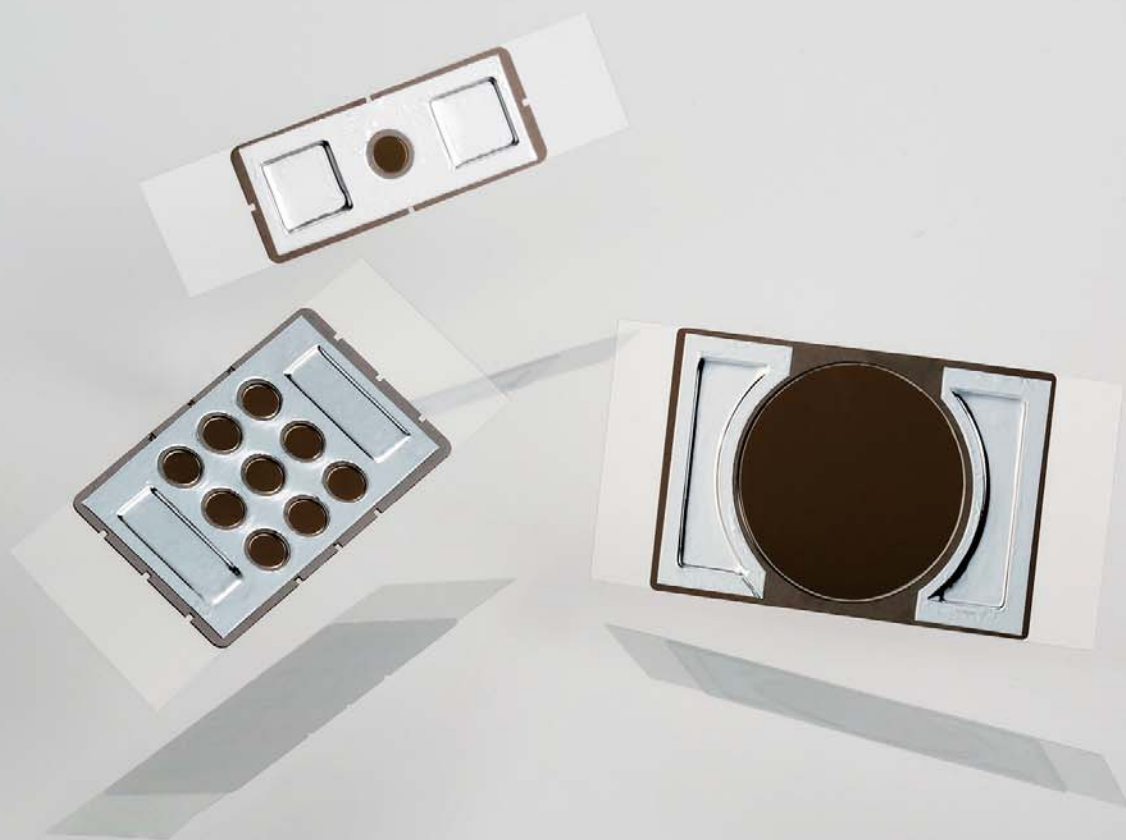
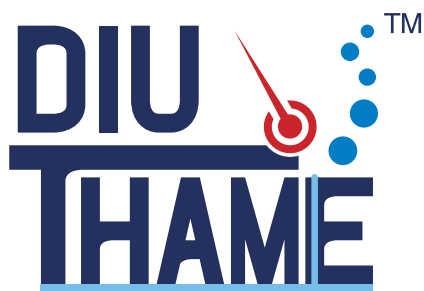


# Ionization-assisting substrates

Desorption Ionization Using Through Hole Alumina MEmbrane



**HAMAMATSU**  
PHOTON IS OUR BUSINESS



DIUTHAME ensures high repro in your mass spectrometry tasks!



Medicine care



Basic research

Hamamatsu offers ionization-assisting substrates called DIUTHAME that support ionization in mass spectrometry in place of matrix that is currently used for MALDI (Matrix-Assisted Laser Desorption/Ionization) and also eliminate the cumbersome sample pretreatment process. DIUTHAME brings high reproducibility and ease-of-handling to mass spectrometry by serving as a completely new tool that can readily be used by all MALDI mass spectrometer users.

