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PAPER IN FOREFRONT

Field-based ion generation from microscale emitters on natural and artificial objects for atmospheric pressure mass spectrometry

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Abstract Field-based ion generation is described for ambient mass spectrometry. The technique allows the analysis of endogenously expressed chemicals and exogenously applied compounds directly from the cuticle of live insects in real time. Cuticular hairs serve as electric field-enhancing structures and play a key role in ion generation. Artificial emitters such as graphite whiskers or sharp metal tips replicate this effect.

Keywords Ions · Field-based ionization · *Drosophila* · Mass spectrometry · Microstructured emitter

Abbreviations

AP	Atmospheric pressure
APCI	Atmospheric pressure chemical ionization
DART	Direct analysis in real time
DESI	Desorption electrospray ionization
ESI	Electrospray ionization
FBIG	Field-based ion generation
FD/FI	Field desorption/field ionization
HC	Hydrocarbon
HC1	*Z*-11-Hexadecenyl acetate
HC2	*Z*-11-Hexadecen-1-ol
HC3	*Z*-11-Hexadecenal
IR	Infrared
IT	Ion trap
MALDI	Matrix-assisted laser desorption/ionization
MS	Mass spectrometry
PESI	Probe electrospray ionization
Q-TOF	Quadrupole time-of-flight

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Introduction

Mass spectrometry (MS) is widely used in many areas of the natural and life sciences as an analytical tool. Recently, a number of ambient ionization methods have been developed that allow open-air analysis of intact specimens and require only minimal sample preparation. Techniques such as desorption electrospray ionization [1, 2] and direct analysis in real time (DART) [3, 4] are particularly useful for the investigation of organisms, e.g., insects, since both allow living objects to be analyzed [5, 6].

Using DART–MS, hydrocarbons (HCs), several of which serve as communication cues, could be profiled from the cuticular surface of living fruit flies [7]. To that end, the insect body was probed with a metal pin which was then introduced into the DART ion source. Changes in cuticular HC expression were observed in the same animal before and after social interaction. However, direct analysis of the fly in the ion source was not possible using this method due to the harsh experimental conditions. To investigate whether it might be possible to analyze cuticular

HCs from living insects by scanning the cuticle with a laser, we used an atmospheric pressure infrared matrix-assisted laser desorption/ionization (AP–IR–MALDI) source in conjunction with an ion trap (IT) mass spectrometer [8]. Remarkably, ions were generated from the insects solely under the influence of the electric field generated between the MALDI sample plate, in which the animals were mounted, and the counter electrode on the instrument. We currently explore this effect in more detail [9, 10] as is explained below, but we wish to disseminate the information quickly so that more scientists can take advantage of its high analytical potential. In order to distinguish the observation from traditional field desorption/field ionization (FD/FI), which is carried out at much higher voltages in vacuum, we refer to this observation using the term field-based ion generation (FBIG).

Experimental

CantonS *Drosophila melanogaster* were raised on autoclaved yeast–sucrose–agar food at 25 °C. For preparation of samples, fruit flies were anesthetized by brief exposure to cold. Using fine forceps, individual flies were taped to the respective holder using double-sided stickers (G304, Plano Wetzlar, Germany). Mated and unmated female flies were not differentiated. Dead flies or flies which had been stored for several days at −20 °C could also be used.

