Ionization-assisting substrates

Desorption Ionization Using Through Hole Alumina MEmbrane

DIU THAME®

HAMAMATSU

PHOTON IS OUR BUSINESS

DIUTHAME® ensures high reproducibility and accuracy in your mass spectrometry tasks!

DIUTHAME is …

… an ionization-assisting tool developed by Hamamatsu for MALDI TOF-MS (time-of-flight mass spectrometry). DIUTHAME streamlines the ionization process in mass spectrometry by replacing the matrix used in MALDI, and totally eliminates the cumbersome pretreatment of samples needed up till now.

Medical care
Drug discovery

Basic research

Food

Material science

Places where DIUTHAME is used

Research institutes with MALDI mass spectrometers

Hospitals, research centers

Universities, educational institutions

Business enterprises

Examples of compatible samples

Compatible with a wide range of samples

Liquid samples

Biological samples

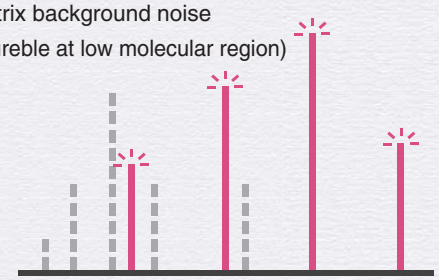
Dry samples

DIUTHAME can be used for a wide range of samples in any situation.

DIUTHAME is an acronym for “Desorption Ionization Using Through Hole Alumina Membrane”

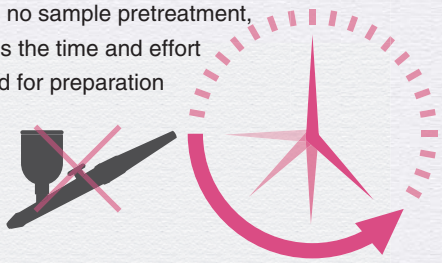
Features

No matrix background noise
(Measureble at low molecular region)

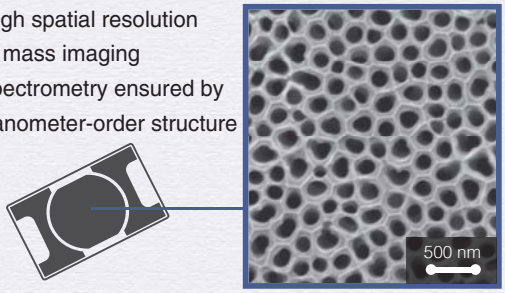


High reproducibility
with minimal variation
no matter who does
the measurement

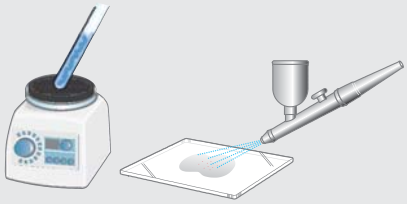
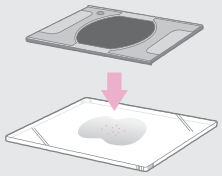
Needs no sample pretreatment,
slashes the time and effort
needed for preparation



High spatial resolution
in mass imaging
spectrometry ensured by
nanometer-order structure

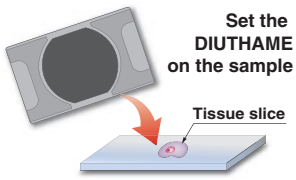


How DIUTHAME differs from matrix ionization

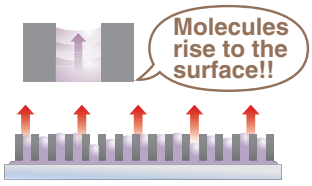
Measurement using matrix	Item	Measurement using DIUTHAME
Matrix preparation → Apply matrix 	**Preparation for measurement**	DIUTHAME is placed on sample *In mass spectrometry imaging 
Generates matrix-derived noise	**Background noise (Low molecular region)**	Generates virtually no noise
Depends on worker skill	**Reproducibility**	Good
Depends on matrix	**Spatial resolution**	High resolution due to micropore structure
Allows high-sensitivity measurement of macromolecules	**Ionization of large molecules**	Sensitivity is somewhat lower than matrix in macromolecular regions

Ionization process using DIUTHAME

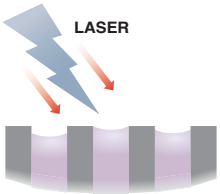
1 Place the DIUTHAME on the sample



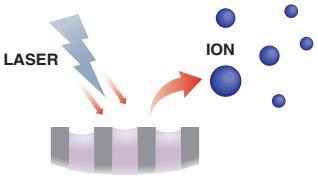
2 Wait until the molecules in the sample rise to the surface by capillary action



