Retraction for Analyst:

Retracted article: Air Flow-Assisted Ionization Imaging Mass Spectrometry: Design and Performance of a New Imaging Mass Spectrometry Method

Yi Chen, Fei Tang, Jiuming He, Zhigang Luo, Xiaohao Wang and Zeper Abliz


We the authors Yi Chen, Fei Tang, Jiuming He, Zhigang Luo, Xiaohao Wang and Zeper Abliz hereby wholly retract this Analyst article. This article contains an optimization of a key factor which improves the sensitivity of the reported method significantly, however recent experiments have shown that this improvement is not repeatable. Though the method developed is successful and the major part is repeatable, we still haven't sufficient confidence in the results of the optimization. Therefore this Analyst article is being retracted in order to maintain the accuracy of the scientific record.

Signed Yi Chen, Fei Tang, Xiaohao Wang, Tsinghua University, Beijing, P.R. China, Jiuming H, Zhigang Luo and Zeper Abliz, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, P.R. China, November 2012.

This retraction is endorsed by May Copsey, Editor. Retraction published 15 November 2012.
This is an Accepted Manuscript, which has been through the RSC Publishing peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, which is prior to technical editing, formatting and proof reading. This free service from RSC Publishing allows authors to make their results available to the community, in citable form, before publication of the edited article. This Accepted Manuscript will be replaced by the edited and formatted Advance Article as soon as this is available.

To cite this manuscript please use its permanent Digital Object Identifier (DOI®), which is identical for all formats of publication.

More information about Accepted Manuscripts can be found in the Information for Authors.

Please note that technical editing may introduce minor changes to the text and/or graphics contained in the manuscript submitted by the author(s) which may alter content, and that the standard Terms & Conditions and the ethical guidelines that apply to the journal are still applicable. In no event shall the RSC be held responsible for any errors or omissions in these Accepted Manuscript manuscripts or any consequences arising from the use of any information contained in them.

Yi Chen*, Fei Tang*, Jiuming He, Zhigang Luo, Xiaohao Wang and Zeper Abliz

Introduction

The imaging mass spectrometry (IMS) technology is a molecular imaging technique which has experienced a rapid development in recent years. It features high chemical specificity, parallel detection, and microscopic imaging capabilities. The IMS image contains information of hundreds or even thousands of different molecules (atoms) in the samples, and both molecular and spatial information can be obtained by analysing a single sample.1,4

IMS generally includes four steps: preparation of samples, mass spectrometry scanning, quality analysis, and data processing.5 The important factors affecting the quality of imaging are the sensitivity of ionisation, preparation of samples, spatial resolution, and speed of sampling. Significant progress has been made in the past 20 years in IMS techniques based on a variety of ion sources including secondary ion mass spectrometry (SIMS), matrix-assisted laser desorption ionization (MALDI), desorption electrospray ionization (DESI), etc.6–12

In particular, the DESI-IMS technique has enabled imaging to be carried out in the ambient conditions and has greatly increased the application scope and applicability of IMS.13 Thus, it is one of the most important and popular IMS technology. But in comparison with other techniques, such as MALDI-IMS, the sensitivity and the resolution of DESI-IMS still need to be improved.

MALDI-IMS, where a laser is used to complete sample desorption and ionisation, features very high spatial resolution.14 Since its introduction in the late 1990s, MALDI-IMS has witnessed a phenomenal expansion.15 However, as its ionisation occurs in the vacuum environment, the imaging area of a sample is limited due to geometric constraints of the MS instrument.16–18

The AFAI-IMS technology described in this paper uses air flow assisted ionization (AFAI).19 AFAI is an ambient ionization method which works in the ambient conditions, as other ambient ion sources do. The adoption of assisted airflow improves the sensitivity of the IMS and increases the resolution of this imaging technique as well. In this paper, AFAI-IMS is used for the imaging of one sample with a large area in a single measurement. The area of the sample is 150 × 100 mm².

Experimental set-up

The design of the AFAI-IMS is shown in Fig. 1. It includes the following sections: AFAI source, high-precision imaging platform, synchronisation circuit of mass spectrometer, and data processing program.
Air Flow Assisted Ionization (AFAI) is a new ambient ion source. As shown in Fig. 1, it includes a stainless steel ion transport tube (with an internal diameter of 3 mm, an external diameter of 4 mm, and different lengths, typically 500 mm), a homemade PMMA refluence tube (with an internal diameter of 16 mm and a length of 60 mm, connected to the mass spectrometer), and a pump (modified from a vacuum cleaner, Kardv, GS-1232, 1300 W, Beijing, China).

