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SYSTEM AND METHOD FOR SAMPLING

FIELD

The present invention relates to systems and methods for sampling, in particular to systems and methods wherein the sampling is based on ultrasound assisted
5 removal of material from the sample for feeding the material to a detecting means. The invention relates also a detecting means comprising the system.

BACKGROUND

Analysis of samples via mass spectrometry (MS) rely on desorption and ionization methods including matrix-assisted laser desorption ionization (MALDI), desorption
10 electrospray ionization (DESI), laser ablation electrospray ionization (LAESI) and secondary ion mass spectrometry (SIMS). Each method performs at best with certain types of analytes and are used for different applications, although there is no universal ionization method that fits for all analytical purposes. Each of the method works either in ambient or in vacuum conditions but none of the current
15 desorption methods operates well with sample immersion. Since biological samples e.g., cell cultures and tissue sections are often stored at liquid medium their mass spectrometric analysis may be laborious. Accordingly, there is still a need for further systems and methods for sampling.

SUMMARY

20 The present invention is based on the observation that at least some of the problems related to sampling material from samples immersed in a fluid can be avoided or at least alleviated by creating controlled focused cavitation at predetermined positions within a sample. According to the present invention cavitation created by high-intensity focused ultrasound waves (HIFU) removes material from interface between
25 the sample and the immersion fluid. The combination of acoustic streaming and flow field induced by transport of fluid into a feeding means such as a sampling capillary close to the ultrasound focus allows capturing removed material and transporting it for further analysis.

Accordingly, it is an object of the present invention to provide a system for sampling
30 material to a detecting means, the system comprising

- a housing for a sample and a fluid for immersing the sample,
- a transducer configured to emit focused ultrasound waves towards a target on interface between the sample and the fluid for removing material from the sample to the fluid and for producing an acoustic stream in the fluid, and
- 5 ○ a feeding means comprising a first end configured to be positioned between the transducer and the interface, and a second end configured to be connected to the detecting means, and fluid transport means between the first end and the second end, the feeding means configured to transport at least part of removed material to the detecting means.

10 It is also an object of the present invention to provide a detecting means comprising system of claim 1.

It is still an object of the present invention to provide a method for feeding material from a sample to a detecting means using the system of claim 1.

Further objects of the present invention are described in the accompanying
15 dependent claims.

Exemplifying and non-limiting embodiments of the invention, both as to constructions and to methods of operation, together with additional objects and advantages thereof, are best understood from the following description of specific exemplifying embodiments when read in connection with the accompanying
20 drawings.

The verbs “to comprise” and “to include” are used in this document as open limitations that neither exclude nor require the existence of un-recited features. The features recited in the accompanied depending claims are mutually freely combinable unless otherwise explicitly stated. Furthermore, it is to be understood
25 that the use of “a” or “an”, i.e., a singular form, throughout this document does not exclude a plurality.

BRIEF DESCRIPTION OF DRAWINGS

Figures 1 and 2 illustrate exemplary systems of the present invention.

Figure 3 illustrates snapshots of a process for removing material from a sample
30 according to an exemplary non-limiting embodiment of the present invention.

Figure 4 illustrates frames of a video acquired with a stroboscopic Schlieren imaging setup. A) without suction of liquid into the sampling capillary and B) with suction flow into the capillary. The width of the capillary is 1.58 mm.

Figure 5 illustrates relevant m/z-peaks and abundances of a marker-related ions of several adjacent HIFU-excitations on a sample surface (spot spacing 1 mm). First
5 three sonication spots (SN 1-3) were performed at slightly lower intensity, then from the next two samples (SN 4-5) with higher intensity, and for the last four samples (SN 6-9) the sampling capillary was translated 2 mm closer to the sample surface.

Figure 6 illustrates an image of an exemplary sample before (A) and after (B)
10 sampling using system of the present invention.

Figure 7 illustrates exemplary MS analysis of material removed from a target on a sample using the method of the present invention. Vpp 900 mV; Spots 1-3 RF gain = 60%; spots 4-6: RF gain = 50%.

Figure 8 illustrates the effect of the ultrasound intensity on an exemplary sample.
15 Vpp = 900 mV; spot 9: RF gain 50%; spots 10-12 RF gain = 30%.

DESCRIPTION

According to one aspect the present invention concerns a sampling system for transporting material removed from a sample to a detecting means. An exemplary system 100 is shown in figure 1. The system comprises a housing 101 for fluid 102
20 and a sample 103, a transducer 104, and a feeding means 105.

The transducer is configured to emit focused ultrasound waves towards a target on interface 103a between the sample and the fluid. An exemplary transducer suitable for the system is a focused piezoelectric transducer. An exemplary operating frequency is 12 MHz. The intensity of the ultrasound at the target is preferably at
25 least 1 W/cm² to allow removing material from the sample and for producing an acoustic stream in the fluid. An acoustic stream is a steady flow in a fluid driven by the absorption of high amplitude acoustic oscillations.

The feeding means shown in the figure comprises a first end 105a configured to be positioned between the transducer and the interface, a second end 105b configured
30 to be connected to the detecting means 106, and a fluid transporting means 107

between the first end and the second end. An exemplary fluid transporting means is a pump.

The first end should be in close proximity to the interface when the system is in operation. The distance **h** between the interface 103a and the first end 105a is preferably 1.5-5 mm. An exemplary distance is 2 mm. This warrants effective transportation of the material from the fluid to the detecting means. A typical first end comprises a capillary tube. If the distance is too short, the ultrasound beam is distorted by the capillary and the cavitation threshold is not exceeded at the focal point. If the distance is too long, the sampling flow volume rate should be increased to effectively collect removed material. Problems might also arise due to increased sample volume i.e., sensitivity might decrease.

According to a preferable embodiment the system also comprises means 108 for adjusting the distance **h** between the interface 103a and the first end 105a. An exemplary means 108 comprises manual micro-meter screws.