3 Irradiate a laser beam onto molecules that have risen to the surface



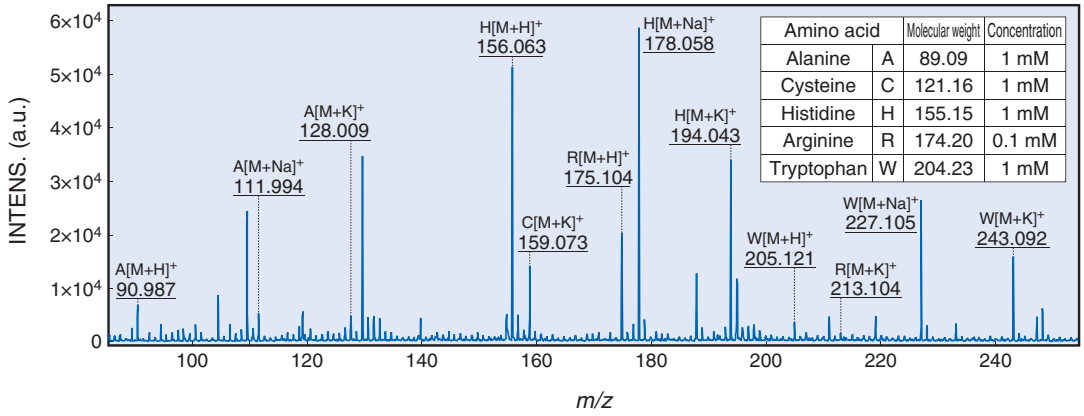
4 The target molecules are desorbed and ionized due to the effect from the fine convexo-concave structure



Mass spectrum measurement examples
Amino acid/Peptide mix

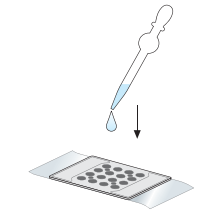
Measurements were carried out in cooperation with Associate Professor Yasuhide Naito,
The Graduate School for the Creation of New Photonics Industries

Measurement example 1 | Amino acid

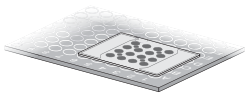


Amino acid		Molecular weight	Concentration
Alanine	A	89.09	1 mM
Cysteine	C	121.16	1 mM
Histidine	H	155.15	1 mM
Arginine	R	174.20	0.1 mM
Tryptophan	W	204.23	1 mM

Measurement method



①The mixed sample was dropped 1 µL from above the DIUTHAME.



②Remove the film and attach the DIUTHAME substrate onto the measurement plate.

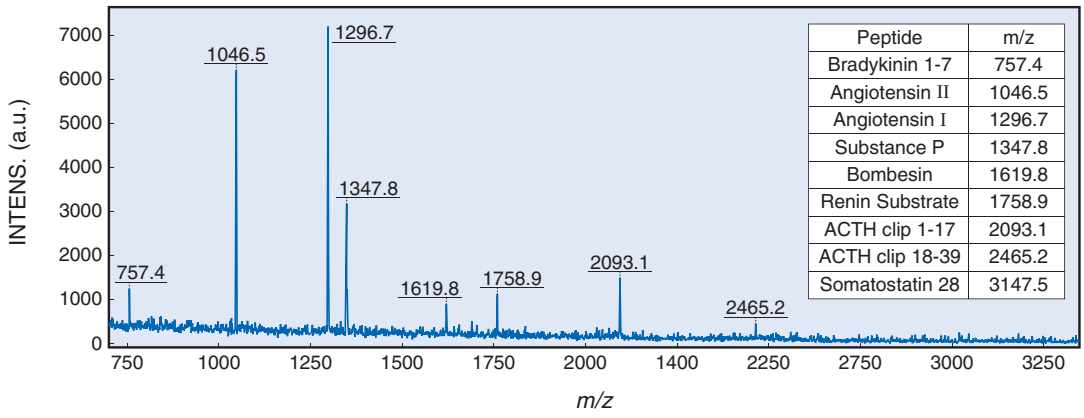
Measurement sample details

Dissolve 5 amino acids in pure water and mix them.

▼ Measurement condition

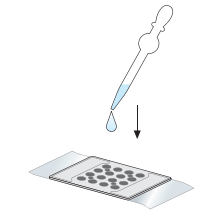
Measuremenr sample	Mixture of 5 kinds of amino acids
Ion mode	Reflectron, positive ion mode

Measurement example 2 | Peptide mix



Peptide	m/z
Bradykinin 1-7	757.4
Angiotensin II	1046.5
Angiotensin I	1296.7
Substance P	1347.8
Bombesin	1619.8
Renin Substrate	1758.9
ACTH clip 1-17	2093.1
ACTH clip 18-39	2465.2
Somatostatin 28	3147.5

Measurement method



①The mixed sample was dropped 1 µL from above the DIUTHAME.

②Remove the film and attach the DIUTHAME substrate onto the measurement plate.

Measurement sample details

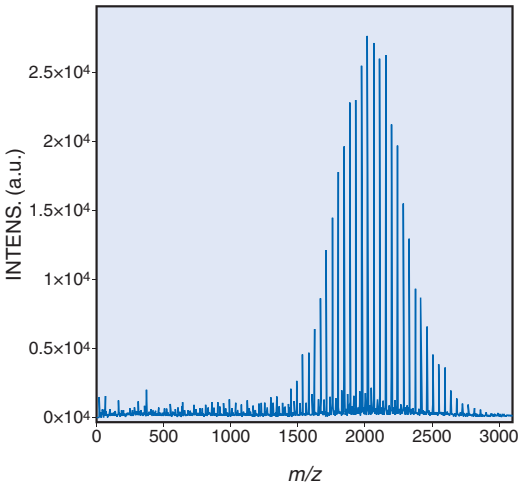
Peptide mix (in 125 µL pure water): Cationizing agent =1: 1
Peptide mix: Bruker, Peptide Calibration Standard Ⅱ
Cationizing agent ⇒CitAc (Citric acid) 5 mg/mL:
DHC (Diammonium hydrogen citrate) 5 mg/mL=1: 1

▼ Measurement condition

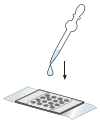
Measuremenr sample	Bruker, Peptide Calibration Standard Ⅱ
Ion mode	Reflectron, positive ion mode

Mass spectrum measurement examples
Polyethylene glycol 2000 / Polystyrene / Insulin

Measurement example 3 | Polyethylene glycol 2000



Measurement method



①The mixed sample was dropped 3 µL from above the DIUTHAME.

