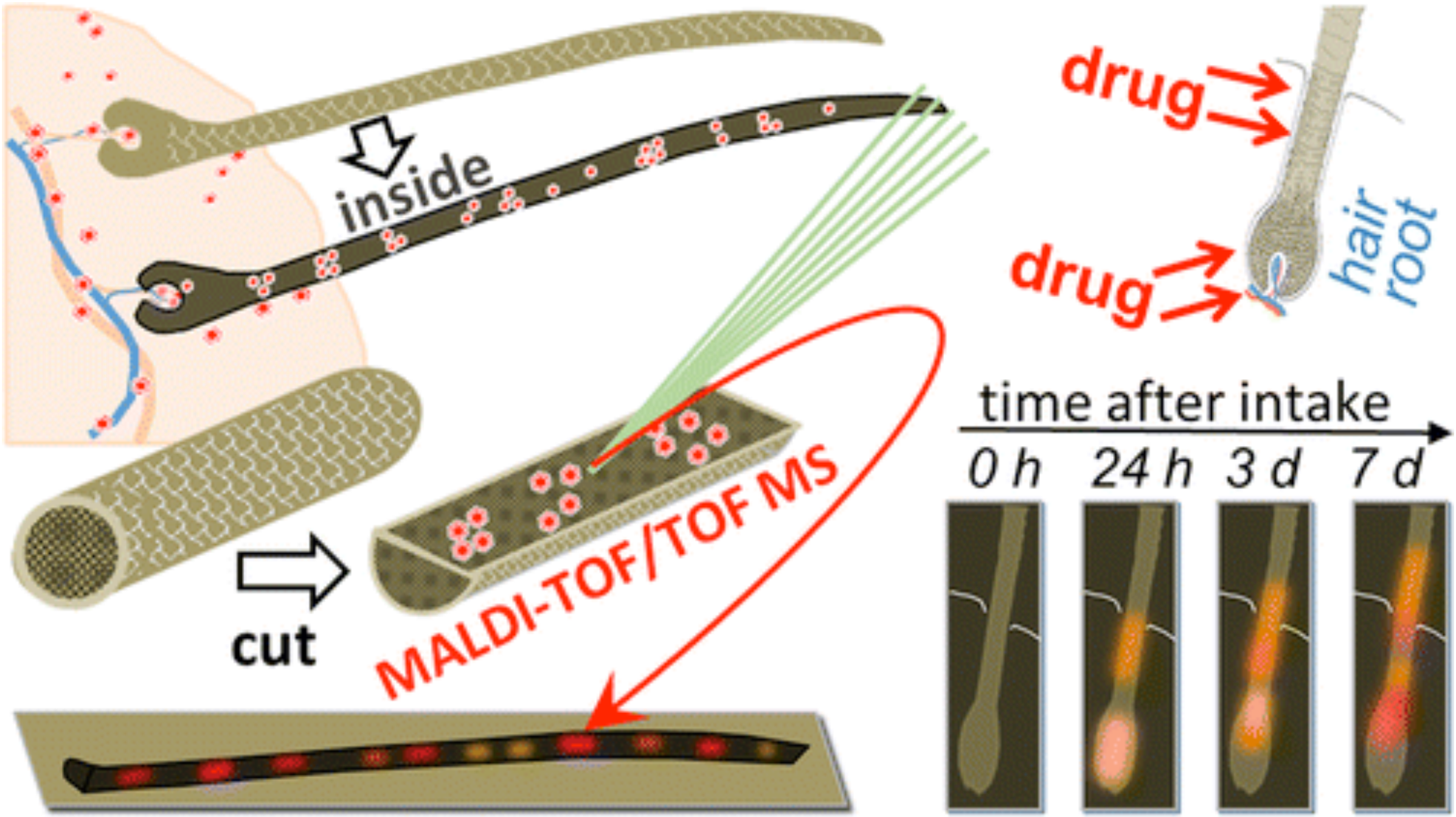

Hair imaging

IMS in hair analysis



Anal. Chem. 87, 11, 5476-5481

IMS in hair analysis



Anal. Chem. 87, 11, 5476-5481

Hairs are important material to monitor history of drug administration.

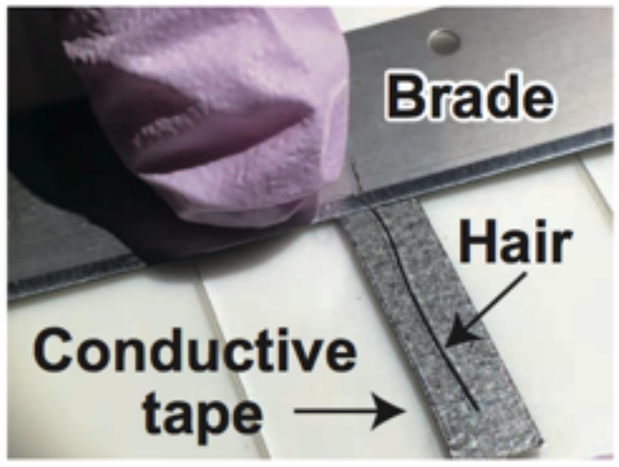
Drug imaging in hair samples

In horse racing, doping test is also important.

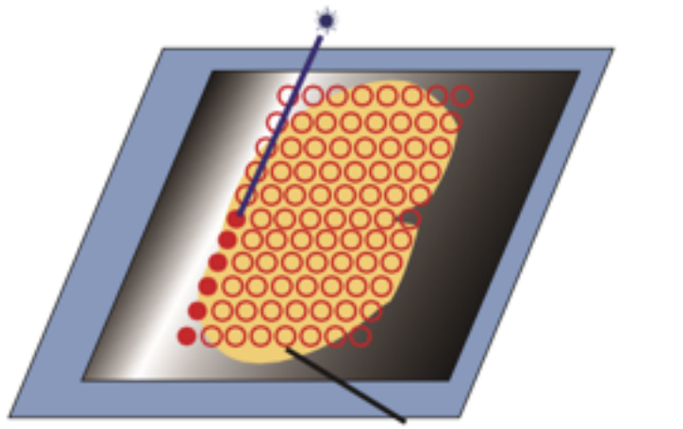
Horse hair is very long, therefore LC-MS detection is laborious.

