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Miniaturizing sample spots for matrix-assisted laser desorption/ionization mass spectrometry

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Abstract

The trend of miniaturization in bioanalytical chemistry is shifting from technical development to practical application. In matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS), progress in miniaturizing sample spots has been driven by the needs to increase sensitivity and speed, to interface with other analytical microtechnologies, and to develop miniaturized instrumentation.

We review recent developments in miniaturizing sample spots for MALDI-MS. We cover both target modification and microdispensing technologies, and we emphasize the benefits with respect to sensitivity, throughput and automation.

We hope that this review will encourage further method development and application of miniaturized sample spots for MALDI-MS, so as to expand applications in analytical chemistry, protein science and molecular biology.

Keywords

Acoustic ejection; Hydrophobic surface; Induction-based fluidics; Mass spectrometry; Matrix-assisted laser desorption/ionization (MALDI); Microdispensing; Miniaturization; Nanovial; Piezoelectric dispensing; Sample preparation

1. Introduction

Ultra-small events occur in most cellular and pathological processes. From a practical perspective in chemical analysis, limitations of sample amounts (e.g., in clinical sampling, protein expression and cellular extraction) are significant obstacles in meeting the goals of proteomics, genomics, and related sciences and in examining the events occurring on a small scale in a cellular milieu. The ability to miniaturize samples and their handling is therefore an important goal in analytical chemistry. For example, the development of microstructures and microfluidics is essential in downscaling samples for chromatography, electrophoresis and electrochemical chips [1].

Mass spectrometry (MS), as a highly sensitive analytical technique, is an area where miniaturization can flourish to meet the demands for higher sensitivity and easy interfacing

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with other techniques. Ultra-low-volume sampling and microfluidic interfacing using electrospray-ionization MS (ESI-MS) is but one example [2–4]. Matrix-assisted laser desorption/ionization (MALDI), complementary to ESI, offers several advantages over ESI, including easy mass-spectral interpretation, higher contaminant tolerance, opportunity for repeated data acquisition from the same sample, and parallel deposition of samples from multiplexed microchannels. Miniaturization in the MALDI-sample preparation is therefore a requirement for higher sensitivity and further applications. As foreseen by Karas et al. [5], limits of detection (LODs) in the sub-ng range should be attainable, pending the development of suitable preparation techniques.

To date, important progress has been made in miniaturizing MALDI-sample preparation. DeVoe et al. and Foret et al. [6,7] discussed liquid-phase interfacing and the use of microfluidic technologies for MALDI-MS, and some efforts aimed at miniaturization. In this review, we focus on miniaturization of MALDI-sample spots with a review of the current methodologies and applications. We attempt to be comprehensive, but we apologize if we overlook some developments or applications. Our goal is to encourage practitioners in the field of MALDI to improve their measurement capabilities.

2. Methods

2.1. Minimizing sample dispersion by target modification

2.1.1. MALDI targets with small channels or vials—Collecting the sample of interest in small channels or vials on a MALDI probe is perhaps the most direct approach to miniaturizing MALDI spots. Indentations in the target are often made by etching or drilling.

Jespersen et al. [8] utilized pL-volume vials etched in silicon wafers and found a significant improvement in the absolute LODs for proteins. These “pL vials” allowed the sample-spot area to be reduced to 0.01 mm² and the sample volume to 250 pL.

Solouki et al. [9] described microdeposition of a few amol of peptides and phospholipids onto small indentation spots (< 1 mm²) on a probe.

Later, when combining with a piezoelectric microdispensing technique, Little et al. [10] and Laurell et al. [11–16] achieved sample deposition into individual etched wells on silicon surfaces for DNA analysis.