②Remove the film and attach the DIUTHAME substrate onto the measurement plate.

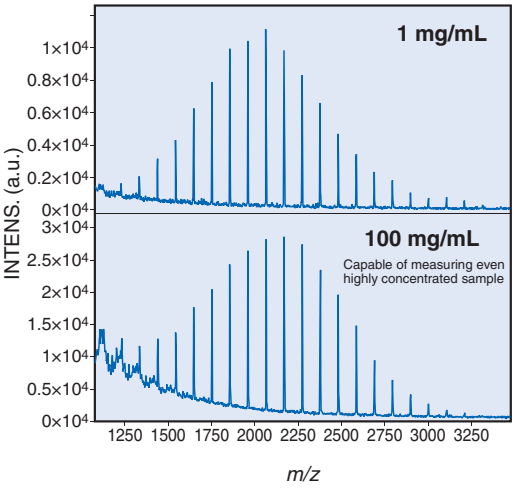
Measurement sample details

PEG2000(1 mg/mL in ACE): NaTFA(1 mg/mL in ACE)=10: 1
ACE: Acetone

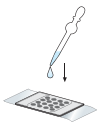
▼ Measurement condition

Measuremenr sample	Polyethylene glycol 2000: 1mg/mL
Ion mode	Reflectron, positive ion mode

Measurement example 4 | Polystyrene



Measurement method



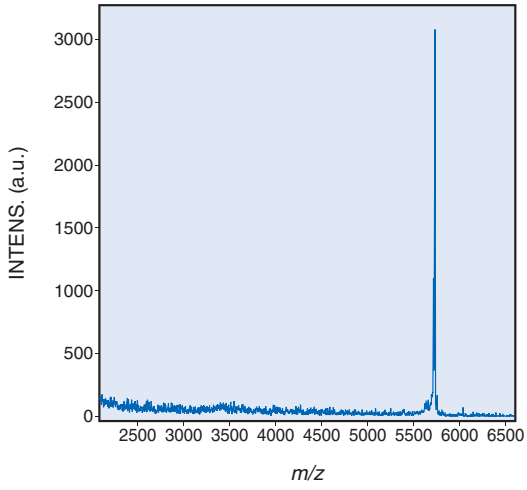
①The mixed sample was dropped 3 µL from above the DIUTHAME.

②Remove the film and attach the DIUTHAME substrate onto the measurement plate.

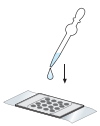
▼ Measurement condition

Measuremenr sample	Polystyrene in THF
Ion mode	Reflectron, positive ion mode

Measurement example 5 | Insulin



Measurement method



①The mixed sample was dropped 1 µL from above the DIUTHAME.

②Remove the film and attach the DIUTHAME substrate onto the measurement plate.

Measurement sample details

Insulin: DHC(5 mg/mL): CitAc(5 mg/mL)=2: 1: 1
DHC: Diammonium hydrogen citrate
CitAc: Citric acid

▼ Measurement condition

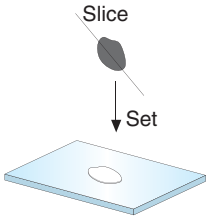
Measuremenr sample	Insulin ([M+H]$^+$, m/z 5733.6): 0.5 mM
Ion mode	Reflectron, positive ion mode

Mass spectrometry imaging measurement examples
Black rice

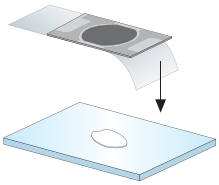
Measurements were carried out in cooperation with Designated Assistant Professor Keiko Kuwata,
The Institute of Transformative Bio-Molecules Nagoya University

Measurement method

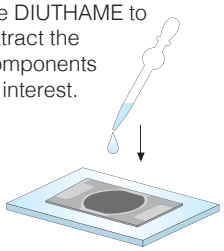
① Set a slice of black rice on an ITO glass slide.



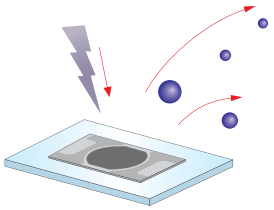
② Remove the film and place the DIUTHAME on the black rice.



③ Drop 2 µL of "70 % AcCN / 30 % H_2O" solution from above the DIUTHAME to extract the components of interest.

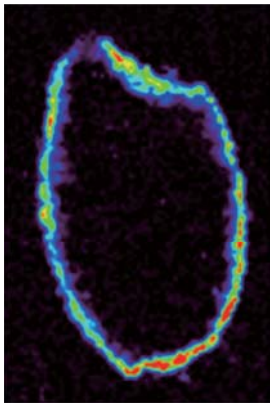


④ Start measurement after the sample dries.



