



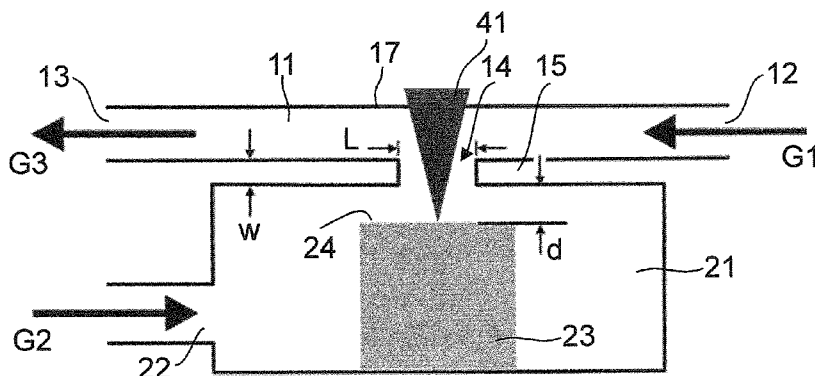
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(54) **Title:** LASER ABLATION CELL**FIG. 2**

(57) **Abstract:** A laser ablation cell (1) comprises a flow channel (11) having an essentially constant cross-sectional area so as to ensure a strictly laminar flow in the flow channel. A sample chamber (21) is provided adjacent to a lateral opening (14) of the flow channel. A laser beam (41) enters the sample chamber (21) through a lateral window (16) and impinges on a surface (24) of a sample (23) to ablate material from the sample. The sample may be positioned in such a distance from the flow channel that the laser-generated aerosol mass distribution has its center within the flow channel. This leads to short aerosol washout times. The laser ablation cell is particularly well suited for aerosol generation in inductively coupled plasma mass spectrometry (ICPMS), including imaging applications.

TITLE

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Laser ablation cell

TECHNICAL FIELD

10 The present invention relates to a laser ablation cell, to an ablation apparatus and an inductively coupled plasma (ICP) ion source employing such a laser ablation cell, and to a method of using such a laser ablation cell.

PRIOR ART

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Inductively coupled plasma mass spectrometry (ICPMS) provides accurate quantitative information on major, minor, trace, and ultra-trace elements of industrial, geological, environmental, and biological samples. In ICPMS, an aerosol sample is carried by a carrier gas stream to a so-called ICP torch. In this torch, the gas is subjected to intense high-frequency electromagnetic fields, which lead to the formation of a plasma by induction. The ions from the plasma are then extracted into a mass spectrometer, where they are separated on the basis of their mass-to-charge ratios.

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ICPMS can be coupled with laser ablation (LA) to ablate material from a solid sample so as to create the aerosol required for ICP. Ablation may be carried out directly in the ICP torch, or the sample may be placed in an external laser ablation cell upstream of the ICP torch, and the aerosol created by laser ablation is transported to the ICP torch by the carrier gas stream. For example, J. Pisonero *et al.*, "High efficiency aerosol dispersion cell for laser ablation-ICPMS", *J. Anal. At. Spectrom.*, 2006, **21**, 922-931 demonstrated a laser ablation cell (the so-called HEAD cell) for which the aerosol ejection direction is parallel to that of the carrier gas. Another laser ablation cell design based on a similar principle is demonstrated by D. Asogan *et al.*, "An open, non-contact cell for laser ablation-inductively coupled plasma-mass spectrometry", *J. Anal. At. Spectrom.*, 2009, **24**, 917-923.

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Since the first half of 1990s, attempts have been made to use laser-ablation ICPMS (LA-ICPMS) as a chemical imaging tool by scanning the laser spot over the sample surface. Many studies have demonstrated the potential imaging capabilities of LA-ICPMS based on a considerable variety of hard and soft samples. Most of these studies showed an effective spatial resolution of approximately 5-100 μm . However, some applications, such as diagnostic analysis of tissue sections, requires higher spatial resolution, e.g. to visualize cell-to-cell variability. The effective spatial resolution is determined by the laser spot size convoluted with the system dispersion. The system dispersion is in turn often dominated by a compromise between the aerosol washout time after each laser shot and the scanning speed. The longer the washout time, the more overlap will occur between signals originating from neighboring sample spots if the scanning speed is kept fixed. Therefore, aerosol washout time often is one of the key limiting factors for improving resolution without increasing total scan time.

The fastest washout time can be achieved by in-torch ablation, resulting in single shot signal durations of a few milliseconds. However, in-torch ablation is limited to very small samples, and scanning of the laser spot is very difficult to realize with in-torch ablation. Therefore, for imaging applications, external laser ablation cells are generally employed. However, even with the best known cell designs, washout times are often on the scale of seconds, and short washout times under 100 milliseconds are hard to achieve.

SUMMARY OF THE INVENTION

In a first aspect, the present invention provides a laser ablation cell that has the potential of achieving short aerosol washout times. Such a laser ablation cell is specified in claim 1. Further embodiments of the invention are laid down in the dependent claims.

Accordingly, a laser ablation cell is provided, comprising a flow channel having an inlet for feeding a carrier gas to the flow channel and having an outlet. A lateral opening is provided in a first wall portion of the flow channel, and a lateral window is disposed in a second wall portion of the flow channel opposite of the lateral opening. A sample chamber is provided adjacent to the lateral opening. The sample chamber is configured to receive a

sample in a manner to enable a laser beam to enter the sample chamber through the lateral window and the lateral opening and to impinge on a surface of the sample, so as to ablate material from the sample surface and to create an aerosol. The sample chamber has an inlet for feeding a sheath gas to the sample chamber.

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The inlet and outlet of the flow channel may be connected to tubing having essentially the same cross-sectional area as the flow channel itself. In this manner, the flow channel essentially acts like a single piece of tubing. The ablation cell of the present invention may therefore be considered to have a “tube cell” design. By minimizing variations of the cross-sectional area (and preferably also of the cross-sectional shape) of the flow channel, this “tube cell” design allows maintaining an essentially laminar flow pattern in the flow channel, avoiding turbulences as far as possible. Furthermore, the design allows positioning the sample sufficiently close to the flow channel that a major proportion of the laser-induced aerosol plume is introduced directly into the flow of the carrier gas. These measures significantly reduce dispersion. In practice, the present cell design allows reducing the washout time to below 30 ms (full width at 1% maximum) and minimizing the tailing of the sample washout. This improvement is observed for elements across the entire atomic mass range.

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The flow channel preferably has a cross-sectional area that is essentially constant or at most varies weakly. In particular, preferably the cross-sectional area of the flow channel is essentially constant in the vicinity of the lateral opening. The cross-sectional area of the flow channel may be regarded to vary at most weakly if any variations in cross section do not significantly disturb laminar flow. In particular, the cross-sectional area may be considered to vary at most weakly if the variation of the average diameter of the flow channel is less than 1.5 mm per 1 mm length along the tube axis, preferably less than 0.5 mm per 1 mm length along the tube axis, more preferably less than 0.2 mm per 1 mm length along the tube axis for any cross-sectional plane along the flow channel. It is preferred that the flow channel does not form a pronounced constriction at the lateral opening so as to avoid pronounced suction effects, as in a Venturi tube, and that the flow channel does not widen up significantly in the vicinity of the lateral opening so as to avoid that the carrier gas is pushed into the sample chamber by the resulting positive pressure difference.

In absolute numbers, the cross-sectional area may take a wide range of values, depending on laser spot size and laser energy. The mean diameter of the flow channel (calculated as $d = 2\sqrt{A/\pi}$, where A is the cross-sectional area) may range, e.g., from 50 micrometers to 5 millimeters, preferably from 200 micrometers to 5 millimeters.

The angle between the inlet and the outlet of the flow channel is preferably at least 160° (to be precise, between 160° and 200°), more preferably at least 170° (to be precise, between 170° and 190°). In other words, the flow channel is preferably essentially straight or bent by not more than 20° or better not more than 10° in any direction. An arbitrary direction of the sheath gas inlet relative to the direction of the flow channel may be chosen. Preferably, the sheath gas inlet extends perpendicular to the transversal direction.

The flow channel and the sample chamber are separated by a separating wall, which forms the first wall portion of the flow channel in which the lateral opening is arranged. In order to allow the sample to be positioned sufficiently close to the flow channel, the separating wall preferably has a minimum thickness of less than 500 micrometers, more preferably less than 200 micrometers. It should be noted that the thickness of the separating wall may vary along the circumference of the flow channel; the separating wall will normally have its smallest thickness immediately adjacent to the opening between the tube and the sample chamber, and the thickness will increase away from the opening in a plane perpendicular to the tube axis. The thickness may further vary along the length of the flow channel.