● Example: Difference in

Steps when using  
DIUTHAME

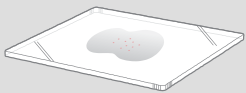
Steps when using  
a matrix and airbrush

# ducibility and accuracy



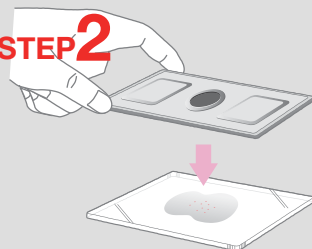
## preparations for frozen section measurements of mouse brain

**STEP 1**



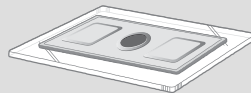
Place the sample on an ITO glass slide

**STEP 2**



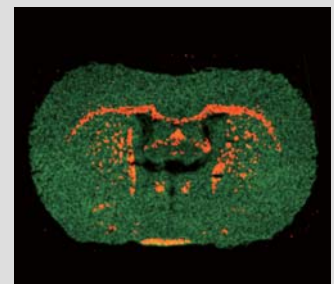
Place the DIUTHAME on the sample

**Now just wait until the sample thaws and dries!**



Thawing & drying

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**End of measurement**

**STEP 1**



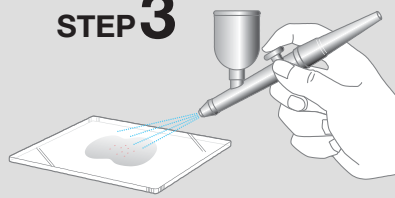
Place the sample on an ITO glass slide

**STEP 2**



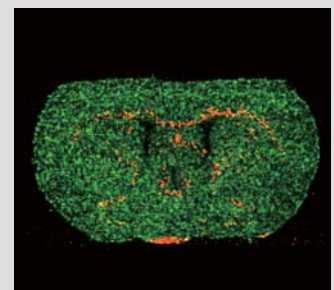
Prepare the matrix

**STEP 3**



Spray-coat the matrix, and let it dry

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\* See page 7 for details on these measurement examples.



## Features

### NO matrix required

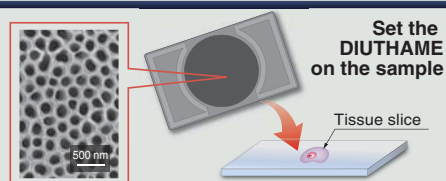
- NO matrix background noise
- High reproducibility with minimal variation no matter who does the measurement
- NO sample pretreatment required
- High spatial resolution in imaging mass spectrometry ensured by nanometer-order structure

## How DIUTHAME differs from conventional MALDI

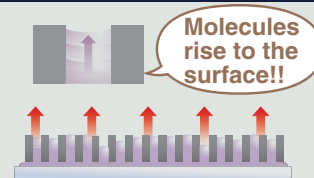
Item	DIUTHAME	MALDI
Background noise	None	Matrix noise appears
Ease of handling	Easy	Expertise is required
Reproducibility	High	Not so high
Spatial resolution	High	Not so high
Measurement of high molecules	Possible depending on samples	Possible

## Ionization process using DIUTHAME

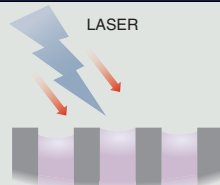
**1** Place the DIUTHAME on the sample



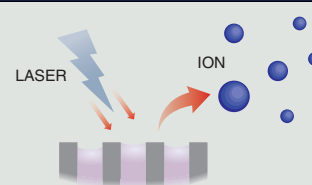
**2** Wait until the molecules in the sample rise to the surface by capillary action