FBIG–MS (originally termed FLIE–MS for fly ion emission [11]) was performed with a quadrupole ion trap $Esquire_{3000}$ (Bruker Daltonics, Bremen, Germany) equipped with interchangeable electrospray (ESI) and AP–MALDI (MassTech Inc., Burtonsville, MD, USA; Agilent, Waldbronn, Germany) ion sources and a Q-TOF Premier instrument with a Z-spray ESI source (Waters Corp., Manchester, UK). For the IT experiments using the AP–MALDI source, instrumental parameters were essentially adopted from settings for UV–MALDI–MS except for the ion charge control acquisition time (200 ms). The UV laser was kept in standby to allow the use of the target control software for positioning of the flies. Sample observation in real time was possible via the standard CCD camera. A voltage of −2.5 to −4 kV was applied to the entrance capillary that also served as counter electrode for ion extraction, while the sample support was held at ground potential. Conductivity of the holder was not critical, and measurement was also possible when the flies were fixed to glass slides. For fixation of the flies, stainless steel targets were milled ~1 mm in depth to hold the flies directly or microscope slides, which were taped to the metal using conductive stickers (G3357, Plano). When dissected body parts were investigated, the standard Agilent gold-coated sample plate was used. The signal intensity depended on the position of the flies in front of the capillary, and spectra were taken at locations of maximum signal intensity. In order to enable greater precision in the positioning of the fly in three dimensions, the nano-ESI sources of both mass spectrometers were modified. For use of the commercial IT off-line nanoelectrospray source, a syringe needle was cut to a length of 15 mm and used as holder (Fig. 1). The end of the tube held the fly in a number of variations (taped to a small plate, anti-static black conductive Teflon coated fiberglass tape (CSHyde Inc., Lake Villa, IL, USA) or Plano stickers). The distance between body parts of the fly and the counter electrode varied from one to several millimeters. The nanoelectrospray source of the Q-TOF Premier was modified accordingly.

For scanning electron microscopy the fruit flies were mounted on aluminum specimen stubs with electrically conductive carbon (Plano) and subsequently rotary shad-

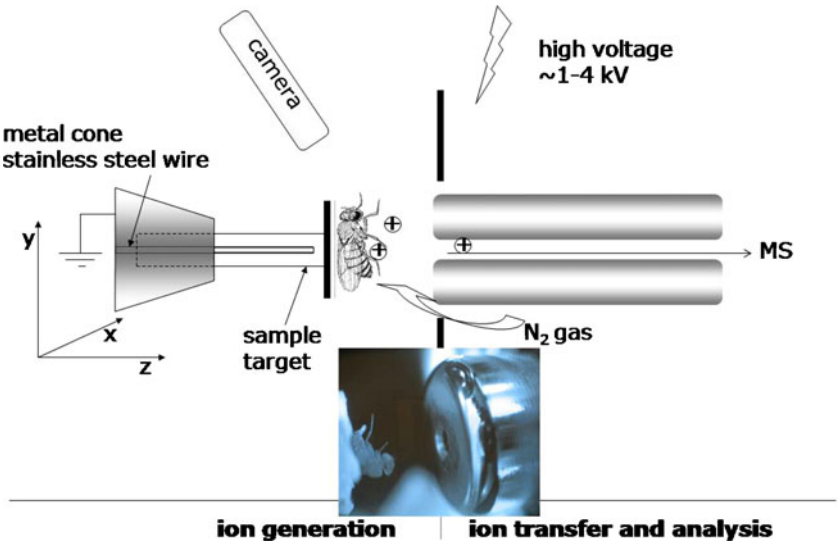


Fig. 1 Schematic of the general design of an FBIG source using an adapted nanospray source [11, 12]. For the investigation of living fruit flies, the insects were taped to a holder and exposed to an electric field maintained between the holder and the entrance capillary of the mass spectrometer. In other experiments, the flies were replaced by microstructured artificial emitters (e.g., a sharp tip or a classical field emitter), allowing the analysis of samples applied to these surfaces. The photograph illustrates the positioning of the sample in front of the ion trap entrance capillary

owed with 3 nm Pt/C at an elevation angle of 65° to obtain sufficient electric conductivity at the surface. Secondary electron micrographs were taken with the "in-lens" type S-5000 high-resolution field-emission scanning electron microscope (Hitachi Ltd., Tokyo, Japan) at 30 °C.

Results and discussion

Modified nanoelectrospray sources of both the ion trap and Q-TOF Premier allowed optimal positioning of the insect in front of the entrance capillary. The general layout is depicted in Fig. 1. An adapter allows the fixation of the fly on its wings while it is exposed to an electrical field. Normally, a stream of nitrogen from the mass spectrometer entrance accompanies measurements on these instruments. FBIG did not require gas flow for ion emission though the gas stream could be used to avoid contamination of the analyzer. When a lower gas temperature of 30–45 °C was used, the animals lived through the length of the measurement. Under such conditions, successive MS interrogation of living insects with intermittent breaks of several hours was possible (Electronic supplementary material Fig. 1).