Measurement example | Mass spectrometry imaging of black rice

Sample



m/z 920 (Phosphatidylcholine)

<Microscopic image>*

* After taking mass spectrometry imaging, a microscopic image was captured from above the DIUTHAME by using a microscope.

▼ Measurement condition

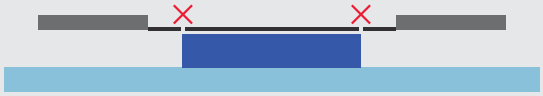
Ion mode	Linear, positive ion mode
Laser pitch	50 µm

Point | When measuring a dry sample

The effective surface of DIUTHAME is a very thin film only a few microns thick and so is easily damaged by samples. It must be handled carefully using the correct procedure during preparation for measurement. Use caution since a dry sample having an uneven or irregular surface or that is too thick will often scratch the active surface of DIUTHAME. To prevent this from happening prepare samples that are as thin and flat as possible.

✕ Sample surface has irregularities.

✕ Sample is too thick
(100 µm or more is usually too thick*)

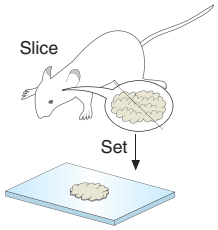


*Appropriate thickness depends on the sample.

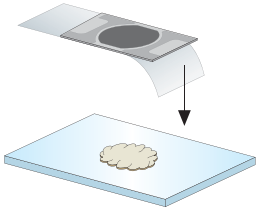
Mass spectrometry imaging measurement examples
MOuse brain

Measurement method

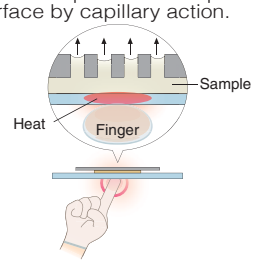
① Set a slice of frozen mouse brain on an ITO glass slide.



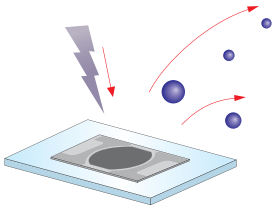
② Place DIUTHAME on the mouse brain slice before it thaws.



③ After the sample thaws with fingertip heat the components of the sample will soak up to the surface by capillary action.



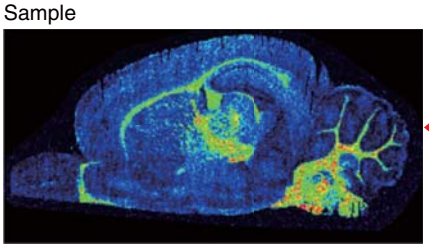
④ Start measurement after the sample dries.



Do this work in a cryostat

Measurement example 1 | MS imaging result of one sample taken by positive and negative ion mode

Sample

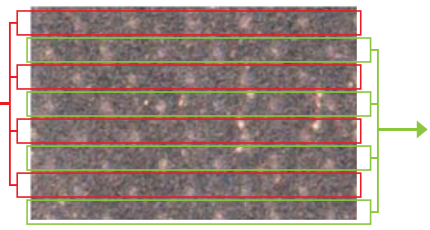


m/z 848.6 [PC(38:4)+K]⁺

▼ Measurement condition

Ion mode	Positive ion mode
Mouse brain slice thickness	20 μm
Laser pitch	50 μm

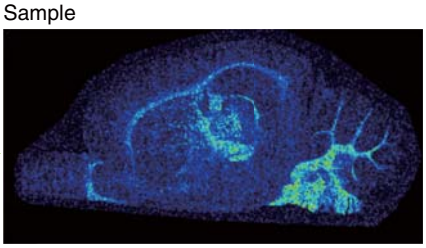
Laser irradiation marks on the DIUTHAME



<Optical image>

*Before making measurements using mass spectrometer, an optical image was captured from above the DIUTHAME.

Sample



m/z 890.7 [ST(d18:1/C24:0)-H]⁻

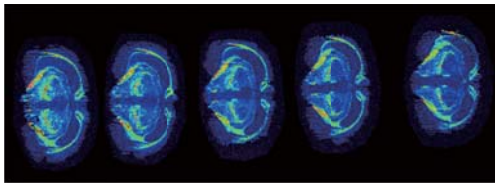
▼ Measurement condition

Ion mode	Negative ion mode
Mouse brain slice thickness	20 μm
Laser pitch	50 μm

Measurement example 2 | High-reproducibility measurements using one DIUTHAME substrate
(5 sample slices were arrayed and measured)

<Optical image>

Sample



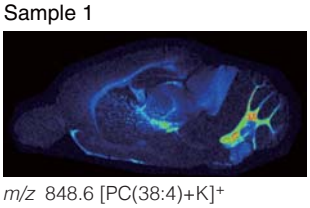
m/z 848.6 [PC(38:4)+K]⁺

▼ Measurement condition

Mouse brain slice thickness	30 μm
Laser pitch	70 μm

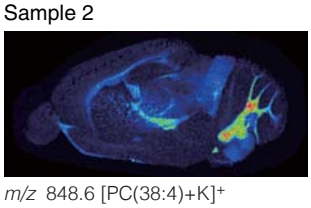
Measurement example 3 | Low variation result for each DIUTHAME
(Three frozen tissue sections were obtained from mouse brain continuously)

