Ultrasound-Based Surface Sampling in Immersion for Mass Spectrometry

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We present a surface sampling method for the chemical analysis of liquid-immersed solid samples. Liquid immersion allows samples to be kept in a hydrated state. We employed cavitation generated by high-intensity focused ultrasound for localized material removal. The appropriate transducer-sample distance was determined using the actuating ultrasound transducer prior to sampling, allowing sonication in focus despite surface height variation. We demonstrate the proposed surface sampling method on water-submerged glass cover slides painted with permanent markers, achieving sampling with tunable spot size down to 500 μm. The removed and collected material was transferred for chemical analysis by electrospray ionization mass spectrometry, which showed mass peaks characteristic to the permanent markers.

I. INTRODUCTION

Mass spectrometry (MS) is applied for chemical analysis due to its high specificity and sensitivity. MS methods, particularly traditional liquid-chromatography MS (LC-MS), often require extensive sample preparation prior to analysis, including homogenization and dissolution of the solid sample into a solvent. Due to homogenization, all spatially dependent chemical information is lost. For this reason, different surface sampling methods allowing direct sampling from the analysis surface are being developed. Especially, when the sampling is coupled with MS, specific spatially-registered chemical information of the surface is obtained. This combination provides a label-free technique for chemical surface analysis.

Analysis of sample surfaces by MS currently relies on a set of desorption ionization methods. The desorption processes are based on ablation by a high-energy laser1-3, extraction by a solvent-spray4, or desorbing secondary ions by an energetic ion beam5-7. These methods are suitable for either ambient or vacuum conditions. Additionally, solid-liquid extraction (SLE) can be used with spatial control via liquid bridge coupling applied with two capillaries8-11, or with a sampling tip12. However, none of these existing methods are designed for operation in liquid immersion. Many biological samples are preferably kept in their natural aqueous environment to prevent cell deterioration or death. Therefore, direct sampling in liquid immersion would be beneficial. We propose that this could be achieved with a focused ultrasound (FUS)-based method.

The use of ultrasound (US) has gained interest in the field of MS in ionization13-15, sampling16, nebulizing17,18 and acoustic droplet ejector19-21 methods. Laser ultrasonics has also been employed for surface sampling, namely by combining laser-induced acoustic desorption (LIAD) with electrospray ionization (ESI-MS)22. Perhaps more conventionally, ultrasound has been utilized in sample preparations for MS. For example, ultrasound-assisted extraction (UAE)23-25 has been employed in solid-liquid and liquid-liquid extraction26. Another example is an acoustic-cavitation-based extraction method known as focused ultrasonic solid-liquid extraction (FUSLE), which has been utilized in food and environmental sciences27-29. The working frequency of FUSLE is approximately 20 kHz. From a sampling perspective, FUSLE offers limited capabilities for reducing the spot size. The focusing of US waves is only done by wave guiding through a tapered ultrasonic horn (probe end size of few mm). Therefore, we propose using MHz-range focused ultrasound (FUS), relying on focused wave propagation, for localized sampling with improved spatial resolution. In addition, FUS offers pulse-echo measurement capability and, by extension, US imaging by scanning over the sample.

High-intensity focused ultrasound (HIFU) can generate localized cavitation. Briefly, in the context of HIFU, acoustic cavitation is a process in which bubbles, either present in the liquid or formed by the high-intensity pressure wave focused to a confined volume, begin to oscillate in either a stable or transient manner. Phenomena associated with transient cavitation are a result of sudden bubble collapses, which can produce micro-jetting, shock waves, localized heating, high local pressures, and the formation of chemically active species30. These phenomena are widely employed for material removal and modification in e.g. cavitation-based machining31,32 and sonochemistry32. In biomedical applications, HIFU-generated cavitation is applied for, e.g., eroding kidney stones33 and destroying tumour tissue with high spatial precision and minimal adverse effects to surrounding tissue34. We propose that cavitation generated by HIFU...
could also be employed for surface sampling.

Acoustic streaming\textsuperscript{35} is a phenomenon present in HIFU beams. The principal cause of acoustic streaming is the acoustic nonlinearity of the immersion medium arising from the dissipation of ultrasound energy into the medium by viscous and scattering phenomena, resulting in a second order flow-field\textsuperscript{36}. Together with the properties of the immersion medium, the ultrasound excitation parameters, e.g. amplitude and US field shape, have an impact on the induced acoustic streaming field\textsuperscript{36}. Consequently, acoustic streaming is taken into account in the proposed sampling method.

Here we demonstrate surface sampling with a high-intensity focused ultrasound (HIFU) method in liquid immersion as a first proof-of-concept study. We used thin layers of permanent marker ink on glass cover slides as analytes to be detected in the chemical analysis. We built a custom-made US transducer to generate a focusing US field and studied the effect of US excitation amplitude on the removed area of the ink layer. We also developed a liquid handling method to collect the removed material during the HIFU excitations. Subsequently, we ran electrospray ionization mass spectrometry (ESI-MS) analysis on the collected liquid samples. Through this effort, we aim to upgrade the toolbox of surface sampling methods.

II. MATERIALS AND METHODS

A. Reagents and Materials

The following chemicals were used in the MS analyses: methanol (LC-MS Chromasolv\textsuperscript{TM}, Honeywell, Germany), acetonitrile (ACN) (LC-MS Chromasolv\textsuperscript{TM}, Honeywell, Germany), and formic acid (98-100\% for analysis ESMURE\textsuperscript{®}, Merek KGaA, Germany). Permanent markers of three different colours were used to prepare samples: black (M1), blue (M2) and red (M3) (Sharpie\textsuperscript{®} fine-point permanent markers, Sanford, Oak Brook, IL, USA). Purified water from a Milli-Q water purification system (Millipore, Molsheim, France) was used.

The sample markers were applied on standard glass cover slides (VWR\textsuperscript{®}, 631-1554), which were cleaned by sonication in solvent acetone (HPLC Chromasolv\textsuperscript{TM}, VWR chemicals, Pennsylvania, USA), and methanol for at least 5 min. The glass trough holding the water-submerged samples was also cleaned with ACN and Milli-Q.