Hair samples

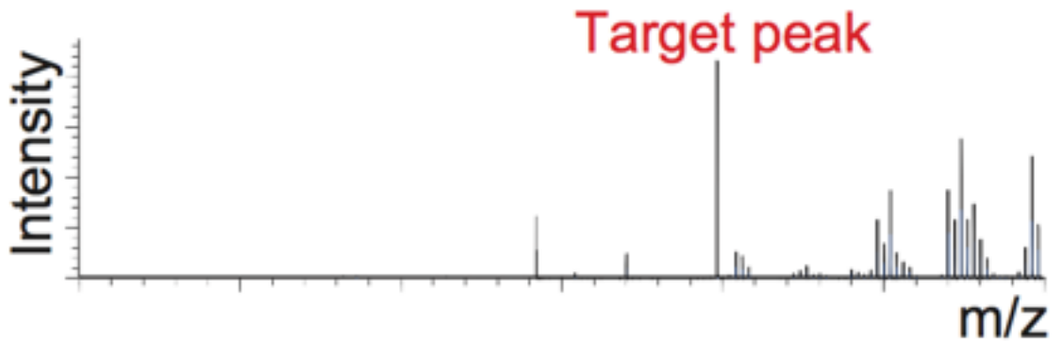
Hair sectioning and matrix application



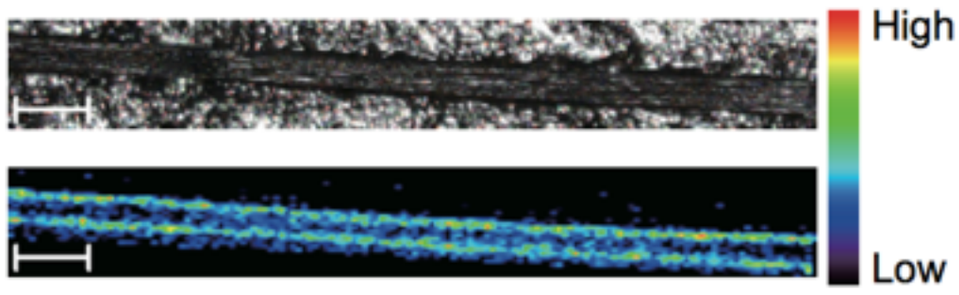
Ionization by laser irradiation



Extraction of ion intensity from obtained mass spectra

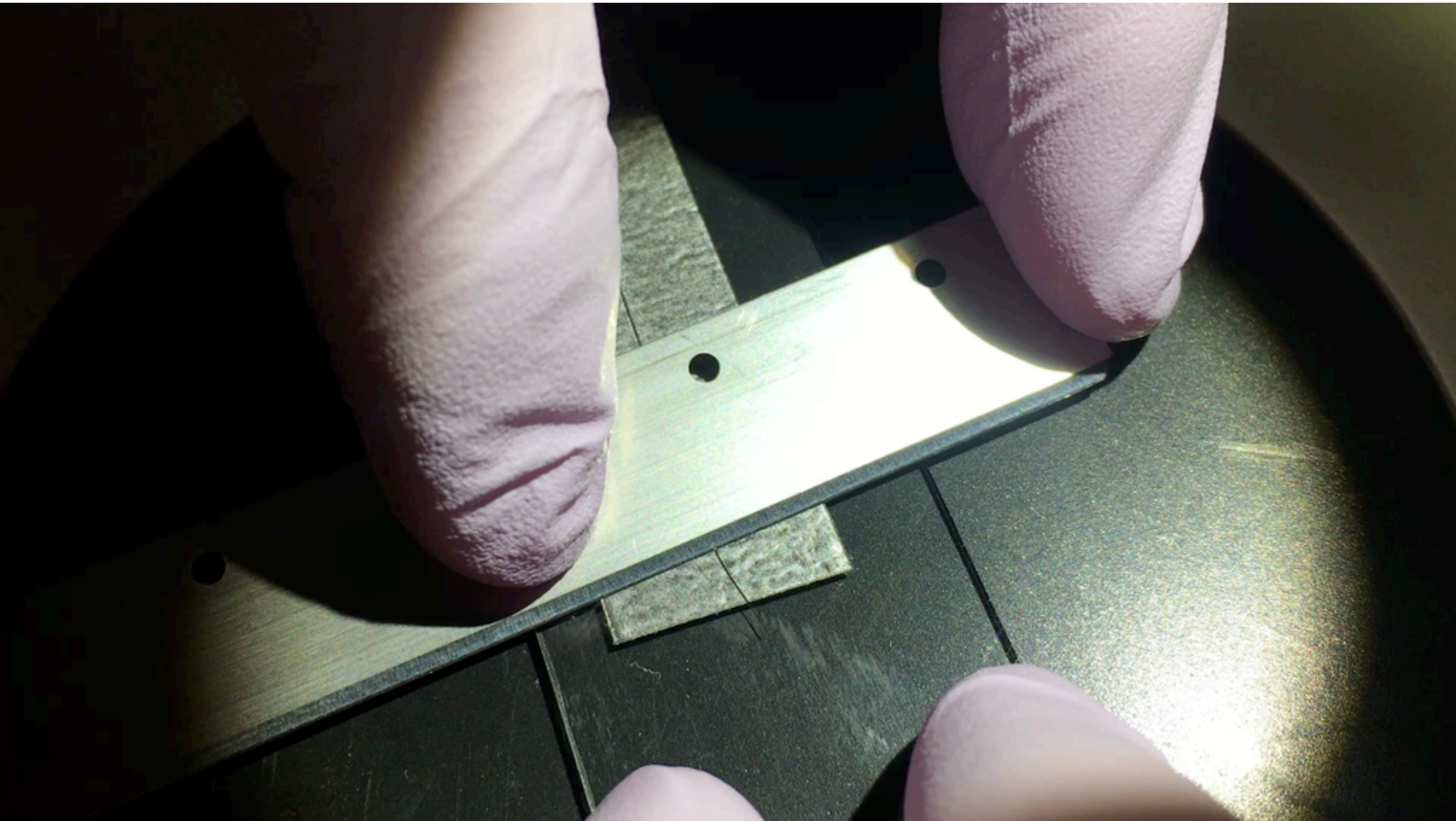


