

哪个辣椒更辣？

辣椒哪里更辣？



辣椒梗？

辣椒皮？

辣椒筋？

辣椒籽？

哪里更辣？

成像质谱显微镜在植物研究中的进展

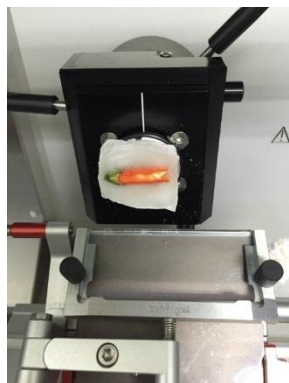
岛津分析测试仪器市场部
陈丹

Create New Synergies

— Best for Our Customers —



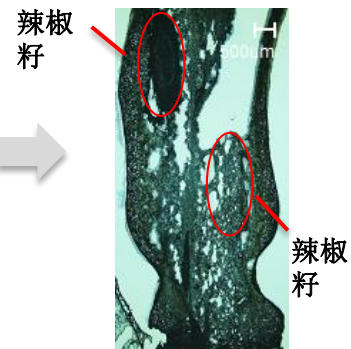
辣椒素在辣椒中是如何分布的？



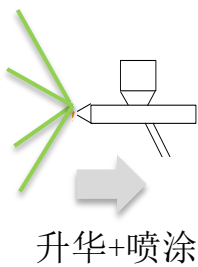
1) 冰冻切片机制作切片



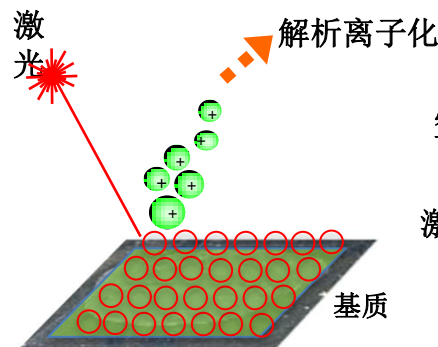
2) 切片转移到导电载玻片



3) 拍摄显微镜图像



4) 涂覆基质



5) 多点质谱分析

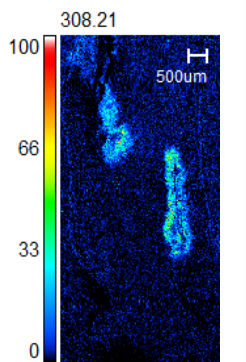
空间分辨率

激光斑点直径

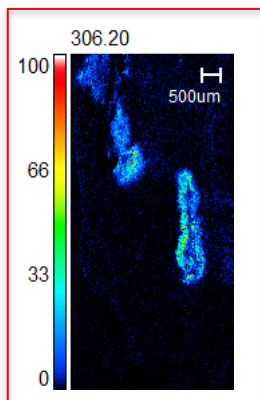


辣椒素在辣椒中是如何分布的？

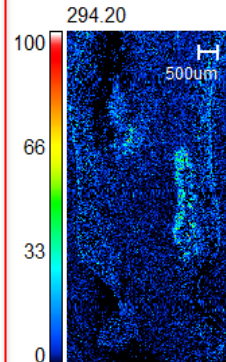
MS



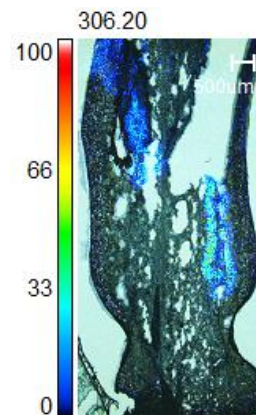
[Dehydrocapsacin +H]



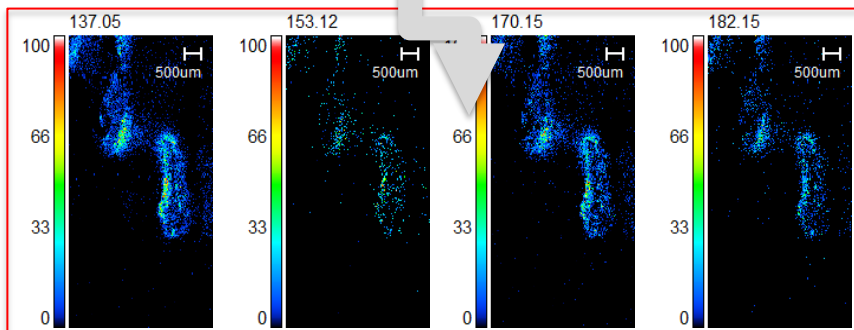
[Capsaisin+H]



[Nonivamide+H]



MS/MS



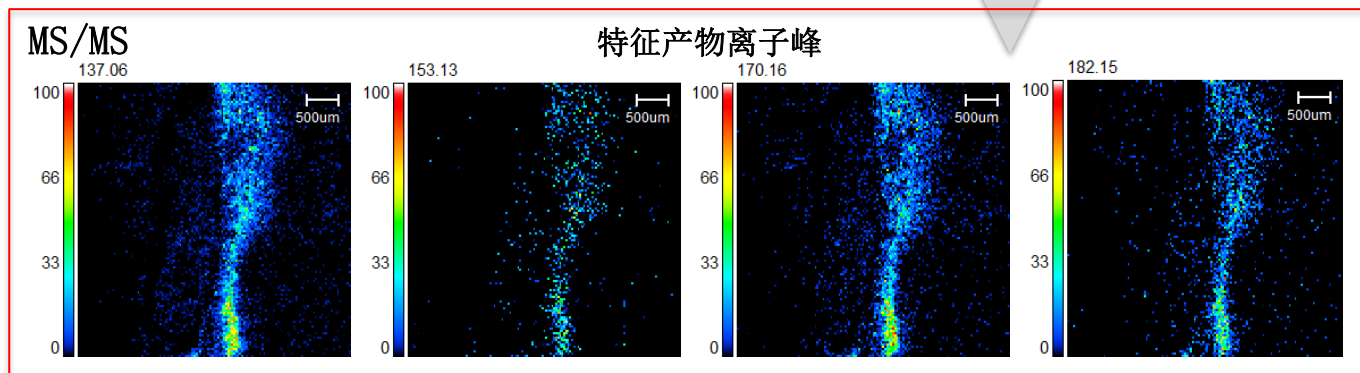
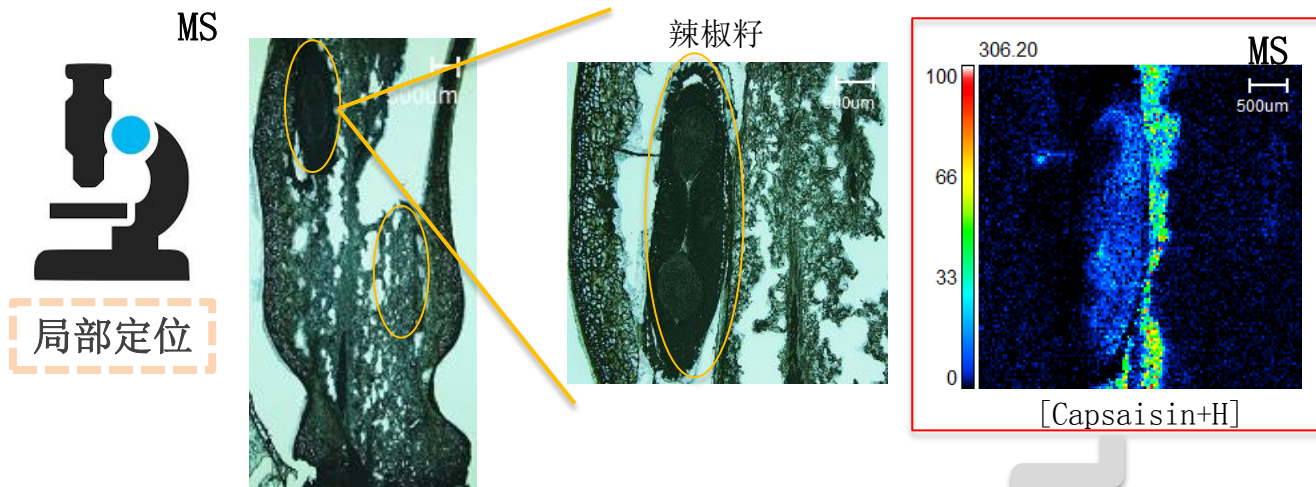
特征产物离子峰

