

# User interview 01

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Designated Assistant Professor

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DIUTHAME makes us think,  
"Anyway, let's give it a try."

You just place it on the sample.  
Really that's all you do,  
just place it!

Dr. Kuwata is conducting sample measurement and analysis while maintaining and operating mass spectrometers and other analyzers at Nagoya University.

Our special thanks for sharing part of your valuable time with us today.

Well, let's get started with the interview. Tell us, Professor, exactly what kind of research and work are you doing?

I work as chief coordinator at the Molecular Structure Center which is one of the ITbM research centers.

At the Molecular Structure Center we maintain and operate large analytical instruments including state-of-the-art mass spectrometers. As one research goal of ITbM, we dedicate ourselves to daily intensive work in creating "transformative bio-molecules" that truly have great ripple effects throughout fields relating to chemistry and biology.

On the other hand, our large analytical instruments are available to outside institutions for doing joint research with a focus on proteomics. More specifically, each year we accept more than 300 requests for measurements and analysis including pretreatment as well as requests exceeding 2,000 samples. We are constantly aware of the need to return measurement results having a high success rate in response to those requests. Along with this type of joint research, we are actively doing product development with a large range of companies and also making efforts to improve equipment performance for better research results.

Well then, do you make any special use of particular animals or samples in researching a designated field of application?

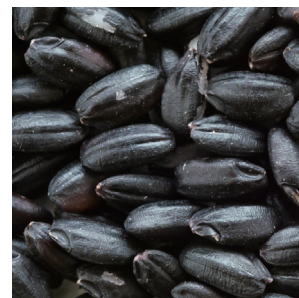
Making effective use of the equipment is at the top of our list, so we have no particular restrictions. A diverse range of plant and animal samples are shipped to us from both Japan and overseas.

Measurements of mouse brain tissue were ultimately the most numerous among recent measurements using DIUTHAME, but prior to using mouse brains we made measurements with foodstuffs such as black rice which is cheap and easy to obtain. I would like to start using DIUTHAME in the field of botany from here onward.

Frozen mouse brain



Black rice



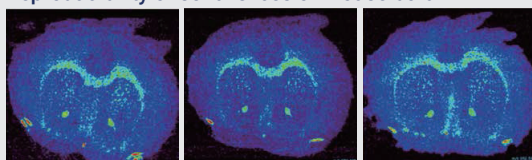
You have used DIUTHAME most often in measurements of mouse brains. Well, if you have any impressions about trying out DIUTHAME in measurements of such animal tissues, would you please tell us about them?

Using DIUTHAME along with animal tissue samples is truly the best possible pairing. I think this is the best possible combination among all samples we have dealt with.

In imaging mass spectrometry, it is often necessary to use organic solvent in order to extract compounds from a sample.

However, if the sample is wet material such as a tissue section and you are using DIUTHAME, the compound will rise to the surface of the DIUTHAME substrate due to capillary action, so the compound extraction by organic solvent is not needed. Besides that, since there is no spraying of the matrix, resolution is not determined by crystal size and, in the case of animal tissue sections, the apparent spatial position resolution seemed to be higher than when using a matrix for imaging mass spectrometry. Other benefits are that this setup process is not complicated and the reproducibility is good.

#### Reproducibility of serial slices of mouse brain



First time

Second time

Third time

near  $m/z$  850

Mouse brain slice thickness: 50  $\mu\text{m}$

Laser pitch: 50  $\mu\text{m}$

As can be seen from the above images, there are hardly any changes in the even after repeated measurements. The high reproducibility is one of the great features that DIUTHAME offers.

#### Did you try imaging mass spectrometry before finding out about DIUTHAME?

We did attempt it, but this is in fact quite difficult for an amateur. I wanted to do imaging mass spectrometry before I even heard about DIUTHAME. But there were some really tough technical issues to overcome. In 2013, we got someone to lend us a sprayer for a week and tried using it to detect trace amounts of hormone in the occipital region (back of the head) of a quail. However, either the amount of hormone was too tiny or our pretreatment was wrong, so we failed to detect it. After returning the sprayer, I tried using an air brush by hand but I couldn't find the time to do it, and this work got shelved for some two years.