Ion intensity map



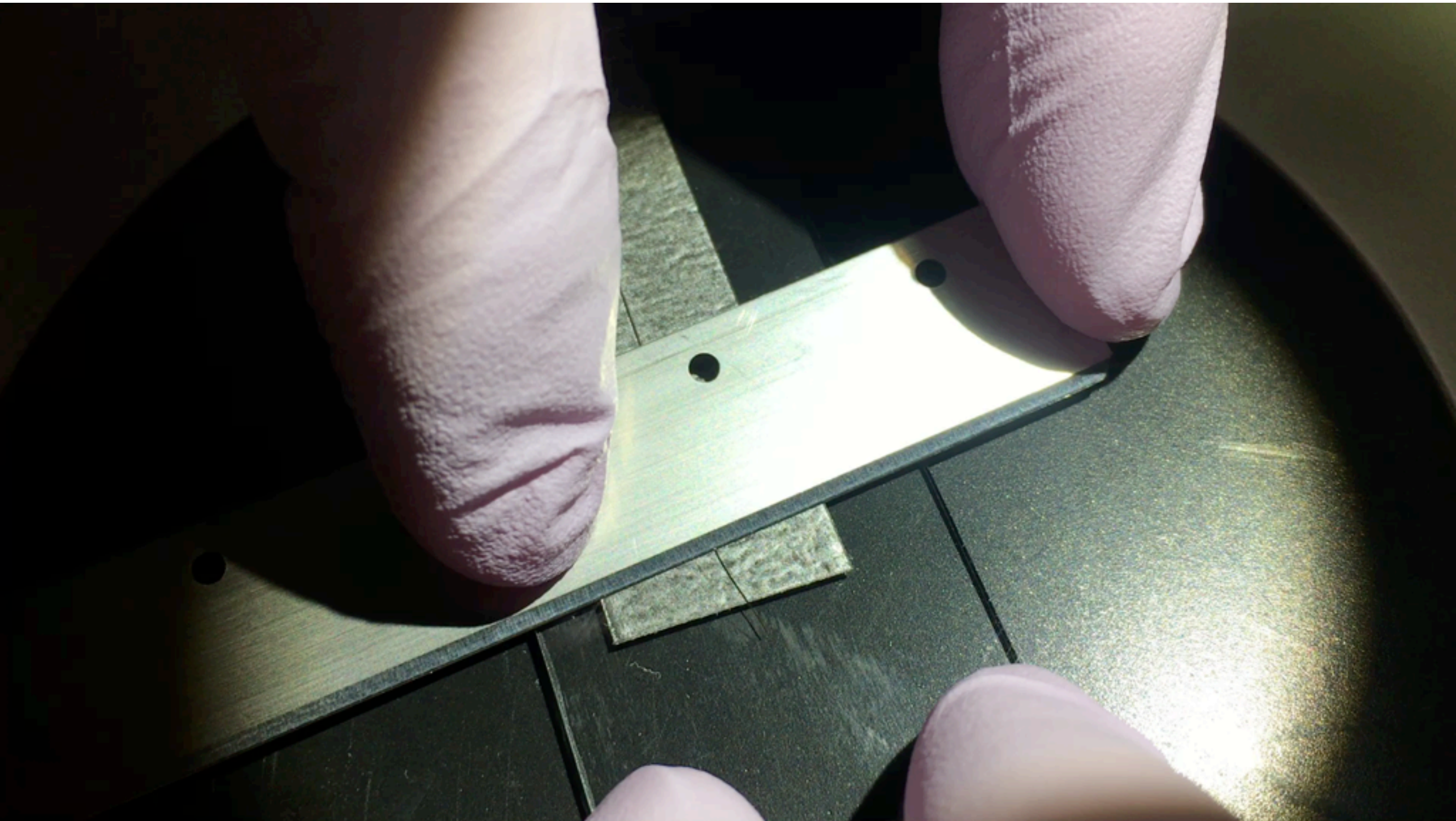
How to make sections

Previous method



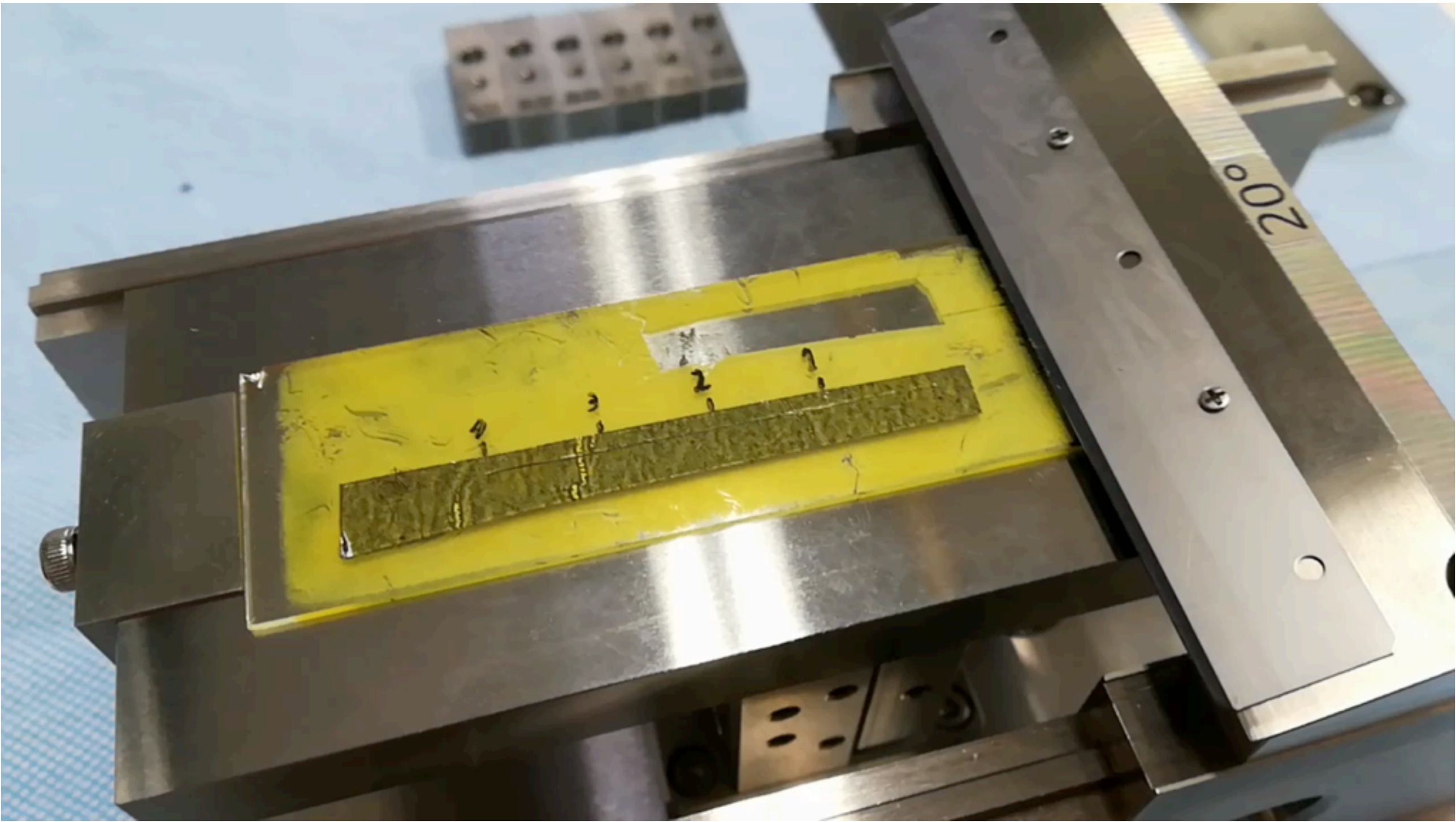
How to make sections

Previous method



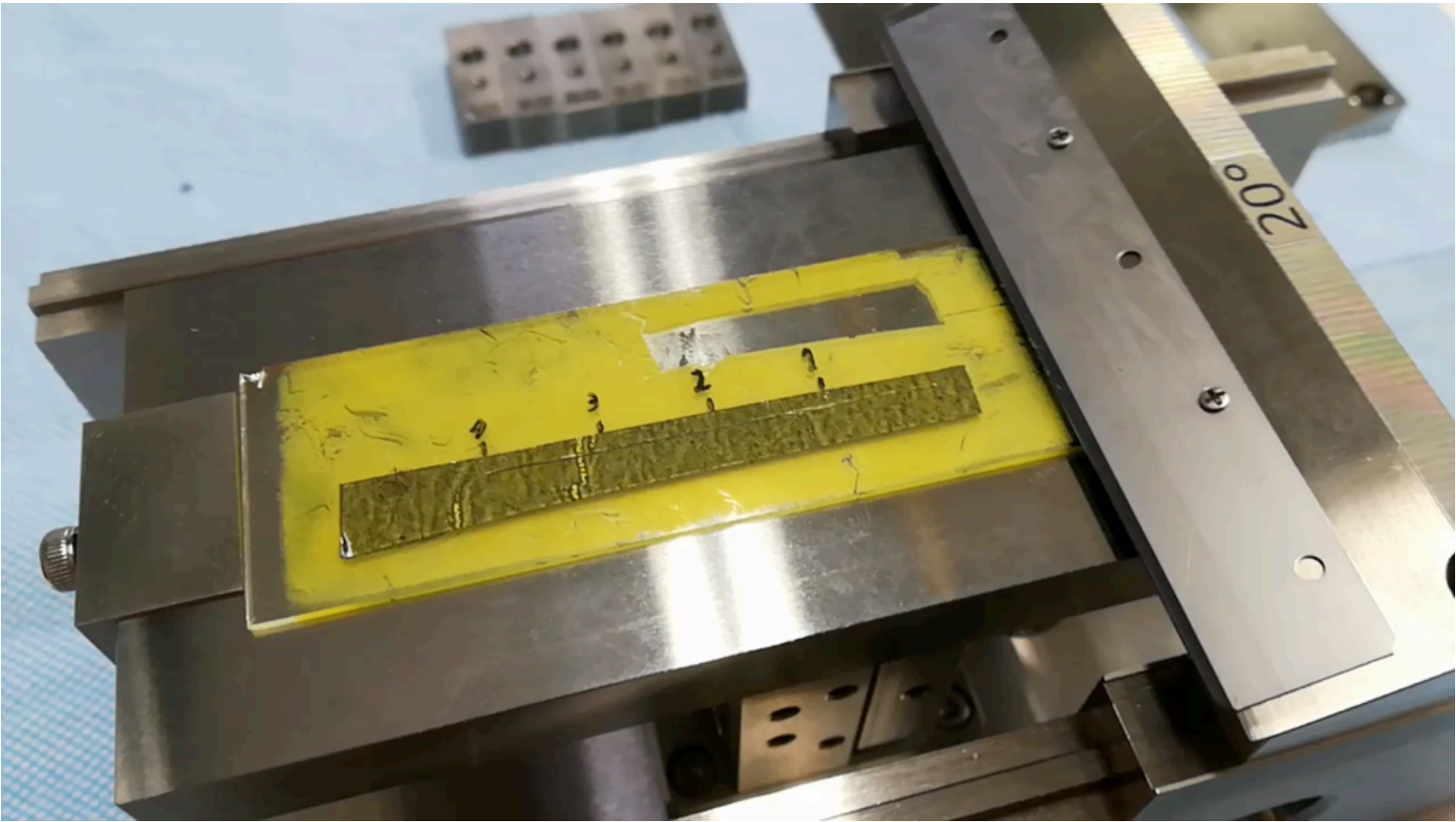
Recent development of hair sectioning

