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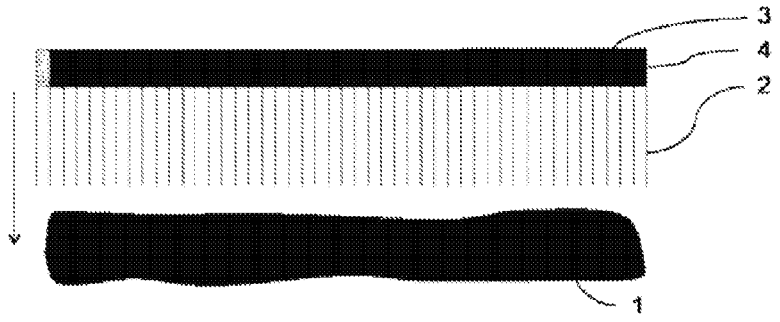


Figure 1

(57) Abstract: The invention relates to devices and systems for use in the slicing, separation and possible disposal of slices of a sample, such as a bodily tissue sample. The invention relates to a multi-blade chopper and a guide track comprising knobs along its surface designed to preferably operate in couple in order to cut and separate/dispose a sliced sample in an easy and quick manner. The chopper can be connected in some embodiments to means for electrically activate its cutting elements for working as electrodes so that the passive electrical characteristics of the sample can be analysed upon the cut.

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Device for high- throughput sample slicing and characterization

Technical Field

[0001] The invention relates to devices, systems and methods for preparing a sample to be analysed.

Background Art

[0002] Maintaining a patient's own tumour tissue viable outside his body (ex vivo) allows performing various tests that otherwise would not have been possible on the patient himself without compromising his health. However, when tissue is resected from the body, blood flow ceases, thereby cutting off the cells from their supply with nutrients. Although tissue may be placed into sugar-and protein-rich culture medium, only cells up to a maximum distance of 200µm from the tissue surface may survive due to a limited nutrient and oxygen diffusion rate (which also corresponds to the maximum cell-capillary distance in the human body). Therefore, it has become a common practice to suspend the cells that constitute the tissue. This has the advantage of putting each of them in direct contact with culture medium as well as manipulating them with ease. However, this ultimately also leads to the destruction of the tissue microenvironment with its specific structure and organization, its loss of stromal-epithelial interaction and cellular heterogeneity. A further strategy consists in slicing tissue samples thin enough so that diffusion distances remain short. Hence, all cells constituting the tissue are resupplied with nutrients. This also carries the advantage of a preserved tissue architecture, which in turn reflects in an experimental response, which is closer to the clinical outcome.

[0003] In order to obtain multiple slices of uniform thickness and assuring their viability for further cultivation, it is of paramount importance to perform the cutting procedure and subsequent transfer to the culture vessel in a very quick and reproducible way, possibly with minimum manual effort (to reduce not only time, but also costs and risks both of cuts for the operator and of contamination for the sample).

[0004] Several slicing methods are currently employed. A vibratome uses a vibrating razor blade that cuts tissue, which is glued to a metal block and immersed into an ice-cold buffer bath. The section floats in the buffer once the blade has crossed the tissue and can be removed for further analysis. This method is precise and allows obtaining sections down to several tenths of micrometers. However, it requires skilled personnel and is time consuming. A longer cutting procedure might result in decreased cell viability. This technique is also unsuitable for sectioning tissue samples obtained from a core biopsy where a relatively thin (1/2mm) but long (2cm) tissue cylinder may be obtained.

[0005] A further option to section tissue is by using a tissue chopper where a razor blade descends vertically and sections tissue similar to a guillotine. The tissue is placed on a moving stage and several cuts are possible at various positions. Although this procedure is simple and quick, it is difficult to obtain sections of less than 1mm in practice, and each slice must be picked singularly for further analyses with the risk of altering the structure thereof.

[0006] A further issue of tissue slices is related to the heterogeneity of the explanted tissue specimen. In fact, the tissue might be biopsied at the margin of the tumour resulting in a mix of normal and cancerous tissue. Alternatively, the tissue might be partly obtained from the core of the tumour where cells are often necrotic. Thus, one biopsy may contain different regions of the same tumour that are distinguished by whether they are normal or cancerous tissue and healthy or dead. In addition, the obtained tissue cylinder might vary in width along the biopsy needle depending on how well the biopsy was performed. These parameters will impact subsequent experiments and their interpretation. Currently, tissue sections need to be examined prior or after the experiment by means of microscopy or biochemical assays (e.g. MTT assay) to account for this issue.

[0007] As alternative to one single blade performing serial cuts on a sample, there is the possibility of having a multiplicity of parallel blades producing

multiple slices simultaneously. Many possible solutions have been presented with this approach.

[0008] Patent document CN 103707329 discloses an adjustable cutter for shaping and cutting biological soft tissues. The adjustable cutter comprises a knife handle, knife rests, a blade and fixed bolts, wherein the knife handle is in an inverted U shape, the two ends of the knife handle are respectively and fixedly connected with the knife rest, the blade can be fixed at the bottom of the two knife rests through the two fixed bolts, and the two knife rests connected on the knife handle are in rectangular shapes and are set to be in parallel.

[0009] Patent document CN 102636370 discloses a device used for rat brain tissue slicing and layering. The device comprises a slicing module, provided with a plurality of cutter grooves with equal spacing, where fresh rat brain tissues are placed. The device is characterized in that the slicing module is embedded in a rectangular cavity on an operation desk, a cutter with a tool rest is arranged above the slicing module, two blades which are mutually parallel and vertical downwards are arranged below the tool rest, spacing between the two blades is just equal to distance between two cutter grooves of the slicing module, the cutter is connected with the upper end of a vertical arm, and the lower end of the vertical arm is connected with a mechanism which can move along the front length direction of the operation desk for positioning.

[0010] Patent document EP1652478 discloses a tissue cutting that comprises: a handle part; a head unit comprising a plurality of blades arranged so as to extend substantially parallel with a longitudinal direction of the handle part at intervals and a blade holding portion for holding the plurality of blades; and a head holding part located at the distal end of the handle part, for holding the head unit. A second object of the invention is a tissue cut assisting device which comprises: a base; a pair of guiding members disposed on the base, for guiding a cutting operation of the biological tissue using the tissue cutting device; a mounting member mounted on a predetermined position between the pair of guiding members, for placement of the biological tissue. A third aspect of invention is intended

for accommodating a head unit of a tissue cutting device, such accommodation housing comprises: a head unit receiving portion for receiving the blades of the head unit so as to face a bottom part but not to contact with an inner face of a bottom part.

[0011] Patent document US7673545 discloses a tissue slicing device comprised of parallel circular blades coaxially connected to a rotary shaft. The blades are separated by gaps. A guide member is supported by a hinge adjacent the blades. The device includes a tissue carrier which is comprised of a base member and parallel plates extending from a top side of the base member. The plates are spaced to correspond with the gaps between the blades. Holes in the plates are aligned to define a tissue chamber for receiving a tissue sample. When the tissue carrier is positioned on the guide member and the blades rotated to drive the tissue carrier along the guide member, the tissue chamber is driven into the blades so the tissue sample is simultaneously cut into a plurality of slices.

[0012] Patent document US2005095666 discloses a tissue slicing apparatus comprising a tissue cutting means comprising a series of juxtaposed blades linked to a support. The apparatus may further comprise a cradle adapted to grip a tissue sample while it is cut with the tissue cutting means. The cradle can help to prevent the tissue sample deforming during sample preparation, even using an untreated or fragile sample, such as a prostate.

[0013] Patent documentWO0127586 discloses a method in which a combination of at least two platy knives, formed of ceramics, having the same linear or curved edges, and held in parallel to one another at specified intervals with their edges also kept parallel to one another, are used to slice a biological tissue block into a preset thickness to obtain biological tissue slices. The invention also discloses pressing members each having a specified thickness capable of being inserted between the knives that are respectively inserted into the gaps between the knives to push out the biological tissue slices from between the knives.

[0014] Patent document WO02088668 discloses a device for manufacturing thin slice pieces of bio-tissue, which includes a loading table, on which there is

provided an upper lid that has slits formed therein for permitting the passage of blade members therethrough. The thin slice pieces of bio-tissue may be obtained by having a living-body specimen loaded on the loading table of a specimen transfer part and moving the living-body specimen toward the blade members and then cutting it off. Those thin slice pieces of bio-tissue may alternatively be obtained by having a living body specimen loaded on the loading table of a fixed specimen-retaining part and moving the blade members in an up-and-down direction. Furthermore, in order to take out the thin slice pieces of bio-tissue from the slicer, it is possible to use a film frame having pasted thereon a film that is to be inserted into the slits.

[0015] Patent document WO0037918 discloses an apparatus for sectioning of irregular tissue blocks in slabs with section planes in the same orientation as any scanning plane used in CT, MRI or PET scanning. The machine consists of an array of long razor blades in a frame that can be lowered through the action of crank. The razor blade frame is set into vibration by a pneumatic vibrator.