When the system is in operation, the sample is immersed in the fluid, and the transducer emits high intensity focused ultrasound (HIFU) waves 109 towards a target on interface between the sample and the fluid. The HIFU induced cavitation erosion removes material from the sample at the target. Acoustic streaming induced by the focused ultrasound beam near acoustic hard boundary causes the removed material to move away from the interface, i.e., along the y-direction of the coordinate system 199. An exemplary removed material particle is illustrated in the figure by a star marked by a reference number 110.

The transducer needs to operate at an intensity at the focal spot which is preferably higher than 1 W/cm², more preferably 10 W/cm² or higher. The frequency of the transducer should be at least 20 kHz, preferably between 1 MHz and 15 MHz. Higher frequencies, up to 1 GHz can be used. Higher frequency provides a smaller focal spot for more localized sampling. For example, for a 12 MHz transducer with a numerical aperture of 0.85, the cavitation erosion pit radii ranges from 20 μm to 200 μm depending on the sample and ultrasound parameters. Increasing the frequency increases the cavitation pressure threshold so higher frequencies require tighter focusing and/or higher driving voltage of the piezo.

Breaking the sample cohesion, e.g., aluminum surface, requires higher acoustic pressure amplitude and higher total energy than breaking down adhesion of the sample, e.g., a thin film on a hard substrate. For aluminum in water the pressure amplitude at the focus should exceed 10 MPa, preferably higher, such as 20-25 MPa. The cavitation probability increases as the amplitude increases so higher amplitudes provide higher erosion/extraction efficiency. As the amplitude gets higher the erosion area increases as the part of the field that exceeds cavitation threshold increases. Accordingly, the spatial resolution of the method depends on the amplitude. The cavitation threshold is function of frequency i.e., the higher frequency the higher is the cavitation threshold. For breaking the adhesion, 15 MPa suffices and tuning the amplitude allows tuning the extraction area. The cycle count of the ultrasonic bursts should be preferably between 20 and 80 or more to provide high enough cavitation probability. The pulse repetition frequency (PRF) should be tuned in such a manner that the transducer does not overheat. For example, for a water-immersed 12 MHz single-element piezoceramic, maximum of 50W – 100W of forward electric power is suitable.

The target can be of any geometric shape. A particular target is a point on the interface. Another target is a line on the interface.

According to a particular embodiment the system comprises means 111 for moving the focal point of the ultrasound waves. This can be done e.g., by moving the transducer or by moving the housing. According to an exemplary embodiment the system comprises means 112 configured to move the transducer in x,y,z-directions of the coordinate system 199 e.g., by translation stages controlled with manual micro-meter screws. This allows positioning the interface at the focal point of the ultrasound waves. According to another embodiment the transducer is a phased array transducer that is configured to tune the focal distance. The focal point adjustment can be assisted by placing a hydrophone or the like in the housing.

According to another embodiment the moving of the focal point of the ultrasound waves is done by a means 113 configured to move the housing in x,y,z-directions, at least in x,z-directions of the coordinate system 199. The movement can be performed by placing the housing onto a CNC-router table operated by a microcontroller.

According to a particular embodiment the system comprises means 114, such as a computer program to assist with selecting the target based on an ultrasound image. According to an exemplary embodiment the program determines areas of the sample surface that are harder than a predetermined hardness level and instructs
5 the means 111 to move the focal point by moving the transducer above those areas one by one. Then, the HIFU induced cavitation erosion removes material from the selected sites, and the system feeds the removed material to the detecting means for analysis. This approach may be used to find abnormalities such as cancers in biological samples. According to another preferable embodiment the means 114 is
10 also configured to instruct the means 108 to adjust the distance h between the interface and the first end and/or to instruct moving the holding means.

According to one embodiment the image is an image imputed to the computer program. According to another embodiment the system comprises imaging means 115 such as a second transducer configured to produce the image which is then
15 transferred to the computer program. According to another embodiment the transducer 104 is configured to produce the ultrasound image. Ultrasound imaging is well known in the art.

According to still another embodiment the system comprises means 116 for controlling properties of the fluid. Exemplary properties are pH of the fluid,
20 temperature of the fluid, water content of a non-aqueous fluid, relative humidity of a gaseous fluid, velocity of the fluid.

According to another embodiment the present invention concerns a detecting means 106 comprising the sampling system described above. The detecting means is preferably selected from mass spectrometer, Raman spectrometer, NMR
25 spectrometer, IR spectrometer, UV spectrophotometer, scanning electron microscope, atomic force microscope, quartz crystal balance, dynamic light scattering meter, high pressure liquid chromatograph, x-ray diffractometer, activity assay means, immunoassay means, preferably mass spectrometer, and Raman spectrometer.

30 According to a particular embodiment the detecting means comprises two or more spectrometers. According to an exemplary embodiment the sampling system is connected to a mass spectrometer via a Raman spectrometer.

According to a preferable embodiment the detecting means is an electrospray ionization (ESI) mass spectrometer. ESI is a versatile soft ionization method suitable for many kinds of analytes and can easily be coupled with other separation methods e.g., liquid chromatography (LC) increasing the analytical performance. Different techniques have also developed further from the basis of spraying the solvent with or without analyte at the ion source as in DESI. ESI suits also for wide range of analytes, and it generates either positive or negative polarity of ions. In comparison to other techniques like SIMS or MALDI, ESI stands for more gentle type of ionization method. Generation of unfragmented ions would be considered as an advantage in respect to MS data interpretation of complex samples such as biological samples.

Figure 2 shows another exemplary system 200 of the present invention. The system comprises housing 201 for a sample and a fluid for immersing the sample, a transducer 204, configured to emit focused ultrasound waves towards a target on interface between the sample and the fluid for removing material from the sample to the fluid and for producing an acoustic stream in the fluid, and a feeding means 205 comprising a first end 205a configured to be positioned between the transducer and the interface, a second end 205b configured to be connected to the detecting means and a third end connected to a collecting means 217. The fluid transport means 207 is configured to feed the fluid towards the detecting means 206 and towards the collecting means 217.