Current method



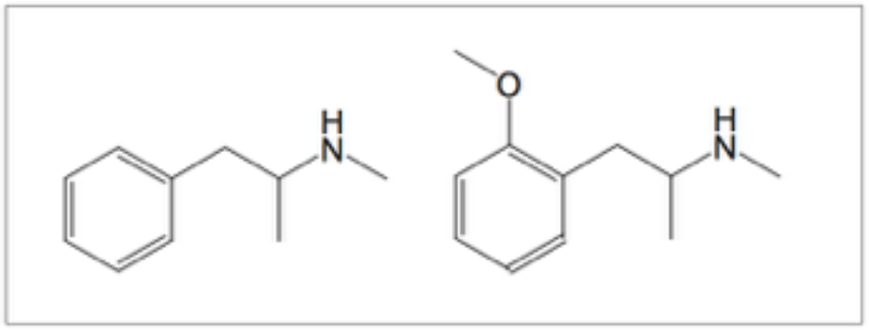
Recent development of hair sectioning

Current method

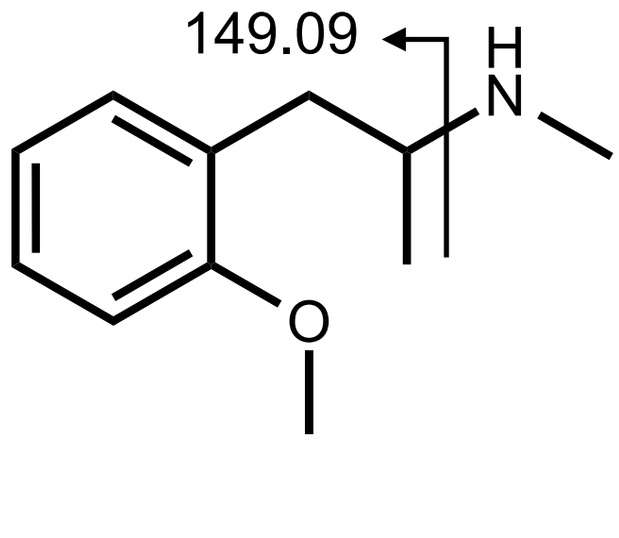


Drug detection in human hair (1) (Methoxyphenamine)

Alternative compound for illegal drug (Methamphetamine)