辣椒素主要分布在辣椒籽上？





辣椒素在辣椒中是如何分布的？



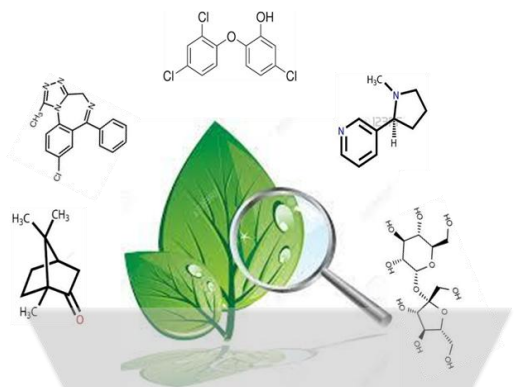
辣椒素主要分布在辣椒籽内侧膜上

原位分子分布可视化时代



质谱显微镜 iMScope *TRIO*

质谱技术与光学显微镜的有机结合



定位
光学显微镜 + 定性
质谱技术

质谱显微镜

2014年分析科学家创新奖**第一名**

评审专家点评：

“light + MS microscopy = **POWER**”



主要内容



1. 成像质谱显微技术的原理和特点
2. 成像质谱显微技术的应用



1. 成像质谱显微技术的原理和特点



成像质谱显微镜构造(iMScope TRIO)

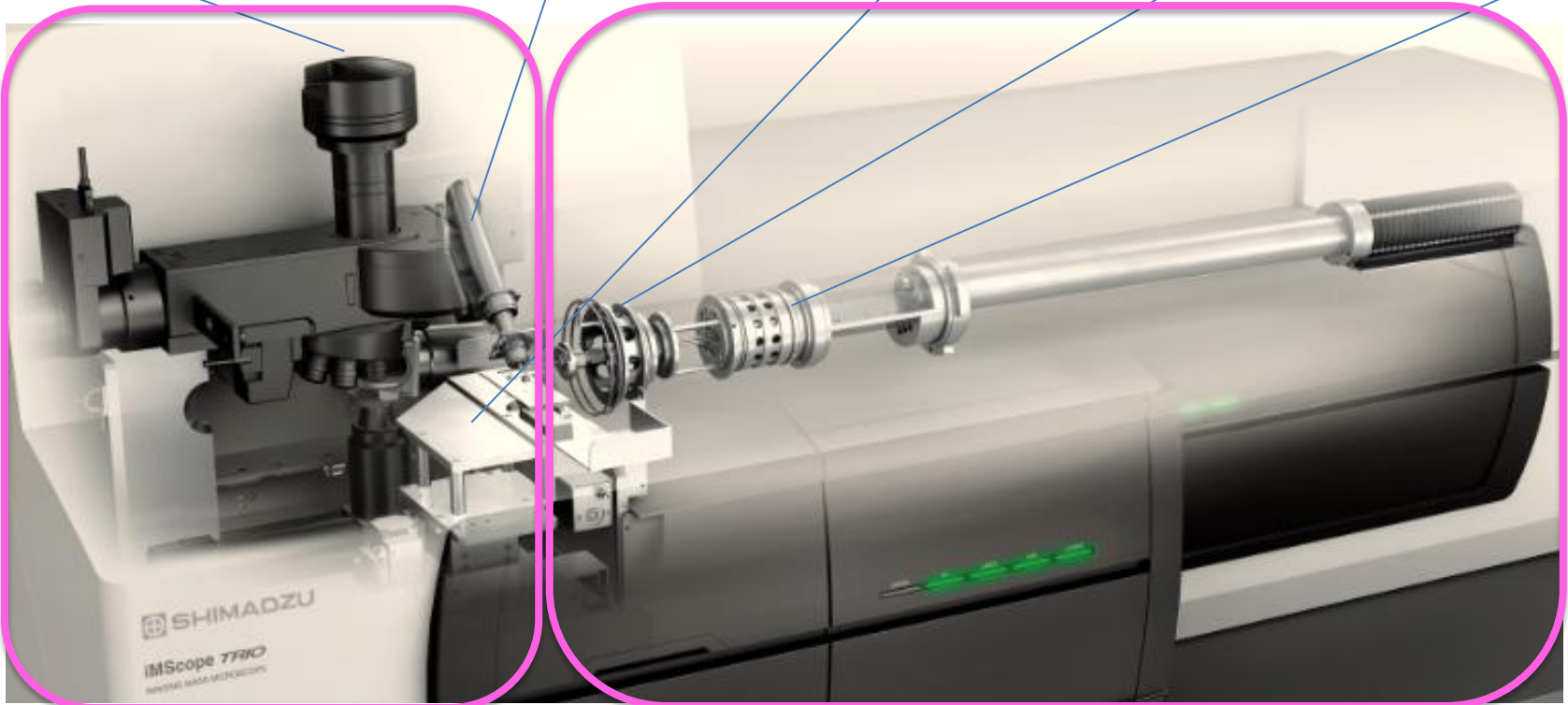
光学显微镜单元
1.25-40倍率

高速激光单元
激光照射直径 5um-200um

样品台
高速, 高精度

离子输送系统
高通量, 高灵敏

四极杆型离子阱
高应用性, 可靠性



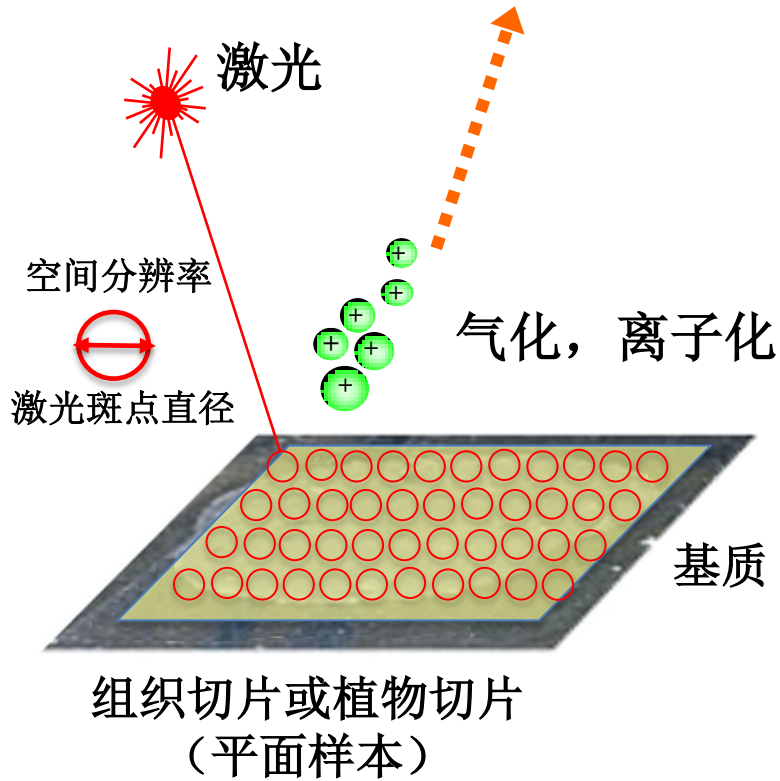
搭载高分辨显微镜的MALDI源

离子阱-反射飞行时间(IT-TOF)质谱检测器

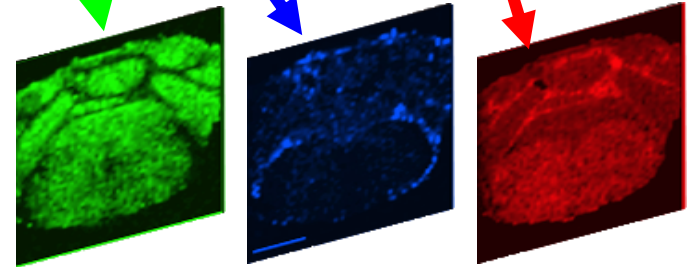
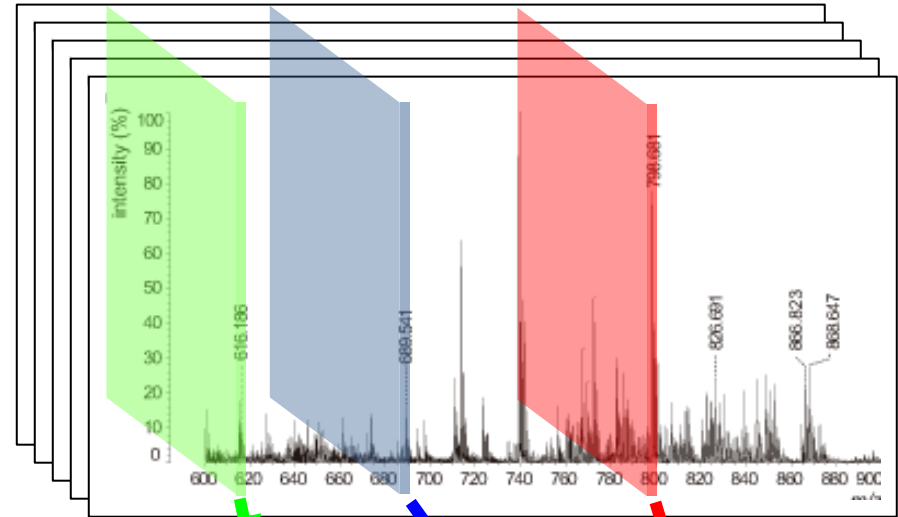
成像质谱显微镜成像原理