The influence of vial geometry was investigated systematically by Laurell, Marko-Varga and their collaborators [11,12], who fabricated the nanovials by anisotropic wet etching of monocrystalline silicon. Inverted pyramidal and shallow nanovials were tested to reveal the relationships of vial depth with mass-resolving power and with mass accuracy. Their experiments showed that samples inside deeper nanovials (> 100 μm) gave lower (by up to a factor of 10) resolution of isotopic clusters than did samples in shallow nanovials. As expected, lower mass-resolving power also gave lower mass accuracy. Their explanation is that the sample-ion cloud has greater length when desorption occurs from matrix-sample crystals spread along the vertical axis of the deep vials. Shallow nanovials (depth of ~20 μm) are a compromise, affording the same resolution of isotope peaks and mass accuracy as achievable with a sample on a planar surface while still maintaining the positive aspects of confining the sample to the vial, thus keeping the spot size small [11].

However, sample-carry-over problems associated with this method make target cleaning demanding, leading to inevitable damage during the cleaning. The advantages of small sample vials do not yet compensate for the frequent need to acquire new nanovial-sample plates. To overcome this problem, Laurell, Marko-Varga, and co-workers [17] developed precise

microfabrication of disposable polymeric high-density nanovial arrays using polymer substrates [e.g., polymethylmethacrylate (PMMA) and polycarbonate (PC)]. A metal master template, fabricated from silicon micromachined arrays and used for injection molding on plastic substrates, can generate a vial density as high as 165 nanovials/cm². This development may be an important advance for the nanovial method, providing low cost and high throughput.

2.1.2. Hydrophobic materials as MALDI-plate surfaces—Hydrophobic materials are an interesting alternative to the standard stainless-steel plates for sample-plate preparation. These hydrophobic interfaces, either attached to or applied by coating a MALDI probe, cause “beading” of the sample droplets due to repulsion, so minimizing the sample area and concentrating the sample on the target. A wide range of commercial hydrophobic films and polymer materials can be used for this purpose [18–33]. Most were designed to repel polar solvents including water, methanol, and acetonitrile, which are commonly used in sample-preparation and separation protocols. Compared to surfaces prepared by probe drilling or etching, films comprised of hydrophobic materials can be easily removed by a simple rinsing step and reapplied so that the target can be reused. These disposable MALDI surfaces obviate the problem of sample carry-over and minimize cross-contamination.

Using a similar principle, Schuerenberg et al. [34] first introduced the prestructured sample probe, which is now available commercially. They coated the sample support, which carries an array of 200 μm gold spots acting as predetermined positions (“anchors”), with a thin layer of hydrophobic Teflon (Fig. 1). After sample/matrix deposition and during solvent evaporation, the droplet shrinks to the localized anchor, as driven by the water-repellent property of Teflon. As a result, the dried sample/matrix becomes localized and concentrated at the predefined adsorption-anchor sites [34–38].

Other plates were developed with similar functionalities, and some are commercially available. Custom-made, prestructured sample surfaces were also developed at low cost in several laboratories [27,30,39].

Representative examples of using surface membranes to reduce spot sizes (Table 1) generally show enhanced sample intensity and sensitivity over a wide range of analyte types. Moreover, the ability of on-probe purification, which distinguishes surface-coating methods from others, obviates the need for laborious pre-purification that leads to sample losses [19,22,28,33,35]. The utility of these surfaces is based on the different affinity of analytes and salts to the hydrophobic surfaces. Different surface materials have additional advantages, determined by various research groups (as summarized in Table 1). More details of this aspect are available in other reviews [40,41].

The main drawbacks of this method are diminished mass accuracy and mass-resolving power when some dielectric materials are used. The effective electric field near the desorption spot may no longer be uniform, and the accelerating potential in the ion source is affected by the dielectric materials that are attached to the probe [20,42]. Reducing the thickness of the films or coatings may alleviate the problem. To achieve a thinner film, a solution of the surface materials could be electrosprayed onto the target plates [41] or the thin film could be stretched by soaking before applying it to the probe [30]. The use of internal calibration and delayed extraction also minimize the peak shifting and afford higher mass accuracy [20,42]. In addition, decoupling ionization and detection should minimize the problem caused by the changed electric fields in the ion source [40]. Another shortcoming of this method is that the crystal-formation rate on the modified surfaces is significantly slower than that on the metal surface because the droplet surface is less exposed [20,30]. The increased drying time is a concern when sample integrity is time sensitive and/or high throughput is needed.