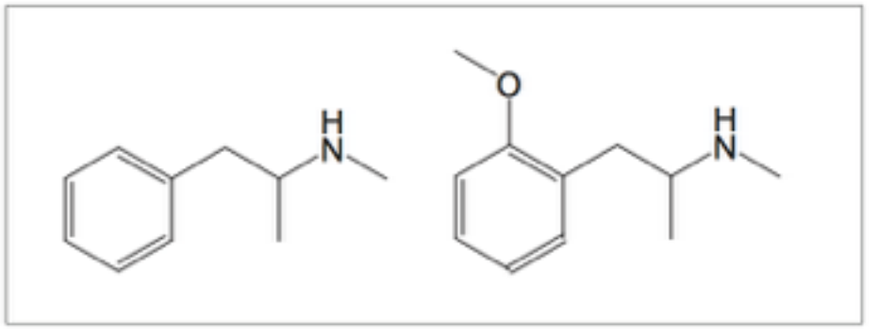
Left: Methamphetamine
Right: Methoxyphenamine



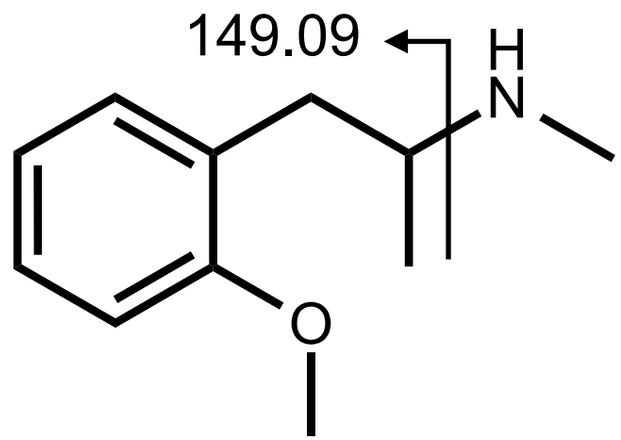
Methoxyphenamine
(Cough medicine)

Drug detection in human hair (1) (Methoxyphenamine)

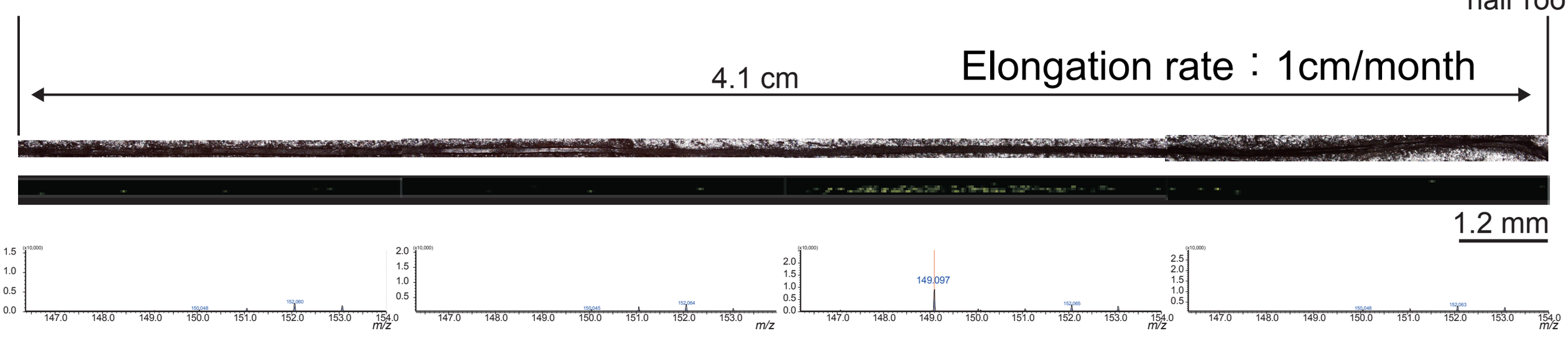
Alternative compound for illegal drug (Methamphetamine)



Left: Methamphetamine
Right: Methoxyphenamine



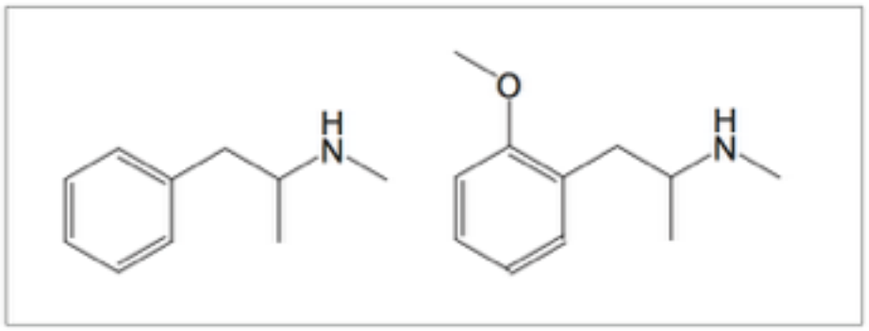
Methoxyphenamine
(Cough medicine)



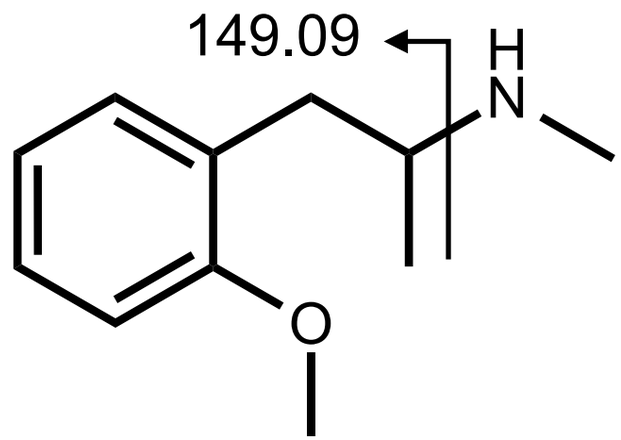
Pixel size: 80 μm x 5 μm

Drug detection in human hair (1) (Methoxyphenamine)