MALDI-QIT-TOF Mass Spectrometry



Mass Spectrum



二维离子密度图

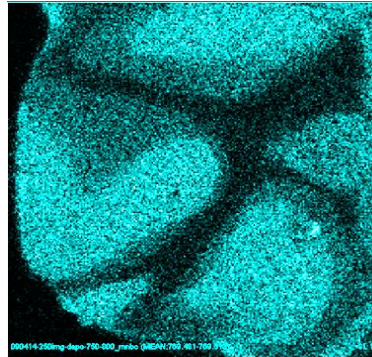
成像质谱显微镜成像结果



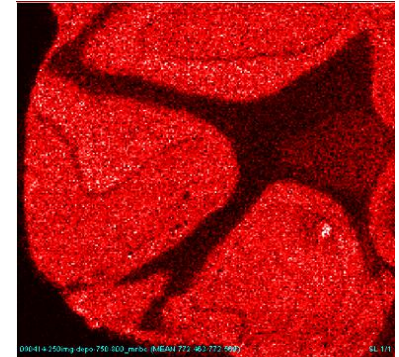
500mm

Optical Micrograph

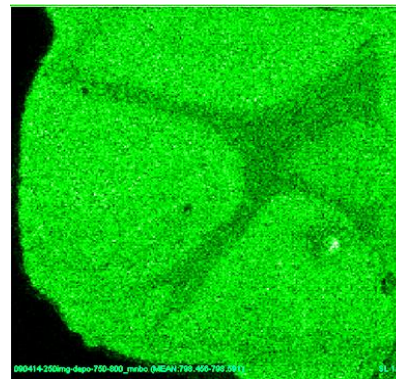
Sample:	小鼠小脑
Matrix:	DHB
Scan Pitch:	10um
Measurement points:	250 X 250 (62,500)



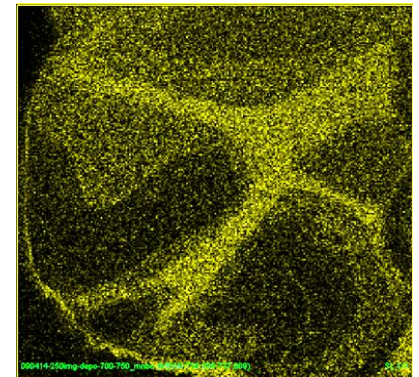
m/z 737.0
鞘磷脂(d18:1/18:0)



m/z 769.5
磷脂酰胆碱(16:0/16:0)



m/z 772.5
磷脂酰胆碱(16:0/18:1)



m/z 798.5
半乳糖神经酰胺(d18:1/24:1)

成像质谱显微镜 iMScope TRIO



特点

- ✓ 光学显微镜与质谱精准融合
- ✓ 高空间分辨率 (5 μ m)
- ✓ 多级质谱 MSⁿ (n \leq 10)成像
- ✓ 超高速分析 (6 像素/秒)
- ✓ 方便的数据解析软件
- ✓ 简便的操作流程



搭载光学显微镜的重要性

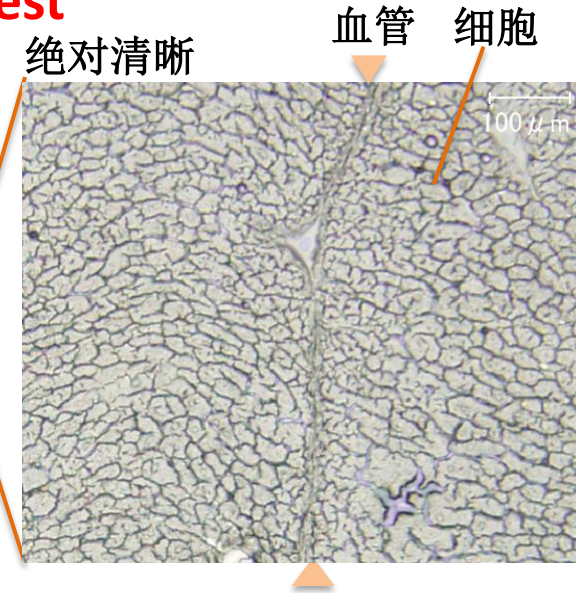
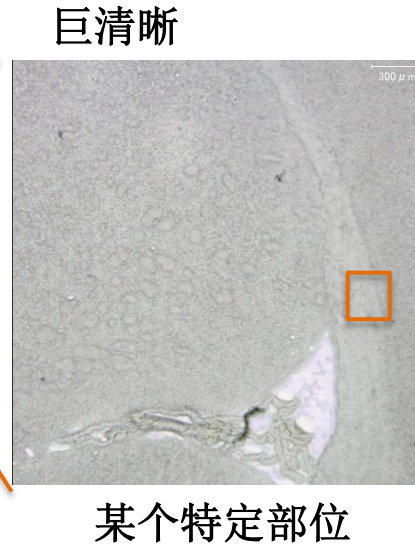


无光学显微镜



模糊

Creation of the region of interest

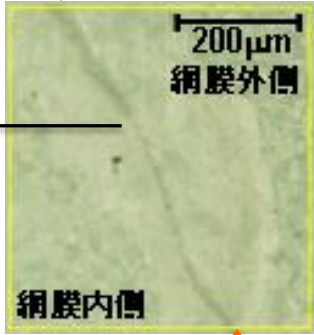


搭载光学显微镜



空间分辨率的重要性

光学图像



空间分辨率

5 μm

15 μm

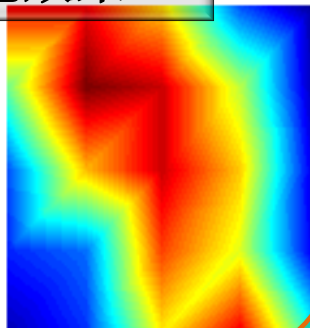
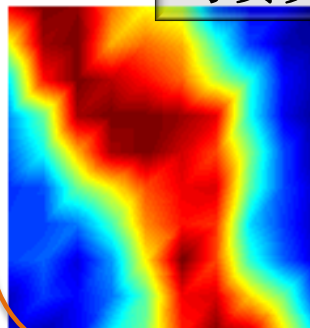
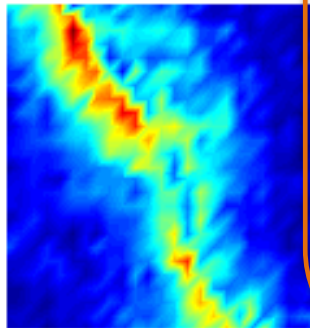
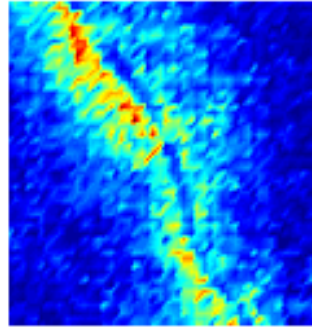
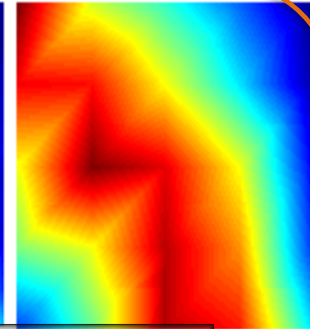
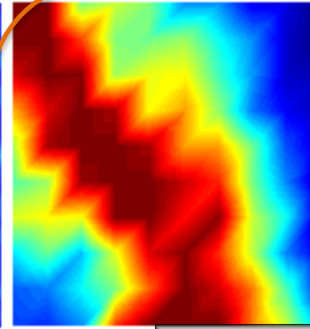
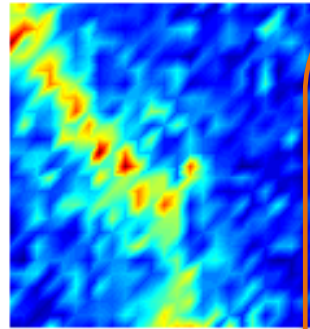
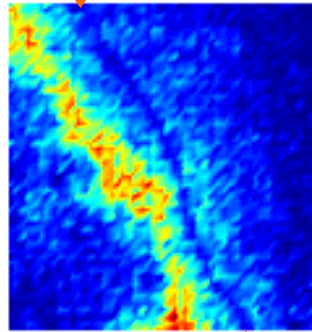
50 μm