A representative FBIG mass spectrum obtained from a living female fly using the Q-TOF is shown in Fig. 2. Mass spectra recorded with IT–MS were comparable in terms of the observed ion series (Electronic supplementary material Figs. 1–7). The spectra were highly complex and in some cases exhibited ions up to ~*m/z* 1,800. Ion series were observed that showed 28 u mass differences. Spectra measured in positive ion mode were typically more complex than those recorded in the negative ion mode (Electronic supplementary material Fig. 2b), presumably due to overlapping series of protonated, sodiated and potassiated molecules. Tentative assignment based on the high mass accuracy of Q-TOF mass spectrometer (Electronic supplementary material Table 1) suggested the presence of a series of oxygen-containing HCs, each being successively elongated by C_2H_4 groups, in protonated and sodiated form. Detailed structural analysis of individual endogenous HCs is in progress.

Both living and previously frozen animals could be used for these experiments with similar results. When individual body parts were dissected and measured under FBIG conditions (Electronic supplementary material Fig. 3), we noted that the legs showed signals of considerably greater intensity than other body regions. Scanning electron microscopy revealed the presence of tiny hairs on the fly body, but in particular on the legs (Fig. 3). These hairs were spaced at ~10–25 μm distance, the radius at the tip was ~80 nm, and they were ~25–30 μm in length (Electronic supplementary material Figs. 8 and 9). We hypothesize that the generation of locally high electric field strengths at the sharp tips of the hairs on the insect body plays a key role in ion formation. A number of experiments have been performed to test this as is summarized in Fig. 4. Data are detailed in the Electronic supplementary material. First, the fly body itself should function as emitter of exogenously applied compounds. Synthetic HC standards were directly applied to intact flies and to specimens that had been washed with solvent to remove endogenous HCs. Compounds such as *Z*-11-hexadecenyl acetate (HC1) and *Z*-11-hexadecen-1-ol (HC2) were detected as protonated and alkali-cationized ions (Electronic supplementary material Fig. 10). Moreover, traditional field emitters containing dendrite whiskers or other similarly structured emitters should promote ionization of analytes as well. These experiments were performed applying a phosphazene

