The field of the invention is atmospheric pressure mass spectrometry (MS), and more specifically a process and apparatus which combine infrared laser ablation with electrospray ionization (ESI).
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FIGURE 4B

Leaf

Stem

Root

m/z

Intensity / 1000 (counts)

(1) Na

(1) K

(1) Na

(1) K

m/z

200 400 600 800 1000
LASER ABLATION ELECTROSPRAY IONIZATION (LAESI) FOR ATMOSPHERIC PRESSURE, IN VIVO, AND IMAGING MASS SPECTROMETRY

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation of U.S. application Ser. No. 13/271,435, filed on Oct. 12, 2011, which is a continuation of U.S. application Ser. No. 12/176,324, filed on Jul. 18, 2008, now U.S. Pat. No. 8,067,730, which claims priority to U.S. provisional application Ser. No. 60/951,186, filed on Jul. 20, 2007, each of the foregoing applications are hereby incorporated herein by reference in their entireties.

STATEMENT OF GOVERNMENTAL INTEREST

Portions of this invention were made with United States government support under Grant Nos. 0415521 and 0719232 awarded by the National Science Foundation and Grant No. DE-FG02-01ER15129 awarded by the Department of Energy. The government has certain rights in the invention.

BACKGROUND

The field of the invention is atmospheric pressure mass spectrometry (MS), and more specifically a process and apparatus which combine infrared laser ablation with electrospray ionization (ESI).

Mass spectrometry (MS) plays a major role in chemical, biological and geological research. Proteomic, glycomic, lipidomic and metabolomic studies would be impossible without modern mass spectrometry. Owing to their high sensitivity and exceptional specificity, mass spectrometric methods also appear to be ideal tools for in vivo analysis in the life sciences. In many of these applications, however, the samples must be preserved in their native environment with preferably no or minimal interference from the analysis. For most of the traditional ion sources applied in the biomedical field, such as matrix-assisted laser desorption ionization (MALDI) or electrospray ionization (ESI), these limitations present serious obstacles. For example, MALDI with ultraviolet laser excitation requires the introduction of an external, often denaturing, matrix, whereas ESI calls for liquid samples with moderate ionic conductivity. As living organisms are typically disrupted by such preparations, there is a great interest in developing direct sampling and ambient ionization sources for in vivo studies.

Rapid advances in recent years have provided a growing number of ambient ion sources. For example, atmospheric pressure infrared MALDI (AP MALDI), capable of producing ions from small and moderate size molecules (up to 3,000 Da), shows promise for metabolic imaging. Small molecules have been analyzed by other methods, including direct analysis in real time (DART), desorption electrospray ionization (DESI), desorption atmospheric pressure chemical ionization (DAPCI) and matrix-assisted laser desorption electrospray ionization (MALDESI). Medium to large biomolecules have also been detected by DESI and on dehydrated samples by electrospray laser desorption ionization (ELDI). Imaging capabilities were demonstrated for DESI on a rat brain tissue section with about 400 µm lateral resolution. Due to the need for sample pretreatment, sensitivity to surface properties (DESI, DART, DAPCI and AP IR-MALDI) and external matrix (ELDI and MALDESI), in vivo capabilities are very limited for these techniques.

An awkward feature of mass spectrometry (MS) is the requirement of a vacuum system. Analysis under ambient conditions would simplify and expand the utility of mass spectrometry.

Takats et al. report a method of desorption electrospray ionization (DESI) whereby an aqueous spray of electrosprayed charged droplets and ions of solvent are directed at an analyte which has been deposited on an insulating surface. The microdroplets from the aqueous spray produce ions from the surface whereby the desorbed ions are directed into a mass spectrometer for analysis. A broad spectrum of analytes was examined, including amino acids, drugs, peptides, proteins, and chemical warfare agents.

Cody et al. report a method they called “DART” wherein helium or nitrogen gas is sent through a multi-chambered tube wherein the gas is (i) subjected to an electrical potential, (ii) ions are removed from the gas stream, (iii) the gas flow is heated, and then iv) the gas is directed at a mass spectrometer ion collection opening. They report that subjecting hundreds of different chemicals to this technique provided a very sensitive method for detecting chemicals, including chemical warfare agents and their signatures, pharmaceuticals, metabolites, peptides, oligosaccharides, synthetic organics and organometalllics, drugs, explosives, and toxic chemicals. Further, they report that these chemicals were detected on a wide variety of substrates including concrete, asphalt, skin, currency, airline boarding passes, business cards, fruit, vegetables, spices, beverages, bodily fluids, plastics, plant leaves, glassware, and clothing.

Shieu et al. report the development of a method called electrospray-assisted laser desorption ionization (ELDI). They report that DESI-MS is limited in that it cannot analyze complex mixtures and there is very little control over the size and definition of the surface area affected by the ESI plume for the desorption of the analyte. They also acknowledge the problem that direct laser desorption is limited to low molecular weight compounds and that lasers desorb more neutrals than ions. Accordingly, they report a combination of ESI and ultraviolet laser desorption (LD) wherein (i) a sample is irradiated with a pulsed nitrogen laser beam to generate laser desorbed material, (ii) this material is then ionized by subjecting it to an electro spray plume, and (iii) the ions sent to a mass spectrometer. This technique is reported to provide sensitivity towards protein detection without sample prep or the use of a matrix. However, their experimental setup shows a stainless steel sample plate upon which aqueous solution of protein was spread and the sample dried. The method was ultimately presented for the analysis of solid samples.