Sample 1



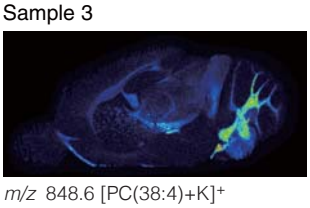
m/z 848.6 [PC(38:4)+K]⁺

Sample 2



m/z 848.6 [PC(38:4)+K]⁺

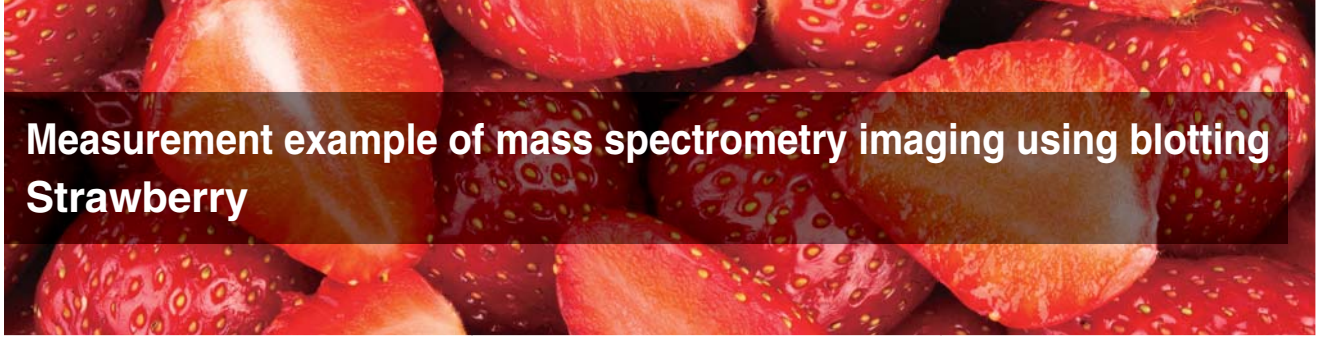
Sample 3



m/z 848.6 [PC(38:4)+K]⁺

▼ Measurement condition

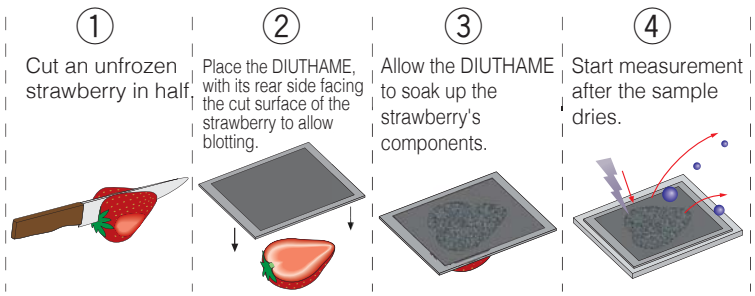
Mouse brain slice thickness	30 μm
Laser pitch	50 μm

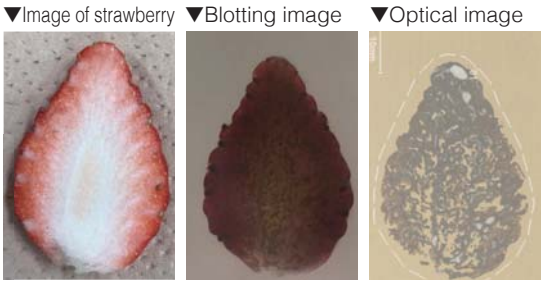


Measurement example of mass spectrometry imaging using blotting
Strawberry

Measurement carried in cooperation with Associate Professor Hirofumi Enomoto, The Department of Biosciences Teikyo University

Measurement method

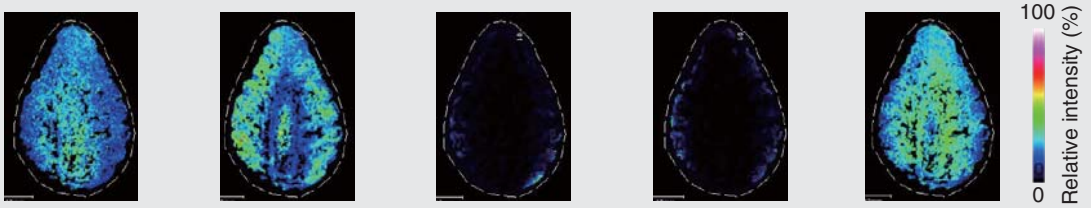




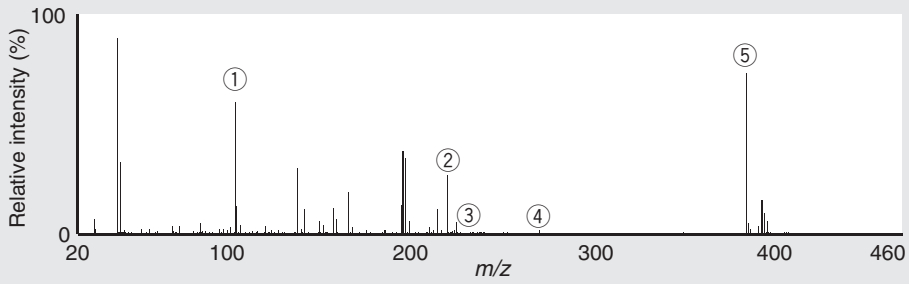
Measurement example 1 | Positive ion mode measurement