100 μm

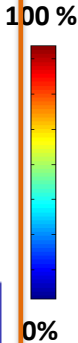
视网膜色素层 10 μm~

m/z 798.54
PC (16:0/18:1) + K

m/z 872.61
PC (18:0/22:6) + K



马赛克效果



PC: 磷脂酰胆碱

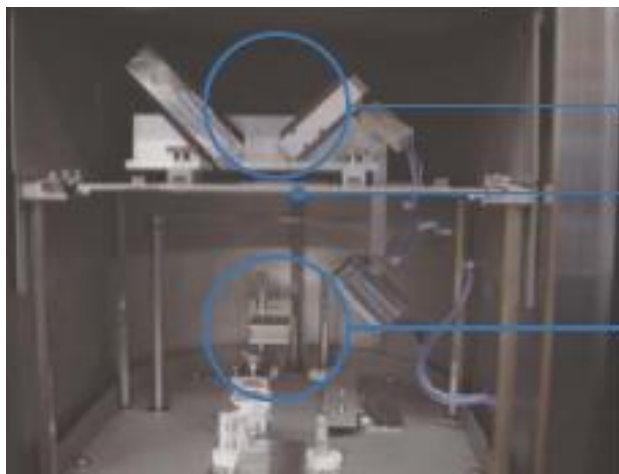


高空间分辨率成像的重要因素

基质升华装置：iMLayer



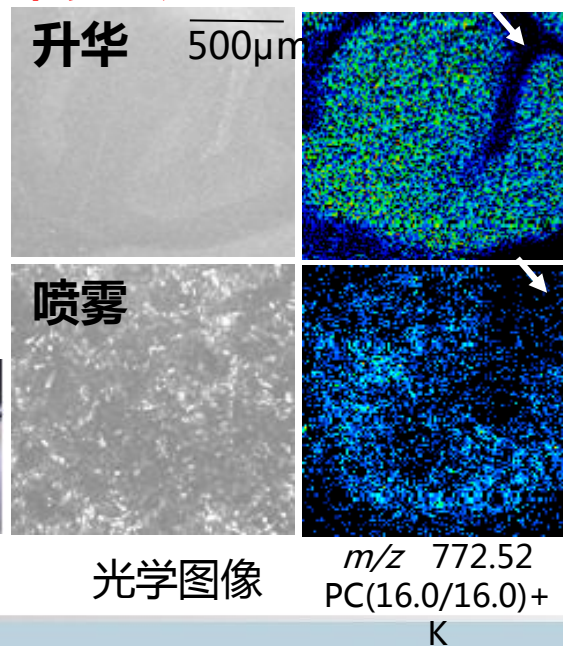
- **结晶颗粒的细微化**
⇒ **使高空间分辨率成像成为可能**
- **前处理过程的自动化**
⇒ **缩短操作时间**
- **搭载基质膜厚控制系统**
⇒ **提高重复性，实现定量**



膜厚测定单元

样品固定单元

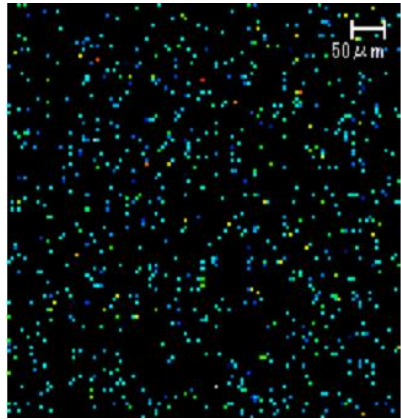
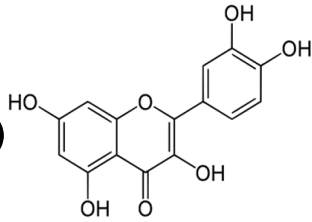
基质盒及加热单元



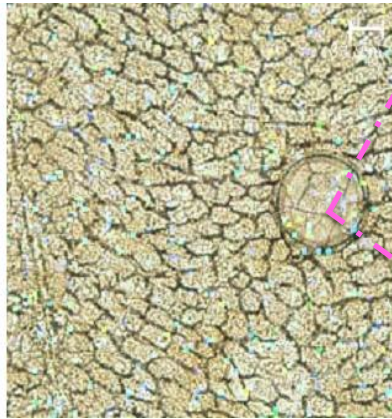
亚细胞水平分析



槲皮素
(quercetin)

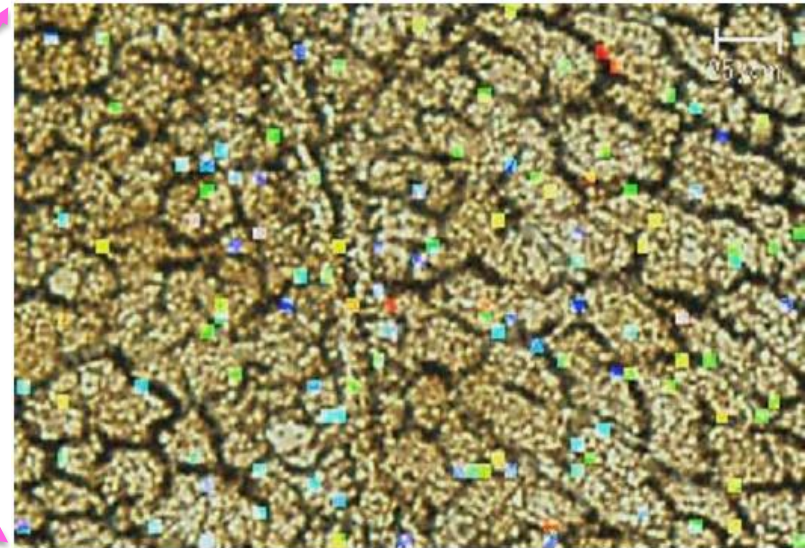


MS/MS image of quercetin
(m/z 269.22 >224.97)



Distribution of quercetin
overlaid onto optical image

药物主要分布在肝细胞的间质



Scale bar:25 μm

- 光学显微镜的分辨率
- 质谱空间分辨率



Where & What?