In order to minimize flow disturbances induced by the lateral opening, the cross-sectional area of this opening should be kept small. On the other hand, it may be desirable to make the opening sufficiently large to enable the laser beam to be scanned over the sample surface without moving the sample relative to the opening. As a compromise, the cross-sectional area of the lateral opening is preferably not more than about 20 mm², more preferably not more than about 7 mm². Expressed relative to the cross-sectional area of the flow channel, the ratio of the cross-sectional areas of the opening and the flow channel is preferably not more than about 5, more preferably not more than about 3, most preferably not more than about 1. In order to enable a laser beam to pass through the lateral opening,

the lateral opening should preferably have a cross-sectional area of at least about 0.01 mm². The width of the lateral opening transverse to the flow channel is preferably less than 80% of the mean diameter of the flow channel, and more preferably less than 50% of the mean diameter of the flow channel. The length of the lateral opening in the direction of the flow channel is preferably not more than five times the mean diameter of the flow channel and more preferably not more than three times or even 1.5 times the diameter of the flow channel.

In order to enable easy sample exchange, the laser ablation cell preferably has a two-part design, comprising a first cell part (in the following referred to as a “cell top”) that houses the flow channel and a second cell part (in the following referred to as a “cell bottom”) that forms the sample chamber. The cell bottom is preferably removable from the cell top for exchanging the sample. The cell bottom is preferably open towards the cell top, i.e., the separating wall between the sample chamber and the flow channel is preferably formed by the cell top rather than by the cell bottom. The terms “top” and “bottom” are to be understood as not defining an absolute orientation of these parts; these terms are only used to better distinguish between the different cell parts, and the laser ablation cell may as well be used in an inverted orientation where the cell top is pointing towards the floor and the cell bottom is pointing towards the ceiling.

The invention further relates to a complete ablation apparatus comprising an ablation cell as described above. The ablation apparatus further comprises a laser, in particular, a UV laser, for shooting a laser beam through the lateral window and the lateral opening and onto the sample surface, and a positioning device for changing the relative position between the sample and the laser beam. The positioning device may comprise, e.g., any of the following: an x-y or x-y-z stage for moving the entire laser ablation cell relative to the laser; an x-y or x-y-z stage for moving the sample within the laser ablation cell while keeping the relative position between the ablation cell and the laser fixed; a beam deflector for deflecting the laser beam while keeping the relative position between the ablation cell and the laser fixed; etc. The positioning device may be employed to scan the laser beam over the sample surface. The resulting aerosol may subsequently be analyzed with respect to its elemental or isotopic composition, e.g., by ICPMS. In this manner, the sample surface may be imaged according to its elemental or isotopic composition. However, the

present invention is not limited to the use of the ablation cell in conjunction with ICPMS imaging and may also be employed in other methods in which short aerosol pulses are required.

- 5 The invention further provides an ICP ion source comprising an ablation cell as described above. The ICP source further comprises an ICP torch connected to the outlet of the ablation cell, and tubing connecting said ablation cell to the ICP torch. Preferably the tubing has a cross-sectional area that is essentially identical to the cross-sectional area of the flow channel of the ablation cell or changes only weakly as compared to the cross-sectional area of the flow channel, so as to maintain a laminar flow with minimum
10 dispersion over the entire length of the tubing.

The invention also encompasses an ICPMS system comprising such an ICP ion source and a mass analyzer coupled to the ion source. The mass analyzer may, e.g., be a quadrupole
15 mass analyzer, a time-of-flight (TOF) mass analyzer, or a sector field mass analyzer, in particular, a Mattauch-Herzog mass analyzer. However, the invention is not restricted to any particular type of mass analyzer.

- The invention further provides a method of operating an ablation cell as described above.
20 The method comprises, not necessarily in the given order:
- placing a sample in the sample chamber such that a surface of the sample faces the lateral opening;
 - feeding a carrier gas to the inlet of the flow channel;
 - feeding a sheath gas to the inlet of the sample chamber; and
 - 25 ablating material from the surface by shooting a pulsed laser beam through the lateral window and the lateral opening and onto said surface.

The direction of the laser beam, the orientation of the lateral window and the lateral opening, and consequently the orientation of the sample may be arbitrary in space. For
30 instance, the laser beam may be directed upwards, downwards, sideways etc., and the sample surface may be oriented in any orientation that allows the laser beam to reach the sample surface.

Each laser pulse will cause a quasi-instantaneous laser-generated aerosol mass distribution (“plume”). Here, “quasi-instantaneous” means a time scale that is much shorter than the time scale of mass transport by the carrier gas stream and the sheath gas stream. The laser-generated aerosol mass distribution is caused by the action of the laser pulse alone, neglecting the normal gas flow of the carrier and sheath gases. This mass distribution is established within less than 1 millisecond after the first interaction of the laser pulse with the sample. The sample is preferably positioned at such a distance from the flow channel that the quasi-instantaneous laser-generated aerosol mass distribution has its center within the flow channel, between the lateral opening and the lateral window. The center of the mass distribution is defined in the usual manner, in the same way as the center-of-mass of a rigid body, integrating over the entire aerosol plume. In this manner, the majority of the aerosol plume is directly injected into the stream of the carrier gas and may be transported away by the stream of the carrier gas with minimum dispersion.

The optimum distance between the sample surface and the center axis of the flow channel will depend on the type of laser, the energy of the laser beam, the type of carrier and sheath gases, and the flow rates of the gases. For instance, for a standard ArF excimer laser with pulses in the nanosecond range, argon as carrier gas at a flow rate of 1.1 L/min, and helium as sheath gas at a flow rate of 0.6 L/min, a distance of about 2 mm has turned out to be optimal. In more general terms, the sample should preferably be positioned in such a manner that the surface of the sample has a distance from the center axis of the flow channel in the range of 0.5 millimeters to 4.5 millimeters for laser pulses in the range of 50 femtoseconds to 50 nanoseconds.

In consequence, the optimum distance between the sample surface and the separating wall that separates the sample chamber from the flow channel will depend on various parameters. This distance may range from less than 50 micrometers to 1 millimeter or more. The distance should be large enough to allow the sheath gas to flow along the surface of the sample and through the lateral opening into the flow channel.

The sheath gas fulfills at least three tasks: it flushes the aerosol in axis with the aerosol injection direction, which helps the uptake of the aerosol particles in the carrier gas stream; it forms a “protection region” above the sample surface and ensures that the ablation is

carried out under a controlled atmosphere; and it increases the flow speed in the flow channel. Preferably the viscosity of the sheath gas is lower than the viscosity of the primary carrier gas. This helps to confine the aerosol in the center of the flow channel and to minimize the aerosol dispersion downstream from the ablation cell. In particular, the carrier gas is preferably argon (Ar). Argon is particularly well-suited for stopping the aerosol expansion before it reaches the walls of the flow channel, and it is also required for an improved instrumental sensitivity in most of the Ar gas based ICP. The sheath gas is preferably helium (He). However, the sheath gas may be replaced by or contain other gases, e.g., hydrogen, nitrogen, or water vapor. Preferably the main proportion of the sheath gas is helium, e.g., at least 50% by volume. At 25° C, Ar has a viscosity of 22.6 µPas, whereas He has a viscosity of 19.8 µPas.

The optimum flow rates of the carrier gas and the sheath gas will depend on a variety of factors, first of all on geometry, in particular, on the cross-sectional area of the flow channel. Secondly, they will depend on the geometry of the lateral opening. However, it is preferred that the volume flow rate of the sheath gas is smaller than the volume flow rate of the carrier gas, in particular, 0.3 to 1.0 times the volume flow rate of the carrier gas. The flow rate of the sheath gas may be adjusted to help to inject the center of the aerosol plume close to the center of the flow channel.

The method of the present invention is particularly suited for chemical imaging. To this end, the above method may comprise scanning the laser beam over the surface and analyzing the resulting aerosol to obtain a chemical image of the sample surface. Analysis may be carried out by mass spectrometry, in particular, by ICPMS, but may also be carried out by any other suitable method.

The method is well suited for the investigation of biological samples, in particular, of tissue samples of human or animal tissue. However, the method is not limited to biological samples and may as well be applied to other kinds of samples.