B. Surface Sampling Setup

The setup consisted of two functional sections: ultrasound excitation and liquid sampling, see Fig. 1. The ultrasound excitation involved a signal generator (Tektronix, AFG31052, Oregon, USA) producing sinusoidal electric signals for alignment and cavitation generation. The signals were amplified with a radio frequency (RF) power amplifier (Amplifier Research, 500A100A, Pennsylvania, USA). A custom-made 7 MHz transducer was used, comprising an epoxy-backed (Bison Epoxy 5 Minutes Blister, Bison International B.V., Netherlands) concave piezo-electric ceramic [Meggitt A/S, type: Pz26 (coupling factors $k_p=0.56$ and $k_t=0.47$), mechanical quality factor $Q > 1000$, $\rho = 7.70 \text{ g/cm}^3$, outer diameter $D_o = 22.5 \text{ mm}$, radius of curvature $R = 17 \text{ mm}$, center hole diameter $D_c = 8 \text{ mm}$] and a 3D-printed enclosure from ABS and PLA. The acoustic pressure field generated with the transducer at low amplitudes was measured at the focal plane, see Supplementary Methods: Ultrasound Field Characterization and Supplementary Figs. 19 and 20.

The electric input signal and the reflected ultrasound echoes were monitored with an oscilloscope (Picoloscope 5203, UK), see Fig. 1(A). A 100X-probe (TESTEK Elektronik GmbH, TT-HV250, Germany) was used to attenuate the electric signal received by the oscilloscope. Echoes from the sample surface were monitored for transducer alignment, i.e., adjusting the transducer tilt angle and transducer-sample distance. The transducer was moved to obtain the strongest echo, from which the time-of-flight (TOF) was determined using a cross-correlation-based method. Specifically, a coded-excitation signal (6-8 MHz linear chirp with a duration of 20 \textmu s) with a peak-to-peak excitation voltage $U_{pp} = 30 \text{ V}$ was used for alignment. At each sampling spot, the TOF was monitored and adjusted to $23.53 \text{ µs} (\pm 0.01 \text{ µs})$, corresponding to a transducer-sample distance close to the geometric focus ($R$) of the transducer ($17.4 \text{ mm}$ for a sound velocity $1483 \text{ m/s}$ in water vs $R = 17 \text{ mm}$).

To generate cavitation ablation, a higher US amplitude was required, and the excitation voltage $U_{pp}$ was increased. Excitations consisted of 2000 burst at the working frequency $f = 7.0 \text{ MHz}$ of the transducer. Each burst consisted of 20 sinusoidal cycles and the pulse-repetition frequency (PRF) was set to 500 Hz. Excitation voltages are specified for each sampling spot in Table I (displayed in section II D). Note, the US frequency corresponds to wavelength $\lambda = 212 \text{ µm}$ in water ($c = 1483 \text{ m/s}$).

The transducer was moved in $x$-, $y$-, $z$-directions by translation stages, which were manually controlled by micrometer screws. Purified water (Milli-Q) was used as the immersion liquid. Degassing was not performed because the glass trough was uncovered and hence provided access to ambient air and a constant exchange of gases between the air and the immersion liquid. The experiments were conducted at room temperature.

C. Liquid Sampling Setup

Sampling of immersion liquid was done in a syringe-free manner by a liquid handling pump (VICI M6, Schenkon, Switzerland), see Fig. 2. Channel A was con-
FIG. 1. A) Controlling the surface sampling setup. B) VICI M6-pump (1) and custom-built ultrasound transducer (2). The transducer and sample (3) were immersed in Milli-Q water in a glass trough (4). The sampling capillary (5), connected to the liquid handling pump (1), was guided through the transducer. Micrometer screws (6) were used to position the transducer.

FIG. 2. Liquid sampling setup. Using a VICI M6-pump with two channels (A, B), a liquid sample was withdrawn into the sampling loop connected to channel A. The sample loop was then infused into an Eppendorf tube by reversing the direction of pumping and withdrawing Milli-Q into the capillary connected to channel B, pushing the sample out of the sampling loop. After each ablation sample, the capillaries were flushed back-to-back with 5 mL of ACN and 5 mL of Milli-Q to waste in order to prevent carry-over effects.

Liquid samples were typically acquired before, during and after HIFU ablations (see Supplementary information: Surface Sampling Experiments). Pre- and post-ablation samples were taken to verify that no carry-over effects occurred and that marker-related ions showed no abundancies in the background mass spectra of the immersion medium. Each sample (volume of 200 µL) was withdrawn into a sampling loop. The sampling loop (200 µL) was then emptied into a 500 µL tube (Eppendorf®, Hamburg, Germany) by changing the direction of pumping (after changing the B-channel bottle to a Milli-Q bottle). A solvent-acid mixture [200 µL, containing 100/0.2 (v/v) acetonitrile (ACN)/formic acid (FA)] was then added into the tube by a pipette to dissolve the captured particles/analytes. After each ablation sample, the sampling capillary line was flushed consecutively with 5 mL of ACN and 5 mL of Milli-Q water at a flow rate of 5 mL/min. In addition, at least one mL of the immersion liquid was then flushed with the liquid handling pump through the sampling loop to waste, to provide a consistent background of immersion liquid in the sampling loop prior to the next HIFU ablation sample. The flushing decreased the possibility of carry-over effects between subsequent samples. After the sampling series, the Eppendorf tubes were either directly transferred for MS analysis or were stored till the next day in a refrigerator (4 °C).

D. Material Removal Experiments

Two surface sampling experiments were performed on different days: The first cover slide is denoted surface S1 (day 1) and the second S2 (day 2). A graphical representation of S1 and S2 is shown in Fig. 3, displaying the adjacent sampling sites on the samples. Surface S1 was covered with black ink (M1). Surface S2 had three adjacent regions with black ink (M1), blue (M2), and black-on-blue. In the black-on-blue region, the black marker (M1) was applied after the blue marker (M2) layer had dried. The two layers were likely mixed due to an expected similarity of the solvents used for the markers. The markers were applied on clean glass cover slides approximately one hour before each experiment. In the
Samples $U_{pp}$ $p_{PN}$ Samples $U_{pp}$ $p_{PN}$
\begin{tabular}{llllll}
S1 #1-5 & 111 & -14 & S2 #13-14 & 55 & -7 \\
S2 #1-3 & 111 & -14 & S2 #15-16 & 55 & -7 \\
S2 #4-6 & 89 & -12 & S2 #17-20 & 44, 44, 49, 55 & -6,-6,-6,-7 \\
S2 #7-9 & 69 & -9 & & & \\
S2 #10-12 & 55 & -7 & & & \\
\end{tabular}

**TABLE I.** Ultrasound excitation voltages ($U_{pp}$) and extrapolated peak-negative-pressures ($p_{PN}$) for each sampling spot (see Supplementary Figure 20 for pressure estimation).

targeted regions of the samples, adjacent sampling spots were sonicated with one mm spacing (indicated by spot number after S1 or S2 e.g., S1 #1). The cover slides were imaged with an optical microscope after the sampling series to quantify the ablation surface area. The imaging was done only after sampling to mitigate pre-contamination of cover slide samples before they were placed in water immersion.