[0016] Patent document WO2004078032 discloses a system for extracting and processing dermal tissue into small particles for purposes of transplantation to a recipient site. The discloses invention includes a tissue processor, which consists of a series of sharpened blades arranged parallel to one another, and a tissue extractor for removing the tissue samples after the tissue processor has processed them. The extractor consists of a series of wires interspersed between the blades, and positioned below the cutting surface of the blades. The wires are extended to a handle at their distal end, and hinged at their proximal end. After processing, the wires can be pulled from between the blades by the handle, which in turn grasps the processed tissue. The processed tissue is then captured by the extractor for easy removal, such as by flushing the extractor, or wiping the extractor.

[0017] Patent document WO2010036931 discloses tissue slicer having a partially open base for permitting the slicing blades to transverse therethrough, a dual pivoting member for activating the sliding blades in a vertical

direction, a blade cartridge with a plurality of blades and a multi-pin specimen holder.

[0018] Notwithstanding the available devices and methods developed in the art to produce multiple slices simultaneously from a tissue sample, there are still issues connected to the preparation of slices for ex-vivo culturing that are still to be addressed.

[0019] More precisely, at least three aspects have not been addressed:

[0020] 1) There is no method that allows for fast characterization of the single tissue slice once obtained. This characterization is fundamental to allow a better comparison of the results coming from different slices. Currently, tissue sections need to be examined prior or after the experiment by means of microscopy or biochemical assays (e.g. MTT assay) to account for this issue. These procedures require further manipulations and/or treatments beside the cutting process (e.g placing the slices on a suitable support for microscopy observation, the biochemical assay by itself); such procedures can introduce errors on the results. In an ideal situation, the characterization should take place right after the cutting process, when the slices are still between the blades, so that they are in the most homogeneous situation possible. Moreover such characterization method should require minimum effort from the operator, and should be highly parallelizable, in order to reduce time, cost, and minimize the sources of possible errors.

[0021] 2) The recovery of the slices from between the blades, minimizing the need for an external operator. Especially in the cases where slices are in the order of magnitude of micrometers, recovering them from between the blades without the risk of altering the three-dimensional architecture of the sample.

[0022] 3) The lack of a simple and quick way to transfer tissue slices to culture wells, thus minimizing to time requirement and the risk of contamination. Once the slices have been recovered from between the blades, it will advisable to have a way to transfer them to the culture plate without the intervention of an operator that manually performs this transfer, one slice at a time. Prior art document such as WO02088668, WO2004078032 and

WO0127586 disclose different approaches to facilitate the recovery of the slices from the blades, but the three of them requires manual intervention for transferring the slices to the culture well.

[0023] The possibility of combining a parallelized cutting process with the characterization of the slices, their recovery and their transfer to the culture wells with a rapid, simple and operator-independent approach will constitute a great advantage in terms of costs and time reduction, leading to the possibility of e.g. implementing protocols on ex-vivo tumour culturing even in low complexity-research facility, such as those of hospital facilities for routine diagnostics, where the high requirements in terms of throughput require sample preparation to be as fast and as simple as possible.

Summary of invention

[0024] The present invention, as described hereinafter and in the appended claims, address and elegantly overcome the above-described drawbacks of the prior art through ad-hoc devices and methods for tissue sample slicing, isolation/dispensing and possibly characterization. It relates to a cutting device for use in preparing slices from a sample, such as a biological tissue sample, and in some embodiments analysing their passive electrical properties, as well as a guide for use in separation and possible recovery of said sample slices. The present invention further relates to a system which combines both devices into an all-in-one apparatus.

[0025] The field of application includes, but is not limited to, the preparation of slices from biopsy samples taken from a subject for ex vivo culturing and analysis.

[0026] The device is characterized by a movable unit comprising several cutting elements, such as blades, able to cut a sample into multiple slices with a single movement. Upon the cut, the cutting elements can be activated to be used as electrodes able to inject an electrical current within the cut slices and analyse, at the same time, the passive electrical properties of each slice comprised between two cutting elements via e.g. an impedance analyser operatively connected thereto.

[0027] Once cut, the slices can be recovered through the movement (e.g., slide) of the movable unit along a guide comprising multiple knobs-like elements conveniently disposed along the guide in various arrangements. The system comprising the tissue chopper and this separating guide device facilitates the recovery for further use of the cut slices.

[0028] In one aspect the invention relates to a chopper for use in preparing slices from a sample, characterized in that it comprises:

[0029] - a support element for placing the sample;

[0030] - a movable head unit placed above the support element; and

[0031] - at least two planar cutting elements aligned in parallel on the same plane, separated by spacers and operatively connected to said head unit.

[0032] In one embodiment, the cutting elements descend simultaneously and vertically on the sample upon activation of the movable head unit.

[0033] In one embodiment, the cutting elements descend one after the other on the sample upon activation of the movable head unit.

[0034] In one embodiment, the cutting elements descend in a diagonal direction on the sample upon activation of the movable head unit.

[0035] In one embodiment, the cutting elements descend in a vibrating mode upon activation of the movable head unit.

[0036] In one embodiment, the chopper further comprises a spring placed above the head unit and operatively connected thereto, the discharging of said spring exerting a pressure on the head unit upon activation so that said head unit descend on the sample.

[0037] In one embodiment, said spring does not fully discharge upon activation, thereby exerting a constant pressure over the head unit towards the underlying sample.

[0038] In one embodiment, the support element comprises a flexible/soft material into which the cutting elements can penetrate, in order to assure proper sample slicing in case of z-misaligned cutting elements, that otherwise could result in some cuts which do not cross the entire tissue thickness.

[0039] In one embodiment, the support element comprises a relief with cavities arranged according to the cutting elements position in order to permit,

upon activation of the head unit, the penetration of these latter into the cavities of a complete cut of the sample.

[0040] In one embodiment, such cavities have angled walls to facilitate the entry of cutting elements.

[0041] In one embodiment, the chopper comprises a cartridge operatively connectable to the head unit and adapted to allocate the cutting elements.

[0042] In one embodiment, the number of cutting elements can vary from two to several thousands.

[0043] In one embodiment, the planar cutting elements are made of a conductive material and the spacers of an insulating material.

[0044] In one embodiment, the chopper further comprises means for electrically connecting the cutting elements to an electrical apparatus adapted so that said cutting elements work as electrodes for analysing the passive electrical properties of the sample through impedance means.

[0045] In one embodiment, each cutting element can be individually addressed as a planar electrode.

[0046] In one embodiment, the cutting elements can be electrically activated in pairs of adjacent electrodes.

[0047] In a further aspect, the invention relates to a guide track for use in separation and possible recovery of sample slices having a length x, a width y and a depth z, comprising at least one knob protruding from said guide and adapted to block a sample slice upon relative movement along the x-direction of the track with respect to a chopper as described above.

[0048] In one embodiment, the guide track comprises a cavity at each edge extending over the whole length x thereof adapted to accommodate the edge of a movable head unit of a chopper.

[0049] In one embodiment, the guide track comprises multiple knobs arranged in parallel along the width y of the track.

[0050] In one embodiment, the knobs can be evenly distributed along the guide track length x and/or the guide track width y.

[0051] In one embodiment, the knobs are adjustable in the x and/or y and/or z direction.

[0052] In one embodiment, the knobs edges are shaped so to avoid blockage of a sample slice obtained through an above-described chopper upon movement of this latter's movable head along the x-direction of the track.

[0053] In one embodiment, the knobs have an arc shape such that a sample slice is pushed inside the knob-arc.

[0054] In one embodiment, the guide track comprises at least one hole placed in front of each knob with respect to the guide track length axis.

[0055] In one embodiment, the knobs are shaped and/or composed of a material such that they can be broken upon the contact of the spacers of the movable head of an above-described chopper moving along the x-direction of the guide track.

[0056] In one embodiment, the guide track further comprises a pillar element arranged so that a knob is allowed to be pulled only vertically through a hole placed in front of the knob.

[0057] In one embodiment, the guide track, particularly a guide track having at least one hole placed in front of each knob with respect to the guide track's length axis, is incorporated on a container's lid, such as a multiwell plate lid.

[0058] In one embodiment, the above guide track is incorporated on a cartridge that can be placed and precisely aligned on top of a container such as a multi-well plate, histology cassette, petri dish or tube.

[0059] In a preferred embodiment, the above guide track is designed so that the above-mentioned holes are aligned with specific portions (e.g., wells) or areas of an underlying container.

[0060] In one aspect, the invention also relates to a multiwell plate characterized in that it comprises a lid having a guide track having at least one hole placed in front of each knob with respect to the guide track's length axis.

[0061] The invention further relates to a system for use in the preparation, recovery and possible analysis of slices obtained from a sample, said system comprising a chopper as described above operatively connected to a guide track as described above.

[0062] In one embodiment, the guide track of the system comprises a ceiling adapted so to accommodate a portion of a chopper as described above.

[0063] In one embodiment, the movable head of the chopper exerts a continuous pressure from the top towards the guide track in order to maintain a constant contact between this latter and the said chopper head.

[0064] In one embodiment, the movable head of the chopper exerts said continuous pressure through a non-fully discharged spring operatively placed above the head unit of the chopper.