BRIEF DESCRIPTION OF THE DRAWINGS

Preferred embodiments of the invention are described in the following with reference to

the drawings, which are for the purpose of illustrating the present preferred embodiments of the invention and not for the purpose of limiting the same. In the drawings,

- Fig. 1 shows a schematic sketch (not to scale) of a laser ablation cell according to the present invention in perspective view;
- Fig. 2 shows a schematic sketch (not to scale) of the laser ablation cell of Fig. 1 in a central longitudinal section;
- Fig. 3 shows a schematic illustration (not to scale) of the laser-generated plume in the laser ablation cell;
- Fig. 4 illustrates, in a schematic manner, simulation results for the gas flow in the flow channel of the laser ablation cell; part (a) shows the mixing distribution pattern between He and Ar, where the ablated aerosol is located at the mixing interface above the lateral opening, the degree of mixing being indicated in gray scale, white representing the highest degree of mixture; part (b) shows the simulated gas flow velocity pattern, flow velocity being indicated in gray scale, white representing the highest flow velocity;
- Fig. 5 illustrates, in a highly schematic manner, a complete LA-ICPMS system employing the laser ablation cell of Fig. 1;
- Fig. 6 shows diagrams illustrating the results of optimizations of the performance of the laser ablation cell by varying (a) the gap space between the sample surface and the lateral opening; (b) the carrier gas (Ar) flow rate; and (c) the sheath gas (He) flow rate; all diagrams show peak width normalized to peak area based on full width at 1% maximum criterion;
- Fig. 7 shows diagrams illustrating the performance of the laser ablation cell as demonstrated by ^{27}Al intensity in a mass spectrometer at various repetition rates: (a) transient signal for a repetition rate of approximately 1 Hz; (b) enlarged view of the bracketed portion of part (a); (c) transient signal for a repetition rate of approximately 10 Hz; (d) transient signal for a repetition rate of approximately 30 Hz;
- Fig. 8 shows diagrams illustrating the characterization of the laser ablation cell for various isotopes; (a) peak width; (b) abundance normalized sensitivity calculated from peak area;
- Fig. 9 shows images obtained for a Pt coated test pattern with an Au film in the

form of letters “ETH” and an overlaid Ag film in the form of letters “PSI”; imaging was carried out by (a) optical microscopy; (b) scanning electron microscopy; (c) and (d) LA-ICP-Quadrupole-MS employing the laser ablation cell of Fig. 1; and

- 5 Fig. 10 illustrates an image of human epidermal growth factor receptor 2 (HER2) distribution in a thin section cut of a breast cancer tissue obtained by LA-ICP-Single-Detector-Sector-Field-MS.

DESCRIPTION OF PREFERRED EMBODIMENTS

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Figures 1 and 2 illustrate, in a schematic manner, a laser ablation cell 1, in the following also called a “tube cell”, according to an exemplary embodiment of the present invention. The ablation cell 1 comprises two parts: a first part or cell top 10 and a second part or cell bottom 20. A tubular flow channel 11 is formed in the cell top 10 and extends from a carrier gas inlet 12 to a mixed-gas outlet 13. In a bottom wall portion 15 of the cell top 10, a lateral opening 14 is formed. In a top wall portion 17 of the cell top 10, a transversal hole is formed and closed by an UV transparent silicon window 16. In the cell bottom 20, a sample chamber 21 is provided. A sheath gas inlet 22 leads to the sample chamber 21. Whereas the sheath gas inlet 22 is shown as extending (anti-)parallel to the flow channel 11, an arbitrary direction of the sheath gas inlet may be chosen. Preferably, the sheath gas inlet extends perpendicular to the transversal direction. A sample 23 is placed in the sample chamber 21, and the cell bottom 20 is mounted to the cell top 10 such that the top surface 24 of the sample 23 is situated below the lateral opening 14.

25 For operating the ablation cell, a carrier gas G1 is fed to the inlet 12 of the flow channel 11, and a sheath gas G2 is fed to the inlet 22 of the sample chamber 21. A UV laser beam 41 enters the window 16, traverses the flow channel 11, exits the flow channel 11 through the lateral opening 14 and impinges on the top surface 24 of the sample 23.

30 Each laser pulse generates an aerosol plume 25, as schematically illustrated in Fig. 3. This plume is the direct result of the action of the laser pulse; the initial mass distribution in the plume immediately after the end of the laser pulse is influenced only very little by the streams of the carrier gas G1 and the sheath gas G2. The design of the laser ablation cell 1

allows placing the center of the laser-generated aerosol mass distribution right in the flow channel, without the need of first transporting the aerosol to the flow channel by the sheath gas stream. The carrier gas G1 and sheath gas G2 then carry away the aerosol towards the outlet 13, where they exit the ablation cell as a mixed-gas stream G3.

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As the carrier gas G1, argon (Ar) is preferred. As the sheath gas, preferably helium (He) is chosen. Ar is beneficial for stopping the aerosol expansion typically occurring in pure He atmospheres. Adding He from the sample container has three advantages: a) this setup flushes the aerosol in axis with the aerosol injection direction, which helps the uptake of the particles; b) He gas forms a 'protection' region above the sample surface and assures that the ablation is conducted under He atmosphere; c) mixing Ar and He already in the tube cell not only avoids the need of a dispersive gas adapter (Ar/He mixing bulb), but also increases the flow speed and gas viscosity comparing to normal setup using only He as carrier gas, hence decreases the aerosol dispersion.

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Figure 4 shows results of computational fluid dynamics simulations carried out using the ANSYS CFX 12 software package (ANSYS Inc., Berlin, Germany). A shear stress transport model for turbulence was considered in the simulation. Simulations were carried out for the following parameters: Length of the lateral opening: $L = 4.5$ mm; width of the lateral opening: 1.5 mm; minimum thickness of the bottom wall portion: $w = 50$ micrometers; total length of the flow channel: 50 mm; diameter of the cylindrical sample chamber: 23 mm; distance between the top surface 24 of the sample 23 and the bottom wall portion 15: $d = 350$ micrometers. Ar flow at the inlet was set to a constant speed of 2.6 m/s (1.1 L/min), while He was simulated using 1.4 m/s (0.6 L/min).

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The mixture distribution of the two inlet gases is shown in Fig. 4 (a). The mixing of the two gases is indicated in gray scale, white being the highest degree of mixture. A sharp interface at the lateral opening 14 is formed by the He flow entering into the Ar flow. Helium significantly dominates the opening region and forms together with Argon a boundary layer. Due to the least degree of mixing of the two gases at the lateral opening 14, and the previous results indicating that laser ablated aerosol penetrates easily in He but not in Ar, it can be assumed that the aerosol is not diffusing into the Argon atmosphere, is therefore not reaching the entire cross section of the flow channel, and remains very dense.

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The initially very sharp interface widens within a few millimeters downstream from the opening. By varying the combination of the inlet gas flows, the height of the boundary layer and accordingly the height of the ablated aerosol can be controlled.

- 5 The simulated gas flow speed distribution is shown in Fig. 4 (b). The Ar inlet flow upstream of the lateral opening 14 represents a typical laminar flow distribution in the flow channel, being the fastest flow in the center of the tube, and decreasing gradually towards the tube wall. Simulating the emergence of the two gases showed no significant turbulence flow. Nevertheless, the calculated Reynolds number (~ 2000) is close to the transition from
- 10 laminar to turbulent flow (2300~4000). However, using a turbulent model indicated a stringent laminar flow. Therefore it can be concluded that due to the absence of turbulences, a defined stopping distance for the laser aerosol close to the center of the tube cell which is matching the highest gas velocity, low aerosol dispersion should be achieved.
- 15 Figure 5 schematically illustrates a complete LA-ICPMS system. The laser beam is generated by a laser 40. The laser ablation cell 1 is mounted on an X-Y-Z stage 5 so as to be able to change the position of the sample relative to the laser beam. The outlet 13 of the laser ablation cell 1 is connected to an ICP torch 6 by tubing 61. The tubing 61 has essentially the same inner diameter as the flow channel 11 of the laser ablation cell 1 so as
- 20 to ensure laminar flow of the outlet gas G3. The ICP torch generates a plasma source by operation of an RF coil 62; it is constructed in the usual manner. ICP torches are well known in the art and do not require further explanations. The ICP torch is connected to a mass analyzer 7 via an ICP source 71. The mass analyzer may be a quadrupole mass analyzer, a time-of-flight (TOF) mass analyzer, a sector mass analyzer etc.
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- Of course, many modifications of the laser ablation cell and the LA-ICPMS setup are possible without leaving the scope of the present invention. In particular, the present invention is not limited to a particular choice of materials for the laser ablation cell, to a particular geometry or size of the sample chamber, to a particular geometry, length, and
- 30 diameter of the flow channel in the ablation cell, to a particular geometry and size of the lateral opening in the ablation cell, to a particular window size or material, to a particular type of laser for ablation, to particular gas types introduced into the ablation cell, etc.

Example

A. Experimental

Manufacture of laser ablation cell

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A cell top 10 was manufactured from a rectangular cuboid of acrylic glass (poly (methyl methacrylate), PMMA). A longitudinal hole of 3 mm inner diameter was drilled through the cuboid along the long axis, forming the flow channel 11. In the top wall portion 17 of the cell top 10, a transversal, slightly elliptically shaped hole with length $L = 4.5$ mm and a width of 1.5 mm was formed and was closed by an UV transparent silica window 16. A lateral opening 14 of similar dimensions as the hole on the top side was formed in the bottom wall portion 15 of the cell top 10. The bottom wall portion 15 was then machined to reduce the minimum thickness w of the bottom wall portion in the region of the flow channel 11 to approximately 50 micrometers. The total length of the flow channel 11 was about 50 mm.