When the number of staff at the Molecular Structure Center increased, we took an opportunity to try again imaging mass spectrometry. We already knew that spraying the matrix would be a tough task, so we first tried detecting compounds by using LDI (laser desorption/ionization). A variety of compounds can be viewed with LDI, but this is a hard ionization method where fragmentation tends to occur and there are compounds that cannot be detected. So we tried coating the matrix. As expected this turned out to be difficult and that was when DIUTHAME appeared on the scene.

So the period where you found out about DIUTHAME was also the period when evaluating use of imaging mass spectrometry, right?

Yes, in November 2016, I found out about DIUTHAME at a conference on mass spectrometry being held at the Hamamatsu Photonics Toyooka Factory. At first I didn't even know how to pronounce the name DIUTHAME.

At the time, I was mainly using LDI as an approach for attempting imaging mass spectrometry, so I was interested in DIUTHAME, yet rather than just assessing imaging techniques, I just felt drawn to it because measurements were virtually matrix-free.

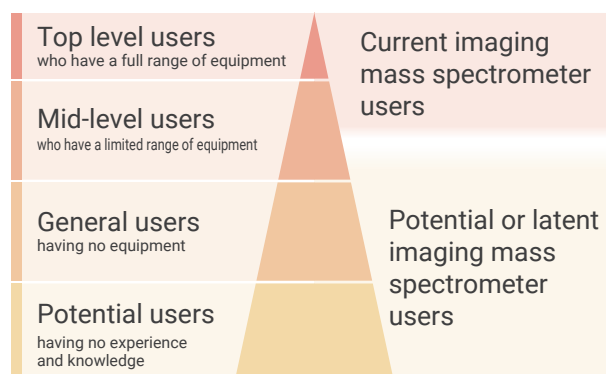
Measuring a wide variety of compounds using MALDI mostly required a lot of screening of matrices. I thought if we could eliminate that step, then things would go a lot smoother, so I asked for a sample of DIUTHAME to try using it.

#### How did things go when you actually tried using DIUTHAME as a mass spectrometer user?

I was surprised how easy it was to make measurements. I think there are a lot of people like me who want to try imaging mass spectrometry yet at the same time almost all of them think it will be really difficult.

Mass spectrometer users include an extremely vast range of persons. For example, only an extremely small portion of the industry's top users possess imaging mass spectrometers and peripheral devices. Ranking somewhat below them are users who, like us, might have a MALDI-TOF mass spectrometer yet don't have dedicated devices or sprayers for imaging mass spectrometry.

Still farther down the list is a huge group that doesn't really understand mass spectrometry but wants to try it even though they don't have any equipment. In that respect, there is a considerably large group of potential users who don't have access to equipment even though interested in imaging mass spectrometry.



I think the easiness and handiness of DIUTHAME will have great appeal in particular for general users.

When a user wants to try some imaging, he or she will have to consider buying a matrix vapor deposition or spray device proposed by equipment manufacturers that cost roughly between 3 million and 10 million yen. Will a general user really say, "Yes, I'll buy it" when offered that high-priced device? Considering the risk that we don't know if the research will succeed or not, most people will feel reluctant to invest that huge amount of money. This isn't surprising because for example if 10 million yen were available, you could buy a quadrupole LC mass spectrometer. Purchasing the LC mass spectrometer would probably make sense since it is generally considered useful for laboratory work.

So you bought the cheap air brush for 30,000 to 40,000 yen but it turned out to be a lot of trouble. Later on when you read the literature, you found out that it's better to use a vapor deposition device before airbrushing in order to obtain a satisfactory image. That was when you thought "What the ...!" and started realizing that in some circumstances the results won't make much sense unless you talk to staff at a lab specializing in mass spectrometry. In our case, we were really fortunate that there was a kind professor specializing in imaging mass spectrometry who told us what to do, so we were broadly able to make the measurements we needed. However, I wondered how many users gave up along the way before being able to make measurements. Also, how many users gave up even before reaching the evaluation stage?