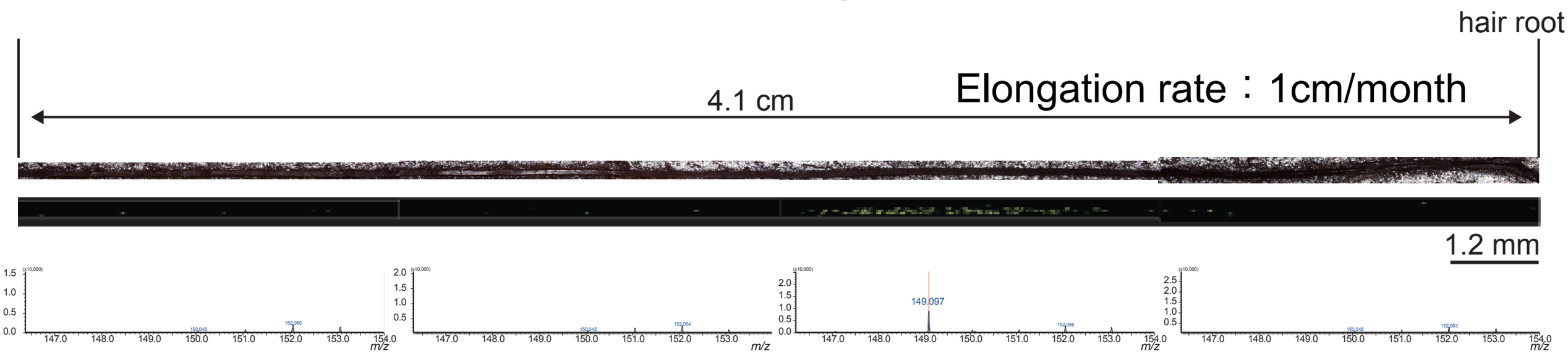
Alternative compound for illegal drug (Methamphetamine)



Left: Methamphetamine
Right: Methoxyphenamine



Methoxyphenamine
(Cough medicine)



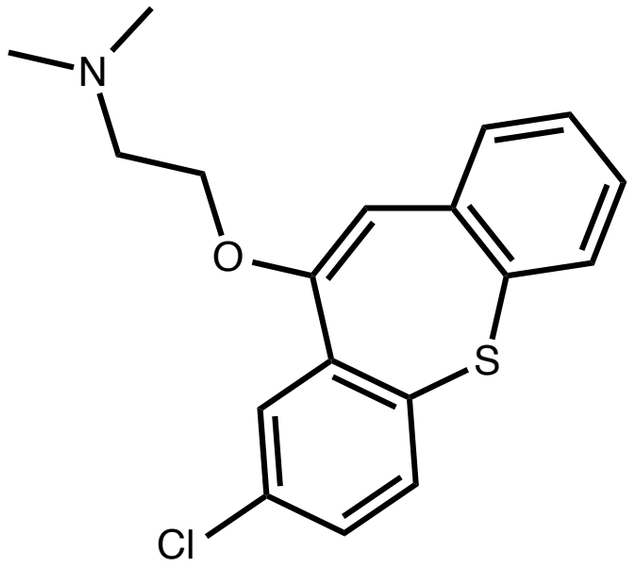
Pixel size: 80 μm x 5 μm

Around 1 cm from hair root, methoxyphenamine was accumulated.
This is correct history of drug administration.

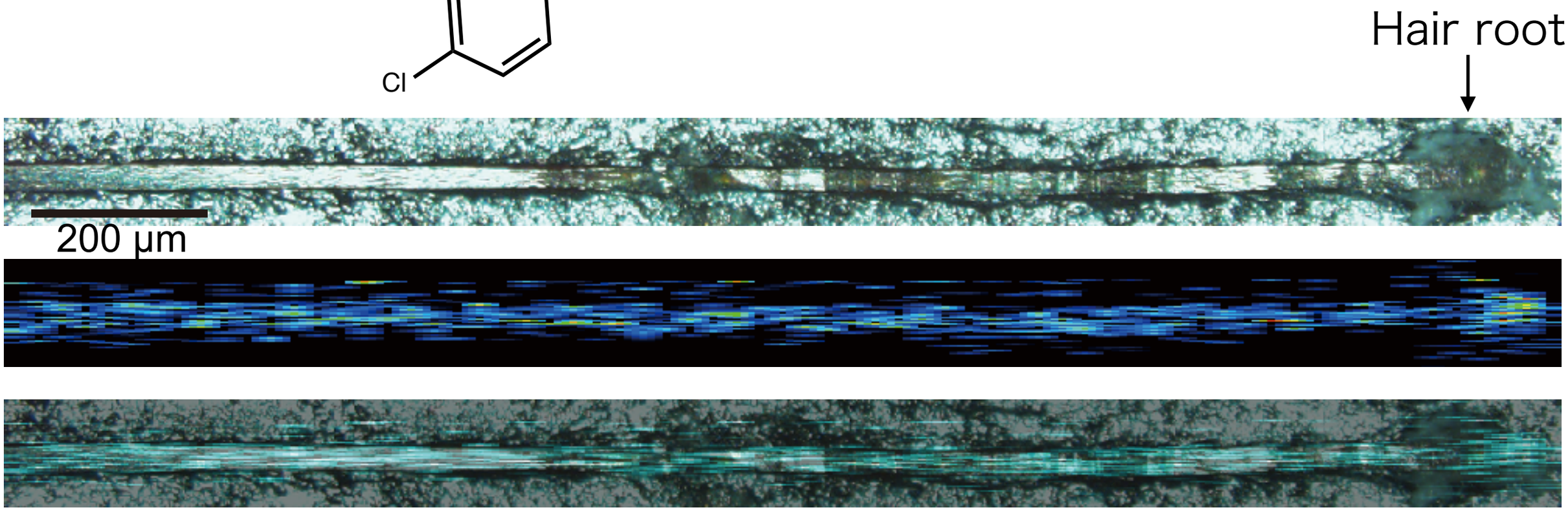
Drug detection in human hair (2) (Zotepine)