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



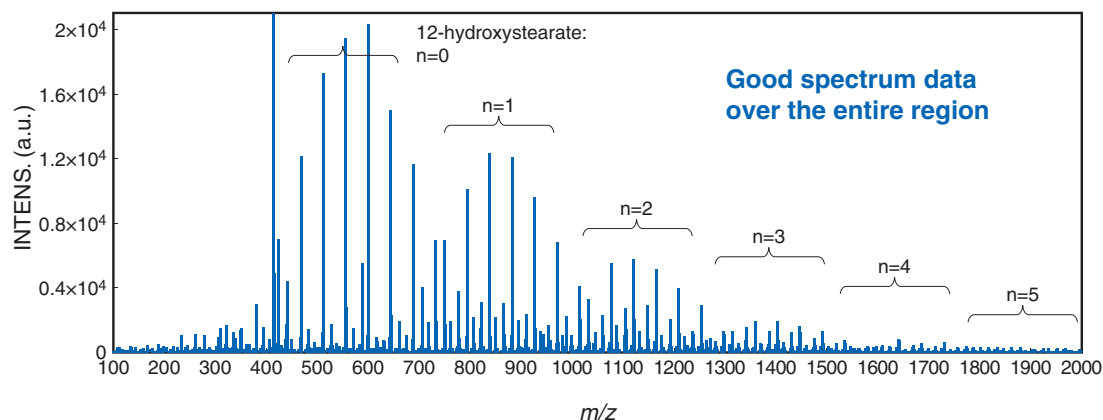
**4** The target molecules are desorbed and ionized due to the effect from the fine convexo-concave structure



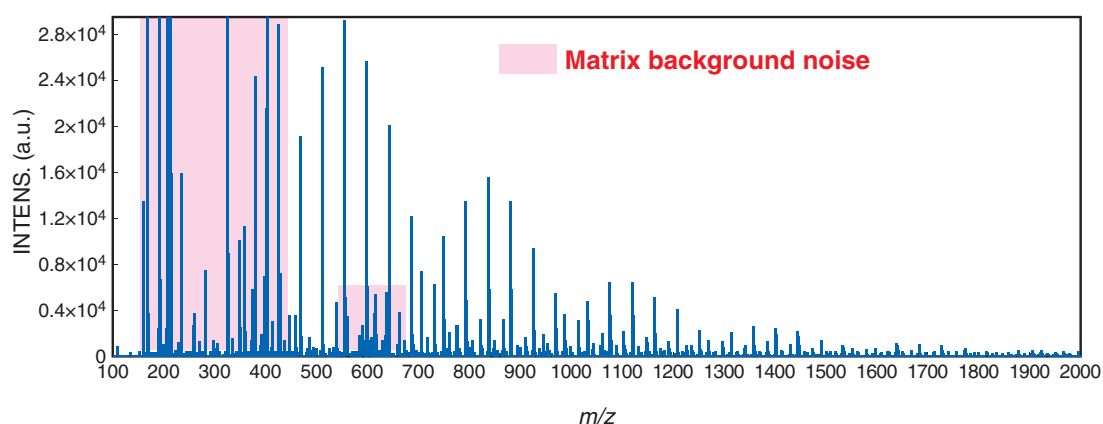
## ● PEO-hydrogenated castor oil

Measurement sample: Cosmetic raw material (Hydrogenated castor oil)

### ● Measurement example of PEO-hydrogenated castor oil using DIUTHAME



### ● Measurement example of PEO-hydrogenated castor oil using MALDI



#### Measurement method

The mixed sample was dropped from above the DIUTHAME.

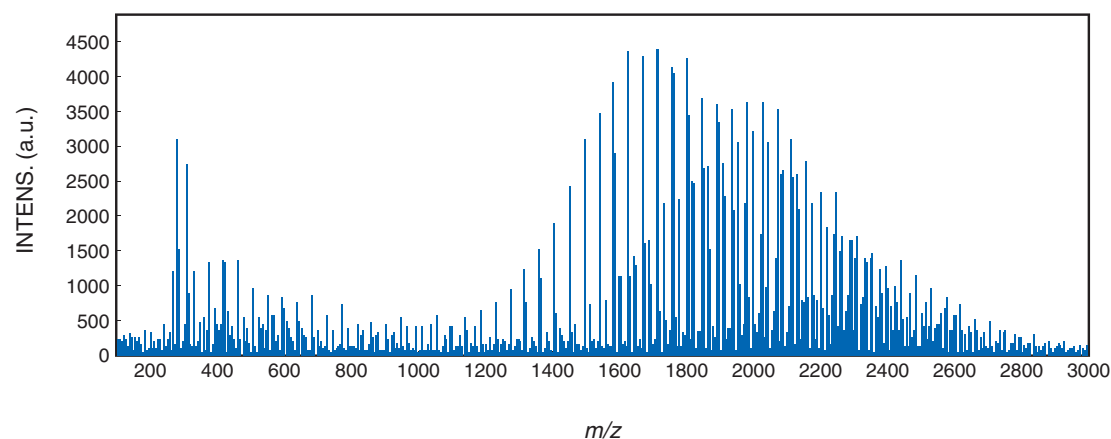
#### Measurement sample details

The sample was dissolved in THF at a concentration of 1 mg/mL. NaTFA was used as the cationizing agent and dissolved in THF at a concentration of 1 mg/mL. The sample was mixed with the cationizing agent at a ratio of 1:10 (v/v).