[0065] In one embodiment, the knobs and/or cutting members lateral edges are shaped so to avoid blockage of a sample slice obtained through an above-described chopper upon movement of this latter's movable head along the x-direction of the track.

[0066] In one embodiment, the spacers exerts a pressure onto the knobs upon the passage of the former above the latter such that the knobs break and fall through a hole, thereby pulling the blocked sample slice with it into the hole.

[0067] In one embodiment, the spacer and the knob have an angled shape on their relative contact surfaces such that a movement of the head unit in the y-direction of the guide track causes the knob to be pushed downwards.

[0068] In one embodiment, at least one element of the system is automated.

Brief description of drawings

[0069] Fig. 1 shows a sketch of the movable head (3) to which the cutting elements (2) are operatively connected. The cutting elements are aligned on the same plane and are separated by spacers (4) to obtain a precise cutting of the underlying sample (1) once the head unit is activated.

[0070] Fig. 2 shows a sketch of a spring (15) used to operate the head unit (3).

[0071] Fig. 2A shows the situation before the cut, when the spring (15) connected to the head unit (3) is still charged and there is enough space between the head unit (3) and the support base (9) to place the sample (1).

[0072] Fig. 4B shows the cut, when the spring (15) is partially discharged and the head (3) is pushed against the support base (9), thereby cutting the sample in slices (7).

[0073] Fig. 3A shows the unfinished slicing of the sample (1) due to z-misaligned cutting elements (2).

[0074] Fig. 3B shows a flexible/soft material (e.g. polymer) (16) that is placed below the sample, into which the cutting elements (2) penetrate. Accordingly, the alignment error will be nullified and the sample slices be well separated.

[0075] Fig. 3C shows the support element (9) comprising a relief with cavities (17) positioned below the cutting elements (2). The cutting elements penetrate into these cavities, and thereby descend deeper than the sample (1). The cavities (17) may have angled walls (18) to facilitate the entry of cutting elements (2).

[0076] Fig. 3D shows the sample (1) contained within a matrix (e.g. hydrogel, polymer) (19). The sample is not in contact with the support element and the cutting elements traverse the entire sample. For additional electrical measurement, this matrix may have a very high resistivity to avoid leak currents bypassing the sample (1).

[0077] Fig. 4 shows a sketch of two adjacent cutting elements (2) working as electrodes and connected to an electrical apparatus (5) to measure the electrical properties of the sample slice (7) situated between said electrodes. The adjacent cutting elements are separated by a spacer (4) made of an insulating material.

[0078] Fig. 5 shows the sketch of the guide track.

[0079] Fig. 5A shows a lateral view where the knob (8) protrudes from the base of the guide track (9) so that when the head (here represented by the section depicting one blade (2) and a spacer (4)) slides over it and moves further the slice (7) is blocked in a specific position by the knob and separated from the head unit.

[0080] Fig. 5B shows the top view of a guide track where multiple knobs (8) are arranged in parallel along the width of the track to recover the single slices (7) from the cutting elements assembly (3). The cutting element assembly slides on the rails (10) of the guide tracks.

[0081] Fig. 5C shows a sketch of the sorting procedure, where upon sliding of blade (2) and spacer (4) over an arc-shaped knob (8), the slice is trapped by the knob, which is placed after a hole (12) on the track. The spacer exerts a pressure onto the inclined surface (13) of the knob (8) such that this one breaks at a specified mechanically weakened position (11) and falls through the hole (12), thereby pulling the sample slice (7) with it in the underlying container (14).

[0082] Fig 6 shows the top view of a knob (8) and cutting members (2) between which the slice (7) is sandwiched, having lateral edges rounded or otherwise shaped (e.g. cone-shaped, triangle) to avoid blockage in case of not perfect y-alignment.

[0083] Fig. 7 shows different means for preventing the tilting of the knob in x-direction during the x-movement of the chopper's head unit.

[0084] Fig. 7A shows a knob with a blocking element at its bottom (20), which is attached at the bottom below the hole. This blocking element pushes against a wall element (21) protruding into the hole, so that when the knob moves in x-direction it limits tilting. The distances indicated by arrows influence the functioning.

[0085] Fig. 7B shows a pillar (22) behind the knob. This pillar prevents the knob from tilting.

[0086] Fig. 7C shows a knob with a support (23) whose breaking point is dislocated further in x-direction, thereby favouring the movement of the knob in z-direction when the spacer is moved over the knob. The distances indicated by arrows influence the ratio of downward force to lateral force.

[0087] Fig. 7D shows knob whose inclined surface (24) has a different angle than the inclined surface of the spacer (25). This avoids blocking of the knob against the spacer when the knob tilts slightly.

[0088] Fig. 8 shows a sketch of a spring (15) used to operate the head unit (3) in the system of the invention.

[0089] Fig. 8A shows the situation before the cut, when the spring (15) connected to the head unit (3) is still charged and there is enough space between the head unit (3) and the guide track base (9) to place the sample (1).

[0090] Fig. 8B shows the cut, when the spring (15) is partially discharged and the head (3) is pushed against the guide track base (9), thereby cutting the sample in slices (7).

[0091] In Fig. 8C the spring (15) still exerts a force on the head (3), thereby pushing it against the guide track (9) so that when the head unit (3) is moved over the knobs (8) the slice (7) can be pushed against the knob (8) and eventually fall in the hole (12).

[0092] Fig. 6 shows how to assure proper sample slicing in case of z-misaligned blades.

[0093] Fig. 9 shows the system implemented on a well plate attachment (28) with a spring-based (15) mechanism (26) for head movement actuation. The sample is introduced through a lateral hole (27).

[0094] Fig. 10 shows the positioning of the well plate attachment (28) on top of a well plate (29). The sectioned samples will fall in the holes (14) of the well plate (29).

[0095] Fig. 11 shows the implementation of the system on a lid (30) of a well plate (29).

Description of embodiments

[0096] The present disclosure may be more readily understood by reference to the following detailed description presented in connection with the accompanying drawing figures, which form a part of this disclosure. It is to be understood that this disclosure is not limited to the specific conditions or parameters described and/or shown herein, and that the terminology used herein is for the purpose of describing particular embodiments by

way of example only and is not intended to be limiting of the claimed disclosure.

[0097] As used herein and in the appended claims, the singular forms "a," "and," and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a cutting element" includes a plurality of such elements and sensors and reference to "a knob" includes reference to one or more knobs, and so forth.

[0098] Also, the use of "or" means "and/or" unless stated otherwise. Similarly, "comprise," "comprises," "comprising" "include," "includes," and "including" are interchangeable and not intended to be limiting. It is to be further understood that where descriptions of various embodiments use the term "comprising", those skilled in the art would understand that in some specific instances, an embodiment can be alternatively described using language "consisting essentially of" or "consisting of".

[0099] In one aspect, the invention relates to a chopper for use in preparing slices from a sample, said chopper comprising at least two planar cutting elements separated by spacers and operatively connected to a movable head unit (Fig.1). In the frame of the present invention, a “chopper”, or “ slicer” or even “cutter” is a mechanical device designed and intended as a cutting tool. In particular, a chopper is a device comprising a cutting element such as a metallic blade used for reduce into slices an object, preferably a soft object. In one preferred embodiment, a chopper is a tissue chopper, and comprises a support adapted for accommodating a sample upon which is conveniently placed a movable head unit comprising a set of cutting elements. As used herein, a “movable head unit” refers to a component that comprises at least the cutting elements and the spacers, said head unit being able to be moved from a position where it is above the sample to a position where it cuts through said sample. The movable head can even be operatively connected to a movable arm that permits the displacement of the former in a z-direction (i.e., from up to down and vice-versa) so that the cutting elements can act on the sample to be sliced. The entire device can be automated so to perform its cutting action without the need for an operator. Those skilled in the art will appreciate how the

possibility to cut simultaneously multiple slices of a predefined thickness gives a clear advantage in sample preparation compared to methods that employ a single cutting element to cut sequentially multiple slices. Not only it saves time, but it also ensures a more precise thickness of the slices.

[00100] For the sake of clarity, the wording “operatively connected”, “operatively connectable” or even “operatively connecting”, as used herein, reflects a functional relationship between two or more components of a device or a system, that is, such a wording means that the claimed components must be connected in a way to perform a designated function. The “designated function” can change depending on the different components involved in the connection; for instance, the designated function of a cutting member is to produce a cut on the sample, and it has to be connected to the head unit such that it is keeping its position while moving in relation to the head unit. In the same way, the designated function of the spacer is that of fixing the distance between two adjacent cutting members. A person skilled in the art would easily understand and figure out what are the designated functions of each and every component of the device or the system of the invention, as well as their correlations, on the basis of the present disclosure.

[00101] As used herein, the term “planar cutting element” refers to a flat sharp tool able to cut a sample. In a preferred embodiment, the planar cutting element is made of a conductive material, in order to be used as electrode. In its simplest embodiment, a cutting element is a blade such as a razor blade. As used herein, the term “spacer” refers to a separator situated between two cutting members. Said spacers determine the distance between the cutting elements and therefore the thickness of the sample slices obtained upon the cut. They can be fixed or, in some embodiments, they can be adapted to select the appropriate distance between the cutting elements, depending on the needs, and their thickness can vary from few micrometers to several centimeters. In a preferred embodiment the spacers are made of insulating material, to act as insulator between two adjacent cutting elements when the latter are electrically activated, as it will be explained below.