Application in medication compliance

- Patients really take medicine correctly or not? -

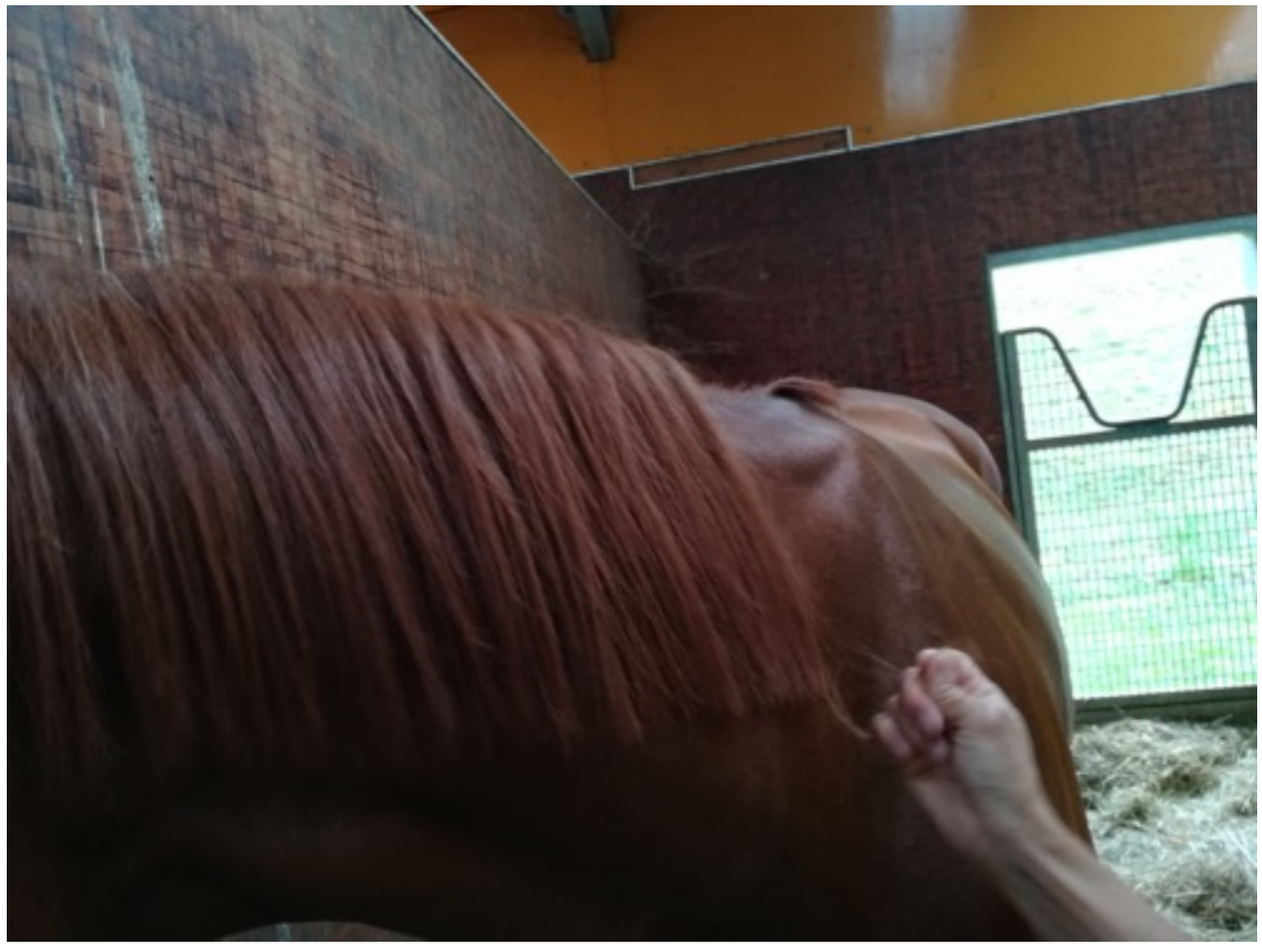


Zotepine :
Drug for anti-depressant



We can find accumulation at hair root
and entirely detect signal derived from zotepine.

Horse hair sampling



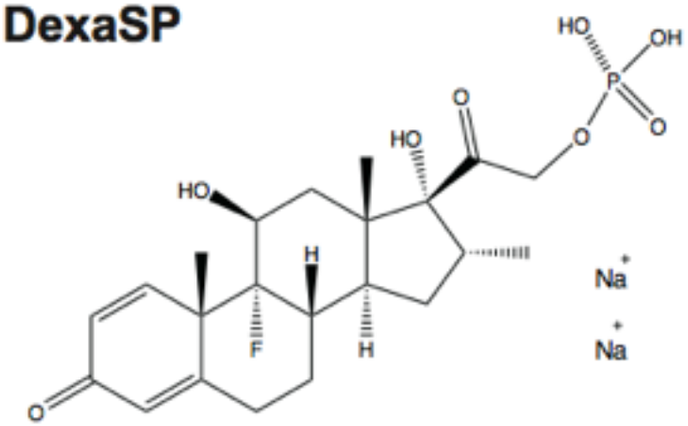
Visualization of dexamethasone sodium phosphate

Shimma S et al.
ICRAV2018

Negative control

In negative control sample (non-dosed DexaSP), signal was not detected at *m/z* 471.16.

DexaSP



Horse hair with 4 cm can be analyzed using IMS. Accumulation at 16.46 mm from hair root was visualized. According to elongation rate of horse hair (2 cm/month), this drug was administered 25 days ago.

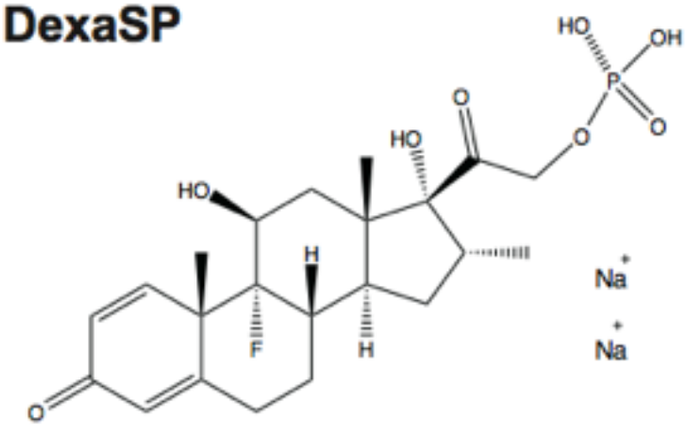
Visualization of dexamethasone sodium phosphate

Shimma S et al.
ICRAV2018

Negative control

In negative control sample (non-dosed DexaSP), signal was not detected at *m/z* 471.16.