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A cell bottom 10 was manufactured from another PMMA cuboid. A cylindrical sample chamber 21 having a diameter of approximately 23 mm was milled into the cuboid. A radially extending hole was drilled into the cell bottom to form a sheath gas inlet 22. The sheath gas inlet extended at an angle of 10° relative to the flow channel 11. A sample 23 was placed in the sample chamber 21. The cell bottom 20 was mounted to the cell top 10 with the aid of four screws (not shown in the drawings). No spacer or seal was required, but a spacer or seal may optionally be provided to better seal of the contact region between the cell top 10 and the cell bottom 20. The top surface 24 of the sample 23 faced the lateral opening 14. The distance d between the top surface 24 of the sample 23 and the bottom wall portion 15 was about 350 micrometers. Thus, the total distance between the sample surface 24 and the center axis of the flow channel 11 was approximately 1.9 millimeters (radius of the flow channel = 1.50 mm, wall thickness $w = 0.05$ mm, distance $d = 0.35$ mm).

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Experimental set-up for tube cell performance, optimization and characterization

An ArF excimer laser system (Lambda Physik, Göttingen, Germany) with homogenized

laser beam profile was coupled to an Agilent 7500cs ICP-Quadrupole-MS instrument (ICP-Q-MS, Agilent Technologies, Waldbronn, Germany). Laser fluence was 17.3 J/cm². In order to improve the confidence level, all data points were derived from 3×3 single shot matrix scans (unless otherwise stated) with laser spot size of 10 μm and spacing of 15 μm between adjacent shots. The transfer tube to the ICP torch consisted of a 3 mm inner diameter PTFE (polytetrafluoroethylene) tubing connected to the mixed-gas outlet 13. A similar tube was used as a feed tube to the Ar inlet 12. The Ar carrier gas flow was adjusted to 1.1 L/min. The 50 cm long transfer tube was directly connected to the ICP torch without changing the diameter. The He sheath gas flow provided through the sample chamber was adjusted to 0.6 L/min. Tube cell performance measurements were carried out using a dwell time of 10 ms. In order to describe the washout of the cell, single isotope ²⁷Al acquisitions during 1 Hz, 10 Hz and 30 Hz laser ablations on NIST 610 reference glass were performed. The ICP was operated at 1470 W and the quadrupole MS was set to 1 point per peak in peak hopping mode.

Optimization of various operational parameters was carried out, including: the gap distance between carrier tube opening and the sample surface; the Ar carrier gas flow rate; and the He sheath gas flow rate. When optimizing one parameter, the other two were set to the ‘pre-optimized’ conditions, based on the preliminary optimization, e.g. gap distance at 350 μm, Ar flow at 1.1 L/min, He flow at 0.6 L/min. The data were evaluated based on the normalized peak width, which is the peak width divided by the total counts collected within each peak (peak area). For each peak, full width at 1% maximum (FW0.01M) was used to determine the peak width. In case the 1% maximum position did not coincide with any data point, a linear interpolation of the nearest two points was applied. For the peak area, all data points within the peak width were integrated and no interpolation was required, since peak tailings contributed to less than 1% of the total counts.

The characterization of the cell for routine analysis was carried out using the same parameters as described above. However, multiple isotopes from low, mid to high m/Q were recorded in different runs. Peak area sensitivities were abundance corrected.

Sample preparation for imaging of “hard” matter

A sample for demonstrating imaging capabilities was produced by a laser-induced forward transfer method. For the preparation of the donor substrate, a high quality fused silica glass was covered with a UV-absorbing triazene polymer (TP) layer, as a sacrificial dynamic release layer (DRL), on which different thin film materials were deposited. In the transfer procedure, a 308 nm XeCl excimer laser beam was imaged on an 'ETH' (or 'PSI') hollow mask and 4 fold demagnified before impacting the back side of the donor substrate. TP-DRL was ablated and the generated shockwave propelling the thin film toward a glass receiver substrate coated with PEDOT:PSS (poly(3,4-ethylenedioxythiophene) blended with poly(styrenesulfonate)). The sample was prepared by a 60 nm thick Au 'ETH' thin layer on bottom and an 80 nm Ag 'PSI' on top (Au/Ag). To control the deposition of the two logos by scanning electron microscope (SEM), a 5 nm Pt thin film was uniformly coated on the receiver substrate after the pattern deposition.

Tissue sample preparation

A formalin-fixed paraffin-embedded human epidermal growth factor receptor 2 (HER2)-enriched breast cancer tissue was sectioned at 6 μm . The sample was processed on the Discovery XT platform (Ventana Medical Systems) under CC1m epitope recovery conditions. Afterwards, the sample was blocked for 30 minutes with phosphate buffered saline (PBS) / 1 % bovine serum albumin (BSA) / 0.1 % Triton X, and incubated with 200 μL ^{165}Ho tagged anti-HER2 at 5 $\mu\text{g}/\text{mL}$ for 50 minutes. The sample was washed three times in PBS / 0.1 % Triton X and dried at room temperature. For antibody conjugation with ^{165}Ho , a commercial MAXPAR antibody labeling kit (DVS Sciences) was employed.

Instrumentation and operating conditions for imaging of "hard" matter

A similar configuration as described for the tube cell characterization was used for the experiments. An area of $852 \times 408 \mu\text{m}^2$ was covered by 10 Hz repetition rate line scans. The distance between successive laser shots and the lateral distance between line scans were both 4 μm , based on a 4 μm laser crater. The actual laser beam size was 1~2 μm . However, a larger affected area was observed, which can be explained by an enlarged heat penetration volume (high thermal diffusion in the metallic thin films, and ns light-material interaction time). Three isotopes, ^{107}Ag , ^{195}Pt and ^{197}Au , were measured in peak hopping

mode with 600 μ s dwell time for each isotope. However, due to an instrument quadrupole-settling time of a few milliseconds for each isotope, the reading of an entire set of isotopes could not be completed in less than 10 ms. Obviously, such a large overhead fraction (low duty cycle) does limit signal quality obtainable during fast, high resolution imaging experiments. Data analysis was based on the integration of each single shot signal (trapezium integration scheme).

Instrumentation and operating conditions for tissue imaging

Tissue imaging was conducted on an Element2 (Thermo Fisher Scientific) ICPMS coupled to an ArF excimer laser at $\sim 1 \mu$ m spatial resolution. The operating conditions were optimized for maximum sensitivity of fast transient signals. Therefore, only ^{165}Ho was recorded. For image analysis, the sample was scanned line by line at a laser frequency of 20 Hz and an image pixel size of $1 \times 1 \mu\text{m}^2$. Dwell time of the MS was set to 50 ms, in accordance with the laser ablation rate applied.

B. Results and discussion

Tube cell optimization

The dependence of the dispersion on the gap between the tube cell bottom and the sample surface is depicted in Fig. 6 (a). The plotted peak widths were normalized to the total counts collected in FW0.01M. A minimum peak width at a gap width of $\sim 350 \mu$ m was observed. Using this optimized gap distance, the Ar and He gas flow rate optimizations were carried out. The corresponding results are shown in Fig. 6 (b) and (c) based on the normalized peak widths. All three optimizations yield an evident minimum of $\sim 10^{-3}$ ms/counts for the normalized peak width. All measurements reported in the following sections were conducted using the optimized values for sample gap and gas flow rates. Depending on different setups, such optimization values may vary.

Experimental evaluation of the tube cell

The tube cell was characterized using a laser frequency of ~ 1 Hz. Typical transient signals

(shown in log-scale) are summarized in Fig. 7 (a). A single washout signal of a major element lasted around 30 ms for FW0.01M. The transient peak has still a slightly asymmetric shape, tailing slightly which is caused by delayed washout of the aerosol. After the peak maxima, signals dropped down for more than 2 orders of magnitude within 20ms, which represents more than 99.98 % of the total integrated signal. The residual fraction of the total signal (0.02 % integrated signal area) was found in the tail of the peak which reached background after 40-50ms. The slope of the second signal decay is different than for the fast washout, indicating a different process, which is suspected to be related to the uptake of redeposited material from the surface. This is most likely due to the flow of the He sheath gas into the tube cell bottom opening, which is parallel to the laser plume injection, which flushes the area around the crater most efficiently. Spacing the single shots by 1 s blank indicates that no further sample removal occurs.

Fig. 7 (b) shows the transient signal acquired at a laser frequency of 10 Hz. The peak width and shape were similar to those signals measured at 1 Hz. Further increase in the laser frequency using a 30 Hz line scan is shown in Fig. 7 (c). The width and shape of the peaks were similar to the signals measured at 1 Hz and 10 Hz. The signal structure indicates that two adjacent peaks cannot be separated to background from each other. However, the overlapping between two successive peaks is less than 1% in intensity. Therefore it can be concluded that even 30 Hz would allow to image concentration differences as large as two orders of magnitude, which makes this ablation cell very attractive for laser ablation imaging. The entire evaluation demonstrates that the washout is significantly improved into the 30-50 ms time range.