Atmospheric pressure laser desorption techniques such as atmospheric pressure matrix-assisted laser desorption ionization (AP MALDI) or electrospray-assisted laser desorption ionization (ELDI) usually require the pretreatment of the sample with a suitable matrix.

Further, it has been difficult previously to study the spatial distribution of chemicals at atmospheric pressure using MS. Lastly, other matrixless methods do not achieve ESI-like ionization. Thus, with other matrixless methods (e.g., DIOS) large molecules cannot be detected as multiply charged species.

The following documents may provide additional context where necessary for fuller understanding of the claimed invention and are incorporated by reference herein in their entirety for references purposes and for determining the level of ordinary skill in the art: U.S. Pat. Nos. 6,949,741 and 7,112,785 by Cody et al.; U.S. Pat. No. 5,965,884 by Laiko et al.; publication on DESI “Mass Spectrometry Sampling Under Ambient Conditions with Desorption Electrospay...

SUMMARY

Mass spectrometric analysis of biomolecules under ambient conditions promises to enable the in vivo investigation of diverse biochemical changes in organisms with high specificity. Here we report on a novel combination of infrared laser ablation with electrospray ionization (LAESI) as an ambient ion source for mass spectrometry. As a result of the interactions between the ablation plume and the spray, LAESI accomplishes electrospray-like ionization. Without any sample preparation or pretreatment, this technique was capable of detecting a variety of molecular classes and size ranges (up to 66 kDa) with a detection limit of about 100 fmol/sample (about 0.1 fmol/ablated spot) and quantitation capability with a four-decade dynamic range. We demonstrated the utility of LAESI in a broad variety of applications ranging from plant biology to clinical analysis. Proteins, lipids, and metabolites were identified, and the pharmacokinetics of antihistamine excretion was followed via the direct analysis of bodily fluids (urine, blood and serum). We also performed in vivo spatial profiling (on leaf, stem and root) of metabolites in a French marigold (Tagetes patula) seedling.

In one preferred embodiment, a process and apparatus which combine infrared laser ablation with electrospray ionization (LAESI) accomplishes electrospray-like ionization. Without any sample preparation or pretreatment, this technique was capable of detecting a variety of molecular classes and size ranges (up to 66 kDa) with a detection limit of about 100 fmol/sample (about 0.1 fmol/ablated spot) and quantitation capability with a four-decade dynamic range. We demonstrated the utility of LAESI in a broad variety of applications ranging from plant biology to clinical analysis. Proteins, lipids, and metabolites were identified, and the pharmacokinetics of antihistamine excretion was followed via the direct analysis of bodily fluids (urine, blood and serum). We also performed in vivo spatial profiling (on leaf, stem and root) of metabolites in a French marigold (Tagetes patula) seedling.

Another preferred embodiment provides a method of directly detecting the components of a sample, comprising: subjecting a sample to infrared LAESI mass spectrometry, wherein the sample is selected from the group consisting of pharmaceuticals, dyes, explosives, narcotics, polymers, tissue samples, and biomolecules, and wherein the LAESI-MS is performed using a LAESI-MS device directly on a sample wherein the sample does not require conventional MS pretreatment and is performed at atmospheric pressure.

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1. Schematics of laser ablation electrospray ionization (LAESI) and fast imaging system (C capillary; SP syringe pump; HV high-voltage power supply; L-N nitrogen laser; M mirrors; FL focusing lenses; CV cuvette; CCD CCD camera with short-distance microscope; CE counter electrode; OSC digital oscilloscope; SH sample holder; L-Er: YAG Er:YAG laser; MS mass spectrometer; PC-1 to PC-3 personal computers). Cone-jet regime is maintained through monitoring the spray current on CE and adjusting the spray parameters. Black dots represent the droplets formed by the electrospray. Their interaction with the particulates and neutrals (red dots) emerging from the laser ablation produces some fused particles (green dots) that are thought to be the basis of the LAESI signal.

FIG. 2. Excretion of the antihistamine fexofenadine (FEX) studied by LAESI mass spectrometry. A 5 μL aliquot of the urine sample collected two hours after administering a Telfast caplet with 120 mg fexofenadine active ingredient was directly analyzed using LAESI-MS. Compared to the reference sample taken before administering the drug, the spectra revealed the presence of some new species (red ovals). Exact mass measurements on dissolved scrapings from a caplet core (see black inset) after drift compensation for reserpine (RES) showed m/z 502.2991 that corresponded to the elemental composition of protonated fexofenadine, [C32H39N04+H]+, with a 7.5 ppm mass accuracy. Analysis of the caplet core by LAESI-MS (black inset) showed fragments of fexofenadine (FEX and FEX) and reserpine (RES) and (2RES). A comparison of the spectra revealed that the other two new species observed in the urine sample were fragments of fexofenadine (FEX and FEX).