2.2. Use of microdispensing devices

2.2.1. Capillary tubes or micropipettes—Deposition of sample spots with nL–pL volumes can be achieved by using fused-silica capillaries or glass micropipettes connected to small-scale syringes. The volume of the deposited spot is often measured by the length of the sample plug in the capillary or controlled by a syringe pump. A C18-packed fused-silica capillary or a modified reserved-phase nano-column can be used as a multi-functional device for microdispensing (nL range), desalting, concentrating and adding matrix; this approach was reported for the analysis of trace levels of peptides and protein digests [43,44].

A nL chemistry station (Fig. 2), introduced by Li et al. [45–47], utilized fused-silica capillaries that were deactivated by silylation to reduce sample adsorption. Excellent LODs can be achieved (~42 zmol substance P) [45].

Moreover, nano-scale protein digestion, peptide mapping, and single mammalian cell analysis are also possible with this approach [46,47]. Karger and co-workers [48] connected a tapered bent capillary to a liquid flow (100–500 nL/min) and deposited continuous thin sample traces of a width comparable to the laser beam used for MALDI. This approach is an alternative to depositing many individual sample spots for on-line MALDI-MS [48] and permits the coupling of capillary electrophoresis (CE) to MALDI-MS [49,50]. Glass micropipettes, pulled from glass tubing to obtain a fine tip with a diameter of several μm , were also employed to deliver the matrix solution in the pL range for direct MALDI analysis of single organelles and cells [51,52].

The microsample-deposition method using simple capillary tubes or micropipettes is simple and low cost. In most cases, no special device is required, and preparation is relatively easy. Nevertheless, this method often requires physical contact between the fluid and the substrate to make the small droplet overcome the adhesive forces at the dispensing tip. Special considerations are required to avoid cross-contamination, clogging, and damage to the tip. Moreover, contact deposition, to some extent, depends on the operator and varies from experiment to experiment, introducing errors and reducing precision. As a result, several contact-free microdispensing methods are available with higher level of automation, and we discuss them in the following sub-sections.

2.2.2. Piezoelectric dispensing—Based on the principle of inkjet printers, piezoelectric dispensers use piezoelectric elements to control the flow of a fluid from dispenser to target. A dispenser is filled with the sample solution, and subsequently sample droplets are ejected onto a MALDI target by voltage or other force. To obtain good droplet formation, the applied voltage requires adjustment, depending on the surface tension and viscosity, which are a function of the composition of sample solution. The drop sizes generated also vary as a result, often in the range of several tens to hundreds of pL. In many cases, trains of droplets are directed to the target, which is located several mm from the nozzle. The pulse frequency, controlled by a pulse generator, determines the sample-deposition rate and makes this automated dispensing approach high throughput.

Various droplet dispensers can be used to deposit MALDI-sample microspots. Several research groups have employed commercially-available piezoelectric dispensers [10,53–59]. A custom-developed piezo-actuated, flow-through microdispenser by Laurell, Marko-Varga and their collaborators [11–15,60,61] features a flow-through cell fabricated by silicon micromachining (Fig. 3). The droplets are formed through a pyramid-shaped nozzle (designed for stable droplet formation) and ejected perpendicular to a gas flow passing through the cell. Its easy connection to analytical microseparation allows high-resolution, high-speed separations to be interfaced with high-frequency pL-sample dispensing for subsequent MALDI analysis (see below for more discussion). Yost and co-workers [62] modified a desk-top ink-jet printer with

piezoelectric heads at low cost so as to deposit an array of high-density matrix spots for MALDI imaging. Commercialized chemical ink-jet printers can also be used to microdispense nL of reagents onto blotted protein bands [63,64] or tissue sections [65,66] for in situ protease digestion followed by MALDI analysis.