Tube cell characterization for broad isotope range

Further characterizations of the tube cell performance are documented in Fig. 8. Peak widths and sensitivities calculated from peak areas are shown for different isotopes from low m/Q (^7Li) up to high m/Q (^{238}U). As seen from Fig. 8 (a), the mean peak widths of all the isotope measurements fall into a narrow range of 30-35ms. The reported signal durations were calculated based on FW0.01M. The standard deviations across the m/Q range are most likely the result of the ablated mass, aliasing effects, and fluctuations due to differences in the gas flow dynamics. Figure 8 (b) shows furthermore the normalized

sensitivities for the peak area, which were determined using 10 μm craters in single shot ablation mode. Compared to commonly used ablation cell setups in single shot mode, the peak area sensitivities improved by a factor of 10. This is not related to improved sample transport efficiency or improved ionization and purely based on the preserved sample density from the ablation site to the ICP.

Fast imaging by sequential Q-MS

The “hard” sample was studied by various imaging techniques. The results are illustrated in Fig. 9. The characteristic details of the pattern were imaged first by optical microscopy (Fig. 9 (a)) and scanning electron microscopy (SEM, Fig. 9 (b)). These images were used to evaluate the quality of the high sensitivity, high spatial resolution LA-ICPMS for ^{197}Au (Fig. 9 (c)) and ^{107}Ag (Fig. 9 (d)). The optical and SEM images indicate that the thin film patterns were not perfect in terms of homogeneity, shape and geometry. However, the sample was considered to be well suited to be analyzed by LA-ICPMS. LA-ICPMS produced highly consistent images with sharp pattern boundaries. The rapid signal change from the thin film to background (or backwards) was considered as an indicator for high spatial resolution of about 1 μm . A scratch was introduced to the left arm of ‘T’ during sample transportation from one to the other laboratory and even this was imaged by LA-ICPMS in Fig. 9 (c) and is consistent with the optical microscope image in Fig. 9 (a) taken as a control picture.

Fast imaging by simultaneous Mattauch-Herzog mass spectrometer

A severe limitation of standard Quadrupole mass spectrometers is their sequential m/Q analyzing scheme. The short signal pulse duration resulting from the low dispersion tube cell limits the recording of multiple isotopes, unless quasi- or simultaneous mass spectrometers are coupled to LA-ICP. Examples of such advanced MS include Mattauch-Herzog MS (MH-MS) and Time-of-Flight-MS (TOF-MS). In order to illustrate the multi-element imaging capabilities of a MH-MS instrument, the same LA-ICP system as described for quadrupole ICPMS was coupled. The images obtained with this system were of similar quality and resolution as for quadrupole MS. It should be mentioned here that an LA-ICP-TOF-MS coupling would be equally suited for such rapid chemical imaging

applications.

Tissue imaging

5 Among the many possible applications of the presently disclosed elemental imaging LA-ICPMS system, it was decided to demonstrate its potential by investigating biomarker distributions in a biological tissue thin section. Such analyses demand, first, low μm resolution to resolve the morphology of and to localize biomarkers within the smallest biological unit, the cell. This information is crucial in the study of biological processes and
10 for comprehensive diagnostic purposes. Second, such analyses demand a short measurement time per pixel, as feasible using the presently disclosed ablation cell; in biological and biomedical analyses typically a large number of samples and large tissue areas ($500 \times 500 \mu\text{m}^2$) need to be analyzed for statistical purposes. Therefore, a breast cancer tissue section was analyzed to investigate the human epidermal growth factor
15 receptor 2 (HER2) statuses of individual cells illustrated in Fig. 6. The image showed HER2 protein highly expressed on the cell membrane. HER2 is a major determinant of relapse free survival time, time to metastasis and overall survival time after an initial breast cancer diagnosis. In the analysis, $\sim 1 \mu\text{m}$ spatial resolution was achieved. Such high spatial resolution and chemical sensitivity allowed a highly precise HER2 determination in the
20 breast cancer tissue. This sub-cellular resolution of an important biomarker for breast cancer analysis may be suitable to guide pathologists in their various treatment options.

CLAIMS

1. An ablation cell (1) for laser ablation of a sample material, the ablation cell comprising:
 - a flow channel (11) having an inlet (12) for feeding a carrier gas (G1) to the flow channel (11) and having an outlet (13);
 - a lateral opening (14) in a first wall portion (15) of said flow channel (11);
 - a lateral window (16) disposed in a second wall portion (17) of said flow channel opposite said lateral opening (14); and
 - a sample chamber (21) adjacent to said lateral opening (14), the sample chamber being configured to receive a sample (23) in a manner to enable a laser beam (41) to enter the sample chamber (21) through said lateral window (16) and said lateral opening (14) and to impinge on a surface (24) of said sample (23), the sample chamber (21) having an inlet (22) for feeding a sheath gas (G2) to the sample chamber.
2. The ablation cell of claim 1, wherein the flow channel has a cross-sectional area that is essentially constant or varies only so weakly that any variations in cross section do not significantly disturb laminar flow through the flow channel.
3. The ablation cell of claim 1 or 2, wherein the inlet (12) and the outlet (13) of the flow channel are oriented to include an angle between 160° and 200°.
4. The ablation cell of any one of the preceding claims, wherein the flow channel (11) and the sample chamber (21) are separated by a separating wall, which forms the first wall portion (15) of the flow channel (11), the separating wall having a minimum thickness (w) of less than 500 micrometers, preferably less than 200 micrometers.
5. The ablation cell of any one of the preceding claims, wherein the lateral opening (14) has a cross-sectional area that is not more than five times the cross-sectional area of the flow channel (11).

6. The ablation cell of any one of the preceding claims, wherein the ablation cell comprises a cell top (10) that houses the flow channel (11) and a cell bottom (20) that forms the sample chamber (21), the cell bottom (20) being removable from the cell top (10) for exchanging the sample (23).
7. An ablation apparatus comprising:
 - the ablation cell (1) of any one of the preceding claims;
 - a laser (4) for shooting a laser beam (41) to the sample (23) through the lateral window (16) and the lateral opening (14); and
 - a positioning device (5) for changing the relative position between the sample (23) and the laser beam (41).
8. An ICP ion source comprising:
 - an ablation cell (1) according to any one of claims 1-6;
 - an ICP torch (6); and
 - tubing (61) connecting said ablation cell to said ICP torch (6), the tubing (61) having a cross-sectional area that is essentially identical or changes only weakly relative to the cross-sectional area of the flow channel (11) of the ablation cell (1), any changes in cross section not significantly disturbing laminar flow through the flow channel and the tubing.
9. A method of operating an ablation cell (1) according to any one of claims 1-6, the method comprising, not necessarily in the given order:
 - placing a sample (23) in the sample chamber (21) such that a surface (24) of the sample faces the lateral opening (14);
 - feeding a carrier gas (G1) to the inlet (12) of the flow channel (11);
 - feeding a sheath gas (G2) to the inlet (22) of the sample chamber (21); and
 - ablating material from said surface (24) by shining a pulsed laser beam (41) through the lateral window (16) and the lateral opening (14) and onto said surface (24).
10. The method of claim 9, wherein each pulse of said pulsed laser beam (41) causes

a quasi-instantaneous laser-generated aerosol mass distribution (25), and wherein the sample (23) is positioned at such a distance from the flow channel (11) that the laser-generated mass distribution (25) has its center within the flow channel (11), between the lateral opening (14) and the lateral window (16).

11. The method of claim 9 or 10, wherein the sample (23) is positioned in such a manner that the surface (24) of the sample (23) has a distance from the center of the flow channel (11) in the range of 0.5 millimeters to 4.5 millimeters.
12. The method of any one of claims 9-11, wherein the carrier gas (G1) has a first viscosity, and wherein the sheath gas (G2) has a second viscosity that is lower than the first viscosity.
13. The method of any one of claims 9-12, wherein the primary carrier gas (G1) is argon and the secondary carrier gas (G2) is a gas that comprises at least 50% helium.
14. The method of any one of claims 9-13,
 - wherein the primary gas (G1) is fed to the inlet (12) of the flow channel (11) at a first volume flow rate,
 - wherein the secondary gas (G2) is fed to the inlet (22) of the sample chamber (21) at a second volume flow rate, and
 - wherein the second volume flow rate is 0.3 to 1.0 times the first volume flow rate.
15. The method of any one of claims 9-14, comprising:
 - scanning the laser beam (41) over the surface (24); and
 - analyzing the resulting aerosol to obtain a chemical image of the surface (24).
16. The method of any one of claims 9-15, wherein the sample (23) is a biological sample, in particular, a tissue sample of human or animal tissue.