FIG. 3. LAESI-MS analysis of whole blood and serum. (a) LAESI-MS spectrum of whole blood without any pretreatment showed several singly and multiply charged metabolites in the low m/z (<1000 Da) region. For example, using exact mass measurements and human metabolome database search, phosphocholine (PC) (see the 20 enlarged segment of the spectrum) and glycerophosphocholines (GPC) were identified. The mass spectrum was dominated by the heme group of human hemoglobin (Herne). Deconvolution of the spectra of multiply charged ions (inset) in the higher m/z region identified the alpha and beta-chains of human hemoglobin with neutral masses of 15,127 Da and 15,868 Da, respectively. A protein with a neutral mass of 19,335 Da was also detected, likely corresponding to the circulating form of guanylin in human blood. (b) Human serum deficient of immunoglobulins in LAESI-MS experiments revealed several metabolites in the lower m/z region. Carnitine, phosphocholine (PC),...
tetradeccenoylcamitine (C14-camitine) and glycercophospho-
choleines (GPC) were identified. Deconvolution of the multi-
ply charged ions observed in the higher m/z region (see inset)
identified human serum albumin (BSA) with a neutral mass
of 66,556 Da.

FIG. 4. In-vivo identification of metabolites in French
marigold (Tagetes patula) seedling organs by LAESI-MS. (a) Single shot laser ablation of the leaf, the stem and the root of
the plant French marigold (Tagetes patula). (b) Deconvoluted mass spectra for the leaf, stem and root of French marigold
(Tagetes patula).

FIG. 5. Flash shadowgraphy with about 10 as exposure
time reveals the interaction between the electrospay (ES) plume and the laser ablation plume (LA) in a LAESI experi-
ment. Pulising spraying regime (top panel) offered lower
duty cycle and larger ES droplets, whereas in cone jet regime
(bottom panel) the droplets were continuously generated and
were too small to appear in the image. As the electrospayed
droplets traveled downstream from the emitter (from left to
right), their trajectories were intercepted by the fine cloud of
particulates (black spots in the images corresponding to 1 to
3 μm particles) traveling upward from the IR-ablation plume.
At the intersection of the two plumes, some of the ablated
particulates are thought to fuse with the ES droplets. The
resulting charged droplets contain some of the ablated mate-
rial and ultimately produce ions in an ESI process.

FIG. 6. (a) LAESI mass spectrum acquired in positive ion
mode directly from a Telfast pill manufactured by Aventis
Pharma Deutschland GmbH, Frankfurt am Main, Germany
(similar to Allegra in the US). The active ingredient antihis-
tamine, fexofenadine (F), was detected at high intensity as
singly protonated monomer, dimer and trimer. Polyethylene
glycol (PEG) 400 and its derivative were also identified dur-
ing the analysis giving oligomer size distributions (short-
dotted curves in black and gray). (b) Excretion of the antihis-
tamine fexofenadine (FEX) studied by LAESI mass spectrometry. A 5 μL aliquot of the urine sample collected two
hours after administering a Telfast caplet with 120 mg fex-
ofenadine active ingredient was directly analyzed using
LAESI-MS. Compared to the reference sample taken before
administering the drug, the spectra revealed the presence of
some altered peaks. Exact mass measurements of dissolved
scrapings from a caplet core (see black inset) after drift compensa-
tion for resonant (RES) showed m/z 502.2991 that corresponded
to the elemental composition of protonated fexofenadine, [C14H26NO4+H]+, with a 7.5 ppm mass accu-

FIG. 7. Identification of explosives by LAESI-MS in nega-
tive ion mode. Dilute trinitrotoluene (TNT) solution was
placed on a glass slide and detected by LAESI-MS (see spec-
trum). In a separate example, shown in the inset, a banknote
contaminated with TNT was successfully analyzed. The solid
square shows the molecular ion of TNT, whereas the open
squares denote its fragments. Peaks labeled B arise from the
ablation of the wetted banknote.

FIG. 8. Analysis of bovine serum albumin (BSA, Sigma-
Aldrich) by LAESI-MS. The dried BSA sample was wetted
prior to analysis. The mass analysis showed ESI-like charge
state distribution ranging from 26+ to 47+ charges. The inset
shows that deconvolution of the charge states gave a 66,547
Da for the molecular mass of BSA.

FIG. 9. LAESI schematics in reflection geometry. Compo-
ent parts are indicated by reference number herein.

FIG. 10. LAESI schematics in transmission geometry.
Component parts are indicated by reference number herein.

DETAILED DESCRIPTION

Referring now to the figures, whereas atmospheric pressure
laser desorption ionization techniques such as atmospheric pressure
matrix-assisted laser desorption ionization (AP-MALDI) or
electrospray-assisted laser desorption ionization (ELDI) usu-
ally require the pretreatment of the sample with a suitable
matrix, the present method does not involve pretreat-
ment of samples at all. As shown herein, the samples can
successfully be analyzed directly or can be presented on
surfaces such as glass, paper or plastic, or substrates
described supra, etc. This offers convenience and yields high
throughput during the analysis.

The LAESI provided herein allows one to study the spatial
distribution of chemicals. In an example, a French marigold
(Tagetes patula) plant in vivo from the leaf through the stem
to the root, FIG. 4(c) was able to be chemically profiled.
The LAESI provided herein achieves ESI-like ionization.
Thus, large molecules can be detected as multiply charged
species. This is shown for the case of bovine serum albumin,
FIG. 8, which was directly ionized from glass substrate.