Number	①	②	③	④	⑤
m/z	104	219	231	271	381
Target	Choline $[M+H]^+$	Hexose $[M+K]^+$	Citric acid $[M+K]^+$	Peralgonidin $[M]^+$	Sucrose $[M+K]^+$
Laser pitch					

Ion image



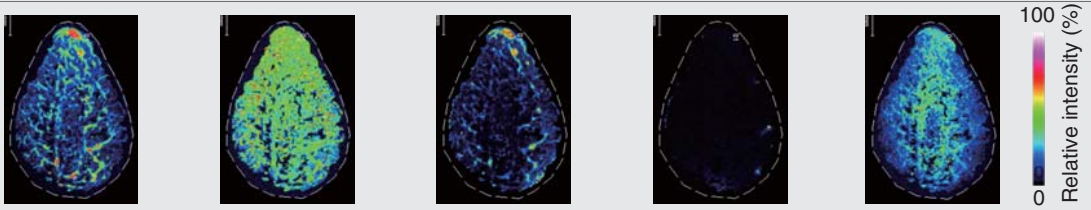
MS spectrum



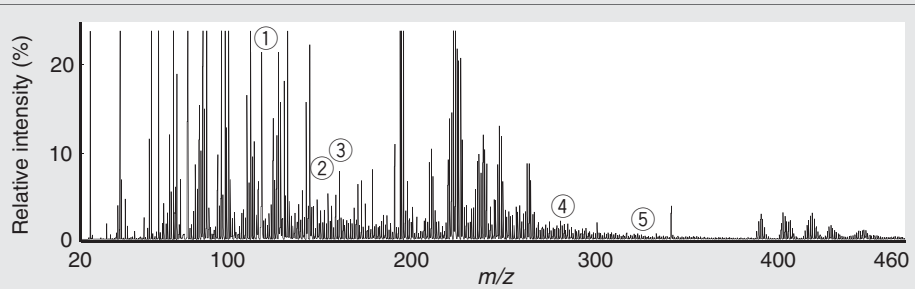
Measurement example 2 | Negative ion mode measurement

Number	①	②	③	④	⑤
m/z	133	179	191	301	341
Target	Malic acid $[M-H]^-$	Hexose $[M-H]^-$	Citric acid $[M-H]^-$	Quercetin and/or ellagic acid $[M-H]^-$	Sucrose $[M-H]^-$
Laser pitch					

Ion image



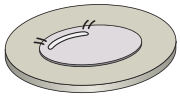
MS spectrum



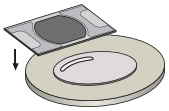
Measurement example of mass spectrometry imaging using blotting
Industrial product

Measurement method

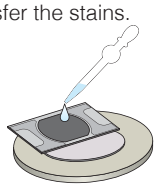
① White stains have occurred on an industrial product.



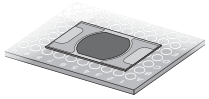
② Place the DIUTHAME on the measurement position (where white stains have occurred) of the product.



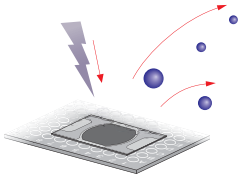
③ Drop 1 µL of solvent from above the DIUTHAME to transfer the stains.



④ Set the DIUTHAME with the transferred stains on the measurement plate.

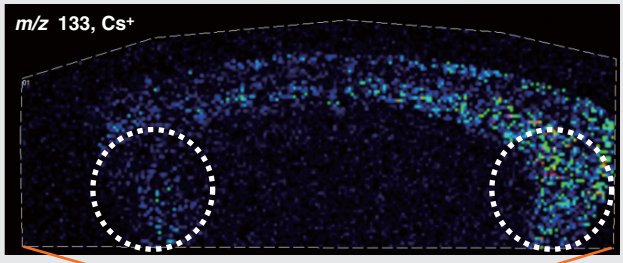


⑤ Start measurement after the sample dries.

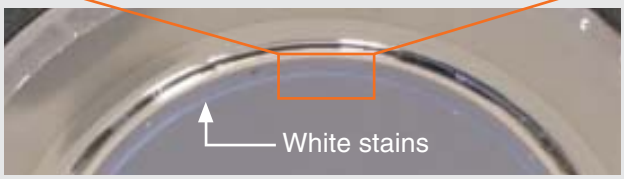


Measurement example | Measurement of a large, thick sample using blotting technique

Ion image



Actual image of measured sample

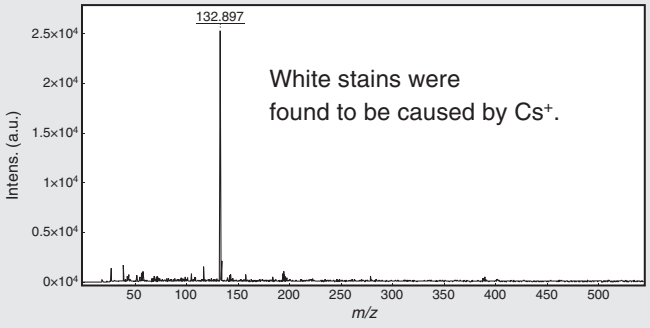


▼ Measurement condition

Ion mode	Positive ion mode
Laser pitch	50 µm
Solvent	HCl: ACN=1: 1

* Area in white dotted line circle shows oozing from solvent drop.