目标物存在于器官/组织的哪个部位？⇒ **Where**

特定部位有哪些组分？⇒ **What**



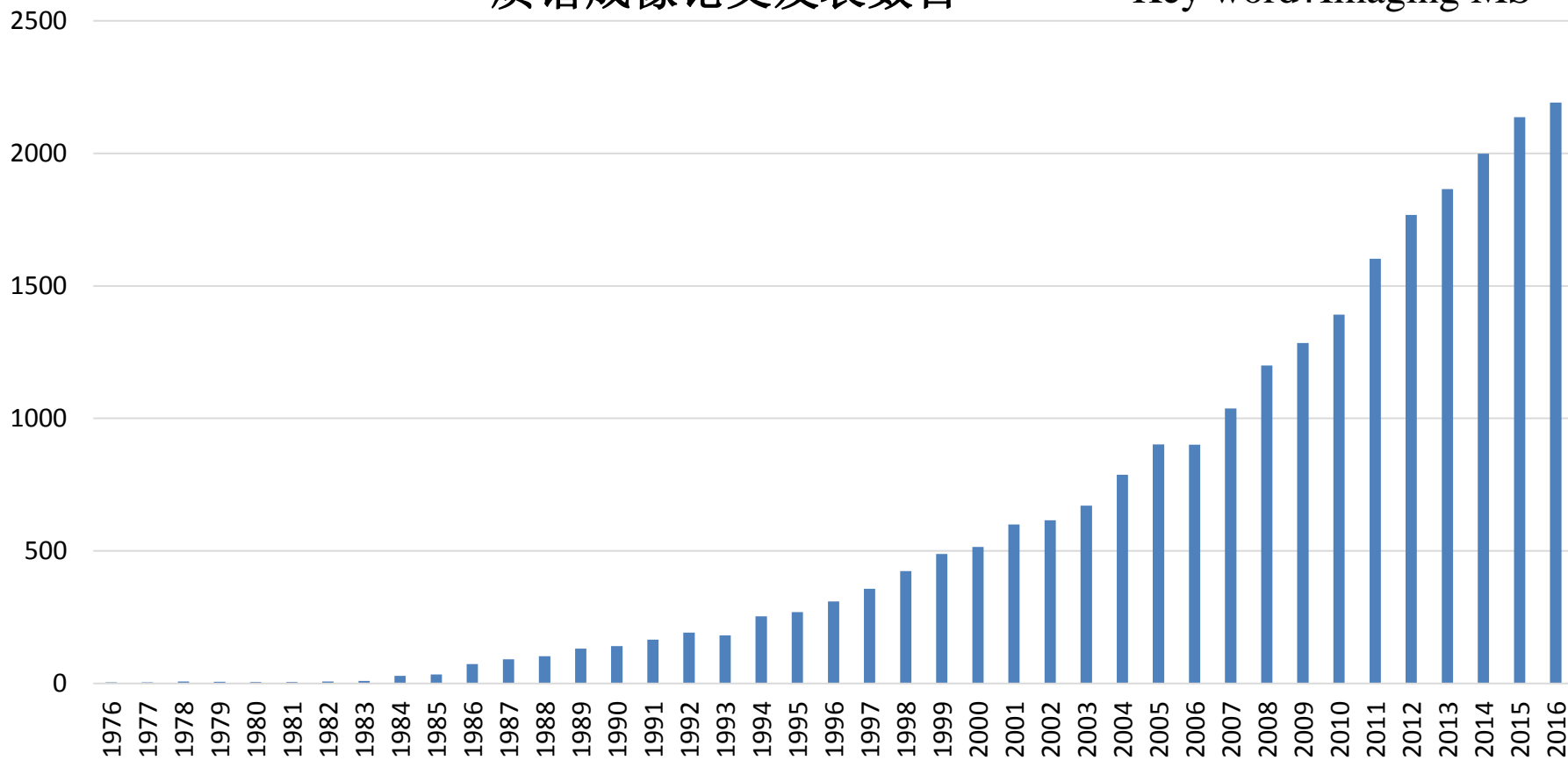
2. 成像质谱显微技术的应用

有关质谱成像论文数目剧增



质谱成像论文发表数目

Key word: Imaging MS



成像质谱显微镜应用

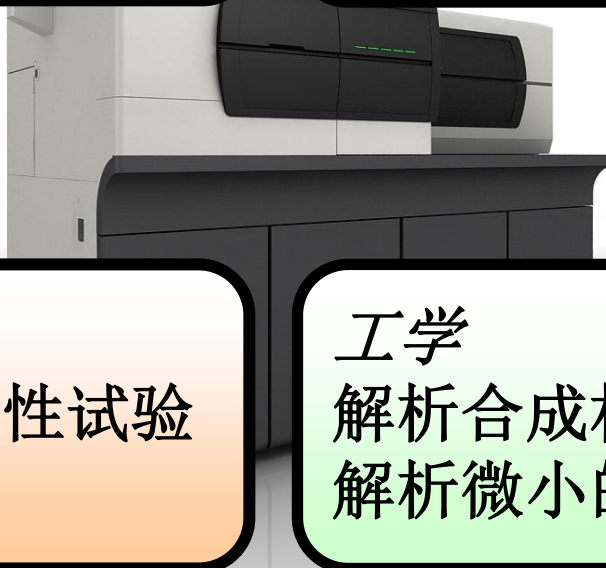


医学

新的生物标志探索
判明疾病机理

农学

食物中成分分布的分析
品种改良



药学

药代动力学/药物安全性试验
新药筛选

工学

解析合成材料微小部分的缺陷
解析微小的有机污染



IMScope—显微质谱成像在农学的意义



通过测定**农产品中特定分子或异物的含量**并观察其**分布**状况，为促进安全、高品质的食品开发、优良农产品品种的开发提供帮助。

- 在食品研究领域，以开发**高质量食品**为目的品种改良工作
- 农业产品中各种**成分分布**的分析（有效促进生长或优化生长条件）
- 加工后食品中的**异物检测**
- **残留化学制剂**的检测

溶血磷脂酰胆碱 (LPC) 在大米中的分布

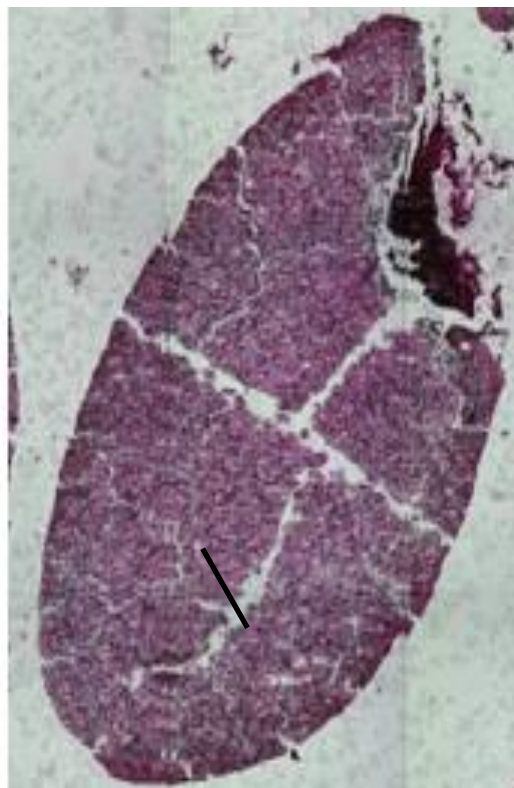


原位检测内生分子分布

无需染色或标记

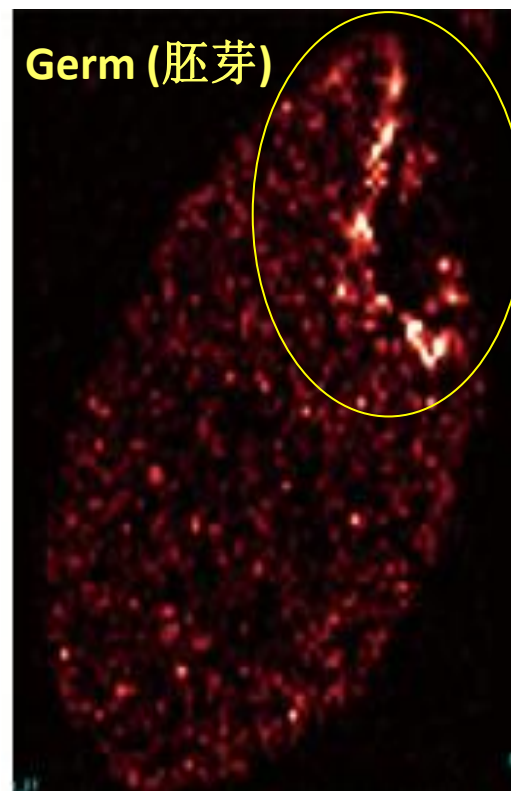
- 农产品品质评价
- 食品安全

光学图像



Endosperm of rice
(大米的胚乳)

多点质谱图像



LPC at m/z 496.293

溶血磷脂酰胆碱 (LPC) 在大米中的分布



Table 2. Lipid profiles of LPC and PC in rice (Hinohikari)

Molecular species	Composition (%)
LPC (1-acyl 16:0)	59.4 ± 4.5
LPC (1-acyl 18:2)	19.6 ± 2.5
LPC (1-acyl 18:1)	14.2 ± 4.5
LPC (1-acyl 18:0)	6.8 ± 1.4
PC (diacyl 16:0/18:2)	19.6 ± 1.0
PC (diacyl 16:0/18:1)	21.0 ± 1.0
PC (diacyl 18:1/18:3)	15.0 ± 1.4
PC (diacyl 18:1/18:2)	26.7 ± 0.7
PC (diacyl 18:0/18:2)	17.8 ± 1.9

Data are represented as means ± SD (n = 5).