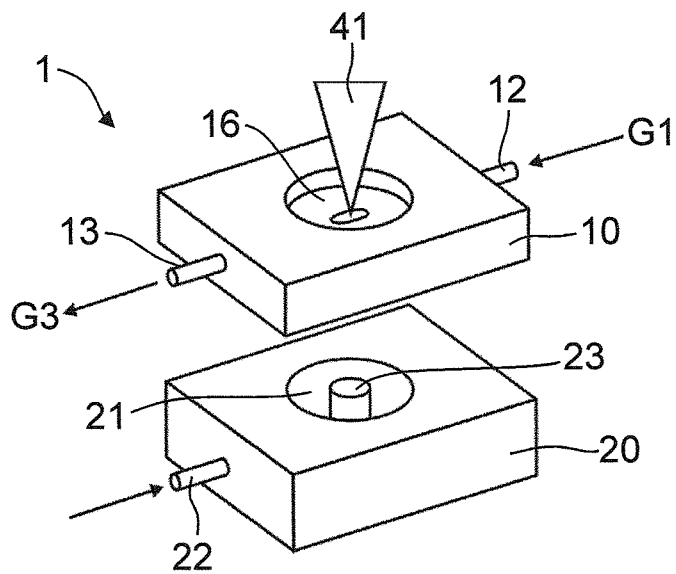


FIG. 1

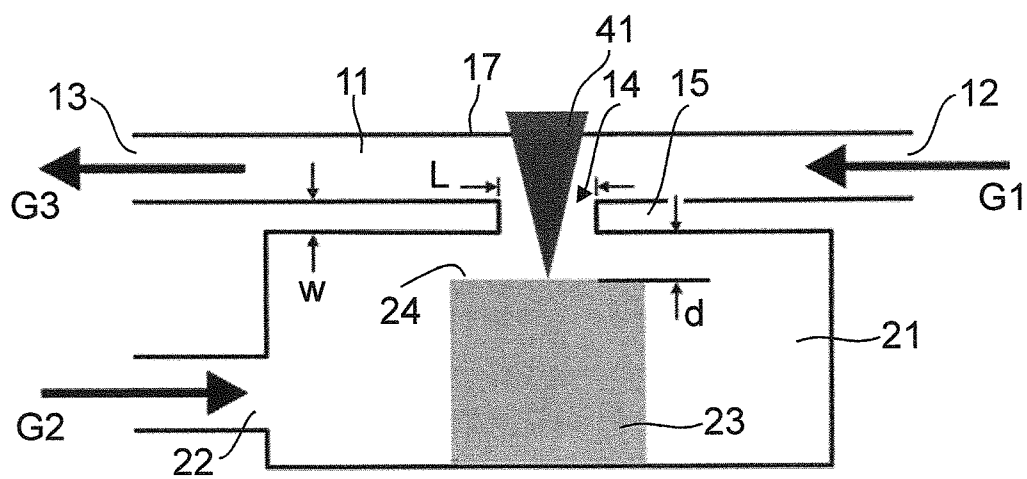


FIG. 2

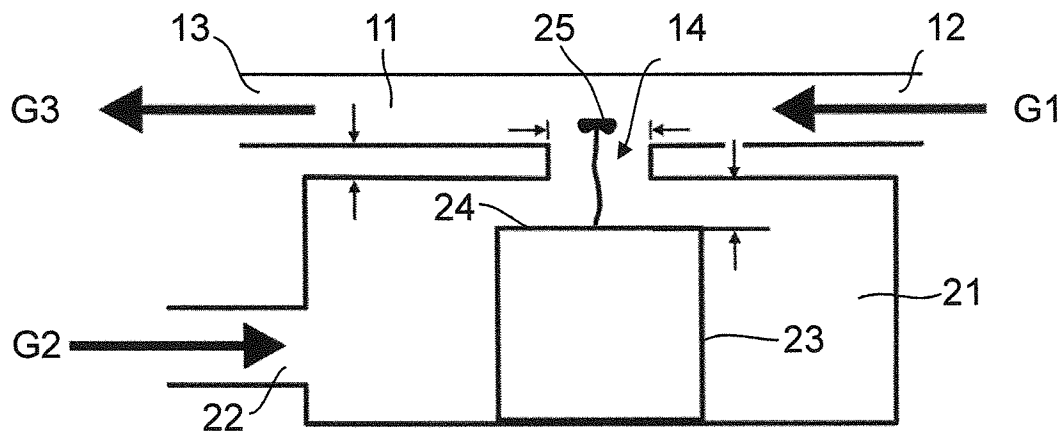


FIG. 3

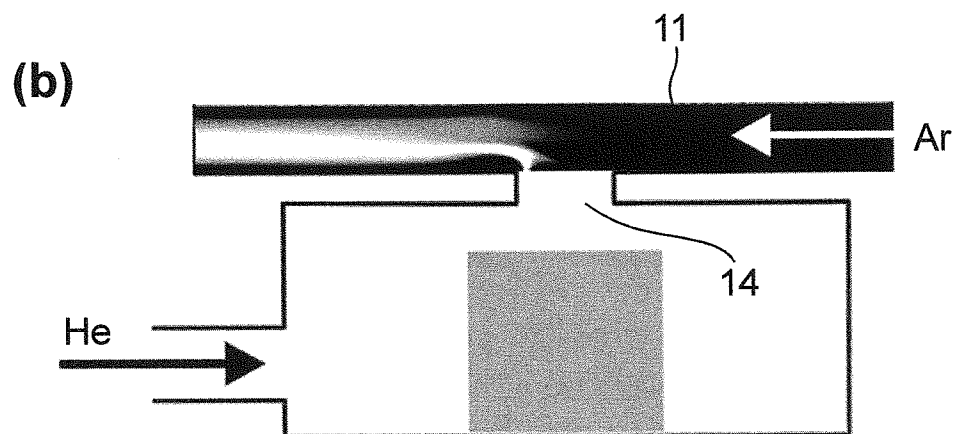
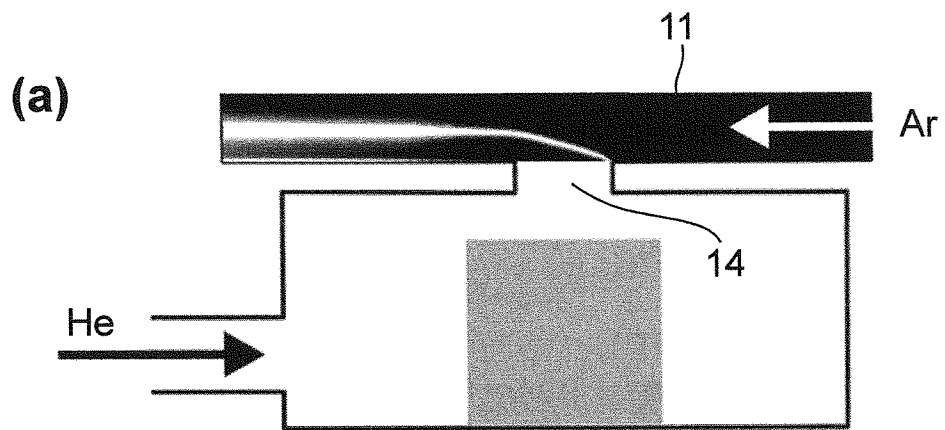