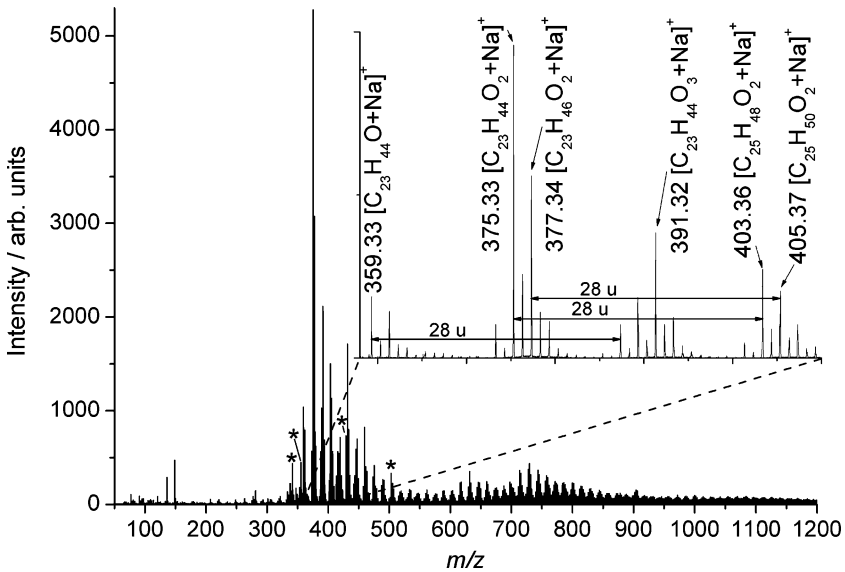


Fig. 2 Q-TOF mass spectrum obtained from a living female fly in positive ion mode. Expanding the area between *m/z* 358–412 (*inset*) visualizes ion series differing by 28 u. Chemical compositions are suggested for selected ions (*inset*: *m/z* 359.3290 $[C_{23}H_{44}O+Na]^+$, 375.3262 $[C_{23}H_{44}O_2+Na]^+$, 377.3400 $[C_{23}H_{46}O_2+Na]^+$, 391.3217 $[C_{23}H_{44}O_3+Na]^+$, 403.3566 $[C_{25}H_{48}O_2+Na]^+$, 405.3671 $[C_{25}H_{50}O_2+Na]^+$; Electronic supplementary material Table 1). Ion signals and series are partially overlapping. Peaks produced from polysiloxane contaminants in the laboratory air are marked with *asterisks*

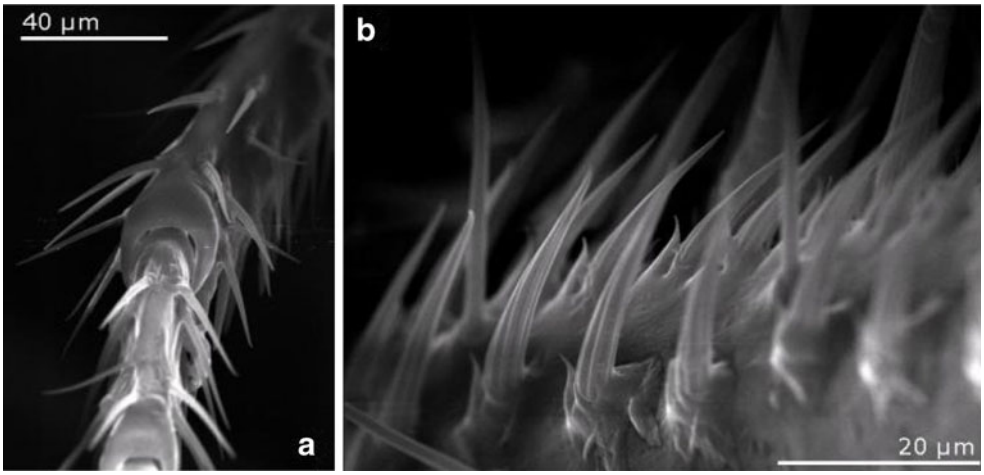


Fig. 3 Scanning electron micrograph images obtained from **a** femoro-tibial joint and **b** a leg of a female fruit fly *D. melanogaster*

reference mixture to commercial FD emitters (Linden CMS, Leeste, Germany [13]; Electronic supplementary material Fig. 11). While these emitters allowed the detection of the prominent ions of this solution, they had the disadvantage of being very fragile and hard to handle.

A more robust emitter would be a single sharp metal tip such as a syringe needle (Electronic supplementary material Fig. 12). When a drop of analyte-containing liquid was placed on its tip, various compounds could be detected, but this particular experiment resembled probe electrospray ionization [14–16], a method that allows ESI measurements from complex samples of peptides, lipids, and oligosaccharides using single sharp emitters like acupuncture needles. Tungsten oxide nanowires have also been used to demonstrate ESI–MS of such biomolecules [17]. These results suggest that the ion generation at ambient conditions using multiple or single-point emitters (like those present in the fly) may contain elements of ESI processes, in particular since the ambient air provides moisture.

A single metal electrode is used in traditional APCI (corona discharge needle). We could show that ions of the oxygen-containing synthetic compounds HC1, HC2, and HC3 (*Z*-11-hexadecenal) can be generated using a syringe needle tip as sample holder (Electronic supplementary material Figs. 10, 12, and 14). The sample could also be placed ~1 mm below the discharge corona created from a clean metal tip, suggesting that volatile compounds were ionized (Electronic supplementary material Fig. 15). Protonated molecules of HC1, HC2, HC3 as well as Nipagin, an aromatic benzoic acid ester that is used as a preservative in fly food, could be detected in this way. Non-polar *Z*-9-tricosene did not produce a signal under those conditions. The faint bluish light of the corona was visible in the above experiments, while it was difficult to assess whether a discharge was produced in FBIG measurements from the intact fly. In general, clean needle tips and to some extent also non-conducting sharp tips cause the ionization of gaseous compounds such as siloxanes from the laboratory air while no discharge is observed (Electronic supplementary material Fig. 16).