The system 200 comprises preferably also one or more of

- means 208 for adjusting distance **h** between the interface and the first end 205a,
- means 210 for moving focal point of the ultrasound waves
- means 212 configured to move the transducer and/or means 213) configured to move the housing,
- means 214 configured to select the target based on an image of the sample.
- imaging means 215 for producing the image of the sample,
- means 216 configured to control one or more of: pH of the fluid, temperature of the fluid, velocity of the fluid, water content of a non-aqueous fluid, relative humidity of a gaseous fluid.

According to another aspect the present invention concerns a method for feeding material from a sample to a detecting means using the sampling system described above. The method comprises the following steps:

- i) immersing the sample into a fluid,
- 5 ii) selecting a target on an interface between the sample and the fluid,
- iii) subjecting the target to focused ultrasound waves wherein intensity of the ultrasound at the target is at least 1 W/cm^2 , preferably at least 10 W/cm^2 , thereby removing material from the sample and producing an acoustic stream in the fluid comprising removed material, and
- 10 iv) feeding at least part of the fluid comprising the removed material to the detecting means.

According to a particular embodiment the method the step iv) comprises feeding at least part of the fluid comprising the removed material to the detecting means.

Particular samples suitable for the method are those which should be preserved and
15 analysed while immersed into a fluid. Exemplary samples are biological samples such as cell cultures, tissue sections, forensic samples, plants, and materials comprising active pharmaceutical ingredients. Further exemplary samples are organic or inorganic solid or semisolid materials that do not dissolve into the immersion fluid, paints, polymers, gel-like materials, and metals.

20 According to a particular embodiment the sample comprises material rich in rare and precious metals (RPM), such as gold.

High-intensity focused-ultrasound-induced (HIFU) inertial cavitation can be used to erode surfaces such as surfaces of PCBs. With a MHz transducer, both imaging to determine ROIs and extracting RPMs is possible.

25 Exemplary immersion fluids are water, buffer solutions such as PBS, and alcohols such as ethanol and methanol, acetonitrile, and their mixtures. Naturally, desalting, or other purification methods may be is needed prior detection.

According to another embodiment the fluid is gas, in particular moist gas.

According to the method a target on the interface sample is subjected to high
30 intensity focused ultrasound. When the sample is solid, the interface is the surface

of the sample. The intensity of the ultrasound should be high enough to remove material from the sample.

The distance **d** between the transducer and the interface is adjusted, if needed by moving the transducer along the +/- y-direction of the coordinate system 199 until the focal point is at the desired position. The energy density of the ultrasound waves should be high enough to remove material from the surface, but low enough to prevent decomposition of the material. Exemplary ultrasonic excitation parameters are piezo voltage amplitude, cycle count in a burst, total amount of bursts and pulse repetition frequency. Exemplary parameters for the method are listed below.

- 10 • HIFU intensity at the focal spot is preferably higher than 1 W/cm², preferably 10 W/cm² or higher.
- The frequency of the transducer should be at least 20 kHz, preferably at least 1 MHz such as 1 MHz – 15 MHz. Even higher frequencies can be used.
- the pressure amplitude at the focus is preferably more than 10 MPa, such
15 as 20-25 MPa.
- The cycle count of the ultrasonic bursts is between 20 and 80 or more.
- Pulse repetition frequency (PRF) is tuned so that the transducer does not overheat. For example, for a water-immersed 12 MHz single-element piezoceramic, maximum of 50W – 100W of forward electric power is suitable.
- 20 • The distance **h** between the first end (such as the tip of a capillary) and the interface should be at least 1 mm, preferably between 1.5 mm and 5 mm.

According to another embodiment the method comprises imaging the sample prior to the step ii) and selecting the target point based on the image.

MATERIALS AND METHODS

- 25 The setup consists of an acoustic excitation part and a liquid handling part. A signal generator (Tektronix AFG31052, UK) was used to generate sinusoidal electric signal, which was amplified with RF power amplifier (Amplifier Research 500A100A, USA). A custom-made transducer (3D-printed) with a concave piezo-electric crystal converted the electric signal into mechanical pressure waves propagating through
30 the immersion bath. The electric signal consists of bursts of a sine wave at $f = 7.0$ MHz and $PRF = 500$ Hz. Ultrasound echoes reflected from the sample surface was

recorded with an oscilloscope (Picoscope 5203, UK) using a 100X- probe for aligning (adjusting the focal distance precisely) with low intensity ultrasound excitation amplitude which is suitable e.g., for imaging modality. A coded excitation signal (frequency modulated chirp sine wave, $f = 6-8$ MHz) was used for cross-correlating the emitted electric signal with the acoustic echo signal resulting in a cross-correlogram, from which the time-of-flight (TOF) of the propagation path was acquired. The transducer was moved along the x,y,z-directions by translation stages controlled with manual micro-metre screws. Milli-Q (Merck Millipore, France) purified water was used as immersion fluid.

10 Fluid Sampling System

Sampling of immersed fluid was performed in a syringe-free manner with a fluid handling pump (VICI® M6, Switzerland). Material for the sampling capillaries was Peek 1/16" (ID of 50 μm). Finger-tight capillary connections (VICI®, Switzerland) were used between the connections. Each sample (sample volume of 200 μl) was withdrawn into a sampling loop before the pump A-channel, which was then emptied into a 500 μl Eppendorf tube by changing the pumping direction and by withdrawing Milli-Q water into the pump's B-channel. The liquid handling pump was controlled by motion control unit (M-Force) from Schneider Electric®. The M-force unit has i/o pins, which were utilized for trigger-functions of programmed (using its own programming language Mcode) pumping actions (infusion and withdrawal at the specific volume rate and volume). When some i/o pin was pulled to ground it triggered the pre-programmed pumping action. The volume rate of sampling was set to 5000 $\mu\text{l}/\text{min}$, which provided good consistency in results of sampling. After each sonicated sample, the sampling capillary line was flushed with 3 ml of MeOH and 3 ml of Milli-Q preventing carry-over effects (solvents are withdrawn through the B- channel into waste).