MS spectrum



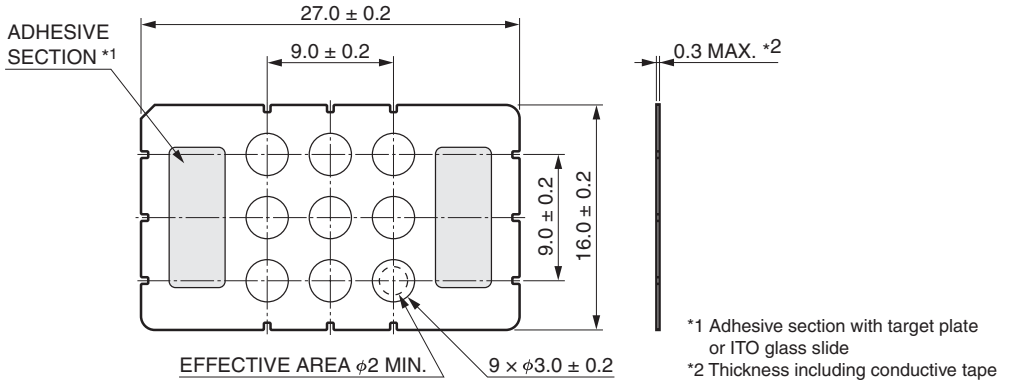
Point | Measurement benefits unique to blotting technique

In mass spectrometry imaging, there are usually limits on the size and thickness of samples that can be placed in mass spectrometers. Therefore, large samples must first be processed or machined to reduce their size or thickness such as by slicing them into thin sections.
In the blotting technique using DIUTHAME, the components on the sample surface are analyzed AFTER being transferred to the DIUTHAME. So there is no need to do extra processing of the sample and mass spectrometry imaging with position information can then start. This will expand mass spectrometry imaging applications to fields where it is difficult to measure samples due to their shape or measurement method.

Dimensional outline
For Mass spectrum

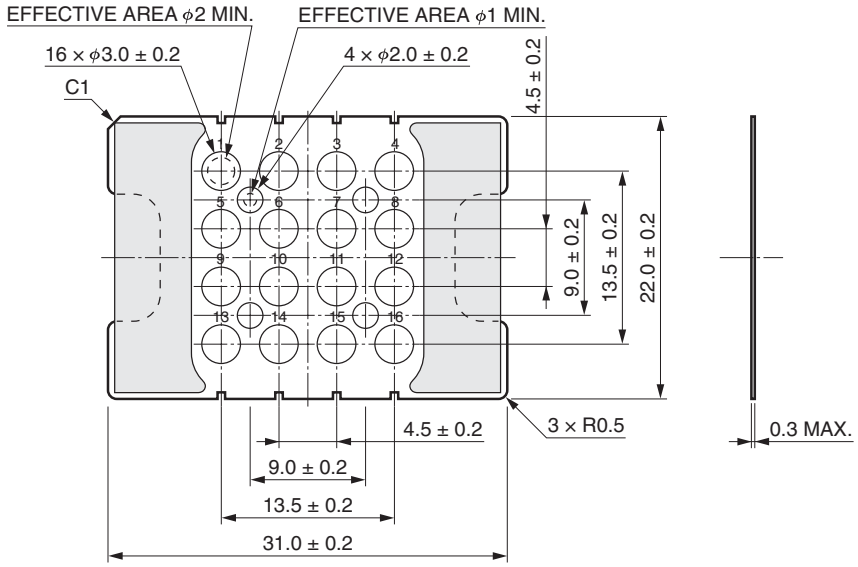
(Unit: mm)

●ϕ3 × 9 ch type
・A14111-3-1 (For mass spectrum)