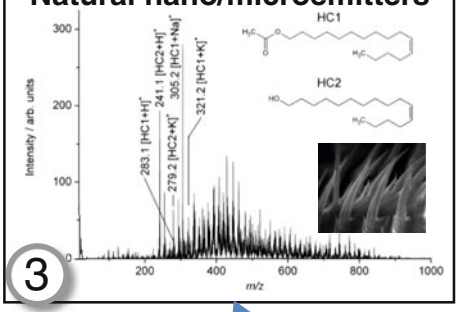
Traditionally, sharp tips, edges, or thin wires are employed to generate electric field strengths sufficient for classical FI and FD [18, 19]. However, these techniques differ considerably from FBIG both in their experimental parameters (use of voltages of 8–12 kV, leading to electric fields of ~10^8 V/cm; ionization in vacuum) and the type of ions detected (intact molecular ions, in exceptions $[M+H]^+$ [20]). While FD/FI is well suited for MS analysis of non-polar molecules, e.g., to determine HCs in crude oil fractions [21], we have not yet detected aliphatic HCs without oxygen with FBIG.

Conclusion

Ions are generated from fruit flies when they are exposed to an electric field. The results indicate that microstructures on the insect body are intrinsic to the process. The geometry of

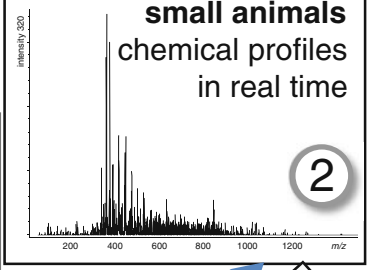
Fig. 4 Overview showing the exploration of FBIG (*1*; Electronic supplementary material Fig. 12) in successive experiments. *2* Following improvement of the ion source with respect to more focused ion collection (small funnel) and control of the source environment (protective gas) behavioral studies on live insects are envisioned. The spectrum was detected after re-measurement of a 3-day-old fly after a 3-h 30-min break (Electronic supplementary material Fig. 1). *3* The use of the insect body as emitter for exogenously applied compounds (HC1, HC2) is possible (Electronic supplementary material Fig. 10a). *4* Similar structures such as traditional field emitters [13] replicate the effect (Electronic supplementary material Fig. 11). *5* Single pointed emitters assist in the investigation of ionization mechanism (Electronic supplementary material Fig. 12). *6* For liquid samples, ESI-type of experiments have been shown. *7* If the applied voltage is high enough to generate a visible corona, APCI-type of experiments are performed. The spectra show the MS/MS of HC2 applied to the needle (Electronic supplementary material Fig. 10) and the MS/MS of the major signal from fly food held ~1 mm under the corona (Electronic supplementary material Fig. 15). *8* Ions can also be observed at comparatively low voltages, e.g., from polysiloxane contaminants present in the laboratory air (Electronic supplementary material Fig. 16)

Natural nano/microemitters



3

MS of live, behaving small animals
chemical profiles in real time



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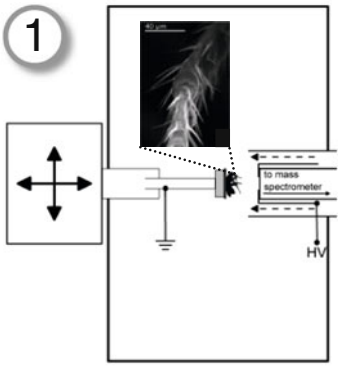
1

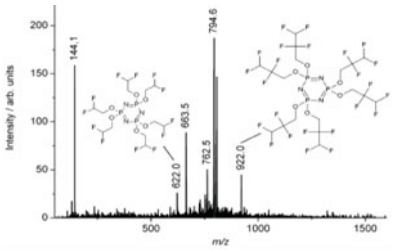


FBIG

Field-based ion generation
- Natural microemitters, 1-4 kV
- Atmospheric pressure
- Optional counter gas
- MS of live insects