Excitation voltages $U_{pp}$ used for cavitation generation are listed in Table I.

**E. Mass Spectrometry Analyses**

Chemical analysis of the samples was performed with an Agilent® Ion Trap (6330, Agilent Technologies, Santa Clara, CA, USA) featuring an ESI source with positive polarity. Samples were introduced to the ESI source by direct infusion using a syringe pump (Harvard apparatus, PHD 2000, Holliston, Massachusetts, USA) at a flow rate of 20 µL/min. The source parameters were: dry gas temperature 325 °C, nebulizer pressure 15.0 psi, and dry gas flow rate 5 L/min. More mass spectrometer parameters can be found in the Supplementary Table 1. The following data acquisition parameters were used for each analysed sample: $t \geq 2$ min run, averaging of five spectra with rolling averaging of two, mass range from 50 to 1000. An average mass spectrum was obtained from the run of each sample with the instrument’s data analysis software (Agilent DataAnalysis for 6330 series Ion Trap LC/MS version 3.4) and the MS spectra were exported into an xy-file for plotting of the data.

Preliminary tests were conducted to find a proper solvent. 50/50/0.1 (v/v/v) ACN/water/FA was found suitable for the used markers. A similar ACN/water/FA solution has been used in earlier marker studies. The corresponding mass spectrum of each marker was then used as the reference spectrum of the marker (black ref and blue ref). The marker-related ions are listed in Table II with a reference to accurate mass measurements by others. Subsequently, an immersion test in Milli-Q water was performed to determine the dissolution tendency of the markers on a painted cover slide surface. A cover slide, identical to S1 and S2, painted with black (M1), blue (M2) and red (M3) regions was placed in water immersion ($V=100$ mL) for up to $t=30$ min periods. Several liquid samples were pipetted at time intervals (0, 5, 15, 30 min) and analysed by MS, see Supplementary Fig. 17. The ion peak from red (M3) marker immediately showed an abundance in the background, thus it was excluded from further experiments.

MS studies (blank tests) were run on the water and solvent-acid mixture [50/50/0.1 (v/v/v) ACN/water/FA] at the beginning of each surface sampling series. Blank tests consisted of five samples before the first ablation sample, ascertaining a sufficiently clean background mass spectrum for the subsequent surface samplings, see Supplementary Figs. 9 and 10.

Further data processing and visualization were done with MATLAB® (The Mathworks, Inc., R2021a, Natick, Massachusetts, United States) after the raw data were exported from the instrument’s DataAnalysis software. In this work, the mass spectra are displayed as raw data without further signal processing.

The ion trap instrument was calibrated prior to the measurement series with a standard Agilent ion trap calibration solution [Agilent LS/MS tuning mix, for ESI (Ion Trap), G2431A] for positive and negative polarity, using the instrument’s control software.

**F. Optical Microscope Imaging**

Cover slide images were obtained with an optical microscope (Nikon eclipse TE300) carrying a Nikon D70 DSLR camera. The microscope featured a 4X objective (Nikon 4X Plan Fluor PhL DL). A calibration image of a Vernier caliper was used as a scale bar. Image analysis was performed with MATLAB to calculate the ablation surface areas. Pixels per area were converted to mm$^2$ according to the scale bar. The method is described in the caption of Supplementary Methods Fig. 3.

**G. Visualization of Surface Sampling Process**

A video visualizing the material removal process was also captured. Another transducer was used in order to avoid unnecessary contamination of the transducer used
for sampling by the high amount of detached ink particulates in the immersion medium. The transducer's geometry matched closely to the one described earlier. It featured a concave piezo-element (Meggitt A/S, piezoelectric material type P226, outer diameter $D_o = 19$ mm, center hole diameter $D_c = 5$ mm and radius of curvature $R = 15$ mm) and was pulsed using frequency $f = 11.5$ MHz, with 20-cycle bursts at 200 Hz PRF. Excitation parameters were: 650 mV input voltage to the RF amplifier with a 65% RF gain. An acrylic container, with clear optical access through a camera objective filter (HOYA HO-PF49, Tokyo, Japan) mounted to its wall, was filled with purified water (Milli-Q). The sample was an aluminium surface painted with two subsequent layers using two permanent markers (bottom: black Sakura Pen-Touch® 130 permanent marker and top: black Centropen® permanent marker). Instead of the liquid handling pump, in this experiment the suction flow was applied by a syringe pump at 1.2 mL/min flow rate. A standard objective (Canon EF-S 18-55 mm, f/3.5-5.6 IS, Tokyo, Japan) with a DSLR digital camera (Canon EOS 550D, Tokyo, Japan) was used to capture the video at 24 fps. Video processing was also performed with MATLAB.

### III. RESULTS

#### A. Visualization of HIFU-induced Surface Sampling

Initial experiments were conducted to visually verify material removal and acoustic streaming generated by HIFU. The material removal and sampling are shown with snapshots from optical video footage, see Fig. 4. These snapshots were taken while applying HIFU on an ink-coated aluminium (Al) plate submerged in purified water. This video captures the release of a black-coloured ink cloud from the surface until it is drawn into a sampling capillary. The upper row (frames #1-4) in Fig. 4 shows the detached ink-particle cloud rising towards the ultrasound transducer after the HIFU was turned on (frame #2). The applied ultrasound induced an acoustic streaming field that transported the ablated particles a few mm away from the surface. When suction was applied with a syringe pump (frame #5), the ink cloud was drawn into the sampling capillary, as shown in the bottom row of the figure. This video is provided in the Supplementary material (see Supplementary Video 1). It should be noted that the particle cloud might be invisible during standard sampling. In the experiment shown here, the amount of material detaching is exaggerated with thick layers of marker ink on the surface.

Another visualization of the ablation process is shown in Supplementary Video 2, imaged with a Stroboscopic Schlieren setup. Under continuous pulsed US, while translating the transducer laterally, the detaching ink particulates were withdrawn into the sampling capillary with the aid of the suction flow, see prevalent streamlines from Supplementary Fig. 2.