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## ● PEO-monostearate

Measurement sample: Industrial surfactant (PEO monostearate)



#### Measurement method

The mixed sample was dropped from above the DIUTHAME.

#### Measurement sample details

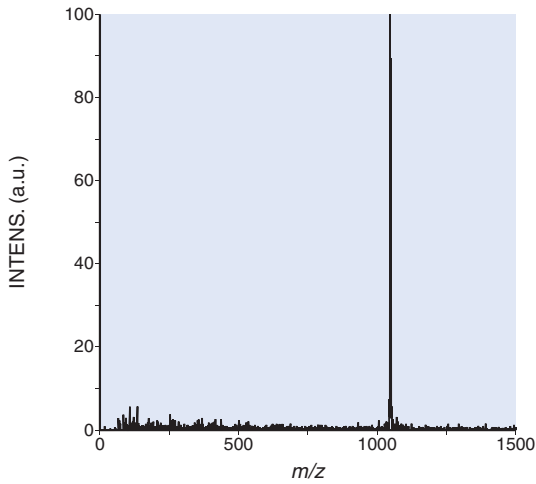
The sample was dissolved in THF at a concentration of 1 mg/mL. NaTFA was used as the cationizing agent and dissolved in THF at a concentration of 1 mg/mL. The sample was mixed with the cationizing agent at a ratio of 1:10 (v/v).

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# Mass spectrum measurement example

## ● Angiotensin II

Measurement sample: Angiotensin II ( $[M+H]^+$ ,  $m/z$  1046.5): 1  $\mu$ M  
 Measurement conditions: Linear, positive ion mode



### Measurement method

The mixed sample was soaked up from below the DIUTHAME.

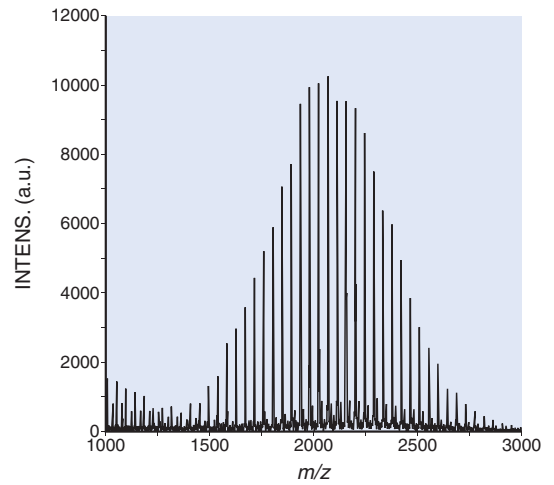
### Measurement sample details

Angiotensin II: DHC : CitAc : ACN=1 : 1 : 1 : 1  
 Angiotensin II: 1  $\mu$ M  
 DHC (Diammonium hydrogen citrate): 0.2 M  
 CitAc (Citric acid): 0.2 M  
 ACN: Acetonitrile

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## ● Polyethylene glycol 2000

Measurement sample: Polyethylene glycol 2000: 1mM in acetone  
 Measurement conditions: Linear, positive ion mode



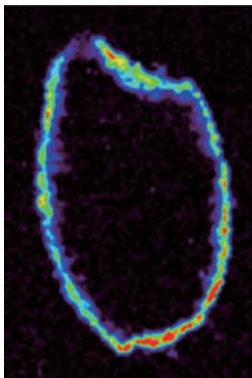
### Measurement method

The mixed sample was dropped from above the DIUTHAME.