DexaSP

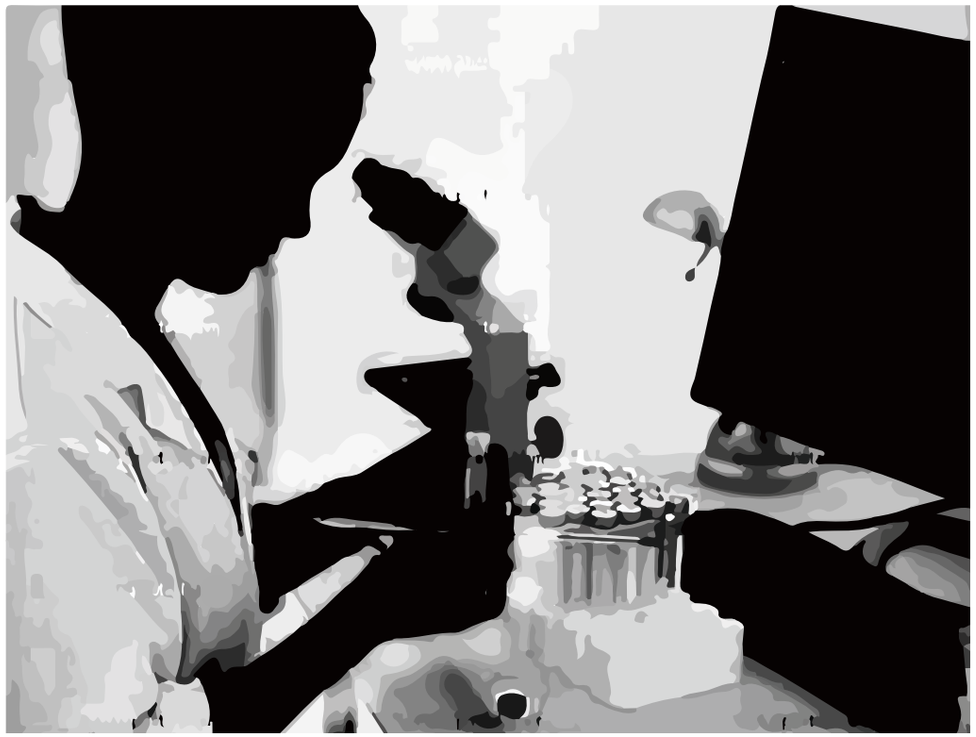


Horse hair with 4 cm can be analyzed using IMS. Accumulation at 16.46 mm from hair root was visualized. According to elongation rate of horse hair (2 cm/month), this drug was administered 25 days ago.

Dosed sample: Total 15-20 mL (1.315 mg/mL) was injected for three days in June.
On July 13, hair sampling was performed.

In this hair imaging results, highest intensity was found at 16.48 mm from the hair root.
This means the injection was performed approximately 24 or 25 days ago.

Summary

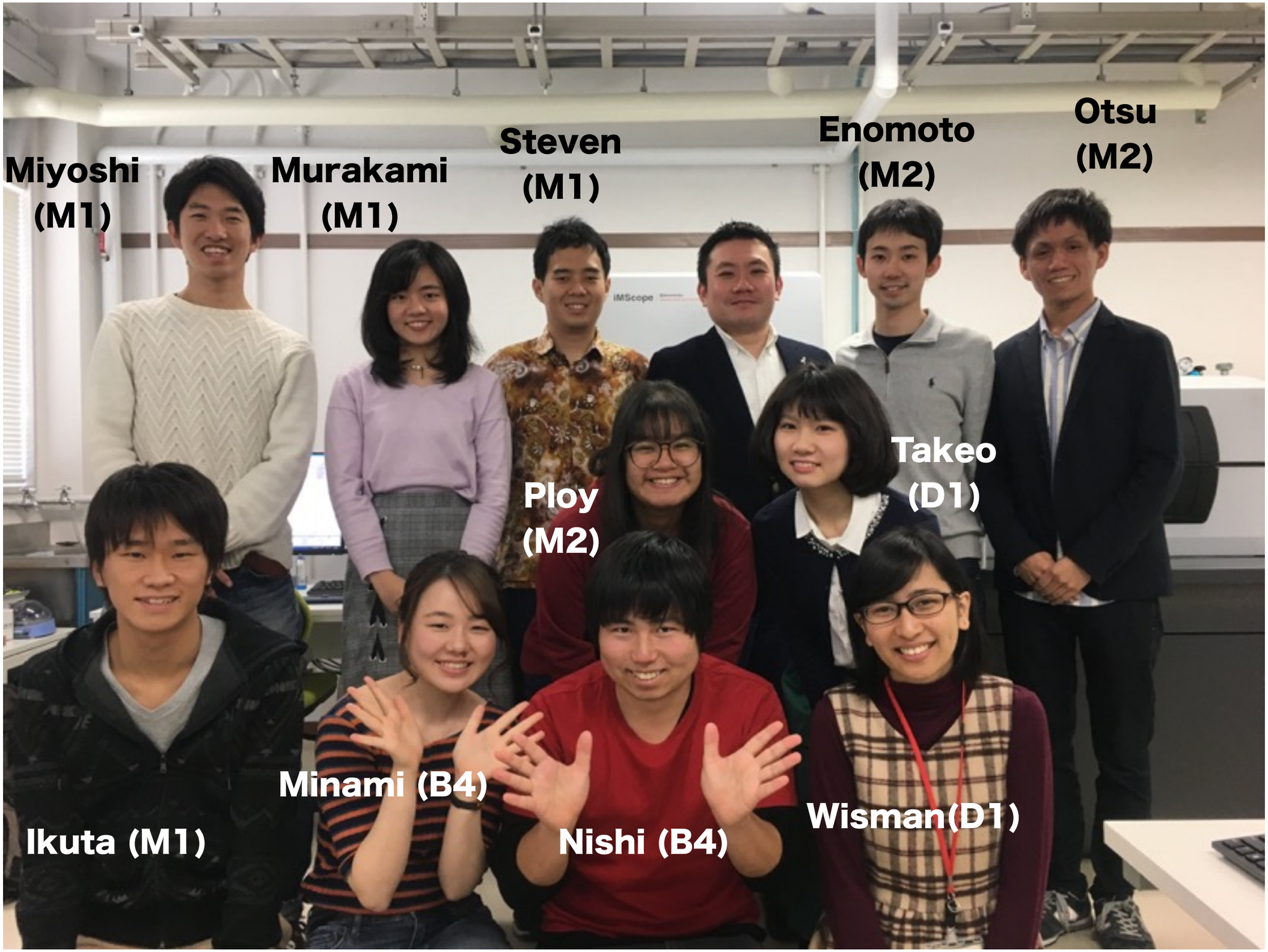


Imaging mass spectrometry

- Novel molecular imaging method
- Instrument, spatial resolution, sensitivity, quantification and reproducibility were improved.
- IMS will be a powerful tool in all research fields as well as medical sciences.

In our laboratory, we challenge to develop applications towards a lot of different research fields.

Acknowledgement



Expectations from clinicians

MADRID 2014 ESMO congress

Needs confirmation, but an exciting opportunity to study intra-tumoral drug concentrations AND distribution

- Include in Phase I studies to demonstrate "therapeutic" tumoural concentrations achieved
- Correlate with efficacy outcomes
- Incorporate in clinical drug development

Expectations from clinicians

MADRID 2014 ESMO congress

Needs confirmation, but an exciting opportunity to study intra-tumoral drug concentrations AND distribution

- Include in Phase I studies to demonstrate "therapeutic" tumoural concentrations achieved
- Correlate with efficacy outcomes
- Incorporate in clinical drug development

Hurdles to overcome

1. Reproducibility
2. Timing for tissue sampling after dosing