2.2.3. Droplet charging by induction or polarization—A non-contact liquid-transport approach based on inductively charging the droplet is called “induction-based fluidics (IBF)”. Gross, Sauter, Harmon and their co-workers [67–70] introduced and evaluated IBF. An electrically-grounded MALDI plate is located below a sample microsyringe with a capillary tip surrounded by an nL-wave inductor (Fig. 4). After a droplet grows to a specific, accurate volume on the capillary tip, controlled by an aligned micrometer, pulsed charging is applied to the nL-wave inductor, forming a capacitor between the capillary tip and the MALDI plate. The inductively-charged droplet is then dynamically directed to the MALDI target by the electric force. This inductive charging facilitates a launch that does not fragment the droplet due to the low field strength. The outcomes are drops with volumes of pL– μ L. The electric force directs the droplet flight, but buoyancy, drag, and gravity are also important, given that the droplet-deposition process occurs in the atmosphere. Using this technique, Gross and co-workers [68] demonstrated a quantitative relationship between the nL-spot sizes and the MALDI signals, based on investigation using both traditional solid matrices and ionic liquid matrices.

Ericson et al. [71] described a similar spotting process, called “electrically mediated deposition”, in which the droplet forms at the capillary tip that is electrically grounded. At the start of the process, a short negative voltage pulse applied to the MALDI target causes the droplet to polarize. The electric field pulls the droplet to the target surface. The droplet, as in the IBF method, cannot form an electrospray beam because the electric force at the droplet surface is small compared to the droplet size. The pulsed nature of the electric field also minimizes unwanted electrochemical side reactions.

2.2.4. Electrodynamic balance—Agnes and co-workers [54,56,57,72] decreased the size of a levitated droplet by allowing solvent evaporation to occur during its transition from the dispenser to the MALDI plate. Evaporation occurs while the drop is suspended in an electrodynamic balance (EDB) by using two ring electrodes mounted in parallel between the droplet generator and the target plate (Fig. 5). Droplets of sample solution, generated by a piezoelectric droplet generator, are ejected upwards and, as they temporarily levitate between the ring electrodes, evaporation occurs. An AC potential, applied to the ring electrodes, ensures that the radial position of the droplet is restored in the center of the EDB, and a DC potential, applied to the induction electrode and MALDI plate in the vertical orientation, adjusts the vertical positions of droplets in the EDB. This evaporation step requires seconds to remove the majority of the volatile components, during which a non-volatile solvent component (e.g., glycerol) remains to prevent the droplet from completely “drying out”. An increased DC field ejects the droplets from the EDB to a MALDI target. This “wall-less” process directly preconcentrates the material in the droplets by allowing desolvation to occur. To increase the efficiency, multiple droplets can be levitated at the same time.

2.2.5. Acoustic ejection—Acoustic drop ejection (ADE) directs short pulses of focused acoustic energy from the bottom of an open fluid reservoir onto a fluid surface. The pressure of the focused acoustic waves at the liquid surface creates an upwelling, allowing a droplet to be ejected from the liquid surface onto a target suspended above the ejection reservoir (Fig. 6). This process can take place at a high rate, and the volume of the ejected droplets (0.1 pL– >10 μ L) depends on the properties of the focused acoustic transducer, the applied radio-frequency energy, and the properties of the liquid in the reservoir [73]. Larger volumes can be transferred by depositing multiple sample drops.

Caprioli and co-workers [73] applied this technology for matrix deposition to preparing tissue samples for MALDI profiling and imaging. They used a multi-spotter based on acoustic ejection. Matrix application with the spotter offers more precise spot placement and higher throughput than traditional spotting techniques that use pulled capillaries or mechanical pipettes. Moreover, the nozzle-free design of the ejector readily permits use of concentrated matrix solutions in those organic solvents commonly used to prepare MALDI samples, without concern for droplet misdirection or stoppage due to clogging of tips, pins or nozzles [73]. The risk of sample cross-contamination is reduced, given that the wash step is much easier without the complicated transfer mechanism.