#### B. Material Removal Experiments

The results of surface sampling with our custom-made 7 MHz focusing US transducer are shown here. The lateral focal plane of the transducer was measured with low amplitude US ($U_{pp} = 3$ V) [shown in the last frame of Fig. 5(A)]. The acoustic pressure map displayed a full-width-half-maximum (FWHM) of 228 ± 26 µm for the main focus, i.e., main lobe. The calculated side-lobe area had diameter $D = 594$ µm (mean radial distance from the center of the main lobe to side-lobe local maxima plus a mean value of the FWHM/2 of the four hot spots). These areas are overlayed on several spots by the dashed rings in Figs 5(A)-5(D). Further details on the US field characterization can be found in Supplementary Methods and Supplementary Fig. 19. The acoustic pressure was also measured at the center of the focus during an input amplitude sweep ($U_{pp} = 3-25$ V), showing a linear trend of peak-positive pressure $p_{PP} = 0.18 \cdot U_{pp}$ [MPa/V] ($R^2=0.997$) and of peak-negative pressure $p_{NP} = -0.13 \cdot U_{pp}$ [MPa/V] ($R^2=0.997$), see Supplementary Fig. 20.

In Fig. 5(A), surface sampling was performed on black ink (M1) on S1 at five adjacent sampling spots (S1 #1-5). The ablated areas (white areas) featured irregular shapes, which is typical for cavitation-induced erosion. The first region (S1 #1) has a rather confined ablation area and the last four (S1 #2-5) display larger and more irregular areas of ablation, despite unchanged US excitation.

### TABLE II. List of marker-ink-related ions and their literature reference. Both markers, M1 and M2, are Sharpie® fine-point permanent markers. Accurate m/z is a measured value of the molecule obtained with high-resolution mass instrument by Van Berkel, et al.\textsuperscript{37}.

<table>
<thead>
<tr>
<th>Marker</th>
<th>Ink compound</th>
<th>Measured m/z</th>
<th>Accurate m/z</th>
<th>Exact m/z</th>
<th>Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1:Black</td>
<td>Not identified</td>
<td>324</td>
<td>324.245</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M1:Black</td>
<td>Methyl violet 2B</td>
<td>358</td>
<td>358.230</td>
<td>358.228</td>
<td>(C$<em>{24}$H$</em>{27}$N$<em>3$H)$</em>{\pm}$</td>
</tr>
<tr>
<td>M1:Black</td>
<td>Crystal violet</td>
<td>372</td>
<td>372.246</td>
<td>372.244</td>
<td>(C$<em>{25}$H$</em>{30}$N$<em>3$)$</em>{\pm}$</td>
</tr>
<tr>
<td>M2:Blue</td>
<td>Not identified</td>
<td>239</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M2:Blue</td>
<td>Cationic basic blue 7</td>
<td>478</td>
<td>478.322</td>
<td>478.322</td>
<td>(C$<em>{33}$H$</em>{40}$N$<em>3$)$</em>{\pm}$</td>
</tr>
</tbody>
</table>
FIG. 4. Snapshots of HIFU-generated ablation with the proposed sampling method. The images are zoomed into the region-of-interest (ROI), which shows the sampling capillary and the sample surface. The transducer is located approx. 17 mm above the sample surface and the capillary-to-surface distance is 4 mm. When the transmitted HIFU-pulse reaches the surface, the high-intensity pressure-wave oscillations at the focus cause localized cavitation-induced ablation, producing a cloud of removed material (frame #2). Following the removal, a material cloud ejects towards the transducer due to acoustic streaming (frames #2-5). From frame #5 onwards, additional liquid motion is caused by suction with a syringe pump. The suction enables collection of the ablated material into the sampling capillary (#6-8). This video is shown in Supplementary Video 1. The image contrast was enhanced to visualize the ink cloud, see Supplementary Fig. 1(A) for the original image.

tion parameters ($U_{pp} = 111$ V). The removal areas agreed in dimensions with the diameter of side-lobe area (magenta ring). For the blue ink (M2) on surface S2, the first three ablation spots [S2 #1-3, top row Fig. 5(B)] were produced with a higher excitation voltage ($U_{pp} = 111$ V) than the three subsequent ones [S2 #4-6, bottom row Fig. 5(B)] ($U_{pp} = 89$ V). While no clear differences were observed between the images in Fig. 5(B), an increase in abundancy of marker-related ions was noticed when the lower excitation $U_{pp}$ was used [see Fig. 6(B) and 7(B)]. Similarly, the ablated areas were mostly confined by the side-lobe area (magenta ring), as seen in S2 #6. In Fig. 5(C), three adjacent ablation spots were carried out on the black-coloured (M1) region with a lower US excitation amplitude ($U_{pp} = 69$ V) (S2 #7-9) than used for spots S1 #1-5. The results showed reduction in the ablation area when using the lower US excitation amplitude [Fig. 5(C) and 7(C)]. Especially the second and third ablations, S2 #8,9, showed reduced and more confined sampling areas compared to S1 #1-5. Noticeably, the ablation mostly occurred within the main-lobe area, see S2 #9. Three adjacent HIFU excitations were carried out on the black ink region of S2 (S2 #10-12) with a further reduction of excitation amplitude ($U_{pp} = 55$ V). At this amplitude cavitation was insufficiently induced, therefore no material removal was observed (see Supplementary Fig. 7).

Next, effects of excitation amplitude and consecutive sonications on an ablation area were studied. Two consecutive excitations at $U_{pp} = 55$ V were performed on the black-on-blue region at the two spots S2 #13-14 and #15-16 [Fig. 5(D)]. The secondary US excitations (S2 #14, #16) were expected to resume ablation of the painted layers, thus increasing the sampling spot size. However, the following mass spectrometric analysis revealed only marker-related ions from the first excitations at each spot [S2 #13 and S2 #16, Fig. 6(D)]. The third spot on the black-on-blue region (S2 #17-20) was produced by progressively increasing $U_{pp}$ (44, 44, 49, and 55 V). The expectation was that the sampling area or depth would increase subsequently. At the spot S2 #17, the first excitation was performed with $U_{pp} = 44$ V. Because no visual removal at the surface was observed, a second US excitation was applied with the same voltage (S2 #18). Next, an excitation with $U_{pp} = 49$ V (S2 #19) was applied, still without visual ink removal. A fourth excitation was performed with $U_{pp} = 55$ V (S2 #20), i.e., the same $U_{pp}$ as for S2 #13-16. The ablation areas were expected to resemble S2 #13 and S2 #15. Instead, only a tiny area of ink was ablated, which did not show...
up in chemical analysis (see mass spectra of S2 #17-20 in Supplementary Fig. 16).