Chemical Analysis via Mass Spectrometry

For chemical analysis of the samples Agilent® Ion Trap (6330) with electrospray ionization (ESI) source at positive polarity was used. Samples were introduced by direct infusion using a syringe pump (Harvard apparatus, PHD 2000) at a flow rate of 20 $\mu\text{l}/\text{min}$. Source parameters were as following: dry gas temperature 325 °C, nebulizer pressure 15.0 psi and dry gas flow rate 5 l/min. Acquisition parameters of

ion optics and trap are displayed in Table T2. Data was captured by averaging five spectra and using a rolling average of two. The m/z -range of acquiring data was set to start from $m/z = 50$ to $m/z = 1000$.

The following chemicals were used methanol (LC-MS Chromasolv®, Honeywell, Germany) and formic acid (98-100% for analysis EMSURE®, Merck KGaA, Germany) in the MS analysis. Marker was Sakura 130, black, Japan. The pre-tests black marker tip was dipped into 100% MeOH and diluted (1/100) into 20%, 40%, 60%, 80% and 100% MeOH (with 0.1% formic acid FA). As the MeOH concentration rises from 20% to 60 % higher m/z (especially $m/z = 530$) becomes more abundant, which rises from the improved solubility of the marker molecules to higher solvent content. The higher the MeOH concentration was the higher also becomes the abundance of non-relevant background ion peaks (contaminants e.g., from the ion source and capillaries). Therefore, a suitable solvent for this marker analyses was chosen to be the 60 % MeOH + 0.1% FA (acid for ionization efficiency).

During the sampling process the sampling capillary wash flushed after each HIFU excitation with three ml of 100% MeOH and three ml of Milli-Q water to prevent contamination of adjacent samples. Also, at least one ml of sampling fluid from the immersion bath was withdrawn through the sampling loop to secure the background stability (sampling loop contains a fully representative liquid background before each sample).

The sampling loop (200 μ l) was emptied into a 500 μ l Eppendorf tube. Immediately, a solvent plus acid mixture (300 μ l, containing 100% MeOH + 0.17% FA) was added into the Eppendorf for dissolving captured particles and the cap was closed. Tubes were labelled and stored at refrigerator (-18 °C) until they were analyzed by MS.

Data processing was performed with MATLAB® 2020b after the raw data (x,y) was exported using the Agilent Data Analysis for 6300 series Ion Trap LC/MS version 3.4 software. Generally, m/z spectra display only the raw data without any signal processing, but a simple background subtraction was performed to enhance the peaks arising from the marker for the sample taken during the HIFU excitation, from which the previously sampled (SN, foc. (focused)) mass spectrum was subtracted. If the subtracted signal intensity was negative, it was recorded as zero.

RESULTS

The HIFU generated desorption process

The HIFU generated desorption process was demonstrated by using a marker ink on top of an aluminium surface. Figure 3 shows snapshots of a video image acquired during the process as a function of time. The figure shows a particle cloud of black ink rising towards the ultrasound transducer (transducer's focal distance ca 17 mm) after the high-intensity ultrasound is turned on. The HIFU transducer produces an acoustic stream which drags the desorbed particles along the streaming motion for a couple of mms from the surface (fig 2, top). When the withdrawal of fluid is applied by the liquid handling pump, the ink particle cloud is withdrawn into the sampling capillary 208 (fig 2, bottom). The direction of movement of the adsorbed material is marked in the figure with arrows.

Figure 4 shows frames of a video acquired with a stroboscopic Schlieren imaging setup. Completely black areas (cross sections of sampling capillary and the sample) correspond to optically opaque media and lighter pattern-areas depict backscattered pressure waves from the surface. The drawn dashed lines illustrate the streamlines of detached material under the HIFU field targeted on the solid surface A) without suction of liquid into the sampling capillary and B) with suction flow into the capillary. Notice that the combined effect of acoustic streaming and suction flow into the capillary in B) show changes to the streaming field in comparison to A). A noticeable increase in motion of ablating material towards the capillary is visible in the video footage.

Chemical analysis of ultrasound desorbed surface material

Chemical analysis of ultrasound desorbed surface material was performed by mass spectrometry using electrospray ionization. Liquid samples taken during and after HIFU excitation were analysed. Several adjacent samples were taken within the same samples and the abundance of two m/z peaks (530, 439) corresponding to a marker (Sakura 130) were determined.

The abundance levels of the two marker ions are presented in fig 5. During the 3W (electric power) excitation, the two most relevant peaks (530, 439) showed ca 4 times higher m/z peak height ($S/N = 4$) than the background sample. When the excitation power was increased to 4 W and the distance **h** of the tip of the sampling

capillary from the interface was decreased, the m/z peak abundance was almost doubled.

Removal of Sharpie fine point permanent marker from a sample surface

Sharpie fine point marker (colour black or blue) was applied on top of a microscopy
5 cover slide. The marker was allowed to dry and the slide was immersed into a water
bath. A plurality of spots (spacing 1 mm) were removed by focusing HIFU to the
slide surface using different US intensities. The distance **h** between the sample
surface (here: interface between the sample and the fluid) and sampling capillary
was adjusted to 2 mm. The samples were withdrawn (aspiration of pump) with 5
10 ml/min during the HIFU excitation. Sample volume was 200 μ L. Samples were
infused from the sampling capillary into an Eppendorf tube, diluted with HPLC/MS
solvent ($\text{CH}_3\text{CN} : \text{H}_2\text{O}$; 1:1 (v/v) + 0.1% by vol formic acid) and analysed by mass
spectrometry.