3. Outcomes in MALDI analysis

3.1. Improved sensitivity and lower LOD

Achieving maximal sensitivity with MALDI-MS is an important primary goal. For conventional MALDI-sample preparation, usually an aliquot of 0.3–2 μL of sample solution with matrix is applied to the target, resulting in a relatively large sample area (typically 5–15 mm^2). As a consequence, the sensitivity is reduced because only a tiny portion (0.1–10%) of the area is laser irradiated to generate ions [30,34,51]. The case is even worse when a solvent of low surface tension [e.g., acetone or hexafluoroisopropanol (HFIP)] is used for the sample preparation often of organic compounds, polymers, and membrane proteins [23,25,27]. With these solvents, the sample quickly spreads over a large area and even merges with other spots. Miniaturized sample spots offer a solution to this problem; a major fraction of a spot, or even the whole spot, can be sampled by the laser beam because the dimensions of the laser are nearly identical to, or even larger than, the area of the deposited sample.

Enhanced sensitivity and lower LODs are reported for almost every effort to downsize sample spots. However, the question remains: “Will the LOD always become lower when the spot size gets smaller and smaller?” Although many dispensing techniques can make ultra-small droplets, many researchers work with sample sizes approximately equal to that of the laser beam, achieving a compromise between small spot size and sufficient analyte to acquire several spectra from each spot [10–12,15,17,^{23,34},45,58,60,68].

Li and co-workers [45] pointed out that a minimum analyte density at 5 molecules/ μm^2 on average is required to generate useful MALDI signals, assuming that all the analyte molecules are located close to the surface of the matrix. A sample that is smaller than the laser spot still has to meet this threshold of average analyte density. When the sample-crystal area is too small, it is ablated easily and becomes readily depleted for multiple laser shots, which are preferred to generate reliable spectra with good signal-to-noise [56,68]. Also, in the cases when the droplets are charged by producing them in an electric field, the resulting MALDI mass spectra have good signal-to-noise ratio because analyte co-crystallization with the matrix is promoted, as demonstrated by Agnes, Harmon and their co-workers [70,72].

Miniaturized sample preparation offers on-target sample enrichment, another important aspect for achieving high sensitivity. Multiple depositions of small sample droplets at the same position, interspersed by short delays for complete or partial solvent evaporation, permit accumulation of the analyte in a single small spot. This circumvents problems when the analyte concentration is not sufficiently high to provide reliable analytical data (e.g., where trace amounts of sample are dissolved [68] or dilute eluents from analytical micro separation must be analyzed [11,13,15,60,61]).

3.2. Improved reproducibility

Samples that are prepared in a traditional way frequently suffer because crystallization is heterogeneous and analyte incorporation is variable, necessitating a search for a “sweet spot” within a relatively large spot area. Furthermore, the reproducibility of signals is poor spot-to-spot and sample-to-sample. The precision becomes worse when the analyte concentrations are lower. Spectral reproducibility can be greatly improved when the sample-spot diameter becomes smaller because a larger portion of each sample is simultaneously irradiated with the desorbing laser beam [10,11,15,19,^{20,22,23,25,26,30,32,34,35,43,74}]. The need for the time-consuming search for the “sweet spot” is eliminated, and the analysis can be expedited by automating for high throughput. Furthermore, the signal response for the sample becomes linear with the sample amount [11,24,32,43,68], making it easier to quantify with MALDI.

3.3. Ready interface to analytical microseparations

An important feature offered by any small sample-spot preparation is the opportunity to interface MALDI-MS to small-scale separation techniques. MALDI-spot miniaturization is crucial to maintaining the resolution of the microseparation without sacrificing high speed. Tedious, costly procedures of nano-fraction collection and handling can be circumvented prior to final analysis, and the benefits of small-scale MALDI spotting (e.g., high sensitivity) can be exploited to improve sample throughput. As demonstrated using protein digests and a combined micro-column or capillary LC [14,15,23,57,71], CE [26,31,38,49,50], gel electrophoresis [11,17,21,29,35,36,44] and solid-phase microextraction [61], higher sequence coverage and, thus, better protein identification, can be more readily achieved than by using conventional MALDI-preparation protocols. Improved sensitivity gained by miniaturizing sample spots is surely important. Suppression effects are also reduced compared to using the traditional dried-droplet method [10,23,25,71].