Also provided herein is the use of combined infrared laser
ablation and electrospray ionization (ESI) as a novel ion
source for mass spectrometry under ambient conditions.
Demonstrated herein is the use of LAESI for the direct analy-
sis of a variety of samples from diverse surfaces for small
organic molecules, e.g., organic dyes, drug molecules FIG. 6
and explosives FIG. 7. narcotics, and other chemicals of interest
described herein previously. Furthermore, the utility of the
method for the direct analysis of synthetic polymers and
biomolecules FIG. 4(c) from biological matrixes including
tissues was shown. In vivo analysis of plant tissue was demon-
strated. We confirmed that our technique enabled one to
obtain intact molecular ions of proteins as large as 66 KDa
(Bovine serum albumin) directly from biological samples
without the need of sample preparation or other chemical
pretreatment. One of the most significant applications of this
ion source is in molecular imaging at atmospheric pressure.

Immediate uses are in biomedical analysis including in
vivo studies, clinical analysis, chemical and biochemical
imaging, drug discovery and other pharmaceutical applica-
tions, environmental monitoring, forensic analysis and home-
land security.

The current version of LAESI achieves ionization from
samples with a considerable absorption at about 3 μm
wavelength. Thus, samples with significant water content are best
suited for this technology. This limitation, however, can be
mitigated by using lasers of different wavelengths and/or
sprays of different composition.

EXPERIMENTAL

Materials

Laser ablation electrospray ionization. The electrospray
system was identical to the one described in our previous
study. Briefly, 50% methanol solution containing 0.1% (v/v) acetic was fed through a tapered tip metal emitter (100 μm i.d. and 320 μm o.d., New Objective, Woburn, Mass.) using a low-noise syringe pump (Physio 22, Harvard Apparatus, Hol­liston, Mass.). Stable high voltage was directly applied to the emitter by a regulated power supply (PS350, Stanford Research Systems, Inc., Sunnyvale, Calif.). A flat polished stainless steel plate counter electrode (38.1 mm x 38.1 mm x 0.6 mm) with a 6.0 mm diameter opening in the center was placed perpendicular to the axis of the emitter at a distance of 10 mm from the tip. This counter electrode was used to monitor the spray current with a digital oscilloscope (WaveSurfer 452, LeCroy, Chestnut Ridge, N.Y.). The temporal behavior of the spray current was analyzed to determine the established spraying mode. The flow rate and the spray voltage were adjusted to establish the cone-jet regime. The electrohydrodynamic behavior of the Taylor cone and the plume of ablated particulates were followed by a fast digital camera (QICAM, Qimaging, Burnaby, BC, Canada) equipped with a long-distance microscope (KC, Infinity Photo-Optical Co., Boulder, Colo.). The cone and the generated droplets were back-illuminated with a about 10 ns flash source based on fluorescence from a laser dye solution (Coumarin 540A, Exciton, Dayton, Ohio) excited by a nitrogen laser (VSL-337, Newport Corp., Irvine, Calif.).

The samples were mounted on microscope slides, positioned 10 to 30 mm below the spray axis and 3 to 5 mm ahead of the emitter tip, and ablated at a 90 degree incidence angle using an Er:YAG laser (BiOspot, Bioptic Lasersysteme AG, Berlin, Germany) at a wavelength of 2940 nm. The Q-switched laser source with a pulse length of <100 ns was operated at 5 Hz repetition rate with an average output energy of 3.5 mL/shot. Focusing was achieved by a single planocon­vex CaF 2 lens (f=150 mm). Burn marks on a thermal paper (multigrade IV, Ilford Imaging Ltd., UK) indicated that the laser spot was circular with a diameter of 350-400 μm, and its size did not change appreciably by moving the target within about 20 mm around the focal distance. This corresponded to about 2.8-3.6 L/cm 2 laser fluence that could result in >600 MPa recoil stress buildup in the target.

The material expelled by the recoil stress in the laser ablation plume was intercepted by the electrospay plume operating in cone jet mode and the generated ions were mass analyzed with a mass spectrometer (JMST100LC AccuTOF, JEOL Ltd., Peabody, Mass.). The data acquisition rate was set to 1 spectrum/spectrum. The sampling cone of the mass spectrometer was in line with the spray axis. The ion optics settings were optimized for the analyte of interest, and were left unchanged during consecutive experiments. The LAESI system was shielded by a Faraday cage and a plastic enclosure to mini­mize the interference of electromagnetic fields and air currents, respectively. The enclosure also provided protection from the health hazards of the fine particulates generated in the laser ablation process.

To expose fresh areas during data acquisition, some of the samples were raster scanned by moving them in the X-Y plane in front of the laser beam using an X-Y-Z translation stage. Unless otherwise mentioned, the presented mass spectra were averaged over 5 seconds (25 laser shots). In general, single laser shots also gave sufficient signal-to-noise ratio in the mass spectra. The LAESI experiments were followed by microscope inspection and imaging of the ablation spots on the targets.