So users like us first thought of DIUTHAME as an "anyway, let's give it a try" kind of product. If a wet sample is available, all you have to do is place the DIUTHAME substrate on the sample while being careful not to break the film that is only a few microns thick. When they are in close contact, measurement setup is complete. Depending on the actual DIUTHAME price, many users probably want to try it if not so expensive. How many MALDI mass spectrometers are there throughout Japan, a few hundred maybe? I don't have any detailed information, but if someone says, "you can easily make measurements using DIUTHAME" then an amazing number of users wanting to try it would appear.

**DIUTHAME appearing on the scene makes imaging mass spectrometry more accessible to others. This may mean that more non-expert users will discover that, "Wow, I can get these kind of images!" without a detailed knowledge of mass spectrometers.**

**DIUTHAME has the potential to help those non-expert users carry out their research, doesn't it?**

Yes, that is true. The latent user group spans quite a wide range I think, and I hope more people start using it.

DIUTHAME is ranked as one type of SALDI mass spectrometry which means that fragmentation tends to occur more easily than in MALDI mass spectrometry. Therefore, some materials are viewable while others are not. So if you know the compound you want to detect, then I think a good place to start is to use DIUTHAME before imaging to confirm that your compound alone can be detected.

Also, on samples such as dried food products that require organic solvent extraction, the ideal solvent composition and dropping amount must be determined, so I think in those cases DIUTHAME might not be handy compared to using samples such as animal tissue slices where "only placing" is needed. On the other hand, DIUTHAME offers high reproducibility making it easy to apply for measurements where differences between samples are compared, and this helps make research interesting. Whatever the case, DIUTHAME will be a handy and powerful tool for research work.

**Yes, that's true. We would like as many users as possible to experience the powerful features of DIUTHAME.**

**Are there any impressive episodes you can call to mind that show the benefits of DIUTHAME?**

Previously when I was making measurements using a particular matrix on MALDI, the problem of "stripes" appearing in the image occurred. But when using DIUTHAME, no such "stripes" occurred.

At the time we had no idea of the cause for these stripes which plagued us for several months. The problem eventually turned out to be extremely simple or namely poor electrical conduction. The fact that poor electrical conduction disrupts the acquired image is described in many textbooks, but at the time it was a blind spot that we simply didn't understand. When we at last understood the problem, we thought, "Wow, this problem was actually such a simple thing!" yet at the same time felt, "DIUTHAME is handy to have around!"



**There may be a lot of users who have to face those simple problems, right?**

I'm not sure. Persons who work in labs specializing in imaging mass spectrometry and people who have taken training courses may find these problems solvable by simple common sense. But, persons like us, who start out with the idea of measuring something just because they have the equipment, have a high probability of running into a brick wall because we missed something that an expert would consider obvious.

When measuring with a MALDI mass spectrometer, besides the "stripe" problem I mentioned earlier, we found that when coating the matrix, it might be better to set the matrix upright and coat it rather than lean the slide glass sideways against something at an oblique angle to coat it. If placed sideways the coating might become uneven or lumpy. These small skills aren't learned right away but may come to be known after gradually becoming aware of irregular data or trends obtained in measurements that are carried out over several months.

**In view of all these facts, as you might expect, we would like to see as many users as possible trying out DIUTHAME.**

**As our final question here we ask, what products will appear from here onward and what applications do you see for DIUTHAME in the future?**

First of all, I would like to see the name DIUTHAME spread far and wide in the mass spectrometry field.

This is a rather ordinary wish but I would like the currently used 18 mm diameter DIUTHAME made available in a larger size, for example, big enough to hold one zebrafish that we often use in research.

In other words, it would be nice to have DIUTHAME available in various sizes to match the sample the user wants to measure. This could for example be a size allowing arranging serial sections of a sample. I'd be happy if I had a large size that covers serial sections. Using serial sections would likely allow taking a simulated 3D image.





As far as applications go, there are many botanists working along with us, so I'd like to do research on imaging of plants as well as animals.

Research is less likely to link to results unless we see phenomena having biological significance, so I think it is important for us as users to determine what we want to observe or measure using DIUTHAME, regardless of whether using plants or animals.

**We gained some great insights from hearing your views.**

**Thanks very much.**