FIG. 4

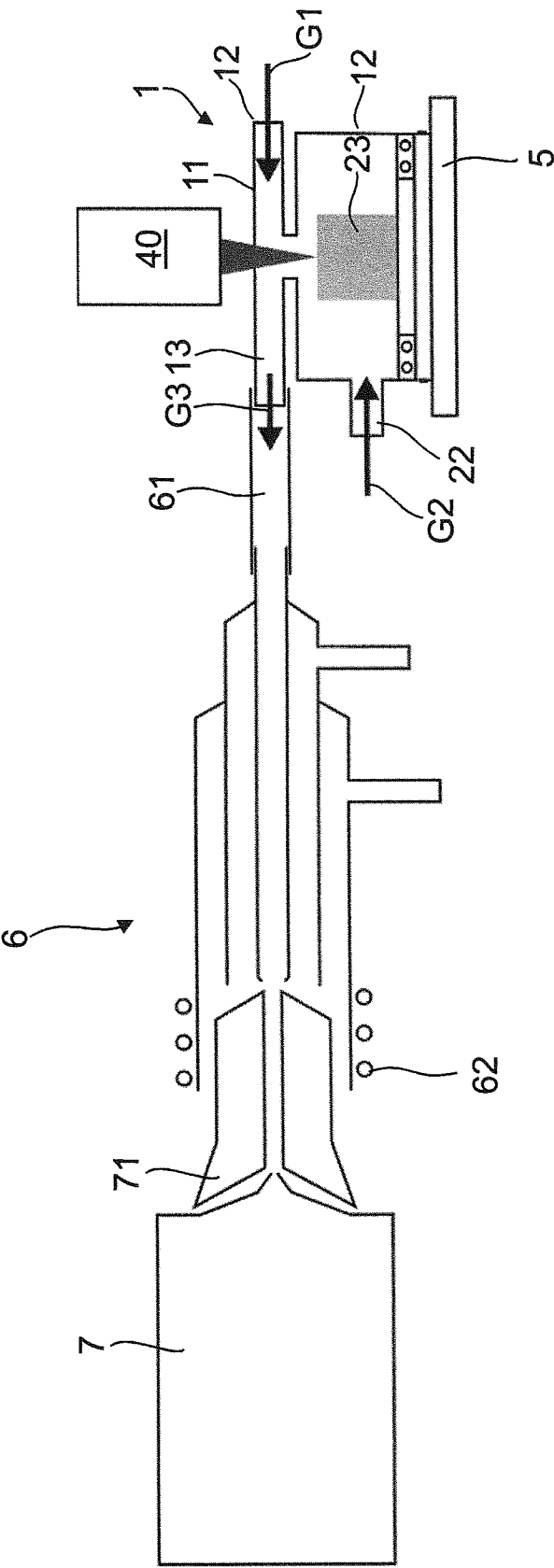


FIG. 5

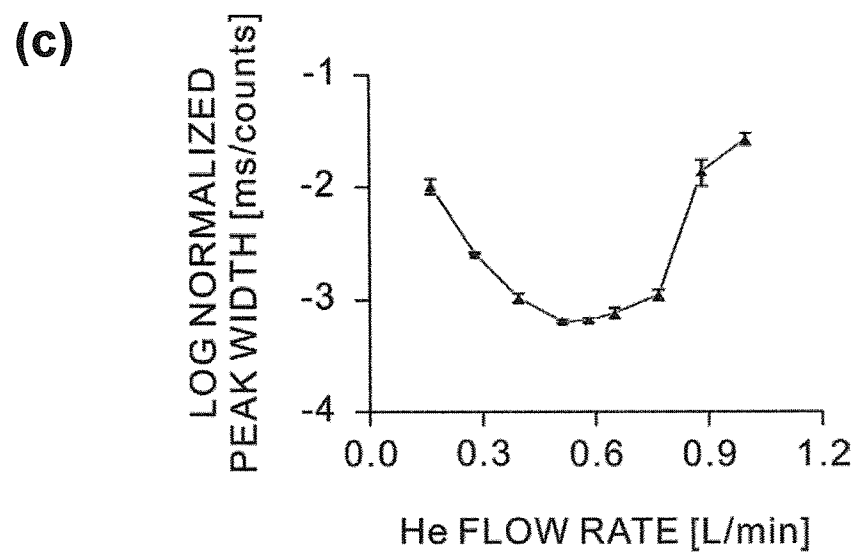
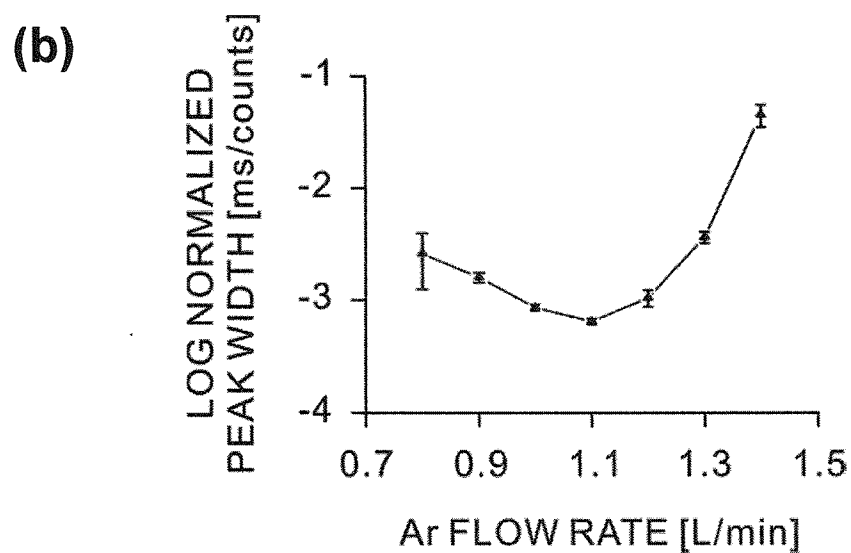
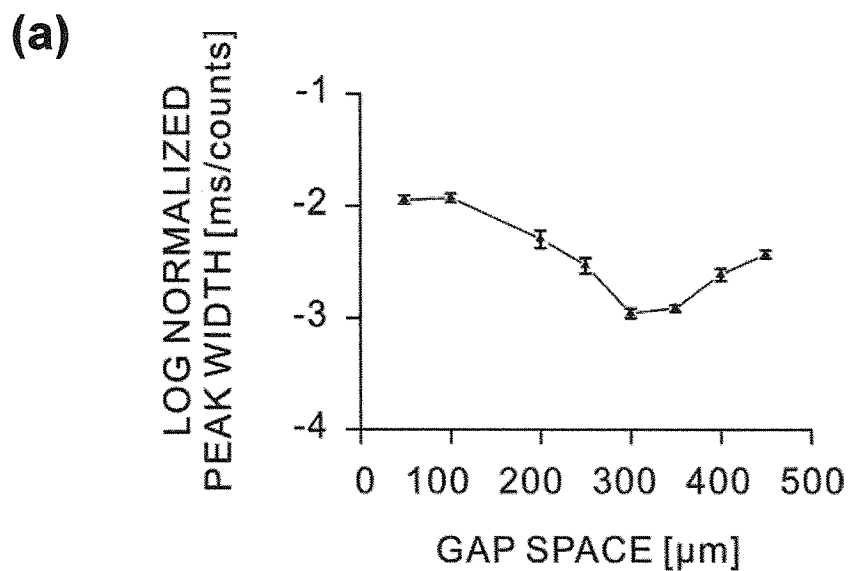