French marigold plant. French marigold (Tagetes patula) seeds were obtained from Fischer Scientific. Seedlings were grown in artificial medium in a germination chamber (model S79054, Fischer Scientific). Two seedlings were removed at 2 and 4 weeks of age, and were subjected to LAESI analysis without any chemical pretreatment. The roots of the plants were kept moist to avoid wilting during the studies. Following the experiment the plants were transplanted into soil and their growth was monitored for up to an additional four weeks to confirm viability.

Results

Positionization in Atmospheric Pressure Infrared Laser Ablation

Laser ablation of water-rich targets in the mid-infrared region (2.94 μm) has been utilized in medical (laser surgery) and analytical (AP IR-MALDI) applications. In these experiments laser energy is coupled into the target through the strong absorption band due to the OH vibrations. Ablation experiments on water, liver and skin revealed two partially overlapping phases. During the first phase lasting about 1 μs, a dense plume develops as a consequence of surface evaporation and further plasticization of the target. This plume contains ions, neutrals and some particulate matter, and exhibits a shock wave front at the plume-air interface. Its expansion is slowed by the pressure of the background gas (air), and eventually comes to a halt and collapses back onto the target. The second phase is induced by the recoil pressure in the target and results in the ejection of mostly particulate matter. Depending on the laser fluence and target properties, this phase lasts for up to about 300 μs. Ultraviolet (UV) laser desorption studies on strongly absorbing targets in vacuum environment indicated that the degree of ionization in the plume was between 10 -4 and 10 -5 . Laser ablation in the IR is likely to produce even lower ion yields due to the lower photon energies, typically lower absorption coefficients, and the copious ejection of neutral particulates. As a consequence, the sensitivity in mass spectrometric applications suffers and the ion composition in the plume can be markedly different from the makeup of the target.

These problems can be alleviated by utilizing the neutral molecular species in the plume through post-ionization strategies. For example, at atmospheric pressure, applying a radioactive emitter (e.g., a 68 Co) or chemical ionization through a corona discharge improved the ion yields for low-mass molecules. In a recent breakthrough, the ELDO method combined UV laser ablation with ESI. Significantly, ELDO did not exhibit discrimination against high mass analytes up to about 20 kDa. Encouraged by the success of ELDO on pretreated and/or dehydrated samples, we sought to develop a new ionization technique for the analysis of untreated water-rich biological samples under ambient conditions. Similarly to AP IR-MALDI, in LAESI mid-IR laser ablation was used to produce a plume directly from the target. To post-ionize the neutrals and the particulate matter, this plume was intercepted under right angle by an electrospay operating in the cone-jet regime. FIG. 1 shows the schematics of the experimental arrangement. We chose the cone jet spraying regime because of its exceptional ion yield and elevated duty cycle compared to other (e.g., burst or pulsating) modes of ESI operation. The sampling orifice of the mass spectrometer was in line with the spray axis. With the spray operating, laser ablation of targets absorbing in the mid-IR resulted in abundant ion signal over a wide range of m/z values. With no solution pumped through the electrified or floating emitter, no ions were detected during the experiments. Conversely, with the spray present but without laser ablation no ion signal was observed. Thus, a DESI-like scenario, or one involving chemical ionization...
through corona discharge at the emitter, did not play role in the ionization process. As we demonstrate after the discussion of concrete applications, LAESI also bears major differences from ELDI in both the range of its utility and probably in the details of ion production.

The figures of merit for LAESI were encouraging. The detection limit for reserpine and Verapamil analytes were about 100 fmol/sample (about 0.1 fmol/ablated spot). Very importantly, quantitation showed linear response over four orders of magnitude with correlation coefficients of R>0.999 for both analytes. No ion suppression effect was observed. We successfully tested the use of LAESI on a variety of samples, including pharmaceuticals, small dye molecules, peptides, explosives, synthetic polymers, animal and plant tissues, etc., in both positive and negative ion modes. Here, we only present some of the examples most relevant in life sciences.

Antihistamine Excretion

Fexofenadine (molecular formula C_{22}H_{29}NO_4) is the active ingredient of various medications (e.g., Allegra® and Telfast®) for the treatment of histamine-related allergic reactions. This second-generation antihistamine does not readily enter the brain from the blood, and, it therefore causes less drowsiness than other remedies. To understand the pharmacokinetics of the active ingredient absorption, distribution, metabolism and excretion (ADME) studies are needed. For example, radiotracer investigations shown that fexofenadine was very poorly metabolized (only about 5% of the total oral dose), and the preferential route of excretion was through feces and urine (80% and 11%, respectively). This and other traditional methods (e.g., liquid chromatography with MS), however, are time consuming and require a great deal of sample preparation. As in the clinical stage of drug development it is common to encounter the need for the analysis of 1,000 to 10,000 samples, high throughput analysis is important. We tested whether LAESI was capable of rapidly detecting fexofenadine directly from urine without chemical pre-treatment or separation.