The AFAI source has an ESI nozzle through which a charged solvent is sprayed on the surface of the sample at a high speed to complete the process of desorption and ionisation. The pump helps to form suction in front of the ion transport tube. After the ionization, the ions are sucked into the ion transport tube. Following the airflow, the ions and other constituents move toward the mass spectrometer orifice. When the samples reach the orifice, the solvent and uncharged molecules are driven by the airflow and move further toward the air outlet, while the ions of the sample move through the orifice into the mass spectrometer under the impact of an electric field.

The ESI sprayer generates the initial charged droplets for the desorption. In this paper, the spray gas was N₂, with a flow rate of 2 l min⁻¹, and a spray voltage of 4,000 V. The spray solution was prepared by mixing methanol and water (4:1, v/v) with 0.1% formic acid, and was delivered to the sprayer by an Agilent LC pump at a flow rate of 10 µl min⁻¹.

The mass spectrometry synchronisation circuit ensures that the mass spectrometer begins simultaneously with the sample scanning, so that each pixel in the imaging map corresponds to the sample. It makes use of the communication port between the mass spectrometer and liquid chromatograph.

The major portion of the synchronisation circuit is a solid-state relay, whose output (2-pin) is connected to the communication port of the mass spectrometer. When the output of the solid-state relay is connected, the communications port of the mass spectrometer receives a short-circuit signal, and the mass spectrometer starts.

Sample preparation

The experiments use three kinds of samples. The first sample which is shown in Fig. 2a is three red stripes (each 3-mm wide). The stripes are printed on glossy photographic paper with an inkjet printer. And the ink was Rhodamine B (m/z 443). This sample was designed for complete the optimisation of AFAI-IMS.

The second sample is shown in Fig. 7a. It is a 150 × 100 mm glass slide with the letter “M” handwritten twice in red Rhodamine B on the left hand side, and the letter “S” handwritten twice in Basic Blue 7 (m/z 478) on the right hand side. This sample is used to compare the processing time of different modes.

The whole-body section of rats (weighing 148 to 170 g) was used to complete the AFAI-IMS experiment of drugs and metabolites. The target drug is antitumor candidate drug S(+)-Deoxycytiduridine (CAT), which was obtained from Prof. Shishan Yu (Institute of Materia Medica, Chinese Academy of Medical Sciences). CAT was prepared as a 3 mg ml⁻¹ aqueous solution in 0.9% NaCl. The rat was administered 10 mg kg⁻¹ CAT via the tails. After 20 minutes, the rats were euthanised with ether overdose and then frozen entirely in dry ice/isopentane and prepared for slicing. A Leica CM3600 cryomicrotome was used to complete the AFAI-IMS experiment.
used to slice the rats, obtaining 20-µm thick rat body tissue sections. The size of tissue sections was 150 × 50 mm, and they were stuck together on a 150 × 100 mm glass slide; the AFAI-IMS system was then used to scan the whole sample.

Sample scanning

A high-precision platform is used to move the samples and complete the sample scanning of AFAI-IMS. It includes two horizontal high-precision electric stages (the X-direction electric stage with a maximum distance of 150 mm and the Y-direction stage with a maximum distance of 100 mm; Beijing Optical Instrument Factory, Beijing, China), a vertical manual stage (with a maximum distance of 4 mm; Beijing Optical Instrument Factory, Beijing, China), controller, homemade levelling device, and installation rack.

There are two modes for sample scanning: linear scanning and point scanning. For both modes, the ESI needle is located in a fixed position above the sample, and the ion transport tube is fixed to form a certain angle with the needle. The X-Y direction stages move the sample, and the ESI needle completes the ionisation of the sample during the process of movement. Under linear scanning mode, the sample is divided into multiple lines, and each scanning completes the data acquisition of one line; the scanning process is continuous. Under point scanning mode, the sample is also divided into multiple lines, and then each line is subdivided into multiple points.

Data processing program

The data of the AFAI-IMS can be collected in two modes: Full Scan mode and Multiple Reaction Monitoring (MRM) mode.

In Full Scan mode, the data of all mass/charge (m/z) ratios within the capacity of the mass spectrometer is collected, obtaining their ion intensity. Each pixel contains the ion intensity of all mass/charge ratios; when an IMS image includes a lot of pixels, the final IMS data set is usually large. In this mode, the data set for each m/z is extracted separately by the data processing program and is placed in the pixel point in one-to-one correspondence so that the IMS image of each m/z is formed.