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# Measurement examples using imaging mass spectrometry

## ● Black rice



$m/z$  920 (Phosphatidylcholine)  
 Measurement conditions:  
 Linear, positive ion mode  
 Laser pitch: 50  $\mu$ m

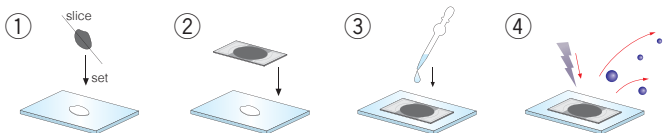
<Microscopic image>



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

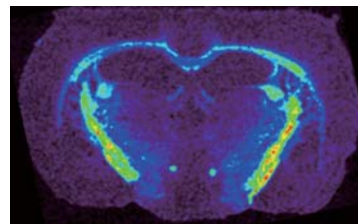
### Measurement method

- ① Set a slice of black rice on an ITO glass slide.
- ② Place the DIUTHAME substrate on the sliced black rice.
- ③ Drop 2  $\mu$ L of "70 % AcCN / 30 % H<sub>2</sub>O" solution from above the DIUTHAME to extract the components of interest.
- ④ After the sample dries, make measurements using imaging mass spectrometry.



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## ● Mouse brain



$m/z$  840  
 Measurement conditions:  
 Linear, positive ion mode  
 Laser pitch: 60  $\mu$ m

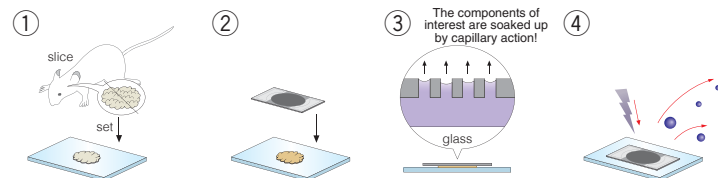
<Microscopic image>



\* Before making measurements using imaging mass spectrometry, a microscopic image was captured from above the DIUTHAME by using a microscope.

### Measurement method

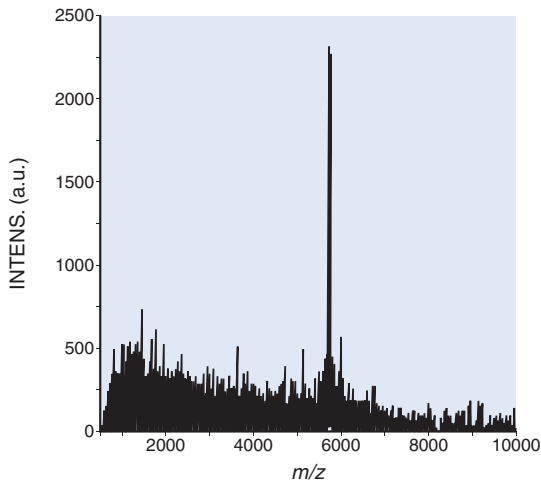
- ① Set a slice of frozen mouse brain on an ITO glass slide.
- ② Place the DIUTHAME on the mouse brain slice before it thaws.
- ③ After the brain slice thaws, the components derived from the sample are soaked up by capillary action.
- ④ After the sample dries, make measurements using imaging mass spectrometry.



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## ● Insulin

Measurement sample: Insulin ( $[M+H]^+$ ,  $m/z$  5733.6): 0.5 mM  
 Measurement conditions: Linear, positive ion mode



### Measurement method

The mixed sample was soaked up from below the DIUTHAME.

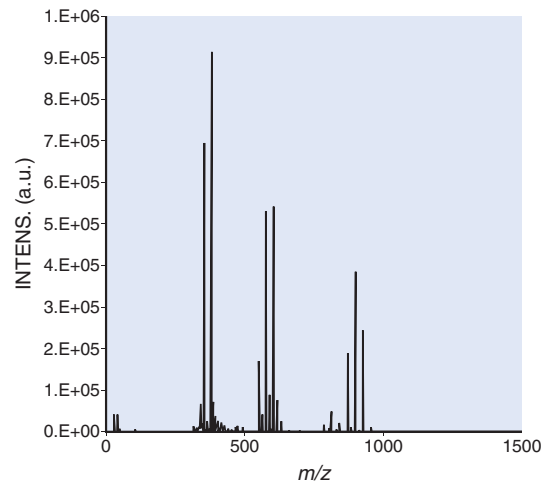
### Measurement sample details

Insulin: DHC : CitAc : ACN = 1 : 1 : 1 : 1  
 Insulin: 0.5 mM  
 DHC (Diammonium hydrogen citrate): 0.2 M  
 CitAc (Citric acid): 0.2 M  
 ACN: Acetonitrile