Figure 6 shows a marker surface before (A) and after (B) subjecting the surface to
15 HIFU. The sample was immersed in a water-bath. The ultrasound transducer and
the sampling capillary is shown in the figure (middle) with reference numbers 404
and 408, respectively. As shown in the figure (B), the HIFU removed five distinct
areas of marker ink from the surface.

Figure 7 shows the MS analysis of materials extracted from six predetermined
20 positions (1-6) of sample surface using the method of the present invention and a
reference (ref; Sharpie fine point marker dissolved in water). As shown, material
from each position of the surface was successfully extracted and analysed. The
material could not be extracted when the transducer was turned off as evidenced by
the dummy mass spectra before and after each excitation, two of which marked in
25 the figure by letters **a** and **b**.

Figure 8 demonstrates the effect of the ultrasound intensity on sample extraction.
When the ultrasound intensity was below cavitation threshold (spots 10-12) the
sample could not be extracted from the sample surface. Analogously, when the
ultrasound intensity was above the cavitation threshold the sample was successfully
30 extracted (spot 9).

The specific examples provided in the description above should not be construed as limiting the scope and/or the applicability of the appended claims.

WHAT IS CLAIMED IS

1. A system (100, 200) for sampling material to a detecting means, the system comprising
 - 5 ○ a housing (101) for
 - a sample and
 - a fluid for immersing the sample,
 - 10 ○ a transducer (104, 204) configured to emit focused ultrasound waves towards a target on interface between the sample and the fluid for removing material from the sample to the fluid and for producing an acoustic stream in the fluid, and
 - 15 ○ a feeding means (105, 205) comprising a first end (105a, 205a) configured to be positioned between the transducer and the interface, and a second end (105b, 205b) configured to be connected to the detecting means, and fluid transport means (107, 207) between the first end and the second end, the feeding means configured to transport at least part of removed material to the detecting means.
2. The system according to claim 1 wherein intensity of the ultrasound at the target is at least 1 W/cm^2 , preferably at least 10 W/cm^2 .
3. The system according to claim 1 or 2 wherein frequency of the ultrasound is at least 20 kHz, preferably at least 1 MHz.
- 20 4. The system according to any one of claim 1 to 3 comprising means (108, 208) for adjusting distance h between the interface and the first end (105a, 205a).
5. The system according to any one of claims 1 to 4 comprising means (111, 211) for moving focal point of the ultrasound waves.
- 25 6. The system according to any one of claims 1 to 5 comprising means (112, 212) configured to move the transducer and/or means (113) configured to move the housing.
7. The system according to any one of claims 1 to 6 comprising means (114, 214) configured to select the target based on an image of the sample.
- 30 8. The system according to any one of claims 1 to 7 comprising imaging means (115, 215) for producing the image of the sample.
9. The system according to any one of claims 1 to 8 comprising means (116, 216) configured to control one or more of: pH of the fluid, temperature of the fluid,

velocity of the fluid, water content of a non-aqueous fluid, relative humidity of a gaseous fluid.

10. The system according any one of claims 1 to 9 comprising means (217) for collecting at least part of the removed material.

5 11. A detecting means (106) comprising a system according to any one of claims 1 to 10.

12. The detecting means according to claim 11 selected from one or more of: a mass spectrometer, Raman spectrometer, NMR spectrometer, IR spectrometer, UV spectrophotometer, scanning electron microscope, atomic force microscope,
10 quartz crystal balance, dynamic light scattering meter, high pressure liquid chromatograph, x-ray diffractometer, activity assay means, immunoassay means, preferably mass spectrometer, and Raman spectrometer.

13. The detecting means according to claim 11 wherein the detecting means is electrospray ionization mass spectrometer.

15 14. A method of feeding material from a sample to a detecting means using a system as defined in any one of claims 1-10, the method comprising

- i) immersing the sample into a fluid,
- ii) selecting a target on an interface between the sample and the fluid,
- iii) subjecting the target to focused ultrasound waves wherein intensity of
20 the ultrasound at the target is at least 1 W/cm^2 , preferably at least 10 W/cm^2 , thereby removing material from the sample and producing an acoustic stream in the fluid comprising removed material, and
- iv) feeding at least part of the fluid comprising the removed material to the detecting means.

25 15. The method according to claim 14 wherein step iv) comprises at least part of the fluid comprising the removed material to the collecting means.

16. The method according to claim 14 or 15 wherein frequency of the ultrasound is at least 20 kHz, preferably at least 1 MHz.

17. The method according to any one of claims 14 to 16 wherein the system
30 comprises means for adjusting distance **h** between the interface and the first end, the method comprising adjusting the distance to at least 1 mm, preferably between 1.5-5 mm.

18. The method according to any one of claims 14 to 17 comprising imaging the sample prior to step ii) and selecting the target based on the imaging.