The IMS image of all values of m/z can be superimposed on one image; this imaging map will distinguish between the m/z (composition of the sample) in accordance with different colours, and the degree of one colour signifies the content level of such m/z.

The MRM mode is utilised to collect the data of only one particular mass/charge ratio; in this mode, the data quantity of imaging will be greatly reduced, and is often only a few thousandths of the data quantity of Full Scan mode, which reduces the complexity of data analysis and shortens the duration of imaging.

The data processing program can also conduct some numerical analyses of IMS images, such as the sum, maximum/minimum values, range, average, and so on.

Results and discussion

Distance between the ion transport tube and orifice

Since AFAI enhances the desolvation process of samples, accelerate the formation of ions, and even avoid the formation of fragment ions to a certain extent, it features remote ionisation and analysis of samples at a high sensitivity.

However, the introduction of assistant airflow causes a serious problem. Due to the airflow, solvents, dust and fragments of samples, especially ablated pieces of tissue in animal slice imaging, might be sucked into the ion transport tube and move to the orifice together with the ions to be detected. Some ablated pieces of tissue may be even larger than the orifice. If the centre of the ion transport tube is directly targeted at the orifice, this part of the large material is possible to be injected into the orifice and blocks it, resulting in greatly deteriorated MS signals. This problem is particularly evident in animal tissue section imaging.

To solve this problem, a distance between the centre of the ion transport tube and the orifice must be kept during the imaging process. The ion transport tube and the orifice are both electrified to a certain level to form a gradient electric field. In this design, the ions are forced to move toward the orifice under the gradient of the electric field, and the dust, solvents, and fragments of sample will not be injected into the orifice; thus, the problem of a blocked orifice is avoided.

Due to the limited role of the gradient electric field, if the centre of the ion transport tube is too far from the orifice, it will reduce the sensitivity of the system. Therefore, the distance between the ion transport tube and the MS orifice needs to be optimized to ensure high detection sensitivity of the present imaging technique and avoidance of introduction of unwanted wastes.

Fig. 2 Experiment on the distance between the centre of the ion transport tube and orifice. The sample is shown in (A) and the distance between the centre of the ion transport tube and the orifice is shown in (B).

The distance between the centre of the ion transport tube and the orifice is d, with a negative value on the up side and a positive value on the down side. The orifice is loaded with a voltage of 800 V, the ion transport tube with a DC voltage of 1,200 V, and the ion spray needle with a voltage of 5,000 V. The voltage gradient ensures efficient transmission of ions into the MS. As shown in Figure 2, the ion transport tube forms a 15° angle to the orifice. Using the experimental setup in Figure 2, the parameter d is optimized and the results are shown in Figure 3.
In Fig. 3b, the centre of the ion transport tube is placed so that it is in line with the orifice ($d = 0$). However, in this configuration the intensity of ions detected by MS is low, resulting in poor imaging quality. The best distance $d$ is 3 mm, under which conditions the intensities of ions detected are the highest. As $d$ was further increased ($d = 4$ or 5 mm), the intensity decreases significantly. Therefore, $d = 3$ mm was selected as the best distance in subsequent imaging analysis.

**Flow rate of the assistant airflow**

As discussed above, the air flow is also important for imaging quality. The relationship between the flow rate of air and the corresponding intensity of ions is shown in Fig. 4. Rhodamine B printed as stripes were used as the analytes for flow rate optimization.

![Graph](image)

**Fig. 3** Experimental results of different distances between the ion transport tube and orifice. (A) Mass spectrometry analysis under different distances ($m/z$ 443); (B) Relationship between total intensity and distance after integrating each peak area.

![Graph](image)

**Fig. 4** Experimental results of different flow rates of assistant air. (A) Analysis spectra under different airflow rates ($m/z$ 443); (B) Relationship between total intensity of ions and rates.

From Figure 4, it can be seen that when the airflow rate is in the range of 5–45 L min$^{-1}$, as the flow rate increases, the intensity increases as well, which suggests that higher flow rate of air will increase the detection sensitivity of this method and will be especially useful in the imaging of complex and large samples. Therefore, in the imaging study followed, an air flow rate of 45 L min$^{-1}$ was used.

**AFAI-IMS of drugs and metabolites in rats’ whole-body sections**

After optimisation, the sensitivity of the AFAI-IMS is improved greatly, and the IMS images of rats’ whole-body section are obtained successfully.

Linear scanning mode was adopted during imaging, which is continuous.