[00102] In an alternative embodiment, the planar cutting elements and/or the spacers are made of a non-metallic material such as polymeric plastic, composite materials, ceramic and so forth, making it possible to manufacture them in a single piece by injection moulding or by machining.

[00103] The chopper of the invention comprises in its head unit more than one cutting elements, preferably but not exclusively aligned in parallel on the same plane. Preferably, tens, hundreds or even thousands of cutting elements can be present in the head unit. Once the head unit activated, the cutting elements descend on the sample conveniently placed below them so that said sample can be cut in several slices in one shot. The cutting elements can descend on the sample simultaneously or one after the other thanks to e.g. joints elements in the head unit permitting a sequential movement of the cutting elements, and can descend with any suitable angle on the sample, such as vertically or diagonally. The cutting elements can be, in one embodiment, allocated into a cartridge that can be operatively connected to the head unit. Said cartridge gives the advantage to be a changeable element that can be substituted as a whole without singularly removing the cutting elements from the head unit.

[00104] The head unit can be activated with any suitable means, such as by hands, with a movable element such as e.g. a movable arm as known in the prior art or, in a particular embodiment, the head unit movement can be produced by a spring operatively connected thereto which is discharged to exert a pressure on the head unit itself (Fig. 2A). Additionally, in order to, for example, ameliorate the quality of the cut, in one embodiment said spring does not fully discharge upon activation, thereby exerting a constant pressure over the head unit towards the underlying sample (Fig. 2B).

[00105] As said, the chopper of the invention comprises a support element for placing the sample. Said support can have any suitable shape and can be made of any material, most preferably of a shape and/or material that do not alter the characteristics of the sample. In case of multiple cutting elements aligned in parallel, one problem that can arise is due to the misalignment on the z plane of such cutting elements (Fig. 3A). This

misalignment can lead to cuts which do not cross the entire tissue thickness. In order to overcome this problem, in one embodiment the substrate element underlying the sample is constituted and/or covered by a flexible/soft material (e.g. polymer), into which the cutting elements penetrate (Fig. 3B) upon activation of the movable head unit comprising them.

[00106] Additionally or alternatively, the support element can comprise a relief with cavities positioned below the cutting elements for e.g. counterbalancing z-misalignments (Fig. 3C). The cutting elements are able to penetrate these cavities, and thereby descend deeper than the sample. In one embodiment such cavities have angled walls to facilitate the entry of cutting elements.

[00107] As used herein, a "sample" is any kind of specimen of a material which is compact enough to retain its shape, but soft enough to be cut with mechanical means. It can be for instance a biological tissue harvested from a human being, an animal or a plant or otherwise deriving from a three-dimensional in-vitro culture, a biomaterial, a nanomaterial, a composite material, a polymer, a gel, a food preparation and so forth.

[00108] In a preferred embodiment, the sample is a biopsy such as a tumour biopsy. In this context, the invention can be conveniently employed for the preparation of slices of said biopsy for subsequent analyses such as e.g. ex vivo culturing or cell extraction as required for adoptive T-cell transfer for immunotherapy of cancer patients. In another embodiment, the sample is a scaffold, loaded or not with cells, that is to be cut and distributed into containers such as (multi)well plates for e.g. 3D culture. In one embodiment, in order to overcome the risk of partial cuts, the sample is contained within a matrix (e.g. hydrogel, polymer etc.) so that it is not in direct contact with the underlying substrate and the cutting elements traverse the entire sample (Fig. 3D).

[00109] A further aspect of the invention relates to a chopper as described above for use in preparing slices from a sample and further analysing their electrical properties through impedance means (Fig. 4).

[00110] In this particular aspect of the invention, the above-described chopper further comprises means for electrically connecting the cutting elements to an electrical apparatus adapted so that said cutting elements work as electrodes for analysing the passive electrical properties of the sample through impedance means. In this context, the cutting elements are substantially further used as electrodes that can be individually addressed in order to inject an electrical current within the cut sample once the cutting procedure has been performed. By injecting a current into each slice of the sample, the passive electrical characteristic of each slice can be analysed so that many different properties thereof can be derived such as internal composition, water content, possible crystalline structure and so forth. Preferably, a set of pre-determined data can be acquired from other samples having known features and compared with the features of the sampled object for comparison. Preferably, in one embodiment, the cutting elements can be electrically activated in pairs of adjacent electrodes. As it will be evident to a person skilled in the art, in order for the cutting elements to work as electrodes, it is mandatory that such cutting element are composed or comprise a conductive material such as a metallic material or a polymeric or composite material able to conduct an electrical current. In the simplest embodiment, the cutting elements in this case are simply metallic blades such as well-known razor blades.

[00111] As used herein the term “electrical properties” refers to the physical conditions that allow an electrical charge to move from one atom to another. “Impedance means” refers to e.g. an apparatus that exploits the concept of impedance in a material to obtain information on the passive electrical properties thereof. Impedance is defined as the opposition that a generic material (e.g. polymer, biological material, electronic component, circuit, system and so forth) offers to alternating and/or direct electric current.

[00112] As used herein, the wording “means for electrically connecting” refers to any possible approach to convey an electrical signal from a component (for instance the cutting element working as an electrode) to another (for

instance the input of the electrical apparatus). Such means includes, and are not limited to, metal wires and specifically designed sockets.

[00113] As used herein, the wording “electrical apparatus” refers to any electronic device that measures current or voltage.

[00114] As used herein, the word “electrode” refers to a conductor element used to provide an electrical current through an object. An electrode is generally a metallic element that conducts electricity toward or away from a conductor in a circuit, said circuit being composed in its simplest embodiment of two electrodes and a material as a dielectric, an electrolyte, or a semiconductor placed in between.

[00115] Thanks to the means for electrically connecting the cutting elements to an electrical apparatus such as an impedance analyser, it is possible to gain information on the electrical properties of each single slice during the cutting process without the need for recovering the slices from the chopper.

[00116] In the context of preparation of biopsy slices for ex vivo culturing, these features represent a quick way to gain information for assessing the heterogeneity of the explanted tissue specimen as outlined in the background art. Briefly, the proportion of cancer and normal cells might change within the biopsied tissue and from one sample to another. Also the sample might vary in biopsy diameter along its length. These variations are accounted for by determining their characteristics (e.g. electrical).

[00117] Once slices of a sample are sandwiched between a multi-blade chopper, the challenge is to retrieve them, maintaining their integrity and possibly transfer them in a fast way which requires minimum effort from an operator. This is a critical point for application where the slices are very thin, a few micrometer, as in the case of slice of a bodily tissue biopsy for ex vivo culturing. For such applications, the slices should not only be sorted one by one, but the process should also minimize the risk of exposing them to possible alteration such as contamination.

[00118] To respond to these needs, a further object of the invention is represented by a guide track for use in separation and possible recovery of sample

slices, said guide track having a length “x”, a width “y” and a depth “z” and comprising at least one knob protruding from said guide adapted to block a slice upon relative movement of the guide track with respect to a head unit of a chopper, such as the one previously described, placed on it (Fig. 5).

[00119] As used herein, the term “guide track” refers to a support adapted to accommodate a chopper, particularly a chopper’s head unit, having multiple cutting elements so that when the head unit of a chopper is placed inside the guide, it can only move in the x direction. For instance, the guide track can comprise two rails protruding from the base support of the guide track or dug into it (i.e., cavities) adapted to be combined with a chopper’s head unit, said rails running in parallel along the entire length of the guide track at a distance matching the distance between the first and the last cutting element of a multi-blade chopper (Fig. 5A).

[00120] As used herein, the term “knob” refers to any kind of element protruding from the guide track that fits in the gap between two cutting elements of a multi-blade chopper. The knob acts as a slice-stopper when the chopper’s head unit slides toward it in the x-direction of the track. Its lateral profile can be either perpendicular to the guide track plane, or the knob can have an arc shape such that the sample slice is pushed inside the knob-arc (Fig. 5C). The guide track in a preferred embodiment is substantially a flat plane, and comprises multiple knobs arranged in parallel along the width y of the track, and these can even be evenly distributed along the guide track length x and/or width y for a better slice separation. Additionally, in some embodiments the knobs are adjustable in the x and/or y and/or z direction in order best fit with the operator’s needs or with the distribution of the cutting elements of a chopper’s head unit slicing on the guide track (i.e. to adapt to different spacing between the cutting elements). By placing multiple knobs on a guide in different x- or y-position, each slice can be retrieved and placed in a specific location on the guide track, facilitating the separation process (Fig. 5B).