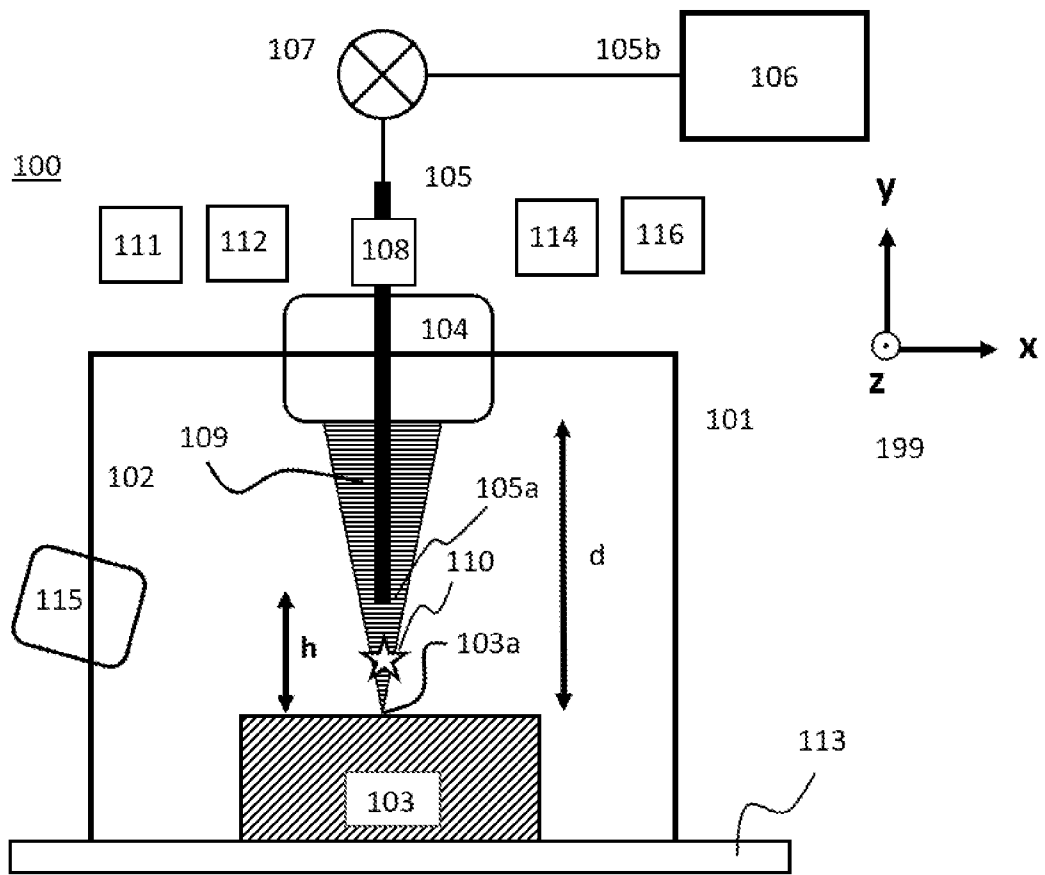


Figure 1

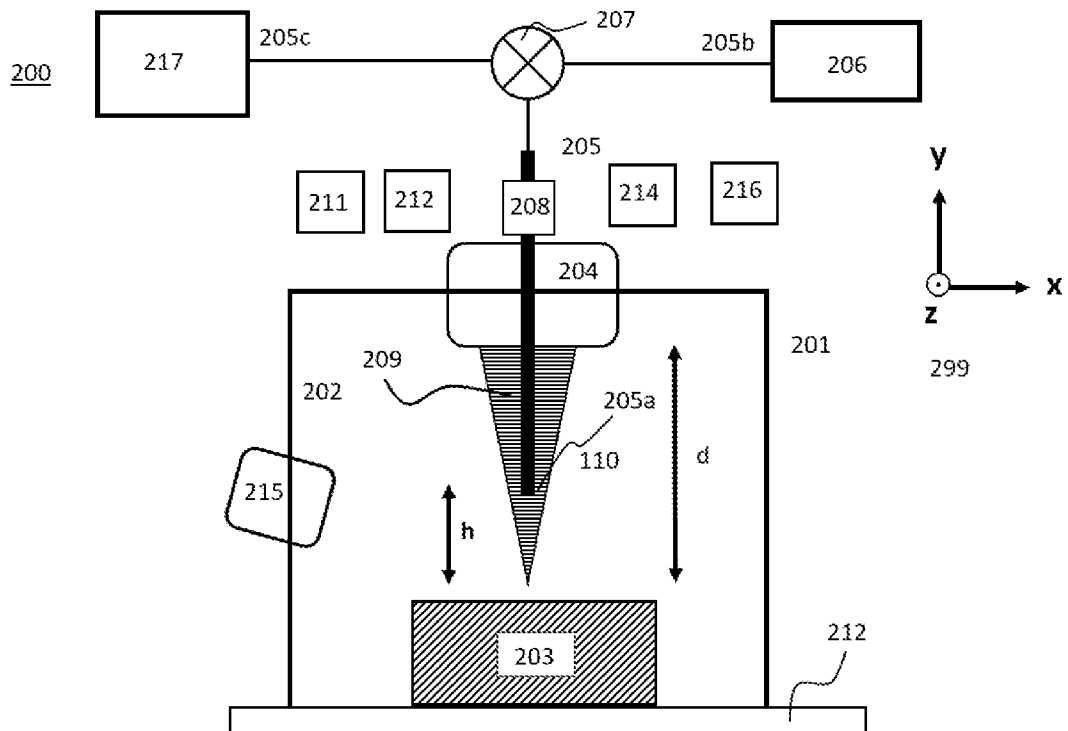


Figure 2

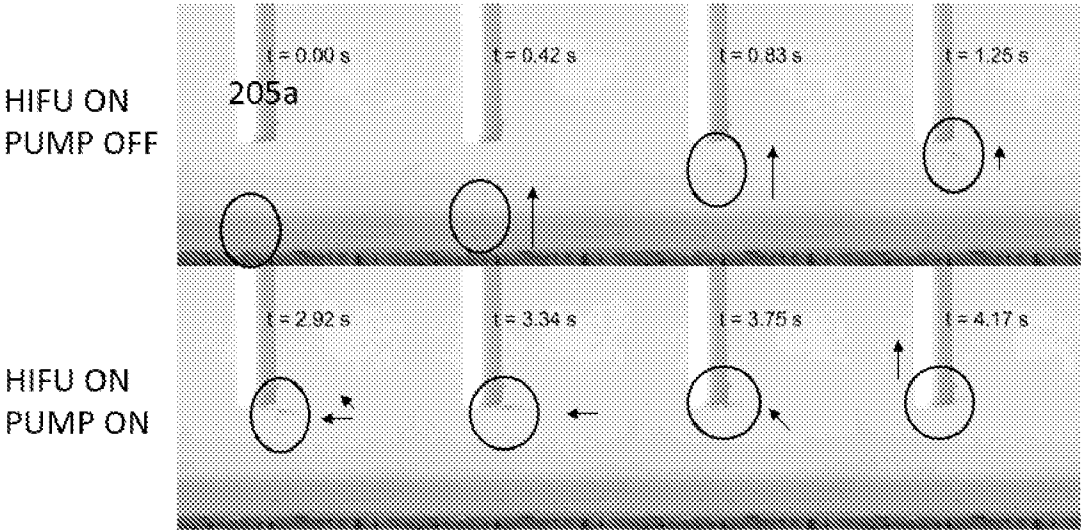


Figure 3

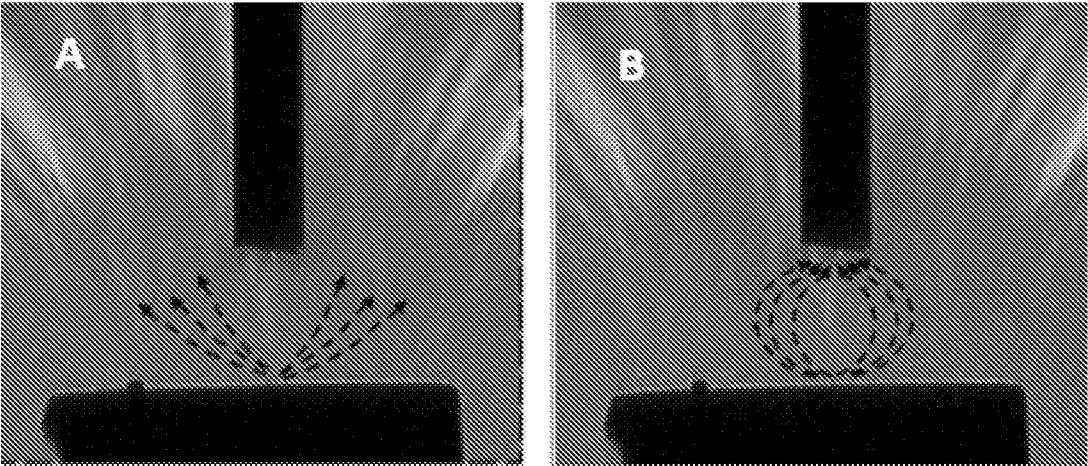


Figure 4

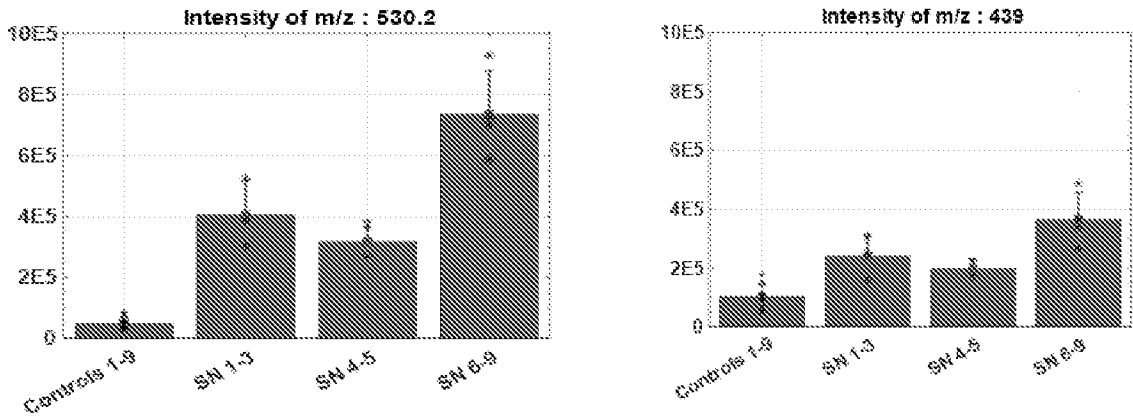


Figure 5

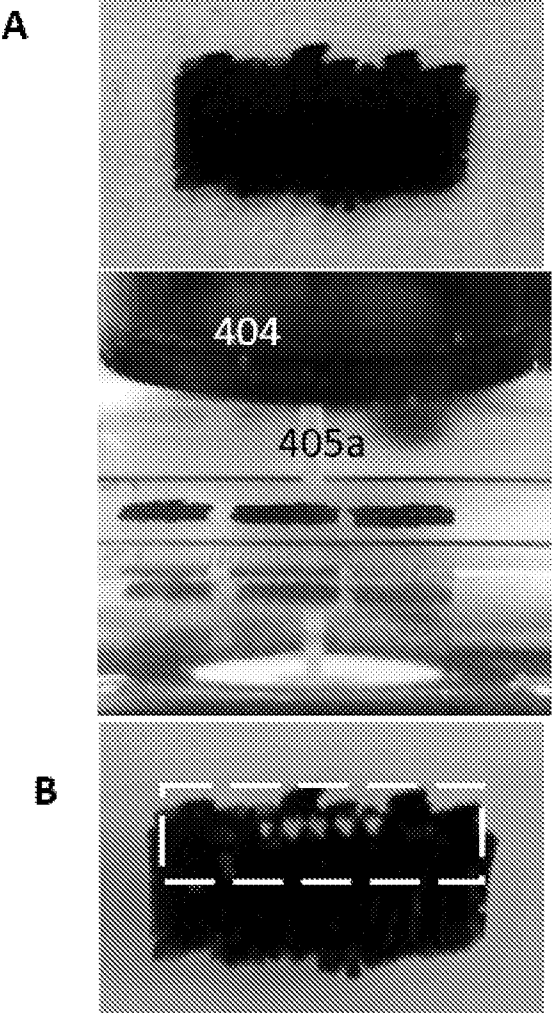


Figure 6

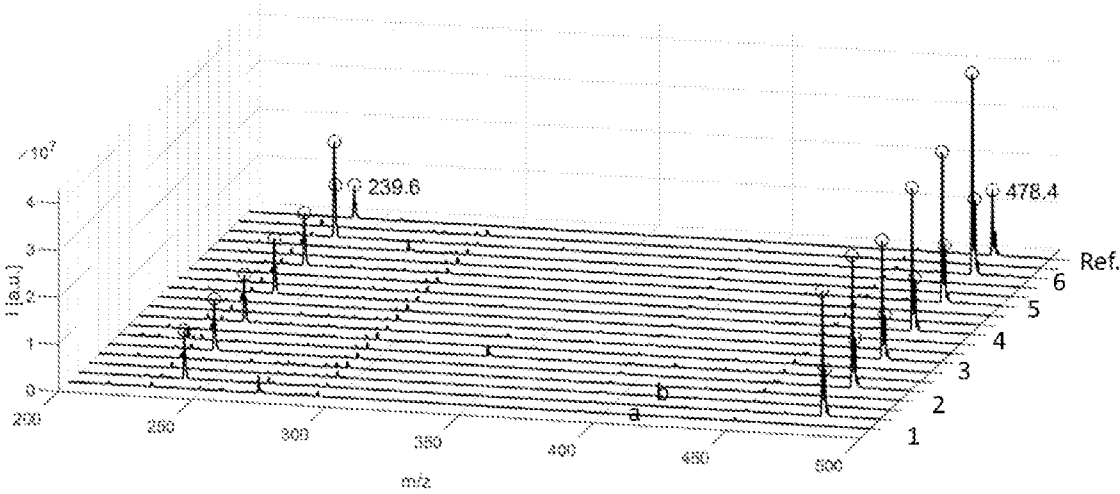


Figure 7

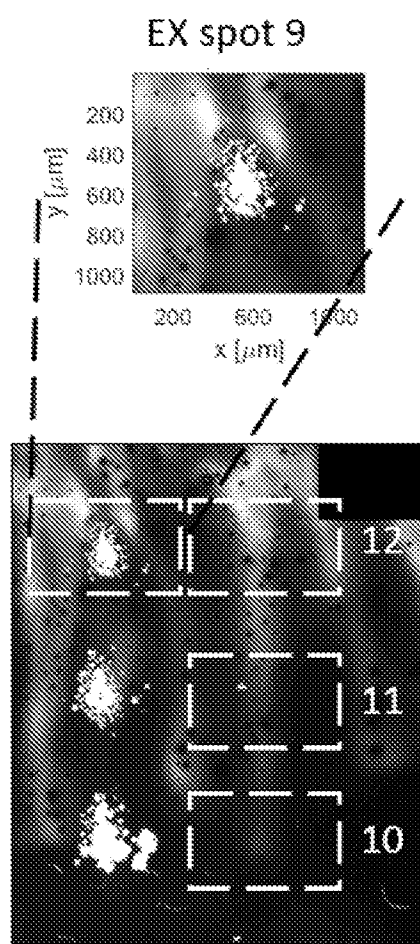


Figure 8

INTERNATIONAL SEARCH REPORT

International application No
PCT/FI2022/050470

A. CLASSIFICATION OF SUBJECT MATTER

INV. H01J49/04

ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

H01J

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2014/283628 A1 (HATTINGH RUAN [GB] ET AL) 25 September 2014 (2014-09-25) paragraphs [0054], [0059]; figure 3C -----	1-18
X	EP 1 125 121 A1 (COVARIS INC [US]) 22 August 2001 (2001-08-22) claims 1,11,12; figure 9 -----	1-18