In consideration of the minimum analysis time of mass spectrometry and minimum speed of the high-precision imaging platform, a pixel size of 200 × 200 µm was selected. The total analysis time was approximately 10 hours, and most of the time was being spent on tissue scanning.

An MRM of parent/daughter transitions in positive ionisation mode is used, which is convenient to track the CAT in the entire rat body. The target $m/z$ values are 364.2. A component of metabolites ($m/z$ 70, which has been detected in blood plasma) is used as a benchmark to obtain the target intensity, that is, the relative intensity of ions of the target drug CAT, so as to complete the mass spectrometry sampling and analysis. Based on
the metabolic pathway study on CAT, another transition (m/z 350.2→70.0) was monitored in the same experiment for its main demethylated metabolite (M1), which, due to few metabolites in the tissue sections 20 min after CAT administration, did not provide a signal above background.22

A homemade data processing program is used for the processing of acquired data, in order to generate IMS images of the whole-body rat tissue section. The IMS image of CAT (m/z 364.2→70.0) is shown in Fig. 5.

![IMS image of CAT](image)

Fig. 5 Optical and AFA-I-IMS image of a rat’s whole-body tissue section (target drug: CAT)

As shown in Fig. 5, from the IMS image in the bottom, it can be seen that the CAT is basically concentrated in the back and hindbrain of the rat.

The intuitive information of the target analyte’s concentration and distribution can be obtained by analysing the IMS images directly. But more accurate, reliable data and conclusions can be obtained by numerical analysis, which is important for drug analysis, diagnosis, and so on. Figure 6 shows the sum and average analysis of the AFA-I-IMS image shown in Fig. 5.

![Numerical analysis](image)

Fig. 6 A data processing program is used for numerical analysis of AFA-I-IMS images. (A) Regions to be analysed; (B) Sum and average of the intensity of respective regions.

Processing time of different modes

The data processing program of AFA-I-IMS is based on Matlab and can operate in two modes (full scan mode and MRM mode); correspondingly, there are two algorithms for analysis and imaging of the sample. In order to determine the difference in the time taken for data analysis in the two modes, different parts of the sample were used for experimentation. The sample is shown in Fig. 7a. The slide is analysed in full scan and MRM modes to obtain the mass spectrometry data. In the data processing program, a clock is set up to obtain the time needed to complete the AFA-I-IMS image in the two modes.
Under full scan mode, additional data screening is required, so the data processing time is much longer compared to the MRM mode, as can also be easily seen in Fig. 7d. In addition, the full scan mode provides the ion intensities of all of the mass/charge ratios, so there is a large amount of data; as the sample size increases, the amount of data will increase further, which will undoubtedly place a burden on data processing programs, making imaging times longer and longer.

It can be seen in Fig. 7d that when the sample size increases, the analysis times of the two modes both increase; yet as the MRM mode provides a smaller amount of data, its analysis time does not vary too much, while the full scan mode witnesses a much faster growth of analysis time.

A comparison of the time of two modes, shown in the Fig. 7d, is offered to illustrate a point that the two modes are complementary and can satisfy the demand of different application fields. In full scan mode, IMS results of all mass/charge ratios can be obtained. The data of this mode are comprehensive, but the processing time is long and data processing is difficult, so it is suitable for pathological diagnosis and other situations in which the mass/charge ratio of the target sample is unknown. The MRM mode allows the data of some specific mass/charge ratios to be obtained, is more sensitive, and requires less processing time and simpler data processing, so it is suitable for situations in which the m/z of the target sample is known, such as drug analysis.

Conclusions

In summary, we have developed a new imaging MS technique AFAI-IMS based on the AFAI source and applied it to the high-resolution imaging of whole-body rat tissue section. Since the introduced air flow enhances sample ionization, the detection sensitivity is increased, which enables the method very suitable for the analysis of complex samples.

Another feature is that our method can be used for the imaging of very large samples. In this study, a rat tissue with an area of $150 \times 100 \text{ mm}^2$ has been successfully imaged at the resolution of $200 \times 200 \text{ mm}$. Meanwhile, we also developed a data processing program to process the data acquired using AFAI-IMS, which can be further used in numerical analysis.

We believe that the AFAI-IMS system has enormous potentials in the MS imaging of large and complicated samples in the field of drug analysis, and pathological diagnosis, etc.

Acknowledgements

This work is financially supported by the National Natural Science Foundation of China (No. 21027013 and No. 81102413) and the Special-funded Programme on National Key Scientific Instruments and Equipment Development (No. 2011YQ17006702).

References