LPC = lysophosphatidylcholine; PC = phosphatidylcholine

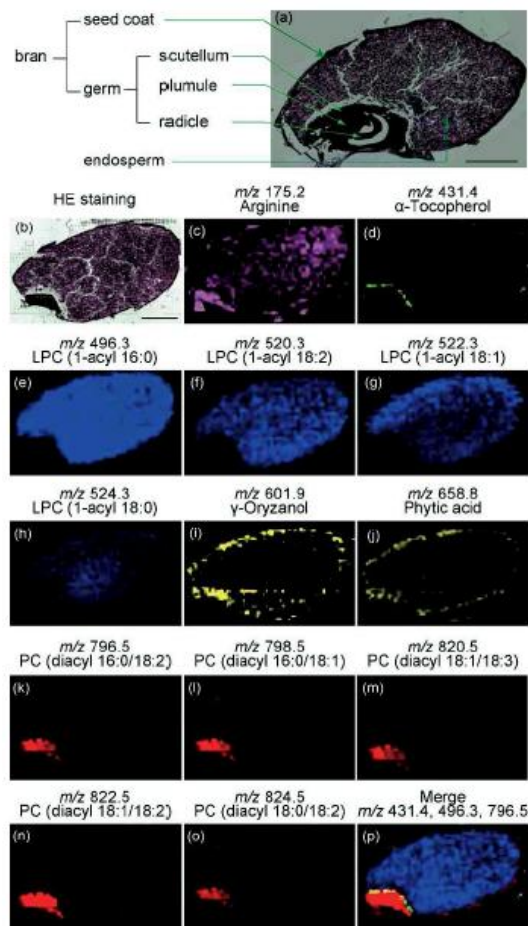


Figure 4. Representative ion images of rice (Hinohikari). Scale bar: 1 mm.

(a) Rice anatomy.

(b) HE-stained rice after MALDI-IMS in the positive ion mode.

(c-o) Ion images in the positive ion mode (purple, green, blue, and red) and negative ion mode (yellow). Each molecule is colored according to the distribution pattern. Contrasting density reflects the intensity of each ion from that molecule.

(p) Merged ion image of m/z 431.4 (green), 496.3 (blue), and 796.5 (red).



植物类：吡虫啉在小番茄机体内的渗透路径

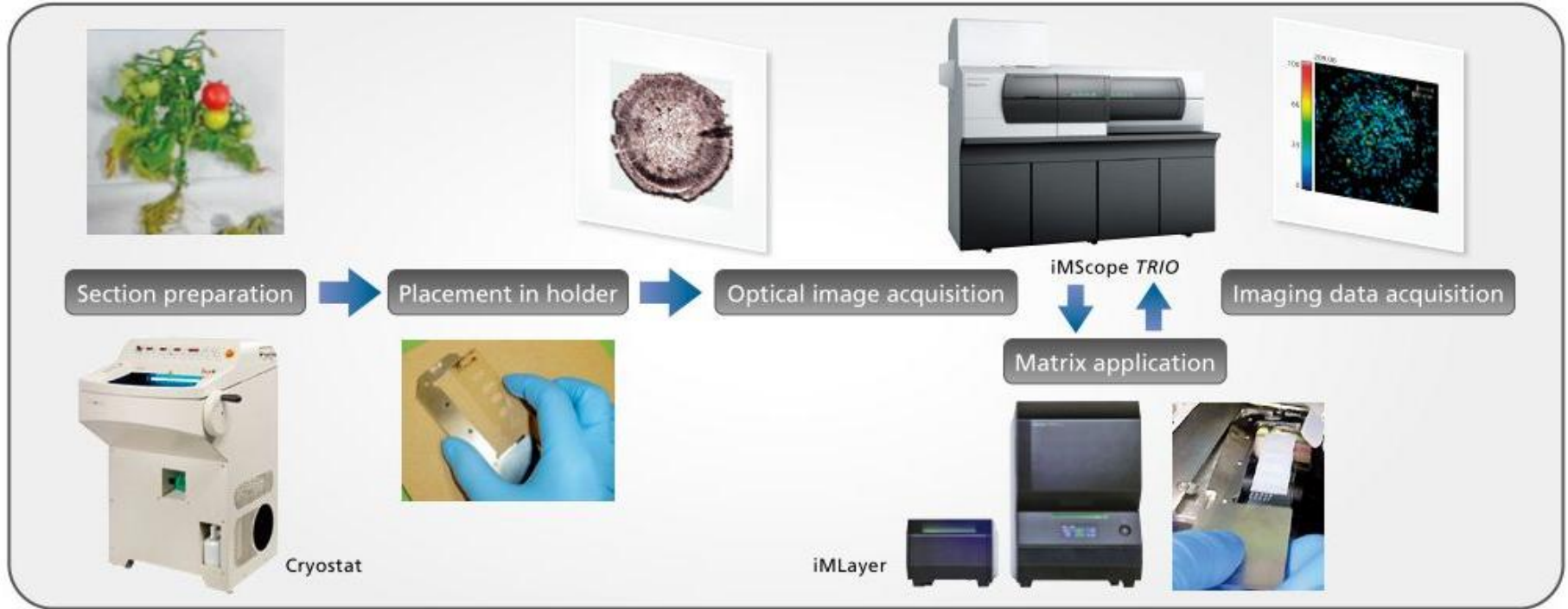


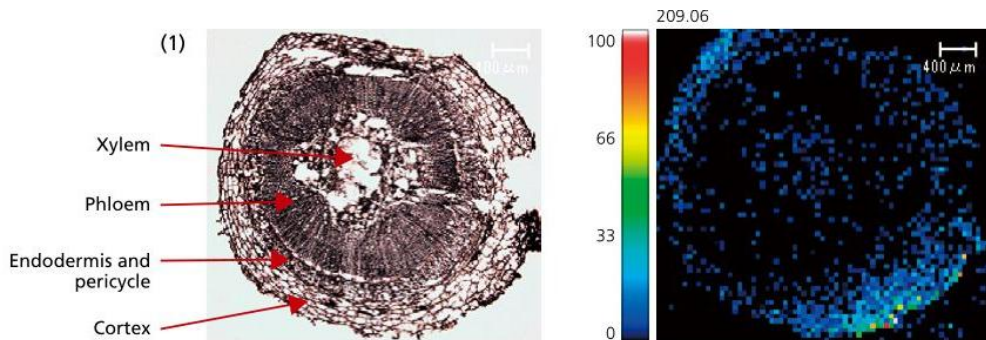
Table 1 Analytical Conditions

Sample	: Micro-Tom tomato plant exposed to 100 ppm imidacloprid for 1 day	Precursor ion	: m/z 255.66
Matrix	: α -CHCA	Laser irradiation diameter parameter	: 2
Measurement mode	: Positive ion mode	Measurement pitch	: 50 μ m