3.4. Improved MALDI imaging and profiling

MALDI imaging allows mapping the two-dimensional distributions and relative abundances of specific molecules in a tissue section to reveal the spatial chemical-specific profile of the original specimen. Tissue sections for MALDI imaging are prepared by carefully depositing matrix solution on top of the tissue surface to ensure that the analyte is properly extracted into the matrix and desorbed without analyte migration. Automated droplet microdispensing is a practical approach for applying a high-density array of matrix spots that is more efficient than conventional manual-deposition, airbrush, or electrospray methods. The goal is to reduce analyte migration on the spot, thereby obtaining high-resolution, high-throughput MS images.

Using an acoustic reagent multi-spotter, Caprioli and co-workers [73] applied small matrix spots of 180–200 μm width on mouse-brain sections and demonstrated that protein migration is limited. The analyte extraction from the tissue can be controlled to some extent by dispensing multiple droplets at varying frequencies onto the same location of the sample.

Similarly, using a modified desk-top ink-jet printer with piezoelectric heads, Yost and co-workers [62] deposited matrix spots (3 pL) with a diameter of a few tens of μm and inter-spot space of 20 μm . These dimensions are significantly smaller than the diameter of laser beam; thus, the spatial accuracy and resolution of MS images are not influenced by analyte migration within these small individual matrix drops.

On-tissue digestion coupled with MALDI imaging can be realized by microdispensing digestion reagents before matrix application to make protein identification more productive [65,66]. Furthermore, the automated preparation process significantly minimizes variation among analysts and improves precision.

3.5. Advanced on-line MALDI analysis

Miniaturization of the sample target simplifies not only static MALDI analysis but also continuous analysis (i.e. “on-line MALDI”) where deposition, desorption and detection by MS occur sequentially in real time [48,59]. The idea of an on-line interface is motivated by the advantage that fewer sample-handling steps reduce the chance for sample losses and result in more effective ultra-trace analysis. Moreover, higher efficiency and throughput should be achieved when the samples undergo on-line processing. An on-line MALDI interface allows handling of small volumes and sensitive analysis of minute amounts of sample.

Karger and co-workers [48,49] connected a fused-silica capillary with a sample flow at 100–400 nL/min for CE, and deposited a thin trace of a solution containing a mixture of analyte and matrix on a rotating quartz wheel or moving tape. The wheel or tape rotates and transports the sample directly to a location for laser desorption.

Murray and co-workers [59] made on-line MALDI analysis capable of repeated use by employing multiple connections, including a piezoelectric single-droplet generator for non-contact sample deposition, a capillary for matrix application, a cleaning system, and a plastic tube to remove waste.

3.6. Other improved applications

3.6.1. On-probe reaction—On-target, small-scale chemical or enzymatic reactions can be conducted by dispensing a small volume of reactants on a MALDI plate followed by direct MALDI-MS analysis [12,16,17,47]. Materials and time can be saved because additional handling and transfer are unnecessary and sample loss is greatly reduced.

3.6.2. Multiple-spot irradiation—When a spot with dimensions that are smaller than that of laser beam can be created and placed in a high-density format, the simultaneous analysis of two or more discrete, but adjacent, sample spots using a single laser can be achieved [39,55]. The approach provides multiplexed sample analysis without concern about ion suppression caused by different co-crystallization with the matrices. Synchronous MALDI measurement is particularly applicable for analytes that do not mix well or for materials in immiscible solutions.

3.6.3. Microextraction combined with MALDI—Li and co-workers [75] demonstrated low-volume liquid extraction of hydrophobic peptides and phospholipids followed by MALDI analysis. The microextraction was achieved by forming an nL-sized droplet containing organic solvents at a capillary tip for extraction from aqueous solutions. The resulting nL droplet containing relatively concentrated analytes can be readily deposited onto a MALDI target for analysis. Sample handling is minimized, and throughput increased with this method.