A Telfast® caplet with 120 mg of fexofenadine (FEX) was orally administered to a healthy volunteer. Urine samples were collected before and several times after ingestion. For all cases, a 5 µL aliquot of the untreated sample was uniformly spread on a microscope slide, and directly analyzed by LAESI-MS. A comparison made between the LAESI mass spectra showed that new spectral features appeared after drug administration. The peaks highlighted by red ovals correspond to the protonated form and the fragments of fexofenadine. Exact mass measurements indicated the presence of an ion with m/z 502.2991 that corresponded to the elemental composition [C_{22}H_{29}NO_4]+ with 7.5 ppm mass accuracy. The measured about 35% intensity at M+1 (see red inset) is consistent with the isotope abundances of this elemental composition. The mass spectra showed the presence of numerous other metabolites not related to the drug. For example, protonated ions of creatinine, the breakdown product of phosphocreatine, were very abundant. In future studies the other numerous metabolites present can be identified through, e.g., tandem MS, for broader metabolomics applications.

For reference, the caplet itself was also analyzed by LAESI (see black inset in FIG. 2). A small portion of the caplet core was dissolved in 50% methanol containing 0.1% acetic acid, and reserpine (RES) was added for exact mass measurements. The black inset in FIG. 2 shows that both the fexofenadine and the reserpine underwent in-source collision activated dis-
tivation species with minimal or no damage to the organism. As an example we utilized LAESI for the in vivo profiling of metabolites in petite French marigold seedlings. The homegrown plants were placed on a microscope slide and single-laser shot analysis was performed on the leaf, stem and root of the plant to minimize the tissue damage.

The acquired mass spectra (see FIG. 4a) revealed various metabolites at high abundances. We identified some of these compounds in a two-step process. Due to the similarity of some metabolites for a diversity of plants, we first performed a search for the measured masses in the metabolomic database for Arabidopsis thaliana (available at http://www.arabidopsis.org/). Then the isotopic distributions of each ionic species were determined to support our findings and also to separate some isobaric species. The list of compounds was further extended by performing LAESI experiments, in which the mass spectra were averaged over about 5 to 10 consecutive laser shots (see FIG. 4b). Several additional compounds were detected, most likely due to the better signal-to-noise ratio provided by signal averaging.

By comparing the mass spectra obtained on the leaf, stem and root we found that certain metabolites were specific to the organs of the plant. The assigned compounds with the location of their occurrence and some of the related metabolic pathways are listed in Table 1. Consistent with the noncova- lent hexose clusters in FIG. 4, both the leaf and the stem had a high glucose and pigment content. However, different types of flavonoids were found in the leaf and the stem. The root primarily contained low-mass metabolites, e.g., saturated and unsaturated plant oils. These oils were also present in the other two organs of the plant. However, the root appeared to be rich in the saturated oils.

Compounds 9 and 11 were detected at surprisingly high abundances. For the latter, however, the database search gave no results. In-source CID experiments proved that 11 had relatively high stability, therefore the possibility of a noncovalent cluster was excluded. Exact mass measurements gave m/z 763.1671 with about 40% M+ isotopic distribution, which corresponded to a C_{29}H_{33}O_{13}Na elemental composition within 4 ppm mass accuracy. Although multiple structural isomers could correspond to the same chemical formula, based on previous reports in the literature on a flavonoid of identical mass, we assigned the compound as the sodiated form of kaempferol 3-O-(2”,3”-di-p-coumaroyl)-glucoside. Tandem MS results on extracts from the stem indicated the presence of several structural features consistent with this assignment. The presence of other kaempferol-derivatives in the plant can also be viewed as corroborative evidence.

After the analysis, microscopy examination of the stem and the leaf revealed circular ablation marks of about 350 µm in diameter (see the insets in FIG. 4). This localized superficial damage had no influence on the life cycle of the seedling. We must emphasize however that due to the ablation by the laser LAESI is a destructive method, thus the size of the ablated area (currently 350-400 µm) needs to be considered as a limiting factor for in vivo experiments. Improvements can be achieved by reducing the size of the ablated areas or applying lower laser irradiances. As the current focusing lens has no correction for spherical aberration, significantly tighter focusing (and much less damage) can be achieved by using aspherical optics.