X	US 2019/157061 A1 (DATWANI SAMMY [US] ET AL) 23 May 2019 (2019-05-23) paragraphs [0101], [0103]; claim 59; figure 1A -----	1-18
X	EP 3 258 480 A1 (MICROMASS LTD [GB]) 20 December 2017 (2017-12-20) claim 14; figure 1 -----	1-18



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

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"P" document published prior to the international filing date but later than the priority date claimed

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"&" document member of the same patent family

Date of the actual completion of the international search

22 September 2022

Date of mailing of the international search report

04/10/2022

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/FI2022/050470

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2014283628 A1	25-09-2014	CN 105190282 A DE 112014001620 T5 GB 2512309 A US 2014283628 A1 WO 2014154501 A1	23-12-2015 21-01-2016 01-10-2014 25-09-2014 02-10-2014
EP 1125121 A1	22-08-2001	AT 381016 T AU 1600000 A DE 69937747 T2 EP 1125121 A1 EP 1875960 A2 US 6719449 B1 US 2004264293 A1 US 2005150830 A1 US 2008050289 A1 WO 0025125 A1	15-12-2007 15-05-2000 04-12-2008 22-08-2001 09-01-2008 13-04-2004 30-12-2004 14-07-2005 28-02-2008 04-05-2000
US 2019157061 A1	23-05-2019	AU 2018374058 A1 CA 3081369 A1 CN 111630378 A EP 3714263 A1 JP 2021504690 A US 2019157060 A1 US 2019157061 A1 US 2021090867 A1 US 2021384023 A1 WO 2019104235 A1 WO 2019104248 A1	18-06-2020 31-05-2019 04-09-2020 30-09-2020 15-02-2021 23-05-2019 23-05-2019 25-03-2021 09-12-2021 31-05-2019 31-05-2019
EP 3258480 A1	20-12-2017	EP 2783385 A1 EP 3258480 A1 GB 2497189 A GB 2527971 A US 2014319335 A1 US 2017263430 A1 US 2018269051 A1 US 2020013605 A1 WO 2013076496 A1	01-10-2014 20-12-2017 05-06-2013 06-01-2016 30-10-2014 14-09-2017 20-09-2018 09-01-2020 30-05-2013