4. Conclusion and future prospects

Highly-sensitive, small-scale sample preparation enhances the capability of MALDI for trace analysis, opening the door to a direct profiling assay using MALDI for small biological structures (e.g., organelles [51]) or for interrogating potential biomarkers (e.g., neurotransmitters and proteins) present in trace amounts in clinical samples.

Meanwhile, the integration of miniaturized MALDI-sample preparation with various microseparation techniques is receiving substantial attention. The combination allows more efficient separation and identification, while minimizing sample consumption. A computer-controlled dispensing and positioning system may provide a future high-throughput, automated MALDI analysis platform.

Also, miniaturization of instrumentation should profit from the ability to prepare high-density sample arrays on small-scale sample targets. This approach may play an important role in the next generation of commercial instrumentation that we expect to be widely deployed in biomedical and clinical laboratories.

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Figure 1.

A prestructured sample support with anchor arrays, as introduced in [34]. On-plate purification is possible, as shown (Reprinted from [40], with permission).

Figure 2.
The set-up of nL chemistry station, as introduced by Li et al. [45] (Reprinted from [45], with permission).

Figure 3.
(A) Cross section of the piezoelectric micro-machined silicon flow-through dispenser. (B) Actuation principle for the microdispenser (Reprinted from [15], with permission).

Figure 4.

Portable induction-based fluidics (IBF) nL spotter for droplet deposition: (a) power supply module; (b) micrometer; (c) nL wave inductor; (d) stainless-steel matrix-assisted laser desorption/ionization (MALDI) plate; (e) electrical wire connected to ground; (f) electrical wire connected to high voltage; (g) syringe holder; (h) syringe with a chemically-treated capillary tip; and, (i) x-y adjustment stage (Reprinted from [68], with permission).

Figure 5. Droplet-levitation system using an electrodynamic balance (EDB). The droplet is trapped between the two ring electrodes for solvent evaporation and then ejected to the matrix-assisted laser desorption/ionization (MALDI) plate (Reprinted from [54], with permission).

Figure 6. Acoustic reagent multi-spotter showing the translational stage, sample and drop-imaging systems, and the acoustic ejector for matrix dispensing (Reprinted from [73], with permission).

Table 1

Examples of hydrophobic surfaces that reduce matrix-assisted laser desorption/ionization (MALDI) spot sizes

^a

Surface materials	Test analytes	Major advantages ^{b,c}	Combined analytical techniques ^c	Ref.
Teflon (poly-(tetrafluoroethylene))	DNA, proteins, peptides, proteolytic products	Higher salt tolerance, on-probe desalting	Gel electrophoresis (Teflon as a transfer membrane)	[18,19,24,33]
Linear synthetic polymers	Protein digests, oligonucleotides	On-plate desalting	Two-dimensional gel electrophoresis (2DGE)	[21,22]
Paraffin wax film (Parafilm)	DNA, protein digests and neuronal tissue releasate	Higher salt tolerance, higher protein-sequence coverage, on-plate purification	2DGE, liquid chromatography (LC), capillary electrophoresis (CE)	[20,28-31]
Hydrophobic foil (DropStop)	Peptides, small organic compounds, intact proteins	Matrix suppression, higher protein-sequence coverage	Capillary-zone electrophoresis (CZE)	[25,26]
3M Scotch Guard	Antibiotics	Easy cleaning and reapplication	Capillary-HPLC	[23]
3M Micro Mate labeling tape	Peptides, proteins, DNA	Salt suppression		[32]
AnchorChip (Teflon prestructured with gold spots)	Peptide mixture, protein digests, oligonucleotides, DNA, PCR products	Higher protein-sequence coverage, on-plate purification	2DGE, CE	[34-38]
Prestructured silicone polymer or silicon wafers	Peptides, protein digests, membrane protein	Higher protein-sequence coverage, reduced ion suppression		[27,39]

^a We omit literature that described other hydrophobic surface materials but does not discuss controlling MALDI spot size.^b This column shows those additional advantages that are not discussed in the text.^c The advantages and the techniques given were emphasized in the corresponding references. They may also apply to other analytical surface materials.