[00121] In a particular embodiment, at least one hole is placed in front of the knobs with respect to the guide’s length axis, so that when a sample slice is

stopped by a knob, it can fall through the hole and delivered it to an underlying collection site (Fig. 5C). As used herein, the term "underlying collection site" refers to the place where the slice falls once it has been recovered from between the head unit's cutting elements. It can be for example, but not limited to, a multi-well plate, a Petri dish, a microscope slide, a storage box with multi slots, tubes, histology cassettes or any other support for analysis or storage of the slices. In this arrangement, the guide track is particularly useful not only for the separation of the different slices but also for dispensing them in a container for further analyses. For example, it could be imagined to process a biopsy sample, separate via the guide track all the slices obtained therefrom after a chopper cut and at the same time immediately dispensing them through the above-cited holes into e.g. wells of a multiwell plate, preferably each sample slice into a different well.

[00122] In one embodiment, the knobs edges are shaped so to avoid blockage of a sample slice obtained through an above-described chopper upon movement of this latter's movable head along the x-direction of the track. For instance, in order to prevent blockage in case of not perfect y-alignment between knobs and cutting elements of a chopper's head unit, in one embodiment the knobs' lateral edges are rounded or otherwise shaped (e.g. cone-shaped, triangle) (Fig. 6).

[00123] In one embodiment, the knobs are shaped and/or composed of a material such that they can be broken upon the contact of the spacers of the movable head of an above-described chopper's head unit moving along the x-direction of the guide track (Fig. 7). This particular embodiment could be advantageous in case of sticky samples that do not easily detach from the knobs once the separation process takes place: by breaking the knobs, these are allowed to fall through a hole, thereby pulling the sample slice with it. The knobs can comprise in some embodiments an inclined upper surface, that is the surface intended to interact with the spacers of a chopper's head unit upon passage of this latter over the track's knobs. This inclined surface can have the same inclination angle of the spacers, but in one embodiment, the knob's inclined surface has a different angle

than the inclined surface of the spacer, avoiding blocking of the knob against the spacer when the knob tilts slightly (Fig. 7D).

[00124] In one embodiment a blocking element is attached at the bottom of the knob below the hole (Fig. 7A). This blocking element pushes against a wall element protruding in the hole, so that when the knob moves in x-direction these two elements avoid tilting.

[00125] Another option to prevent tilting, is the presence of a pillar (Fig. 7B) positioned behind the knob such that the knob cannot be pulled with the spacer in the lateral direction but can only descend vertically through the hole.

[00126] In a further alternative embodiment (Fig. 7C), the breaking point of the knob is dislocated further in x-direction, thereby favouring the movement of the knob in z-direction when the spacer is moved over the knob.

[00127] The sticking of the tissue to the knob might also be prevented by modifying the surface of the knob (e.g. by a suitable surface treatment) or by changing the shape of the surface such that the contact surface between tissue and knob is decreased (e.g. spikes).

[00128] The knobs, as well as all or part of the guide track, can be manufactured with any suitable shape and with any suitable material. For instance, the guide track and/or the knobs can be made of or can comprise a metallic material, wood or preferably an easily moldable material such as a polymeric plastic material. In this latter case, well-known manufacturing methods (e.g. plastic injection, 3D printing and the like) could allow the production of one-piece, possibly disposable and even sterilisable (if needed) devices. This is particularly convenient when a guide track is intended to be coupled with further systems and/or devices such as disposable containers like multiwell plates as previously described.

[00129] In some embodiments, the guide track, particularly a guide track having at least one hole placed in front of each knob with respect to the guide track' s length axis, is directly incorporated on a container's lid, such as a multiwell plate lid, or otherwise on a (disposable) cartridge that can be placed and precisely aligned on top of a container such as a multi-well plate, histology cassette, petri dish or tube. As will be evident to a skilled

person, in a preferred embodiment the above guide track is designed so that the above-mentioned holes are aligned with specific portions (e.g., wells) or areas of an underlying container.

[00130] Advantageously, in some embodiment where the guide track is incorporated on or coupled with a container's lid, the track can conveniently runs in a specific path that results in a precise sorting of the slices in the underlying container. The possibility of integrating the guide on a lid and to design different path for the guide itself result in a fast sorting with minimum external manipulation requirement, so that not only makes the process fast, but also less prone to contamination. In one embodiment, the device is designed and adapted so that the guide track runs in a straight line and the extremities of such device are precisely matching the width and/or the length of a container such as a well plate, to ensure precise alignment of the device to the desired portion of an underlying container (e.g. wells column or rows).

[00131] As will be evident for a skilled person, another aspect of the invention naturally coming for the teaching of the present disclosure relates to the possibility to combine a chopper for use in preparing slices from a sample as the one described above with the disclosed guide track for use in separation and possible recovery of sample slices in one single system for use in slice preparation, separation and recovery, with the further possibility in some embodiments of analysing the passive electrical properties of said slices with impedance means. Some particular embodiments can be further envisioned for such a system that are specifically studied for optimizing the combination of the chopper and the guide track, so to guarantee a fast and reliable cutting/separation/dispensing process.

[00132] For example, in some embodiments, in order to guarantee proper sliding of the cutting elements on the guide, the cutting element edges and/or the knobs edges may be rounded or be of another shape that facilitates their relative movement in x-direction along the guide track.

[00133] When the slices are very thin - micrometer scale – and the sample is not rigid, as in the case for example of a bodily tissue biopsy, the slice could stick on the knob, preventing its fall through the hole. To overcome this problem, in one embodiment the spacers exerts a pressure onto the knob such that this one breaks and falls through the hole, thereby pulling the sample slice with it (fig. 5C). In order to prevent the tilting of the breakable knob in x-direction during the x-movement of the chopper's head unit, different solution can be envisaged as the ones previously described.

[00134] In one embodiment the entire system is incorporated on or coupled with a container's lid (Figs. 8-11), and the guide track conveniently runs in a specific path that results in a precise sorting of the slices in the underlying container. The possibility of integrating the system on a container's lid and to design different path for the guide track result in a fast sorting with minimum external manipulation requirement, so that not only makes the process fast, but also less prone to contamination. For example, In the case of ex vivo culturing of slices taken from bodily tissue biopsy, a system integrated on a multi-well plate could guarantee in one single slide movement the precise sorting of up to 6, 12, 24, 96 slices or more. In one embodiment, the system is designed and adapted so that the guide track runs in a straight line (Fig. 9) and the extremities of such device are precisely matching the width and/or the length of a container such as a well plate (Figs. 10, 11), to ensure precise alignment of the device to the desired portion of an underlying container (e.g. wells column or rows).

[00135] In one embodiment, the guide track of the system advantageously comprises a ceiling adapted so to accommodate a portion of a chopper as described above (Figs. 8, 9). The ceiling can be conveniently placed above the guide track in a specular fashion with respect to it, so to create a sort of channel. The channel can host for instance the chopper's head unit in it so to further block them and stabilizing together all the elements of the system, and preferably comprises also a portion adapted so to accommodate a part of the chopper, or preferably of the head unit, so that these two items can be operatively connected. For example, the ceiling

can comprise a rail for permitting the sliding of the head unit along the guide track base, blocking and stabilizing at the same time the head unit.

[00136] A practical example of this connection is given in Figure 8. For an optimal functionality of the system, a constant and continuous force can be imposed to the top of the chopper's head unit in order to maintain a constant contact between the chopper itself and the guide track, so to facilitate the cut of the sample and the delivery/distribution thereof along the guide track by the chopper's head unit. Said force is exerted by an element such as a spring placed above the head unit, the discharging of said spring exerting a pressure on the head unit upon activation so that said head unit descend on the sample. By adapting the spring's features (size, material, position and so forth), this can be forced not to fully discharge upon activation, thereby exerting a constant pressure over the head unit towards the underlying sample. The spring can be conveniently placed between the chopper's head unit and the guide track's ceiling to perform its action

[00137] Due to the minimum requirement in terms of external manipulation, in some embodiments at least one element of the system can automated, such as for instance the head unit.

Claims

1. A chopper for use in preparing slices from a sample, characterized in that it comprises:
   - a support element for placing the sample;
   - a movable head unit placed above the support element; and
   - at least two planar cutting elements aligned in parallel on the same plane, separated by spacers and operatively connected to said head unit.

2. The chopper of claim 1, characterized in that it further comprises means for electrically connecting the cutting elements to an electrical apparatus adapted so that said cutting elements work as electrodes for analysing the passive electrical properties of the sample through impedance means.

3. A guide track for use in separation and possible recovery of sample slices having a length x, a width y and a depth z, comprising at least one knob protruding from said guide and adapted to block a sample slice upon relative movement along the x-direction of the track with respect to a chopper.

4. A system for use in the preparation, recovery and possible analysis of slices obtained from a sample, said system comprising a chopper of claim 1 or 2 operatively connected to a guide track of claim 3.