### TABLE 1

<table>
<thead>
<tr>
<th>#</th>
<th>Metabolite</th>
<th>Formula</th>
<th>Monoisotopic mass</th>
<th>Measured mass</th>
<th>Organ</th>
<th>Metabolic pathways</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>glucose</td>
<td>C_{6}H_{12}O_{6}</td>
<td>181.071 (H)</td>
<td>181.019 (H)</td>
<td>leaf, stem</td>
<td>glucoseogenesis, glycolysis</td>
</tr>
<tr>
<td>2</td>
<td>2-C-methyl-erythritol-4-phosphate</td>
<td>C_{6}H_{12}O_{6}P</td>
<td>217.048 (H)</td>
<td>217.078 (H)</td>
<td>leaf</td>
<td>methylerythritol phosphate pathway</td>
</tr>
<tr>
<td>3</td>
<td>dTDP-4-dehydro-6-deoxy-glucose</td>
<td>C_{6}H_{16}N_{2}O_{13}P_{2}</td>
<td>547.073 (H)</td>
<td>547.342 (H)</td>
<td>leaf</td>
<td>rhamnose biosynthesis</td>
</tr>
<tr>
<td>4</td>
<td>dTDP-glucose</td>
<td>C_{6}H_{16}N_{2}O_{13}P_{2}</td>
<td>565.084 (H)</td>
<td>565.152 (H)</td>
<td>leaf</td>
<td>rhamnose biosynthesis</td>
</tr>
<tr>
<td>5</td>
<td>kaempferol-3-O-rhamnoside-7-O-glucose</td>
<td>C_{17}H_{33}O_{14}</td>
<td>579.171 (H)</td>
<td>579.173 (H)</td>
<td>leaf</td>
<td>flavonol biosynthesis</td>
</tr>
<tr>
<td>6</td>
<td>kaempferol-3-O-rhamnoside-7-O-glucose</td>
<td>C_{17}H_{33}O_{14}</td>
<td>595.166 (H)</td>
<td>595.171 (H)</td>
<td>leaf</td>
<td>flavonol biosynthesis</td>
</tr>
<tr>
<td>7</td>
<td>linalonic acid</td>
<td>C_{10}H_{16}O_{6}</td>
<td>279.232 (H)</td>
<td>279.153 (H)</td>
<td>stem</td>
<td>fatty acid oxidation</td>
</tr>
<tr>
<td>8</td>
<td>cyanidin</td>
<td>C_{12}H_{13}O_{6}</td>
<td>287.056 (+)</td>
<td>287.055 (+)</td>
<td>stem</td>
<td>oxidation, anthocyanin biosynthesis</td>
</tr>
<tr>
<td>9</td>
<td>kaempferol-3-O-glucoside, kaempferol-3-glucoside</td>
<td>C_{13}H_{16}O_{6}</td>
<td>449.108 (+)</td>
<td>449.109 (+)</td>
<td>stem</td>
<td>flavonol biosynthesis, oxidation, anthocyanin biosynthesis</td>
</tr>
<tr>
<td>10</td>
<td>cyanidin-3- diglucoside, kaempferol-3,7-O-diglucoside</td>
<td>C_{17}H_{33}O_{16}</td>
<td>611.161 (+)</td>
<td>611.163 (+)</td>
<td>stem</td>
<td>flavonol biosynthesis, oxidation, anthocyanin biosynthesis</td>
</tr>
<tr>
<td>11</td>
<td>kaempferol-3-O-(2&quot;,3&quot;-di-p-coumaroyl)-glucoside</td>
<td>C_{20}H_{36}O_{15}</td>
<td>763.164 (Na)</td>
<td>763.167 (Na)</td>
<td>stem</td>
<td>flavonol biosynthesis, oxidation, anthocyanin biosynthesis</td>
</tr>
<tr>
<td>12</td>
<td>methylsalicylate xanthine</td>
<td>C_{3}H_{6}O_{3}</td>
<td>153.055 (H)</td>
<td>152.989 (H)</td>
<td>root</td>
<td>benzenoid ester biosynthesis, xvide</td>
</tr>
</tbody>
</table>
LAESI Mechanism

In the LAESI experiments surprisingly large target-to-spray distances (10 to 30 mm) provided the strongest signal. We also noticed that short distances (e.g., about 5 mm) led to the destabilization of the electrospray, resulting in a significant deterioration of the ion counts.

Following the laser pulse, often material ejection was observed in the form of small particulates. The optimum distance of the ablation spot to the spray axis was established as about 25 mm, but appreciable ion abundances were still measured at 30 mm and beyond. As the area of the laser spot did not change noticeably within about 20 mm of the focal distance, the variations in LAESI signal were not related to differences in laser irradiance.

These observations in combination with fast imaging results on IR-laser ablation can provide some insight into the mechanism of LAESI. At similar laser fluences water and soft tissues first undergo non-equilibrium vaporization in the form of surface evaporation and to a much larger degree phase explosion. After about 1 μs, the expansion stops at a few millimeters from the surface and the plume collapses. Due to the recoil stress in the condensed phase, secondary ejection follows in the form of particulates that can last up to several hundred microseconds. These particulates travel to larger distances than the initial plume. They are slowed and eventually stopped at tens of millimeters from the target by the drag force exerted on them by the resting background gas. The difference between the stopping distance of the primary plume and the recoil induced particle ejection can explain the differences in laser irradiance.

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variations of water content and/or tensile strength in a sample can also lead to changes in LAESI ion yield and influence imaging results.

Based on our understanding of the LAESI mechanism, additional improvements in ion yield can be expected from enhancing the interaction between the laser ablation and the electrospray plumes. For example, tubular confinement of the ablation plume can make it more directed and increase its overlap with the electrospray. Adjusting the laser wavelength to other (CH or NH) absorption bands can introduce additional channels for laser energy deposition, thereby enabling the analysis of biological samples with low water content. The current and anticipated unique capabilities of LAESI promise to benefit the life sciences in metabolomic, screening and imaging applications including the possibility of in vivo studies.

Referring to FIGS. 9 and 10, schematics illustrate LAESI using reflection geometry FIG. 9 and LAESI using transmission geometry FIG. 10 with components labeled. The components are provided in TABLE 2 below and are indicated by reference number.