C. Mass Spectrometry Analyses

The mass spectra of ablation samples were compared with the reference mass spectra [black ref and blue ref in Figs. 6(A)-6(C)] allowing the detection of marker-related mass peaks in the samples. As listed in Table II, the following mass peaks were related to M1: m/z 372, m/z 358, m/z 324. Figures 6(A) and 6(C) show the mass spectra of analysed samples obtained from the surfaces with black ink (M1). The three mass peaks related to black ink showed abundant peaks, see black lines S1 #1-5) in Fig. 6(A) and (S2 #7-9) in Fig. 6(C).

Samples acquired from the blue-ink (M2) region of S2 displayed abundant marker-related ions corresponding to the marker dye: m/z 478 and another, m/z 239, as displayed in Fig. 6(B) (S2 #1-6). No efforts were made to identify the mass peak m/z 239, but it also showed an abundant peak in the reference mass spectrum from the marker sample [blue ref in Fig. 6(B)]. The three adjacent spots pulsed with $U_{pp} = 55 \text{ V}$ excitations (S2 #10-12) did not produce ablation of the black (M1) area, and no marker-related ions were observed in the corresponding mass spectra, see Supplementary Fig. 15.

The adjacent HIFU spots on black-on-blue regions (S2 #13 and S2 #15) revealed ions corresponding to both markers (M1 and M2), see Fig. 6(D). However, the second US excitations (S2 #14 and S2 #16) on top of the earlier (S2 #13 and S2 #15) were expected to continue the removal process further, i.e., increase the ablation area, but none of the marker-related ions were detected from these samples, see Fig. 6(D). The characteristic marker-related mass peaks were not detectable from the pre-ablation samples [gray lines in front of S1 or S2 #1-15 in Figs. 6(A)-6(D)]. Furthermore, carry-over effects, i.e., contamination by previous samples, were not observed in the post-ablation samples (darker gray lines between subsequent ablation samples).

The average abundance levels (heights of mass peaks) of the characteristic ion peaks (m/z 372 (M1) and 478 (M2)) were compared to the averaged abundance of the pre-ablation control samples, see Fig. 7(A)-7(B). The error bars correspond to one standard deviation within each group. These abundancies show that the marker-related ions can be sampled and effectively transferred to mass spectrometric analysis using the proposed method. In Fig. 7(A), the average abundance of samples S2 #7-9 was 68% less compared to that of S1 #1-5. Note that the decrease in abundance agrees with the average 71% reduction in the ablation area [Fig. 7(C)]. In Fig. 7(B), an average 31% increase in abundance of samples S2 #4-6 was noticed in comparison to the average of the first three samples (S2 #1-3), while the corresponding ablation areas decreased by 19% on average [Fig. 7(C)]. This may be a result of increased sampling efficiency due to changes in acoustic streaming during the HIFU excitation.

IV. DISCUSSION

We demonstrated a FUS-based surface sampling method developed for MS analysis. The results displayed features of HIFU-induced transient cavitation, causing localized material removal by ablation. The sampling process was visualized by video frames (Fig. 4) showing the ablated ink-cloud movement. After the detachment of material, its movement was at first caused by acoustic streaming, and next by suction of the liquid into a sampling capillary. The suction by syringe-pump was deliberately delayed for this visualization purpose. How-
FIG. 6. Mass spectrometric analyses from cover slide samples (S1 and S2) obtained with the proposed surface sampling method. The abundant ions in the mass spectra corresponding to the marker ions are marked with red circles (o). Mass spectra of control samples (pre = light gray, post = dark gray) revealed no carry-over effects between the HIFU-induced ablation samples (black lines). A) Five sampled ablation spots from S1 (black M1). Mass spectra show high abundance mass peaks corresponding to marker dye ions at m/z = 372, 358, and 324 (see details in Table II) in the samples collected during the HIFU excitations and no abundance in the control samples above the background level drawn pre and post HIFU excitations. B) Six samples taken during HIFU excitations in the blue region of S2 produced mass spectra matching the marker-related ions at m/z 478 and m/z 239 of M2 (Table II). Successive ablations demonstrate the reproducibility of our surface sampling method within each group: the first three samples (S2 #1-3) with slightly higher US excitation power and the latter three (S2 #4-6) with lower power. C) Three sampled ablation spots (S2 #7-9) show abundancies of black (M1) marker from surface S2. D) Two consecutive HIFU excitations at two different sites on the black-on-blue region (S2). Ions corresponding to black and blue inks were observed for the first excitation in each spot (S2 #13, #15) but not for the consecutive excitation in either sample (S2 #14, #16). Full mass range (m/z 50-1000) of these A)-D) are displayed in Supplementary Figs. 11-14.

ever, when used concurrently, the acoustic streaming and pump-induced flow can complement each other, which was the case when the surface samplings were performed on the cover slide samples.

The microscope images of ablated areas revealed irregularly shaped sampling spots. Here, neither the sampling spot size nor shape was optimized, since we aimed to demonstrate the novel surface sampling method and couple the method with a suitable detector, in this case a mass spectrometer. We will now try to explain the cause of irregularity and link the size of the sampling spots to the dimensions of the generated HIFU field.

The material removal process is based on transient cavitation, which is a stochastic process. The generated cavitation results in hundreds of imploding cavitation bubbles or bubble clouds at random locations within the focal volume of the US transducer. Due to this stochastic cavitation erosion process, the microscope images showed irregularly shaped sampling spots. The focal volume, which spatially restrains the cavitation ablation events,
is connected to the dimensions of the US pressure field at the lateral focal plane. Evidently a more confined bubble cloud will reduce the irregularity of sampling spots, while simultaneously permitting sampling of smaller areas.