5. Use of a chopper as defined in claim 1 or 2 for simultaneously separating a sample into a plurality of slices.

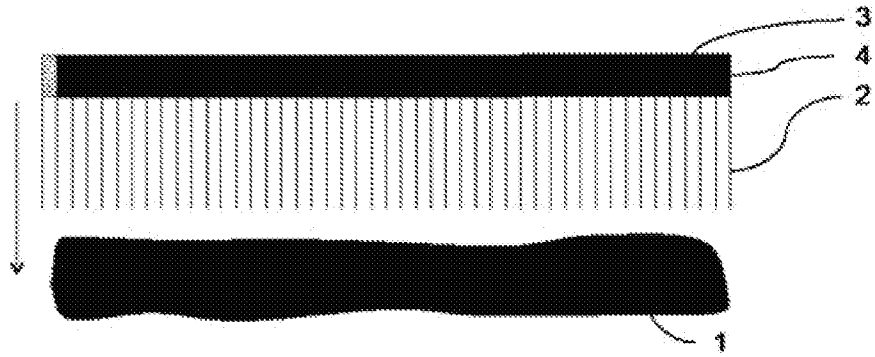


Figure 1

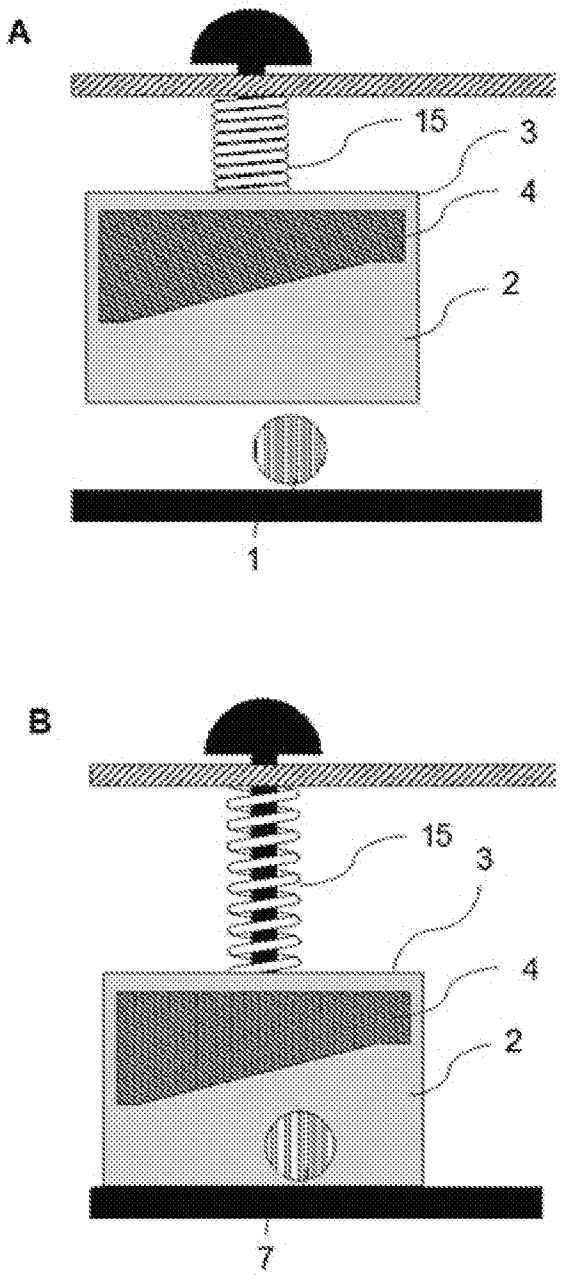


Figure 2

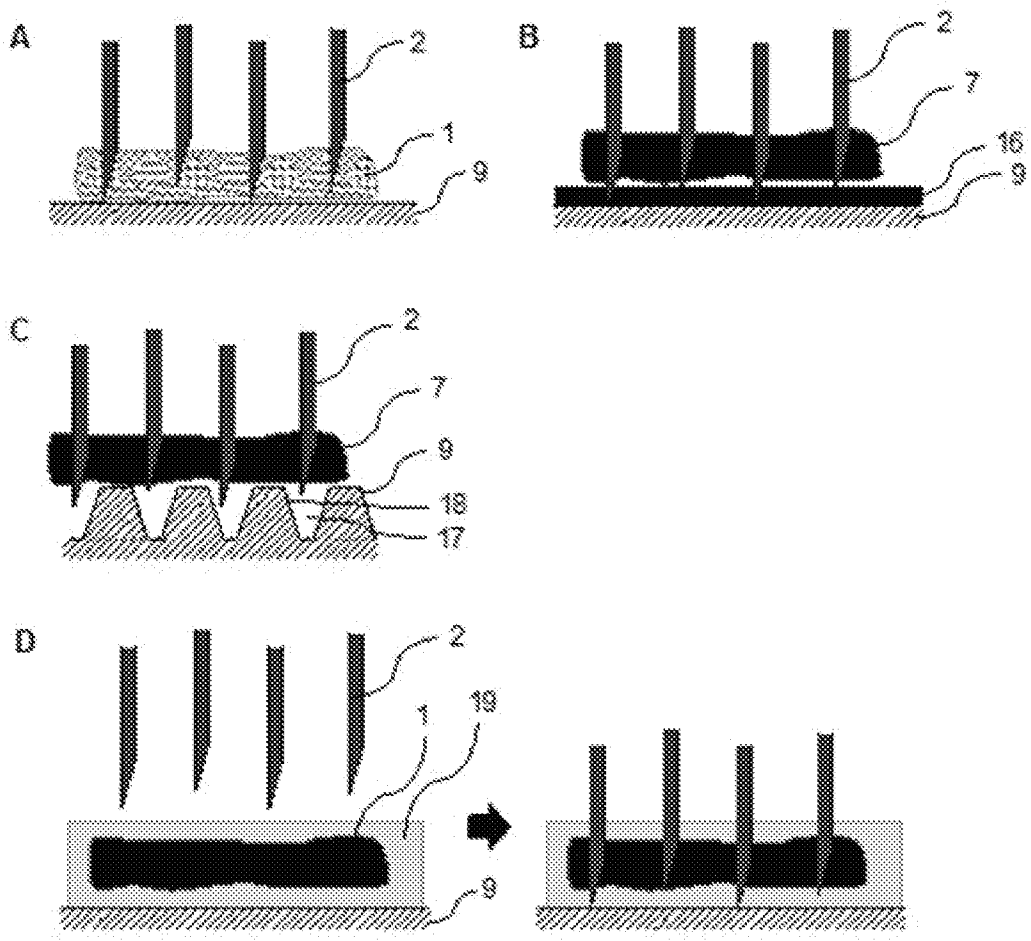


Figure 3

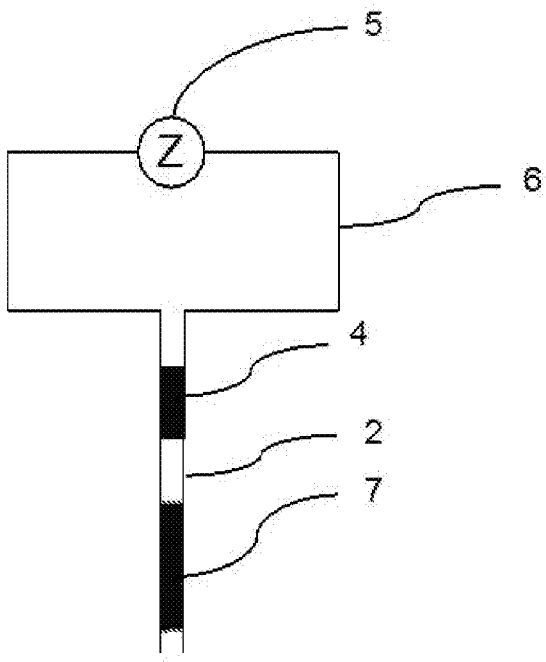


Figure 4

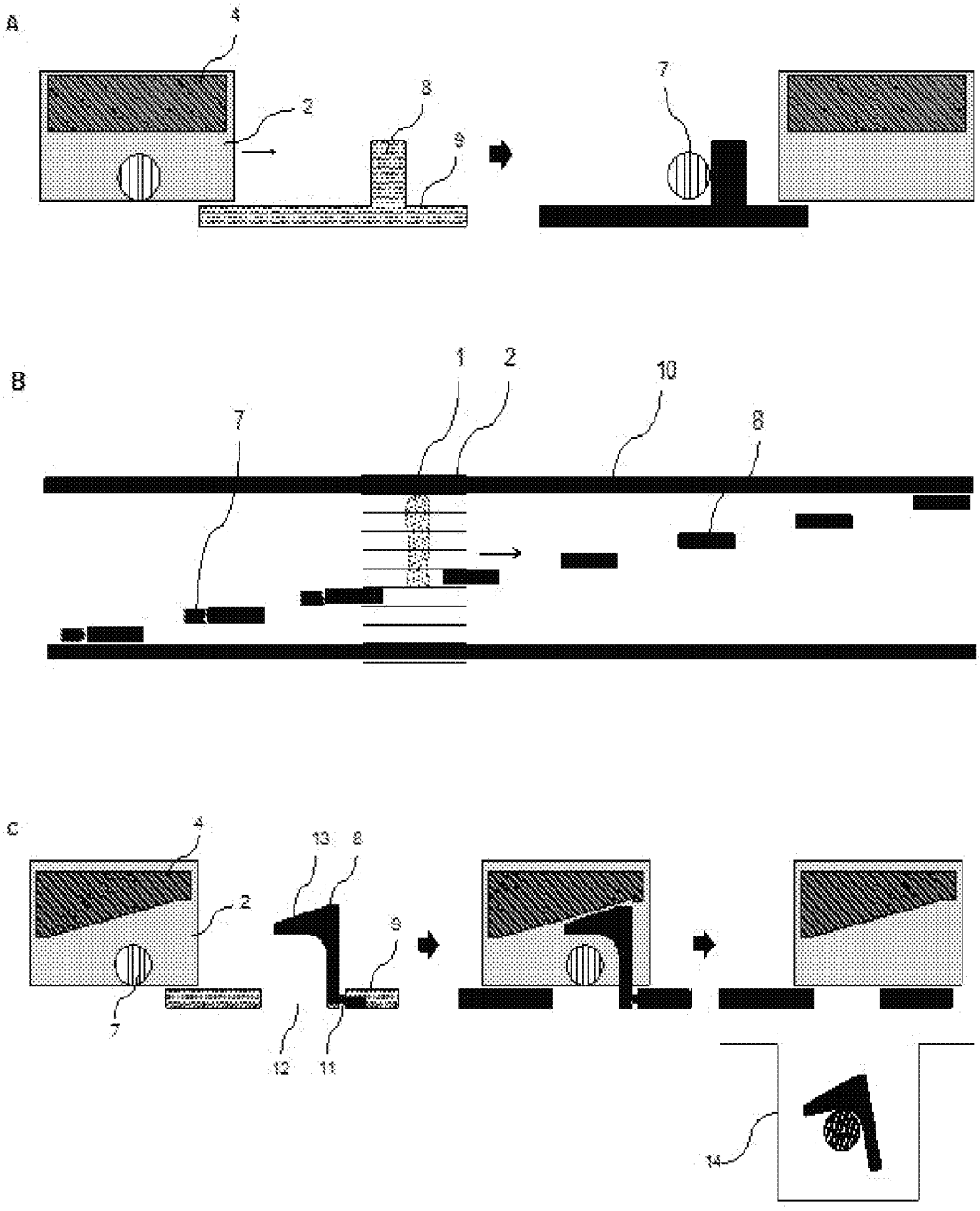


Figure 5

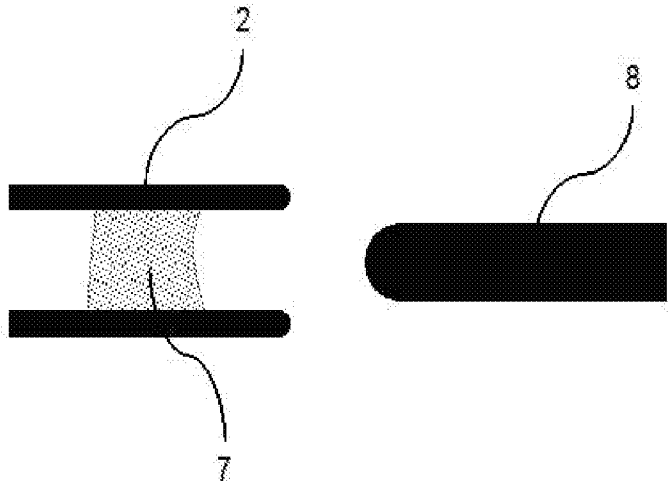


Figure 6

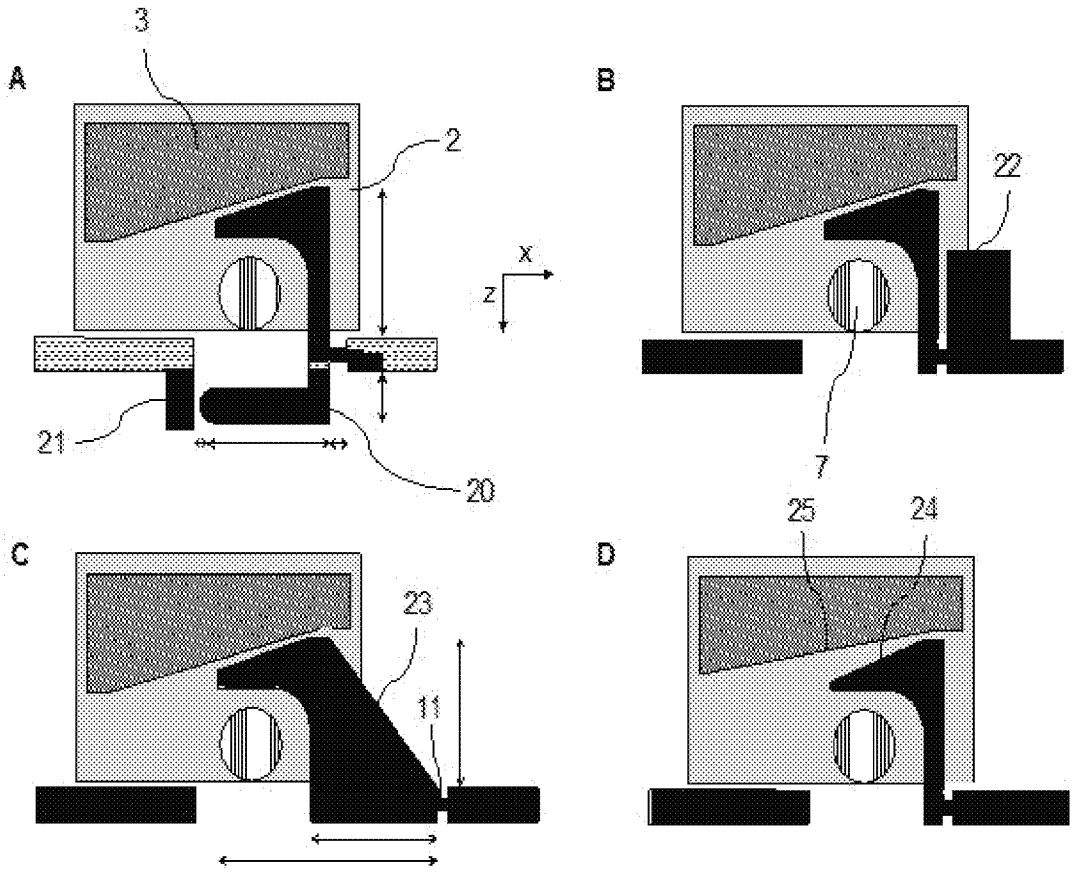


Figure 7

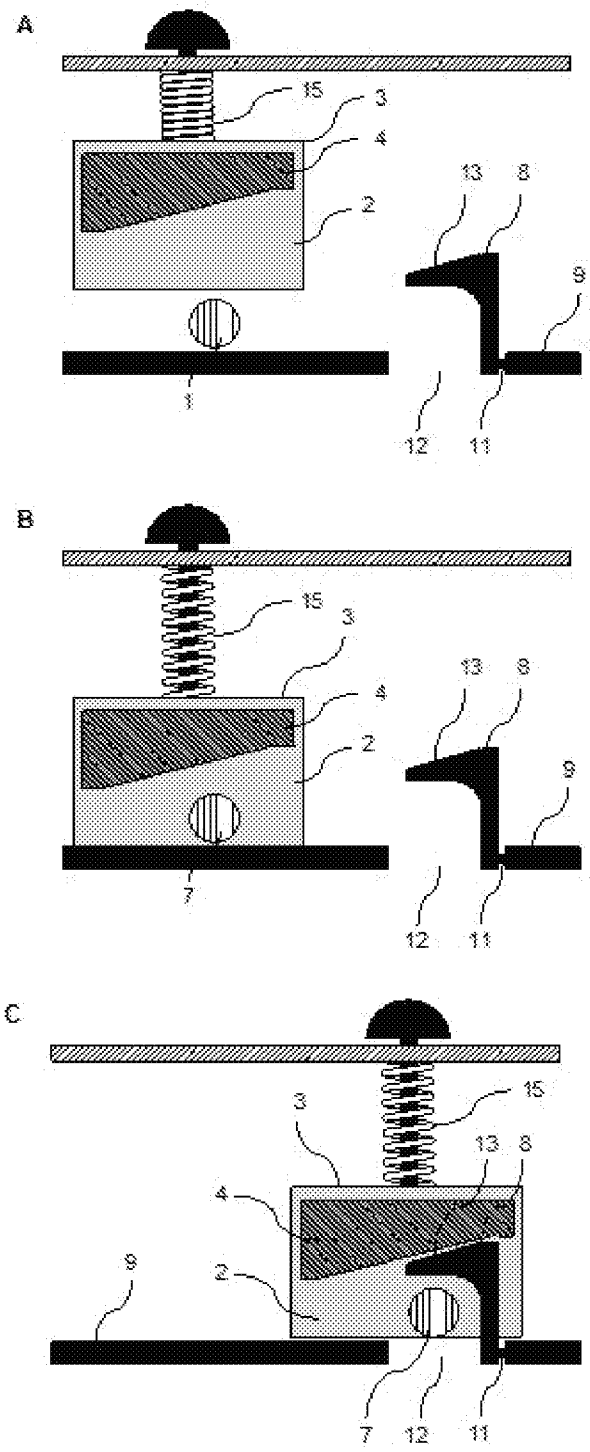


Figure 8

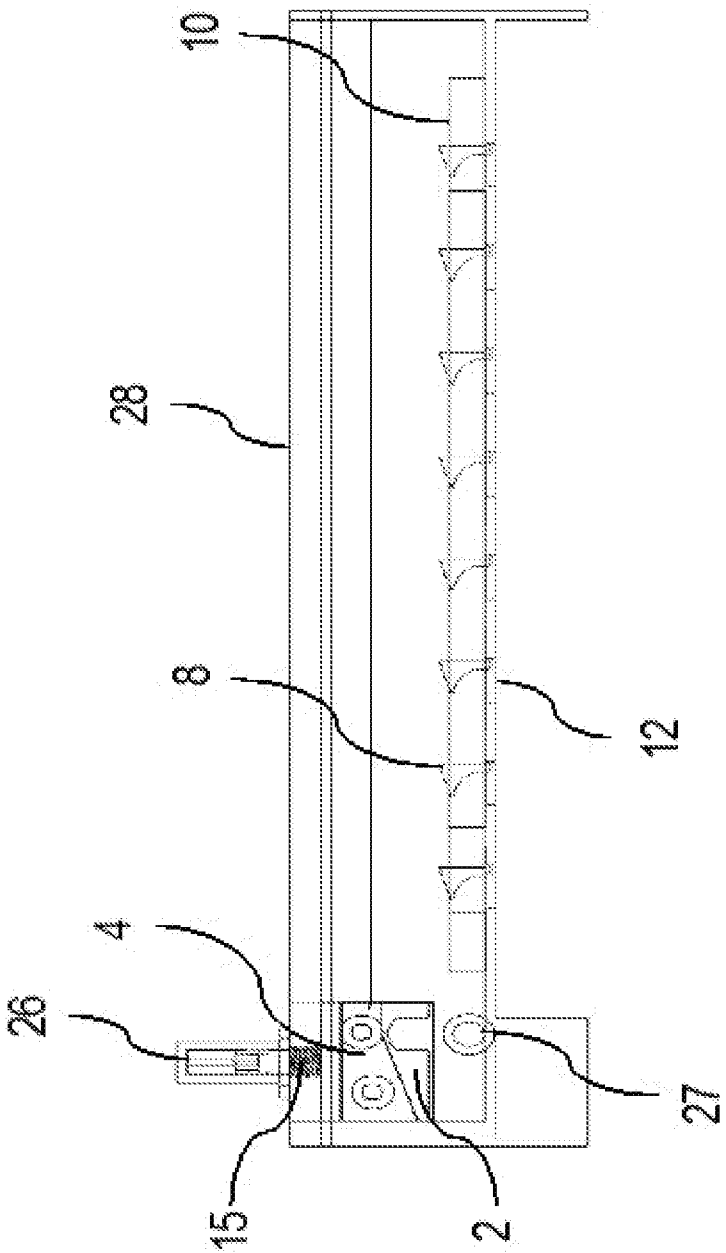


Figure 9

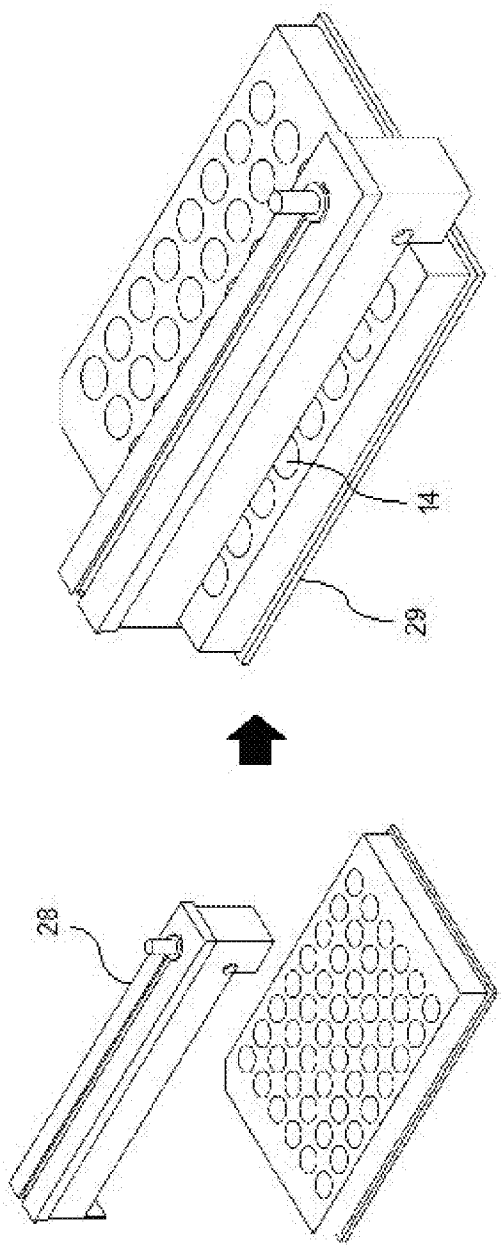


Figure 10

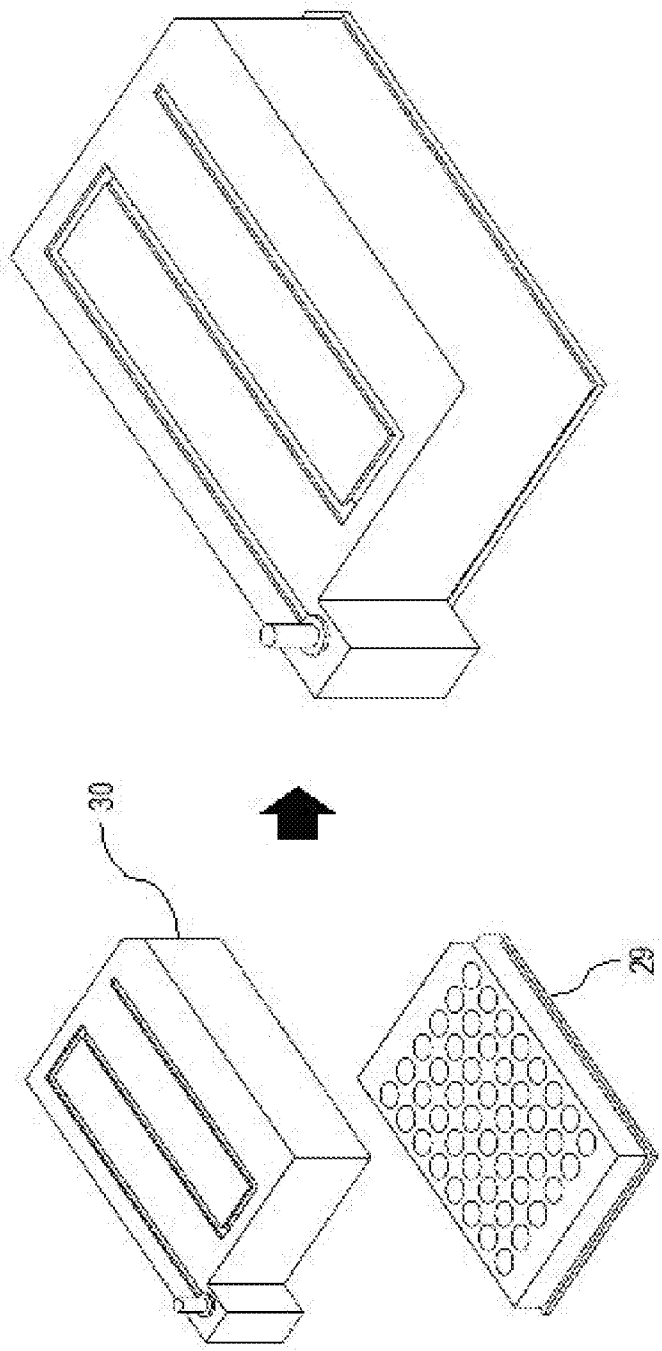


Figure 11

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2016/054212

A. CLASSIFICATION OF SUBJECT MATTER
INV. G01N1/06
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)
G01N

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 Y	US 3 257 884 A (BEST MERLE L ET AL) 28 June 1966 (1966-06-28) figures 1-3 column 1, lines 24-28 column 2, lines 15-38 column 3, lines 4-7	1,3-5 2
X	WO 2010/036931 A1 (UNIV KANSAS MEDICAL CENTER [US]; TAWFIK OSSAMA [US]; MONTGOMERY BROOKE) 1 April 2010 (2010-04-01) cited in the application figure 8 page 11, lines 9-15	1
X	US 7 393 685 B1 (JORDAN ANDREAS [DE]) 1 July 2008 (2008-07-01) figure 1	1

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"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	Date of mailing of the international search report
24 October 2016	04/11/2016

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Dregely, Daniel

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Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2016/054212

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CN 104 142 263 A (XIAN MODERN CHEMISTRY RES INST) 12 November 2014 (2014-11-12) figures 1,2	1
Y	US 5 282 404 A (LEIGHTON STEPHEN B [US] ET AL) 1 February 1994 (1994-02-01) figure 4 column 9, lines 12-19	2

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/IB2016/054212

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