<table>
<thead>
<tr>
<th>FIG. 9: LAESI schematics in reflection geometry</th>
</tr>
</thead>
<tbody>
<tr>
<td>2: electrospray capillary</td>
</tr>
<tr>
<td>4: liquid supply with pump (this component is optional in the nanospray embodiment)</td>
</tr>
<tr>
<td>6: high voltage power supply</td>
</tr>
<tr>
<td>8: counter electrode</td>
</tr>
<tr>
<td>10: oscilloscope</td>
</tr>
<tr>
<td>12: recording device (e.g., personal computer)</td>
</tr>
<tr>
<td>14: infrared laser (e.g., Er:YAG or Nd:YAG laser driven optical parametric oscillator)</td>
</tr>
<tr>
<td>16: beam steering device (e.g., mirror)</td>
</tr>
<tr>
<td>18: focusing device (e.g., lens or sharpened optical fiber)</td>
</tr>
<tr>
<td>20: sample holder with x-y-z-positioning stage</td>
</tr>
<tr>
<td>22: mass spectrometer</td>
</tr>
<tr>
<td>24: recording device (e.g., personal computer)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>FIG. 10: LAESI schematics in transmission geometry</th>
</tr>
</thead>
<tbody>
<tr>
<td>26: electrospray capillary</td>
</tr>
<tr>
<td>28: liquid supply with pump (this component is optional in the nanospray embodiment)</td>
</tr>
<tr>
<td>30: high voltage power supply</td>
</tr>
<tr>
<td>32: counter electrode</td>
</tr>
<tr>
<td>34: oscilloscope</td>
</tr>
<tr>
<td>36: recording device (e.g., personal computer)</td>
</tr>
<tr>
<td>38: infrared laser (e.g., Er:YAG or Nd:YAG laser driven optical parametric oscillator)</td>
</tr>
<tr>
<td>40: beam steering device (e.g., mirror)</td>
</tr>
<tr>
<td>42: focusing device (e.g., lens or sharpened optical fiber)</td>
</tr>
<tr>
<td>44: sample holder with x-y-z-positioning stage</td>
</tr>
<tr>
<td>46: mass spectrometer</td>
</tr>
<tr>
<td>48: recording device (e.g., personal computer)</td>
</tr>
</tbody>
</table>

It will be clear to a person of ordinary skill in the art that the above embodiments may be altered or that insubstantial changes may be made without departing from the scope of the invention. Accordingly, the scope of the invention is determined by the scope of the following claims and their equitable equivalents.

What is claimed is:

1. A laser ablation ionization device comprising: a pulsed, mid-infrared laser to emit energy at a sample to ablate the sample and generate an ablation plume; a translation stage comprising a sample holder, wherein the sample is in the sample holder; a focusing device to focus the laser energy onto a first side of the sample, wherein the ablation plume is generated on a second side of the sample; an electrospray apparatus to produce an electrospray to intercept the ablation plume and generate ions; and a mass spectrometer to detect the ions; wherein the laser energy has a wavelength at an absorption band of an OH group; and wherein the laser energy is coupled into the sample by water in the sample.

2. The device of claim 1, wherein the translation stage comprises a three dimensional stage.

3. The device of claim 1, wherein the focusing device comprises at least one of a lens and an optical fiber.

4. The device of claim 3, wherein the lens comprises one of aspherical optics and near-field optics.

5. The device of claim 1 comprising a beam steering device to steer the laser energy onto the first side of the sample.

6. The device of claim 1 comprising a microscope to view at least one of the sample, electrospray, and ablation.

7. The device of claim 1, wherein the electrospray apparatus is a nanospray apparatus.

8. The device of claim 1, wherein the ablation has no influence on the life cycle of the sample.

9. The device of claim 1, wherein the sample is in its native environment.

10. The device of claim 1, wherein the sample is at least one of ambient conditions and atmospheric pressure.

11. A laser ablation ionization device comprising: a pulsed, mid-infrared laser to emit energy at a sample to ablate the sample and generate an ablation plume; a translation stage comprising a sample holder, wherein the sample is in the sample holder; a focusing device to focus the laser energy onto a first side of the sample, wherein the ablation plume is generated on a second side of the sample; an electrospray apparatus to produce an electrospray to intercept the ablation plume and generate ions; and a mass spectrometer to detect the ions; wherein the laser energy has a wavelength at an absorption band of one of an OH group; and wherein the laser energy is coupled into the sample at the wavelength of the absorption band.

12. The device of claim 11, wherein the translation stage comprises a three dimensional stage.

13. The device of claim 11, wherein the focusing device comprises at least one of a lens and an optical fiber.

14. The device of claim 11 comprising a beam steering device to steer the laser energy onto the first side of the sample.

15. The device of claim 11 comprising a microscope to view at least one of the sample, electrospray, and ablation.

16. The device of claim 11, wherein the electrospray apparatus is a nanospray apparatus.

17. The device of claim 11, wherein the ablation has no influence on the life cycle of the sample.

18. A method of laser ablation ionization comprising: focusing a mid-infrared laser pulse on a first side of a sample; ablating the sample with the laser pulse to generate an ablation plume; intercepting the ablation plume and generate ions; and detecting the ions with a mass spectrometer; wherein the laser pulse has a wavelength at an absorption band of an OH group; and wherein the laser energy is coupled into the sample by water in the sample.

19. The method of claim 18, wherein the laser energy is coupled into the sample by water in the sample.
20. The method of claim 18, wherein the electrospray is a nanospray.