To characterize the US pressure field, we both numerically simulated the focused ultrasound field and measured it by a hydrophone field scan. In our case, the bowl transducer features a hole in the middle, which was accounted for in the numerical model of the pressure field generated by our transducer. This model is valid in the linear (small amplitude) ultrasound regime and we assumed radial symmetry. The model-predicted dimensions for the field were: main-lobe width (FWHM) of 210 µm (approx. one wavelength in water), first side-lobe maximum at 257 µm (distance from the main-lobe center), and the pressure ratio between the first side-lobe maximum and the main lobe $P_{\text{side}}/P_{\text{main}} = 25\%$. The measured US field [see Supplementary Figs. 19(A)&19(B)] featured a slightly broader main lobe FWHM of 228 ± 26 µm. Unexpectedly, the measured US field was not radially symmetric. Instead of a side-lobe ring in the lateral focal plane, it displayed four side-lobe hot spots, which were symmetrically distributed around the main lobe with a radial distance of 214 ± 27 µm from the center. The average peak position deviated by 18% from the model prediction. The side-lobe hot spots displayed a pressure ratio (side to main) of 47% (alternatively, 22% for the extraction planes across the side-lobe minima), meaning the side lobes had higher pressure amplitudes than expected based on simulations. The US field shape along the four lines are displayed in Supplementary Fig. 19B. The cavitation erosion at the side-lobe areas agreed with the locations of hot spots in the measured pressure field [especially, see Figs. 5(A)&5(C)&5(D)]. The deviation from the ideal, radially symmetric field could be attributed to higher-order vibrations of the piezo-element. The most intense pressure amplitude is usually excited by the fundamental thickness extensional (TE) resonant vibration mode of the piezo-element. Beside the TE vibration mode, other resonant vibration modes could be simultaneously excited such as higher-order radial and fundamental circumferential modes. These additional vibration modes of the piezoceramic could explain the deviations from an ideal axisymmetric field. In literature, characterization of different vibration modes of piezoceramics has been limited to annular disk and plate geometries, for which the vibration modes have been numerically obtained and also experimentally verified. In our literature search, we could not find similar results on vibration modes present in a bowl-shaped annular piezo-element coupled to the generated pressure field at the focal plane. The transducer used was built in-house, and therefore some discrepancies from simulations might be due to the manufacturing process. Possible effects may arise from e.g. soldering the electrode wires.

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**FIG. 7.** Averaged marker-related mass peaks A), B) and ablation areas C). Note that the y-axes in A), B) are in logarithmic scale. Marker-related mass peaks showed two orders of magnitude higher mass peak abundancies than in the control samples/background in A) and B). A) Average abundancy of black-marker-related (M1) ions m/z 372 for samples captured during the five adjacent HIFU excitations ($U_{pp}= 111$ V) on S1 and the three adjacent excitations ($U_{pp}= 69$ V) on S2 covered by the black (M1) Sharpie marker. A 68% average decrease in abundancy was recorded between these sampling spots (S1 #1-5 vs S2 #7-9), which agrees with the decreased area of ablation (71%) [see Fig. 7(C)]. B) The average abundancy of blue-marker (M2) specific ions m/z 478 captured during three adjacent spots with $U_{pp}= 111$ V (S2 #1-3) and three adjacent spots with $U_{pp}= 89$ V (S2 #4-6). This resulted in a 31% average increase in abundancy, though the ablated area decreased by 19% [see Fig. 6(C) and Fig. 7(C)]. C) The averaged ablated surface areas within each sampling group. The two spots on the black-on-blue region (S2 #13-14 and S2 #15-16) showed areas of 0.1 mm². The area of the last ablation spot S2 #17-20 was 0.016 mm² and marker-related ions in the corresponding samples were not detected. Error bars are one standard deviation. The individual samples within the groups are displayed with asterisks (*).
non-uniformity of the backing material, or residual pre-
stresses on the piezo-element due to the epoxy curing
process attaching the piezo-element to the 3D-printed
housing. In addition, our simulation model did not ac-
count for the additional vibration modes. Because the
piezo-element was simply modeled as an axisymmetric
2D-line source and was forced to vibrate at the driving
frequency, the boundary conditions corresponded with
the fundamental TE vibration mode. Nonetheless, the
simulated and measured pressure fields agreed reason-
able well in terms of spatial dimensions at the lateral
focal plane in the low-amplitude regime.

The pressure distribution in the high-amplitude regime
will display similar characteristics as seen in the low-
amplitude regime, although with possible changes to fo-
cal beam dimensions (FWHM of main lobe), pressure
amplitudes, and pressure ratio between side and main
lobes. For the sampling process, the high-amplitude
pressure field should exceed a threshold value to induce
cavitation. The cavitation threshold is proportional to
peak-negative-pressure ($p_{NP}$), depends on the immersion
medium, and exhibits strong frequency dependency. We
expect that the threshold would be first exceeded at
the fundamental frequency, because it contains the
highest-amplitude pressure oscillations. The pressure
threshold should also be exceeded first in the main lobe
area/volume. With a higher excitation amplitude, the
nonlinear effects increasingly generate higher harmonics
(multiples of the fundamental frequency) and the result-
ing nonlinear waves can effectively narrow the focus com-
pared to the main lobe of the linear beam. We ob-
served second and third harmonics from the monitored
signals with high amplitude excitations. Their corre-
sponding amplitudes in Fast Fourier transform (FFT)
analysis were 16% and 1%, respectively, of the fundamen-
tal frequency at the 25 V excitation amplitude. Beam
narrowing effects cannot be deduced from the results,
since the total ablation areas were always at least as wide
as the linear main lobe. Nonetheless, nonlinear focusing
should be investigated in order to optimize the size of the
sampling area.

In this study, we only varied the pressure amplitude
(excitation voltage) between sonications. The roles of
the US excitation parameters, such as total burst count,
cycles per burst, transducer-sample distance, PRF and
amplitude will be studied in our following research. From
the surface sampling results with the 7 MHz transducer,
we can conclude that the removal area was affected by the
amplitude of US excitation. The decrease of the excita-
tion voltage from 111 V to 69 V generated a 71% smaller
ablation area on average [Figs. 5 and 7(C)], and a corre-
sponding decrease in abundance was seen in the MS anal-
ysis. It is noteworthy that the decreased ablation area
correlated with the 85% reduction in the cross-sectional
area of the active focal US field (cavitation diminished
in the side-lobe area and occurred only in the main-lobe
area), which were shown in Fig. 5(A) in magenta and red
circles, respectively. Thus, surface sampling with the pro-
posed method allows regulating the sampled area, which
could be optimized to match the sensitivity of a chemical
analysis method.

