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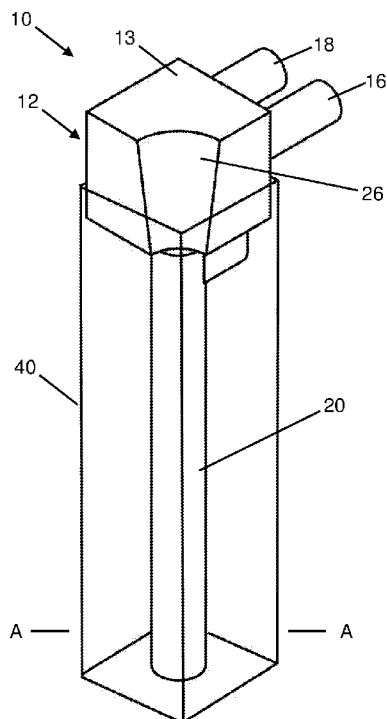


Figure 2

(57) Abstract: A device for mounting a living (or otherwise shape-changing or moving) specimen in a chamber for microscopic examination, the device comprising: a first part for mounting the device in or on the chamber; an inlet port for receiving a flow of a liquid medium; and a second part attached to the first part, the second part being arranged to extend into the chamber in use and to form a substantially straight channel between the second part and one or more walls of the chamber, the channel being for constraining the specimen in use; wherein a first conduit extends from the inlet port, through at least part of the second part, to an outlet hole in the second part, for conveying the liquid medium into the chamber via the outlet hole. Also provided is an assembly comprising such a device mounted in a chamber, and a method of examining a specimen using such a device.



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SPECIMEN MOUNTING DEVICE AND METHOD FOR LIVE MICROSCOPY

Field of the Invention

The present invention relates to a device for mounting a living specimen, to enable
5 the specimen to be examined using a microscope whilst the specimen is alive.
The present invention is particularly applicable, but by no means limited, to the
study of plant root growth using an optical microscope. Other potential
applications include the study of other living organisms such as fungi, worms, etc.,
or other specimens that are not living but which may nevertheless change shape
10 or move over a period of time, such as a growing crystal, or Brownian motion in a
fluid. A method of examining a living, shape-changing or moving specimen using
such a device is also provided.

Background to the Invention

15 In the areas of biological science, plant science, agricultural science, and such
like, there is often a desire to study the growth (or lack thereof) of living specimens
such as plant roots or seedlings using a microscope. For example, a researcher
may wish to study the effects of certain nutrients or other environmental factors on
the growth of a specimen. To do this, the specimen may be sustained in a
20 chamber using a hydroponic perfusion system, and examined using an optical
microscope over an extended period of time (typically several days).

One such approach is described in a paper by Sena *et al.* [1]. The technique
described in this paper uses Light Sheet Microscopy (also known as Single Plane
25 Illumination Microscopy or "SPIM") and a glass chamber having inlet and outlet
holes/ports for a liquid perfusion medium, the holes/ports being located in a wall of
the chamber. A vertical channel to contain the specimen is defined in one corner
of the chamber by a capillary tube, the capillary tube being held in position by a
large number of glass beads which fill the rest of the chamber.

30

It has been found that, in practice, the use of the beads in the above technique is
rather awkward. It also reduces the volume of the liquid medium in the chamber,

as a consequence of which it can be difficult to control the flow of the liquid medium through the chamber. Furthermore, with the above technique, the presence of the holes/ports in the wall of the chamber can lead to breakage of the chamber, as they act as sites of stress concentration and potential sources of brittle failure. The process of connecting or disconnecting lengths of tubing to the ports on the walls can also lead to breakage of the chamber or mechanical failure between the ports and the chamber wall.

There is therefore a desire to improve upon the above technique. In particular, there is a desire for a simpler way of mounting a living specimen to enable it to be examined using a microscope, whilst also allowing the perfusion conditions to be well controlled and reproducible. Importantly, and contrary to other pre-existing systems such as the one described by Calder *et al.* [2], it is often preferable for the growth channel of the specimen to be oriented vertically (rather than horizontally), since this enables samples that naturally grow aligned with the gravity vector, such as plant roots, to be studied in their natural orientation.