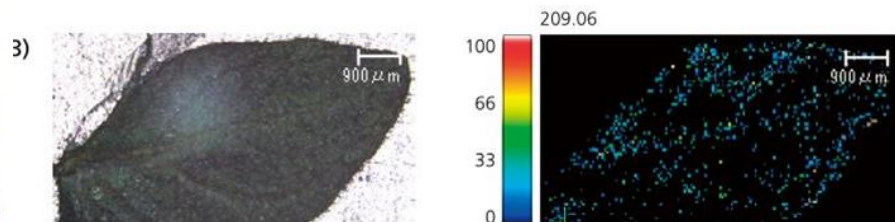


植物类：吡虫啉在小番茄机体内的渗透路径

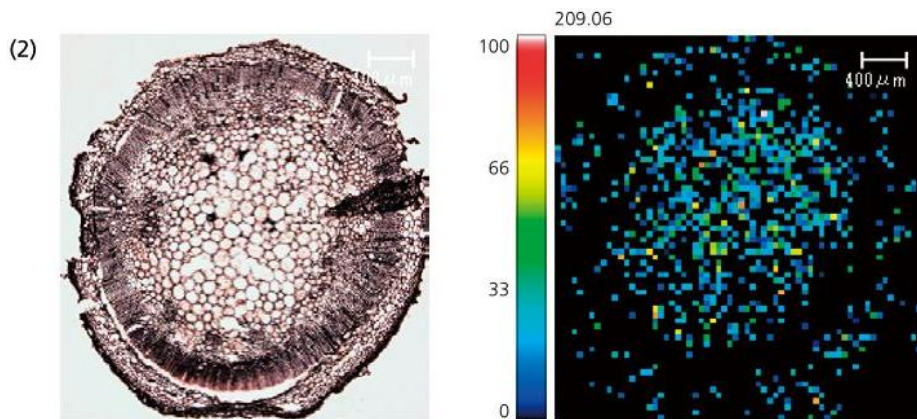
根



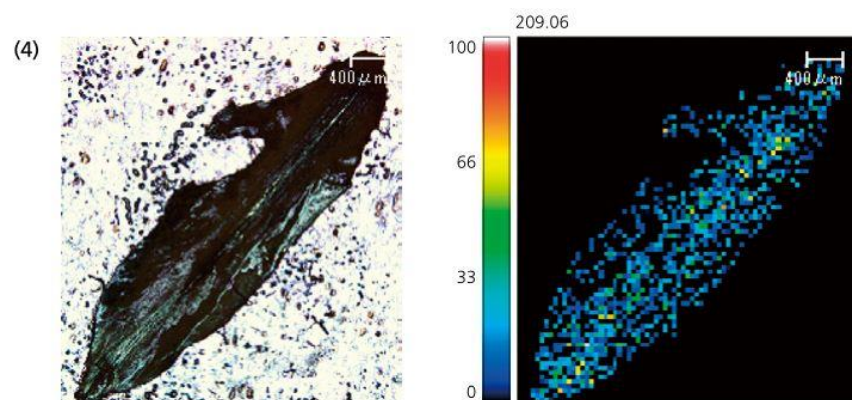
叶



茎



花

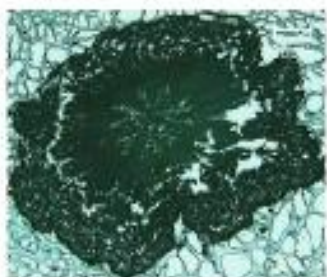


可应用于农药以及环境毒素在植物体内的渗透途径分析



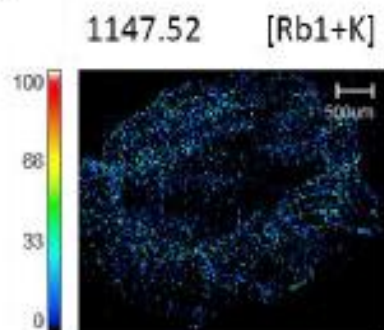
人参中人参皂苷类物质的分布

(A) 光学图像

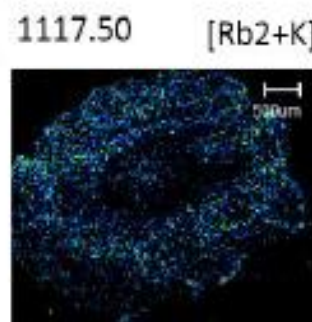


人参根茎切片

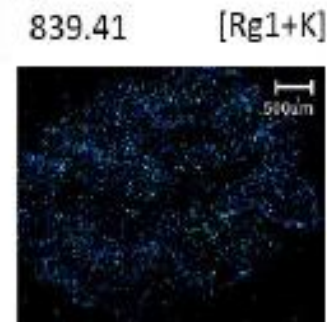
(B) Rb1一级质谱图



(C) Rb2一级质谱图

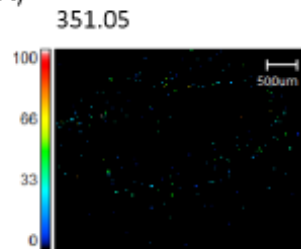


(D) Rg1一级质谱图

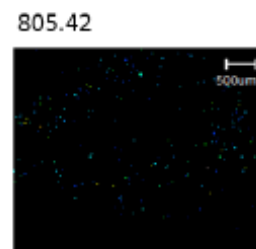


Rb2二级质谱图

(A)



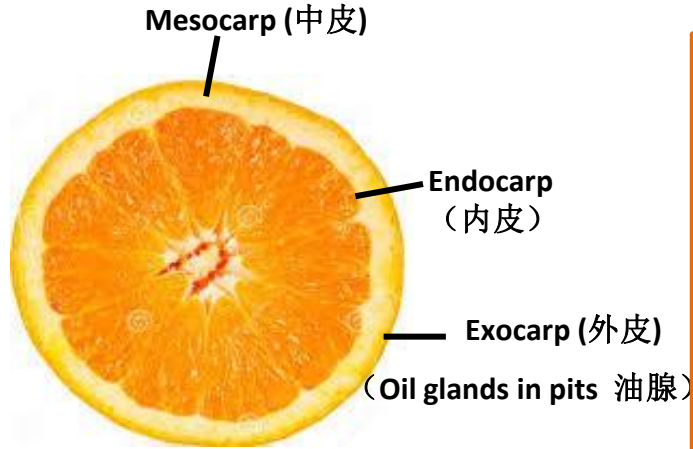
(B)



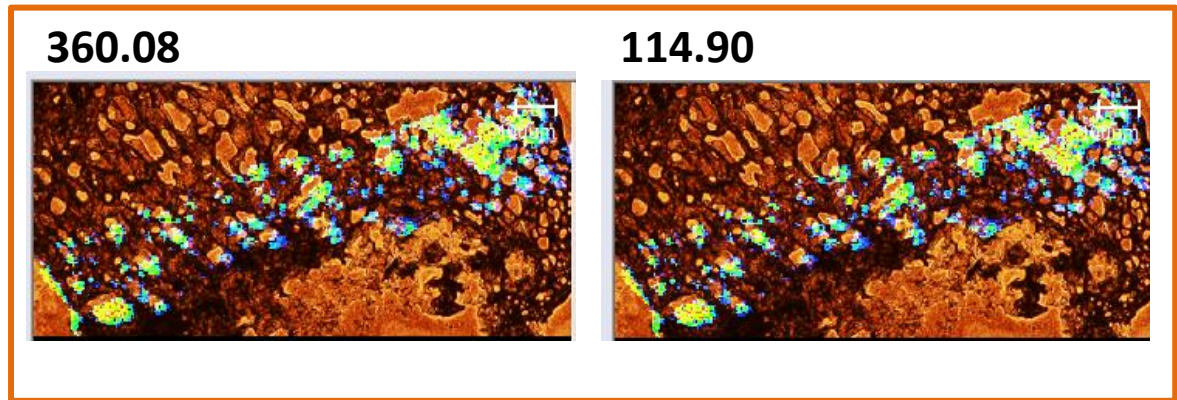
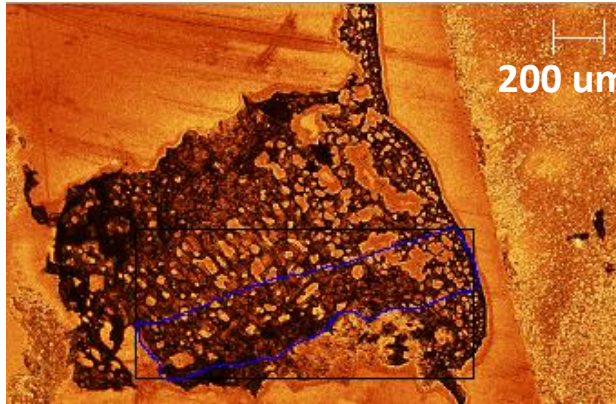
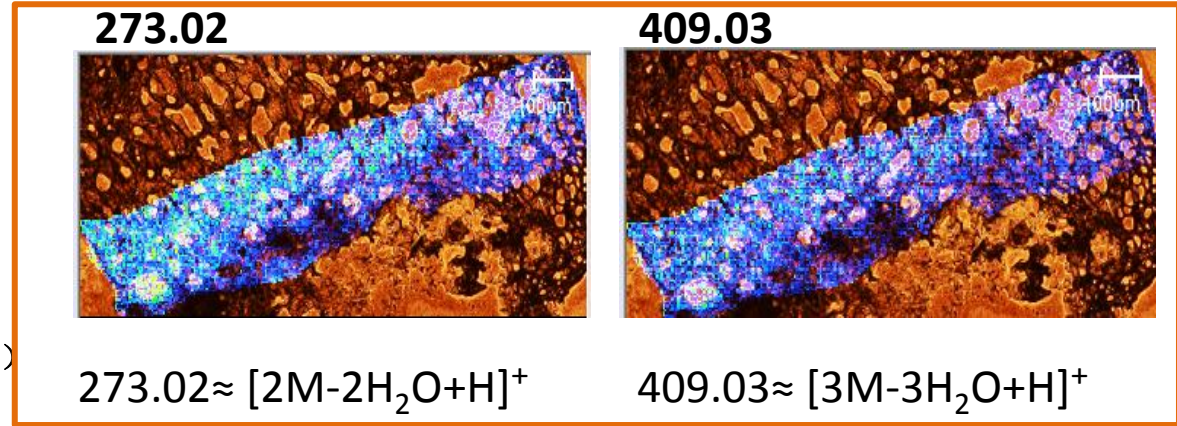
主要分布在人参的韧皮层及其表面，可以鉴别人参品种和产地，同时帮助探索有效成分的代谢通路。