Two phenomena related to cavitation could explain the
non-ideal behaviour in the sampling process with samples
S1 #1-6, S2 #1-6 and S2 #17-20. First, the sampling
spots S1 #2-5 showed larger and more irregular abla-
tion areas and higher mass peak abundancies than the
first S1 #1 spot despite constant acoustic parameters.
This finding could point to the history dependency of
cavitation, i.e., earlier acoustic bursts can have an effect
on subsequent ones. The bubbles formed during the
HIFU excitation at S1 #1 remain in the liquid and could
act as nucleation sites for the subsequent excitations at
S1 #2-5, increasing the probability of cavitation. How-
ever, this was not observed in the second experiment (S2
#1-6), where the ablation areas were similar within the
two groups (S2 #1-3 and S2 #4-6). Second, cavitation
may display hysteresis. If the cavitation threshold is
approached from below by gradually increasing the US
pressure amplitude, a higher pressure threshold is mea-
sured than if the initial acoustic pressure first exceeds the
threshold value and then is gradually decreased. Hystere-
sis could explain the results at the last spot, S2 #17-20,
see Fig. 5(D). The fourth and final HIFU excitation (S2
#20) was expected to produce similar ablation as in S2
#13 and #15. As the results showed, after the set of
excitations with gradually increased amplitudes S2 #17-
20 ($U_{pp} = 44, 44, 49, 55$ V), only minute cavitation pits
formed [see Fig. 5(D) last frame], and the subsequent MS
analysis revealed no clear traces of marker-related ions.
Therefore, to achieve repeatable and consistent surface
sampling, the method would benefit from real-time cav-
itation detection, verifying that the removal process has
started. Fortunately, this can be coupled to the system
by monitoring the backscattered echoes and analysing
the frequency content of the signals, which is a commonly
applied passive cavitation detection method.

In the future, several approaches can be chosen to al-
leviate the cavitation history effects. One approach is to
countrol the gaseous state of the immersion liquid. This
could be obtained with a constant exchange of the immers-
ion liquid with a degassed medium accompanied with
local delivery of nucleation sites for cavitation (e.g. mi-
crobulles) to the focus of the transducer. In this way,
the amount and location of the nucleation sites can be
delicately controlled. Another approach to introduce the
cavitation seeds could be to use a dual-frequency exci-
tation scheme, which has been demonstrated in HIFU
therapies earlier. A higher frequency low-amplitude
burst can be used to generate microbubbles (nucleation
sites), which are caused to collapse with a subsequent
lower frequency high-amplitude burst. Such approaches
could improve the repeatability of the cavitation-based
removal mechanism, albeit with added complexity to the
system.

Due to a large acoustic impedance mismatch between
air and water, a cavitation cloud can cause an energy
shielding phenomenon\textsuperscript{17}, which is similar to the cavi-
tation cloud scattering effect observed in lithotripsy
bursts\textsuperscript{48}. Shielding is likely to occur when the two fol-
lowing conditions apply: 1) the excitation amplitude is
sufficient to produce cavitation in bulk water and 2) the
focus of the transducer is placed too far above the sur-
face. Hence, the formed bubble cloud above the sur-
face acts as a reflecting boundary and protects the sur-
face underneath. A relevant result from studies of a
single collapsing bubble near a rigid boundary is that
the bubble stand-off distance has a significant effect on
surface cleaning efficacy\textsuperscript{49}. A stand-off distance of 0.7
times the bubble’s maximum size at the expansion phase
was determined to be most efficient for cleaning. Fur-
thermore, in the case of cavitation-based surface ero-
sion, maximal surface damage occurs at the stand-off
distance of 1.2. At this separation the resulted micro-
jet formed during bubble collapse has the highest kinetic
energy\textsuperscript{50}. These results suggest that optimal removal
could be achieved when the cavitation bubble cloud col-
lapses with the stand-off distance between (0.7-1.2) above
the surface. However, more recent studies suggest that
maximal surface erosion is obtained when the stand-off
parameter is close to 0.2\textsuperscript{51,52}. How well these results
apply to the case of complex bubble cloud dynamics is
not yet well understood. Nonetheless, transducer-sample
distance evidently greatly affects the erosion potential of
cavitation, and should therefore be controlled with high
precision. For this reason, we used a cross-correlation-
based TOF measurement for the positioning feedback
of the transducer, which is a similar technique that has
been used in imaging with a coded-excitation scanning
acoustic microscope\textsuperscript{53}. While in this study an appro-
priate transducer-sample distance was established exper-
imentally, bubble cloud dynamics and the possible of
effects of shielding on material removal require further
studies, both experimental and theoretical. In fact, we
have already initiated this research by capturing cavi-
tation cloud activity on and above a solid surface with
different transducer-sample distances using a high-speed
camera. The results will be a subject for our follow-up
study.

Acoustic streaming plays a role in the sampling pro-
cess. As shown in Fig. 4, acoustic streaming can cause
desirable particulate transfer towards the surface, al-
though, we could not verify the direction of streaming
during the surface sampling experiments. Recently,
we have noticed that the streaming field is sensitive to
transducer-sample distance, which could be optimized
for sampling purposes. We are currently conducting re-
search on the sampling associated streaming phenomena.
Changes in the acoustic streaming field could explain
that the latter sampling group (S2 #4-6) displayed a
31% increase in abundance when a lower US excitation
voltage was used. Since the streaming velocity is propor-
tional to the square of the pressure amplitude, the corre-
sponding amplitude increase would result in 56% higher
streaming velocity. The higher amplitude excitation also
increases the probability of cavitation in the focus. Be-
cause of the increased streaming velocity and the pro-
nounced cavitation, the ablated particulates could have
sufficient momentum to escape the suction of the sam-
pling capillary. These phenomena could account for the
changes in the sampling efficiency, shown by the increase
in mass peak abundancy [S2 #1-3 vs S2 #4-6 in Figs.
7(B)\&7(C)]. The proposed sampling method may ben-
efit from a supplementary streaming field that directs the
ablated material towards the sonicating transducer (as
visible in Supplementary Video 1), resulting in more ef-

cient sampling.

The presented sampling method works in liquid immers-
on, which is needed to propagate the high-amplitude
US waves and to generate cavitation. A benefit of li-
did immersion is that imaging with FUS can be cou-
pleted with the proposed US-based sampling method. The
US imaging of a surface can reveal regions of mecha-

nical contrast, enabling identification of regions-of-interest for
sampling. Material sampling from the desired location
could then be verified by subsequent US imaging of the
surface. The imaging ability can potentially benefit e.g.
cancer diagnostics\textsuperscript{54}, where the malignant tissue section
can be distinguished from the normal tissue by mecha-

nical contrast. Furthermore, sampling taking place in a
liquid environment can decrease the risk of e.g. biohaz-

ards and nanoparticles ending up in the ambient air.