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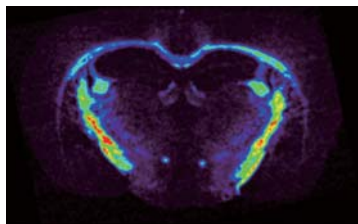
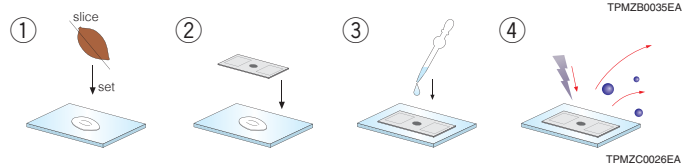
## ● Cocoa raw bean (Triglycerides)

Measurement sample: Triglycerides in cocoa raw beans  
 Measurement conditions: Linear, positive ion mode



### Measurement method

- ① Set a slice of cacao raw bean on an ITO glass slide.
- ② Place the DIUTHAME on the sliced bean.
- ③ Drop 2  $\mu$ L of acetone from above the DIUTHAME to extract the components of interest.
- ④ Irradiate a laser beam onto the DIUTHAME to cause ionization.

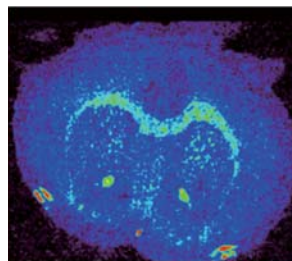


$m/z$  910  
 Measurement conditions:  
 Linear, negative ion mode  
 Laser pitch: 60  $\mu$ m

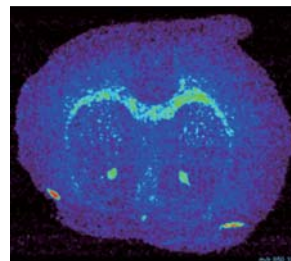
## ● Reproducibility of serial slices of mouse brain (near $m/z$ 850)

**Good reproducibility** >>>

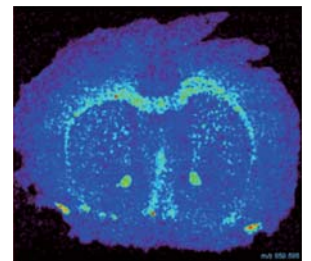
**DIUTHAME** Mouse brain slice thickness: 50  $\mu$ m  
 Laser pitch: 50  $\mu$ m



First time



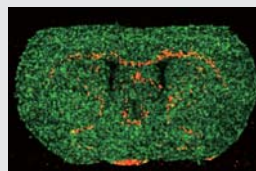
Second time



Third time

## ● Comparison between MALDI and DIUTHAME using mouse brain ( $m/z$ 848)

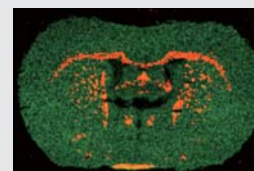
### MALDI



**Tissue section has contracted somewhat**

<Conditions>  
 ■ Mouse brain slice thickness 10  $\mu$ m  
 ■ Matrix conditions DHB 50 mg/ml, 50 % ACN  
 Spray coating  
 ■ Measurement conditions Reflectron, positive ion mode  
 ■ Laser pitch 50  $\mu$ m

### DIUTHAME

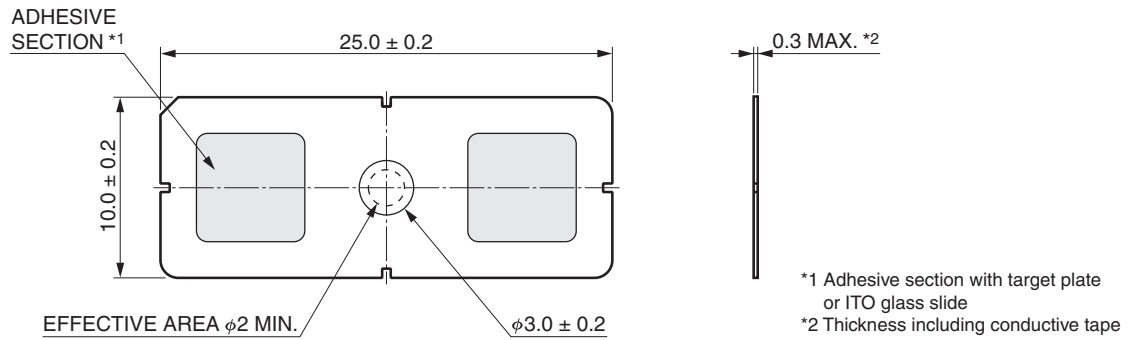


**· Good spatial resolution**  
**· Maintains shape of tissue section**

<Conditions>  
 ■ Mouse brain slice thickness 50  $\mu$ m  
 ■ DIUTHAME size Effective diameter: 18 mm  
 ■ Measurement conditions Reflectron, positive ion mode  
 ■ Laser pitch 50  $\mu$ m