橘子皮中有效成分的分布



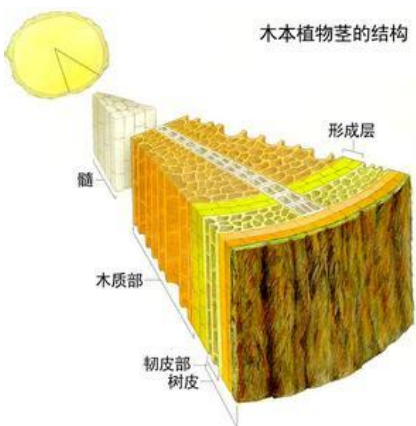
Overlaid optical image and mass image



桔皮中油脂成分可能分布在柑桔皮坑中



黄玛咖中芥子油苷类物质的分布

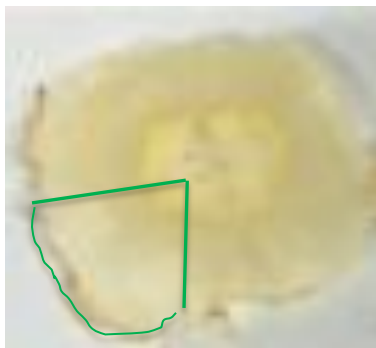


木本植物茎的结构

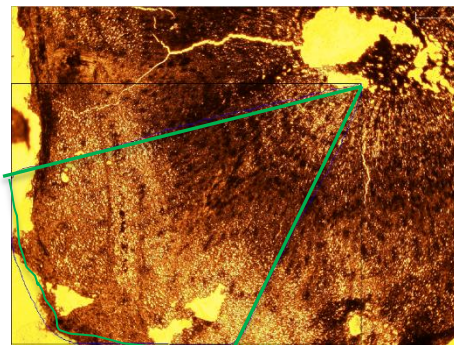
用质谱成像的方法直接观察黄玛咖中不同次级代谢产物在植物组织中的分布特征及其相对含量，对于鉴定其产地和品种并进一步揭示其生物代谢途径具有广泛的利用前景。

通过质谱成像技术可以直观观察到葡萄糖苷类物质主要分布在黄玛咖的木质部

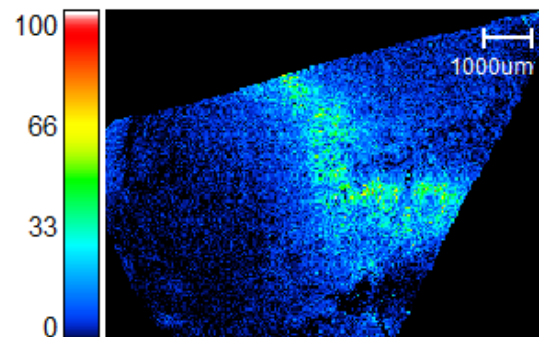
黄玛咖切片



光学图像

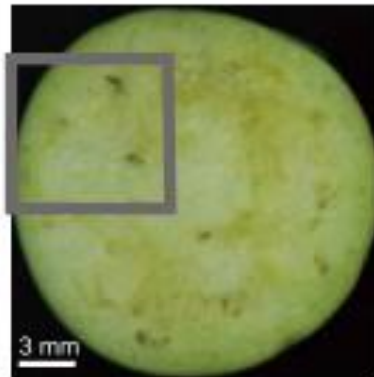


质谱成像





植物类：茄子中GABA以及其他功能性成分分析



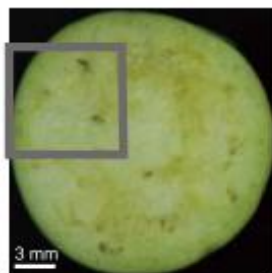
Visualization of spatial distribution of gamma-aminobutyric acid in eggplant (*Solanum melongena*) by matrix-assisted laser desorption/ionization imaging mass spectrometry., [Goto-Inoue N](#), [Setou M](#), [Zaima N.](#),

ANALYTICAL SCIENCES JULY 2010, VOL. 26



植物类：茄子中GABA以及其他功能性成分分析

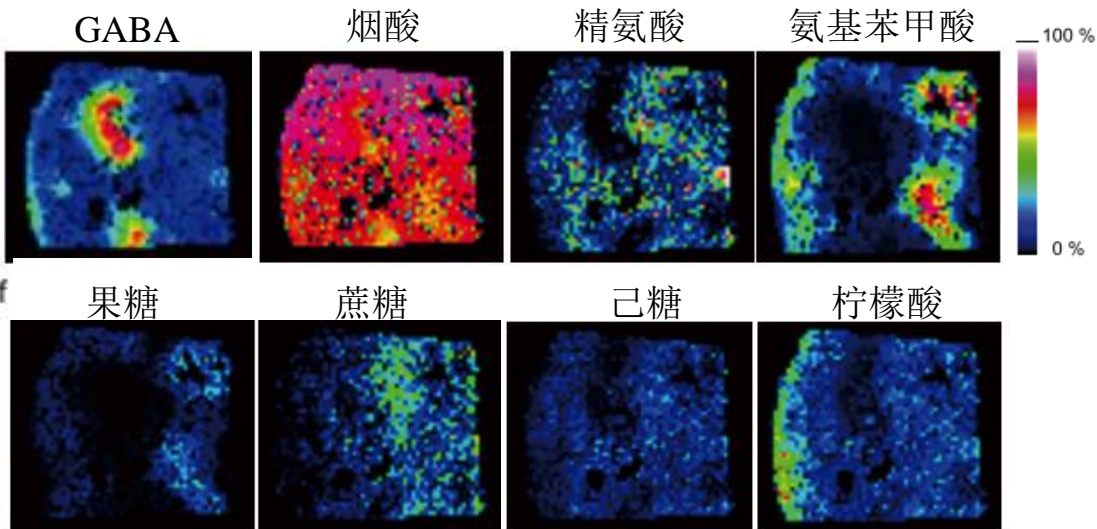
成像分析显示多种生理活性物质的不同分布特征
 如具有维持生理活性机能和降血压作用的 γ -氨基丁酸（GABA），合成多肽/蛋白质的氨基酸，提供能量或参与能量循环的糖、柠檬酸等



Optical image of eggplant section



High-power field of red rectangle area



Visualization of spatial distribution of gamma-aminobutyric acid in eggplant (*Solanum melongena*) by matrix-assisted laser desorption/ionization imaging mass spectrometry., [Goto-Inoue N](#), [Setou M](#), [Zaima N.](#),

ANALYTICAL SCIENCES JULY 2010, VOL. 26

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63. Transcription factor Nrf2 hyperactivation in early-phase renal ischemia-reperfusion injury prevents tubular damage progression [↵]
[Masahiro Nezu](#), [Tomokazu Souma](#), [Lei Yu](#), [Takafumi Suzuki](#), [Daisuke Saigusa](#), [Sadayoshi Ito](#), [Norio Suzuki](#), [Masayuki Yamamoto](#) [↵]
[Kidney International](#) Volume 91, Issue 2, February 2017, Pages 387-401 [↵]

iMScope *TRIO* IMAGING MASS MICROSCOPE



Hybrid of Optical Microscope and Mass Spectrometer (光学显微镜和质谱相结合)

➤ **多种组织切片可视化生物分子分布**

新鲜分离的样品，冷冻样品，石蜡样品



➤ **多种类化合物鉴定**

脂质类，小肽类，小分子代谢产物，药物及其代谢产物，……



➤ **多种成分同时分析**

广范围内进行筛查，进一步鉴定目标化合物

➤ **无需标记，无需指定目标检测**

无需进行同位素或荧光标记和体内成像分析，无需开发抗体



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