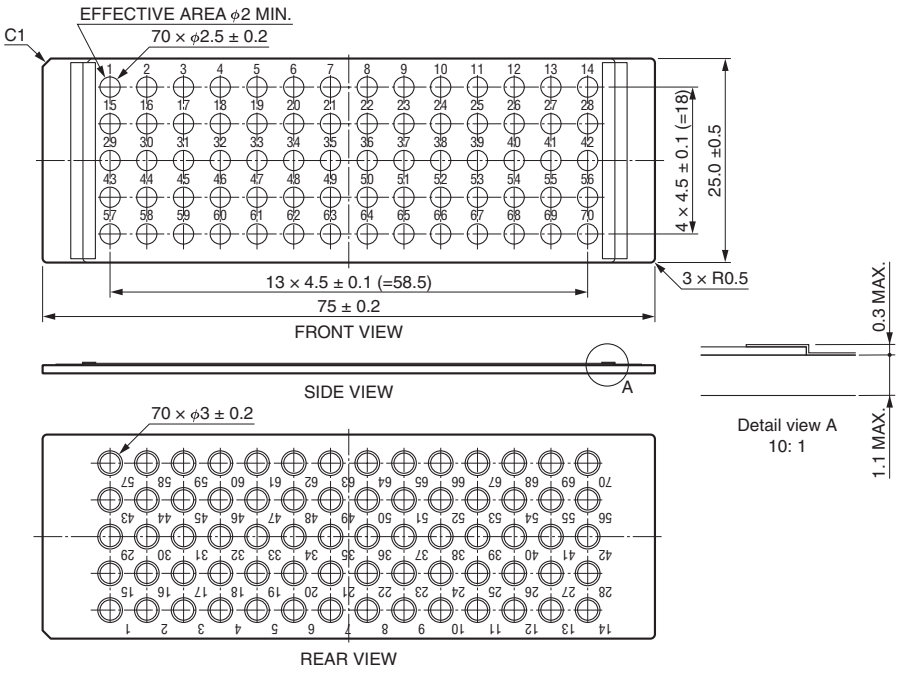


TPMZA0027EA

●ϕ3 × 16 ch type
・A14111-3-2



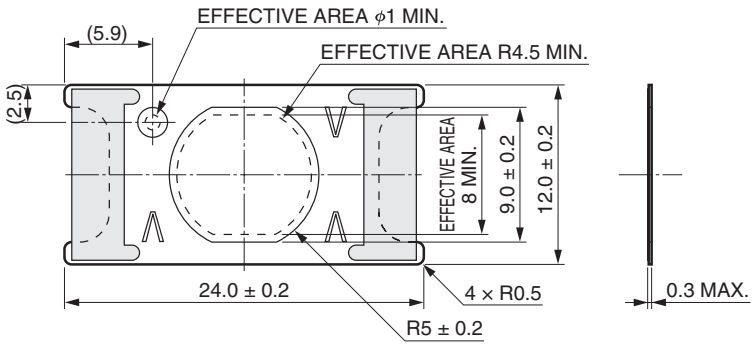
●ϕ3 × 70 ch type
・A14111-3-3



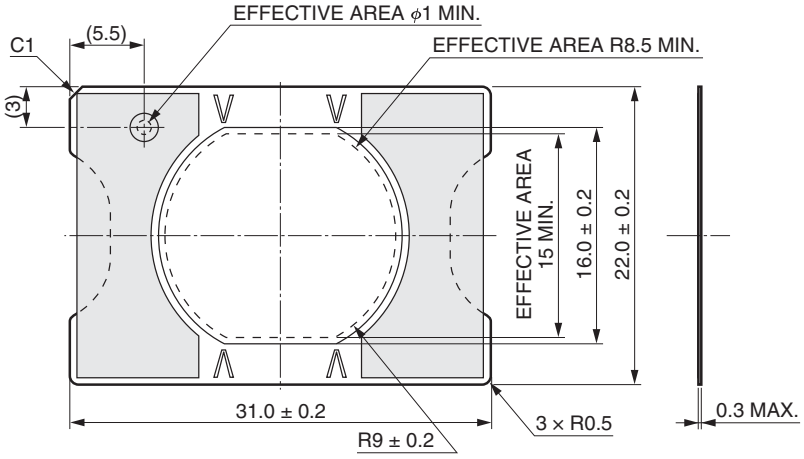
Dimensional outline
For MS imaging

(Unit: mm)

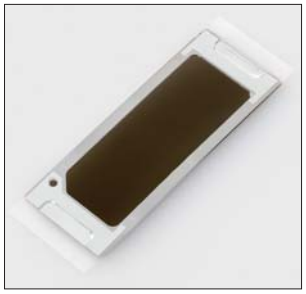
●ϕ10 type
- **A13331-10-1**
- **A13331-10-1B (For blotting)**

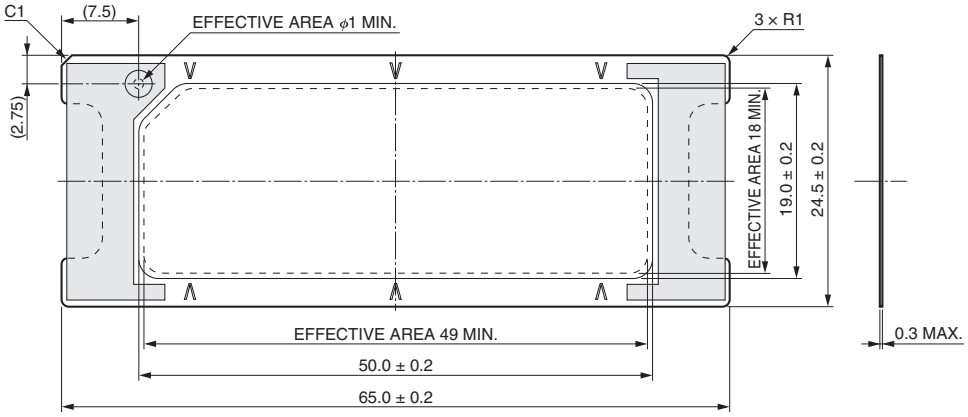


●ϕ18 type
- **A13331-18-2**
- **A13331-18-2B (For blotting)**



●Glass slide size type
- **A13331-5019-1**
- **A13331-5019-1B (For blotting)**





Website information

To find more detailed information about DIUTHAME,
please visit our website via QR code or URL below.

[Main contents on website]

- Application notes
- How-to-use instruction video
- Related literature
- What the developer has to say
- Interview with users

https://www.hamamatsu.com/jp/en/product/optical-components/DIUTHAME/index.html

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