FIG. 6

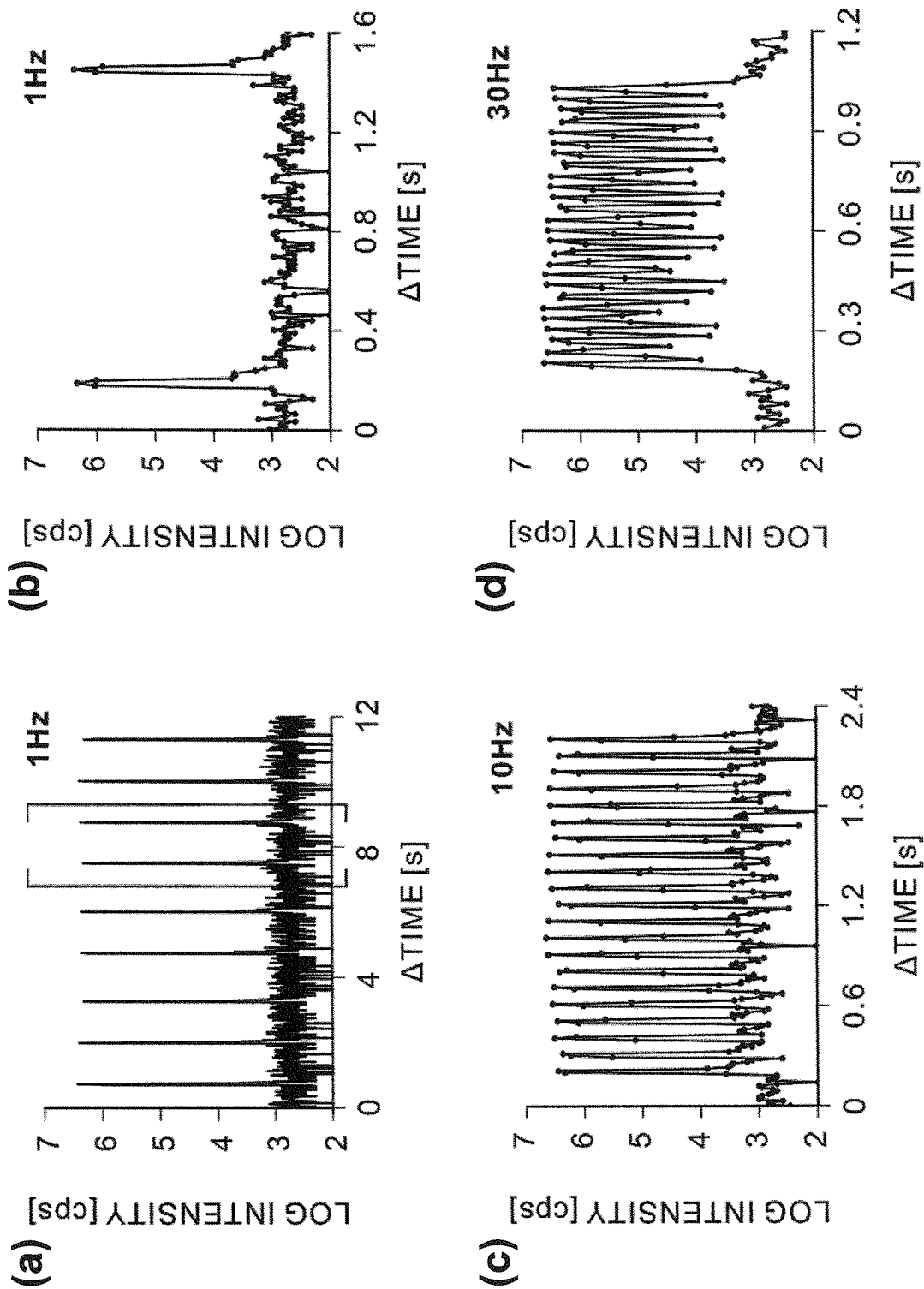


FIG. 7

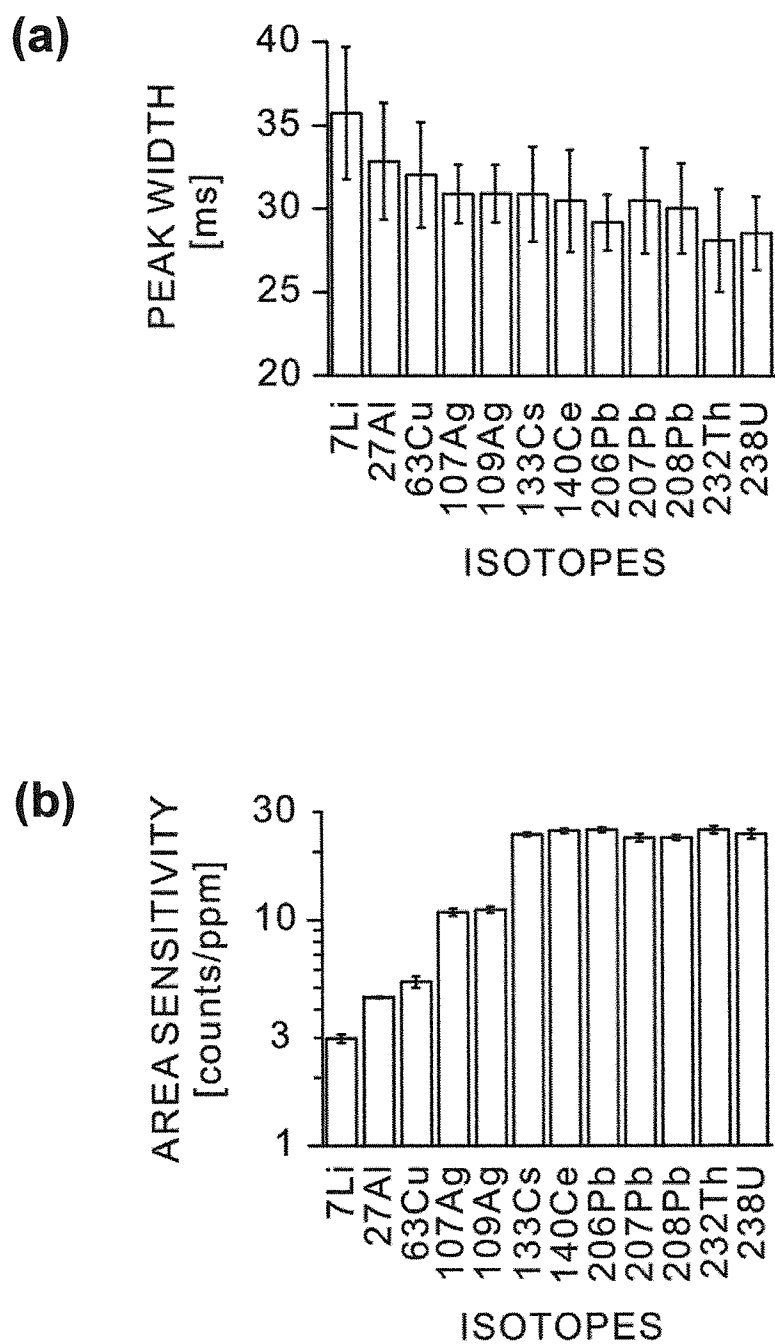


FIG. 8

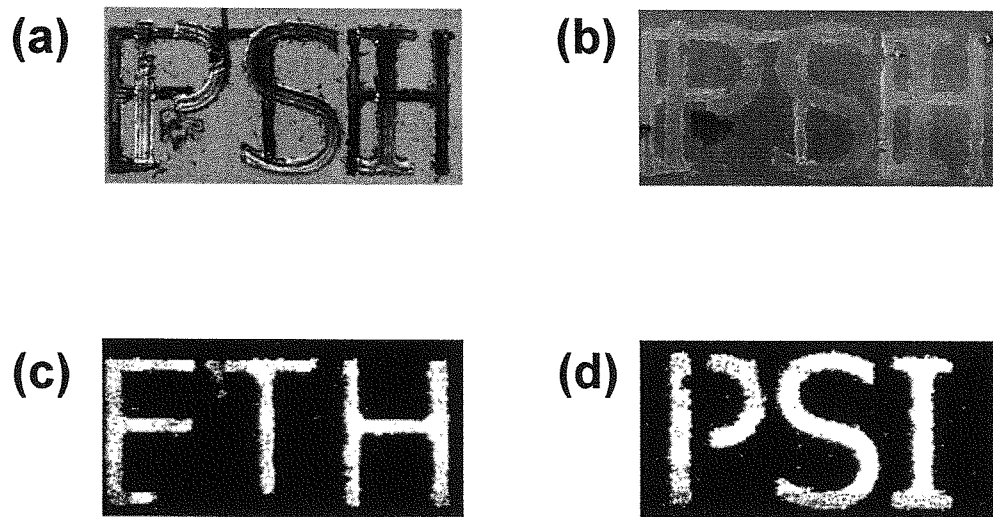


FIG. 9

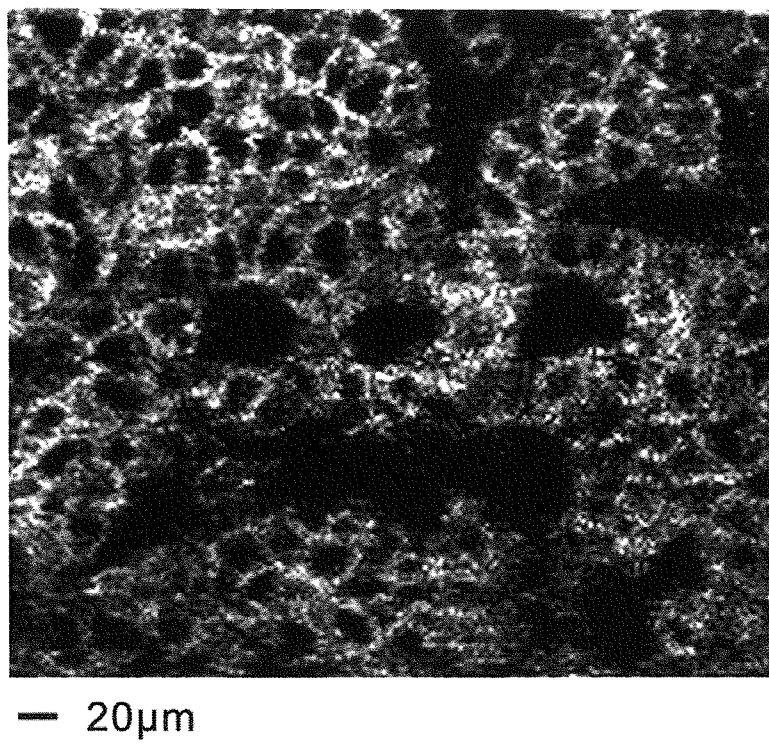


FIG. 10

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2013/056115

A. CLASSIFICATION OF SUBJECT MATTER
INV. H01J49/04 H01J49/10
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 2005/161598 A1 (GUEVREMONT ROGER [CA] ET AL) 28 July 2005 (2005-07-28)	1-7,9-16
Y	figure 1 paragraph [0036] abstract	8
Y	JP H11 201945 A (JEOL LTD) 30 July 1999 (1999-07-30) figure 1	8
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Further documents are listed in the continuation of Box C.



See patent family annex.

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

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

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

10 July 2013

Date of mailing of the international search report

19/07/2013

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
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Authorized officer

Peters, Volker

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2013/056115

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	J. PISONERO ET AL: "High efficiency aerosol dispersion cell for laser ablation-ICP-MS", JOURNAL OF ANALYTICAL ATOMIC SPECTROMETRY, vol. 21, no. 9, 1 January 2006 (2006-01-01), page 922, XP055070673, ISSN: 0267-9477, DOI: 10.1039/b603867k the whole document -----	1-16
A	US 2011/042564 A1 (NAITO YASUhide [JP] ET AL) 24 February 2011 (2011-02-24) figures 1b, 2 -----	1-16
A	FR 2 883 977 A1 (CENTRE NAT RECH SCIENT [FR]; UNIV PAU ET DES PAYS DE L ADOU [FR]) 6 October 2006 (2006-10-06) abstract -----	1-16

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2013/056115

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US 2011042564	A1	24-02-2011	NONE

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			WO 2006106265 A1 12-10-2006