Focussed ion collection
Controlled source atmosphere

AP-MS emitters

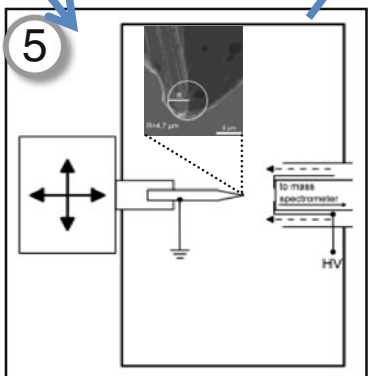


Artificial nano/microemitters
- FD/FI emitters, dendrites
- Tungsten nanowires[17]

Bionic
- New emitter types
- Arrays

4

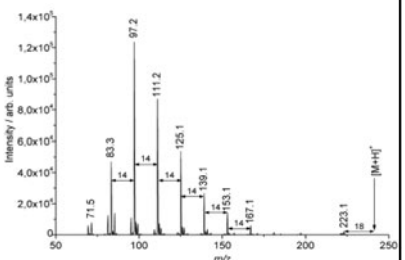
5



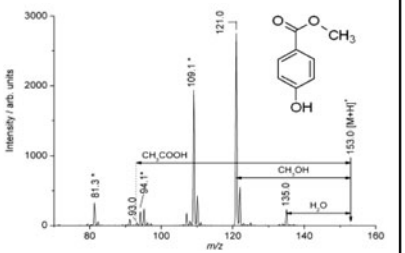
Single sharp tip

Visible corona
- Syringe needle, 2-4 kV
- Volatile compounds
- Desorption?

7



Sample on needle
APCI-like



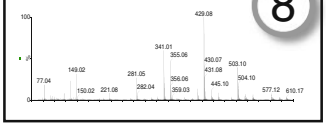
Sample off needle
APCI

PESI [14, 15]
Probe electrospray ionisation
- Acupuncture needles etc.
- Liquid samples
- Tissue profiling [16]

6

No visible corona
0.4-2 kV

8



hairs on fly legs seems to be particularly suited, and it can be replicated by graphite whiskers, tungsten nanowires, or single sharp emitter tips for use in analytical chemistry. The mechanism of FBIG shows features of ESI and APCI and needs to be explored in more detail. It remains to be investigated to which extent FBIG involves true desorption/ionization with transfer of molecules into the gas phase. Different classes of compounds will be analyzed in order to define the specificity of FBIG.

Furthermore, it needs to be explored if gas phase reactions occur in FBIG. Oxidation of saturated HCs has been demonstrated in controlled discharge experiments using voltages >10 kV or a catalyst [22, 23]. So far, we did not find any evidence for such reactions in FBIG, but we note that much fewer oxygen-containing HCs were found when spatially resolved UV–LDI in combination with an orthogonal TOF mass spectrometer was used to map the cuticular HC profile directly from the flies [24].

In the current configuration, the reproducibility from fly to fly was limited in terms of the observed ion patterns and intensities (Electronic supplementary material Figs. 1–7). The strength of individual signals varied with changes of the position of the fly with respect to the entrance capillary of the mass spectrometer—either due to re-positioning of the sample holder by the operator or due to movements of the fly itself. Moreover, the area from which ions are collected is poorly defined. Utilization of miniaturized ion funnels may provide a means to collect ions from a better defined part of the insects (Fig. 4). In addition, providing a controlled gaseous environment in the source will reduce contaminant signals. Such improvements of the ion source with respect to the investigation of small animals will provide a minimal invasive way to monitor the chemical communication of living insects concurrent with behavior.

The phenomenon of FBIG has a number of versatile applications relevant to analytical and material science as well as behavioral biology. It offers an economical and technically simple method for the analysis of oxygen-containing HCs and a number of other small molecules from complex samples using most commercial AP sources.

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