The liquid environment can bring challenges to the
coupling with a mass spectrometer. For example, undes-
ired dissolution of samples into the immersion medium

can complicate sampling. Salts or other unwanted im-
impurities from samples can induce ion suppression and
adduct formation, which decreases the quality of the MS
spectrum. Samples consisting of rapidly dissolving an-
alytes in the immersion liquid, e.g. in water, can be
difficult to analyse with the proposed method. Possi-
ble methods to alleviate this problem include: (1) con-
stant exchange of the immersion medium with fresh li-
quid (complemented with online degassing), (2) covering
samples with a non-soluble coating compatible with MS
and the removal mechanism, (3) replacing the immer-
sion liquid with another, where the analytes of interest
are immiscible, (4) adding a sample purification or sep-
aration step before MS analysis, and (5) decreasing the
temperature of the immersion medium to lower the dis-
solution rate. A combination of these approaches could
also be used. Suggested method (1) could also minimize
cavitation history dependency, as mentioned earlier. For
method (2), for example, Parylene C coating has been
used to prevent the direct liquid extraction of analytes
in another sampling technique\textsuperscript{55}. In this study, a test
with the Sharpie\textsuperscript{®} permanent markers in water immers-
ion showed only minute dissolution (in a 30 min time
frame) of marker-related ions from black (M1) and blue
(M2) coloured areas, whereas a noticeable mass peak
rose immediately from a similar area coloured with red
(M3) marker (consequently we excluded the red marker
from this study). This result is displayed in the Sup-

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We demonstrated the feasibility of the surface sampling method with ink material deposited on a glass surface. Ink samples acted as a sample that might be relevant in forensic or counterfeit applications. We selected permanent markers with previously identified marker-related ions, which allowed us to compare our findings and confirm the MS results. Possible analyte fragmentation could take place due to the cavitational removal mechanism, since inertial cavitation is a relatively violent process at the microscopic level. In addition to the presence of high shear stresses near a cavitating bubble, shock wave generation upon collapse and micro-jetting resulting from asymmetric collapse, cavitating bubbles are known for their high chemical activity. Highly active chemical species can be formed inside an imploding cavitation bubble, hence it has been referred to as a sonochemical hot spot. Sonochemistry is initiated, when very high local temperatures and pressures are reached in the vapour of a collapsing bubble. In aqueous medium, these extreme conditions dissociate water molecules producing oxygen and hydroxide radicals. In a spherically symmetric bubble collapse, energy will focus geometrically at the center of the bubble, thus higher sonochemical activity is observed inside the bubble than in an asymmetric bubble collapse. The asymmetric collapse, which occurs when the bubble collapses next to a solid boundary and forms a micro-jet protruding towards the surface, may yield lower sonochemical activity, however it can more efficiently disperse radicals away from the bubble. The formed oxidative radical species (O and OH radicals) will quickly react with volatile solutes present inside the bubble. A portion of the radicals also reaches the gas-liquid interface, which is a second chemically active region in the presence of the cavitation bubble. Therefore, in the case of a sample containing volatile and dissolving molecules in the immersion liquid, possible sonochemistry should be considered. A small portion of radicals may escape the bubble into the surrounding medium, where the transported hydroxide radicals at the latest will recombine and form hydrogen peroxide, which can further increase chemical activity of the molecules present in the liquid. The constant exchange of immersion liquid would likely also decrease accumulation of chemically active species near the sample surface. In summary, these sonochemical factors play an important role in the next part of our research, when the target is to study the viability of the method with different kinds of materials, e.g., soft materials, such as tissue sections and other biomaterials.

The demonstrated sampling method can be coupled with other analytical detection methods in addition to MS. The collected liquid samples could be analysed by, e.g., UV/Vis spectroscopy, Raman spectroscopy, nuclear magnetic resonance (NMR) spectroscopy, and electroanalytical methods. In this study, we used MS to detect the sampled material because of its sensitivity and specificity. MS analysis could be further improved by using LC-MS and/or a high-resolution MS instrument for an increased analytical selectivity and specificity, which would be needed with biological samples or screening applications. ESI was chosen as the ionization method, but other methods could be used as well.

A similar ultrasound-based surface sampling method for MS has not been presented before. The proposed method adds the capability to attain chemical samples from surfaces placed in liquid immersion. In addition, the method could be complemented with ultrasound imaging to obtain mechanical contrast maps of the surface in order to detect regions-of-interest and to verify sampling at desired locations. The established surface sampling techniques in MS have not been much used for sampling in immersion, excluding FUSLE, but may be beneficial in several fields such as material science, biology and medicine. For example, the capability to directly sample cells from a cell culture and plant or mammalian tissues from a liquid medium could yield many applications in these fields.

V. CONCLUSIONS

We demonstrated a surface sampling method operating in liquid immersion. Surface sampling analytes were detected by MS from sampling spots smaller than 500 μm. The developed sampling method is on par with common sampling techniques (by solvent-spray or liquid extraction-based methods) in terms of sampling spot size, however this method is distinct from existing methods in that sampling take place in liquid immersion at ambient conditions. We suggest that the presented sampling method may be used in online MS surface sampling analysis, permitting direct mass spectrometric sampling with tunable spot size even from uneven surfaces. Furthermore, the obtained chemical fingerprints could be complemented with a mechanical contrast map gained by US imaging prior to and after sampling, verifying that the samples are obtained from the region-of-interest.

SUPPLEMENTARY MATERIALS

See supplementary materials for further details of the methods used to produce this manuscript data and figures. Additionally, supplementary video 1 shows the visualization of the surface sampling process, from which the snapshots shown in Fig. 4 were selected. Supplementary video 2 is a stroboscopic Schlieren video displaying the ultrasound field and material removal process. It also shows the associated particulate streaming phenomena with and without external suction flow.
ACKNOWLEDGMENTS

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AUTHOR DECLARATIONS

Conflicts of Interest


Author Contributions

A.S. had the original idea of the project; A.K., T.K., E.H., A.S. supervised the project; T.S. carried out surface sampling experiments, analysed results, and drafted the manuscript; J.H., P.L., and T.P. provided technical support for the HIFU part; J.M. and A.H. discussed theoretical aspects of the sampling method and contributed to writing the manuscript; R.L. assisted T.S. in the MS analysis part; All authors participated in reviewing this manuscript and accepted the final version.

DATA AVAILABILITY

The datasets supporting the findings in this study are available from the corresponding author upon reasonable request.

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Oscilloscope Picoscope 5203
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VICI M6
Waste

1. 2. 3. 4. 5. 6.
Sample loop
Flushing
Solvent Milli-Q
M-force for motion control, Arduino for triggering
Waste
A B
Eppendorf
Bidirectional liquid handling pump
A) Five HIFU spots

B) Six HIFU spots

C) Three HIFU spots

D) Two HIFU spots

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A) m/z = 372

Abundancy [a.u.]

B) m/z = 478

Abundancy [a.u.]

C) Ablation area

Area [mm$^2$]