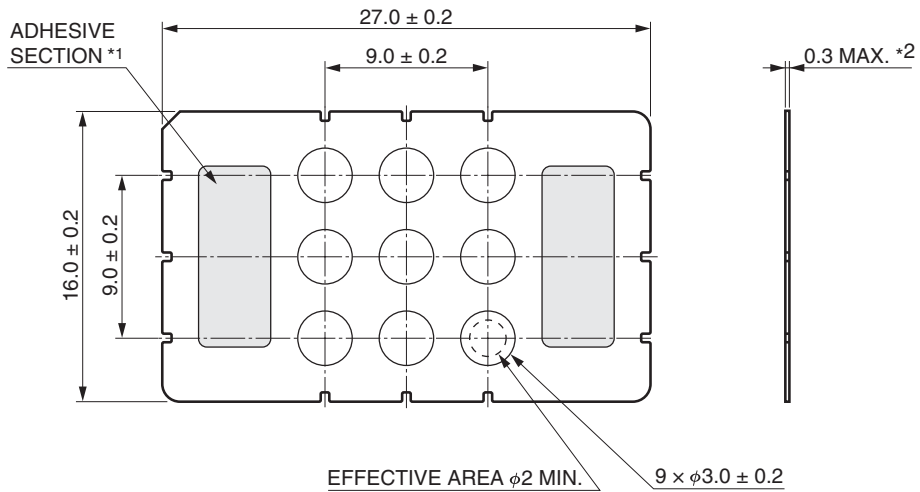
# Dimensional outlines (unit: mm)

## A13331-3-1 (3 mm diameter) For mass spectrum



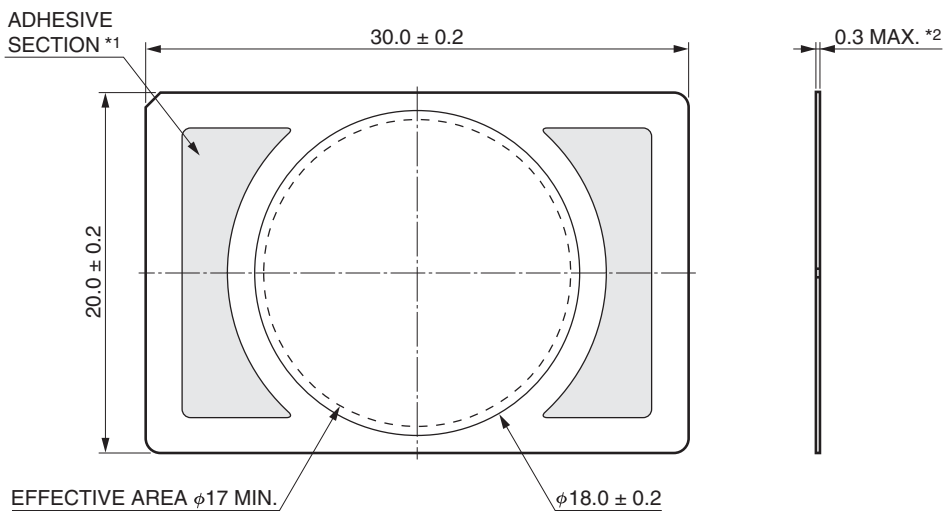
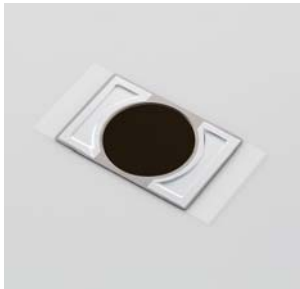
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## A14111-3-1 (3 mm diameter × 9 ch) For mass spectrum



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## A13331-18-1 (18 mm diameter) For imaging mass spectrometry



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