Furthermore, in other areas of research, there may be a similar desire to examine other living, shape-changing or moving specimens using a microscope, under controlled conditions, over an extended period of time.

Summary of the Invention

According to a first aspect of the present invention there is provided a device as defined in Claim 1 of the appended claims. Thus there is provided a device for mounting a living (or otherwise shape-changing or moving) specimen in a chamber for microscopic examination, the device comprising: a first part for mounting the device in or on the chamber; an inlet port for receiving a flow of a liquid medium; and a second part attached to the first part, the second part being arranged to extend into the chamber in use and to form a substantially straight channel between the second part and one or more walls of the chamber, the channel being for constraining the specimen in use; wherein a first conduit extends from the inlet port, through at least part of the second part, to an outlet

hole in the second part, for conveying the liquid medium into the chamber via the outlet hole.

5 The term “inlet port” as used herein should be interpreted broadly, to encompass any inlet through which the liquid medium can be supplied to the device.

By virtue of the device being configured to convey the liquid medium into the chamber via the inlet port, the first conduit and the outlet hole within the device itself, there is no need to use a specially adapted chamber having an inlet
10 hole/port in one of its walls. Thus, contrary to the technique disclosed by Sena *et al.* [1], a readily-available off-the-shelf cuvette may now be used to form the chamber, facilitating the experimental process and reducing costs. Moreover, as a result of not needing to provide an inlet hole/port in one of the walls of the chamber, this mitigates the risk of breakage arising from having a hole/port in the
15 chamber wall, as discussed above. Simultaneously, though, the present device is able to physically constrain a specimen in a substantially straight channel in the chamber, thereby facilitating examination of the specimen. It also facilitates the use of an automated system for automatically tracking and imaging a living (or otherwise shape-changing or moving) specimen over an extended period of time.

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Optional features are defined in the dependent claims.

Thus, the first part may include the inlet port. This provides a convenient location for the inlet port and tubing connections thereto, above the chamber in use.

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Preferably the second part is arranged so as to define said channel between the second part and a corner (i.e. two adjacent walls) of the chamber. This is particularly advantageous for imaging a specimen using Light Sheet Microscopy / SPIM, as it enables the specimen to be readily illuminated and for perpendicularly-
30 emitted fluorescence light to be readily collected for imaging.

In the presently-preferred embodiments the second part is in the form of a shaft. In particular the shaft may be cylindrical, although in alternative embodiments it may have other cross-sectional geometries.

- 5 The shaft is preferably aligned close to or with a corner of the first part, said corner of the first part corresponding to said corner of the chamber.

In certain embodiments the shaft (or second part more generally) may be provided with spacing means, such as one or more collars, arranged to space the second
10 part from one or more walls of the chamber, to ensure free diffusion of the liquid medium from the rest of the chamber to the channel.

In presently-preferred embodiments the second part is arranged such that the channel that is formed is substantially vertical, thereby aligning the specimen with
15 the gravity vector. This enables samples that naturally grow aligned with the gravity vector, such as plant roots, to be studied in their natural orientation. Alternatively, the device may be used to impart a gravitational challenge on a specimen that does not naturally grow in a vertical orientation.

20 However, in alternative implementations (and with the use of any necessary sealing as appropriate, to prevent leaks) the device may be oriented such that channel that is formed is substantially horizontal, or is oriented at any other desired angle.

25 In any event, the device provides a well-defined straight channel for the specimen, and supports controlled fluid flow at the same time.

Preferably the outlet hole is positioned at or near the bottom of the second part, typically close to the bottom of the chamber, so as to cause the liquid medium to
30 flow through substantially the entire depth of the chamber, from the bottom to the top, and thereby induce convection in the chamber and increase mixing of the medium.

Particularly preferably the outlet hole faces away from said channel, thereby reducing the extent to which a specimen in the channel is subjected to turbulence and disturbance arising from the flow of the liquid medium into the chamber.

5

Preferably the device further comprises an outlet port for conveying a circulated flow of the liquid medium away from the device.

10 The term “outlet port” as used herein should be interpreted broadly, to encompass any outlet through which the liquid medium can be removed.

As with the inlet port, by virtue of not needing to provide an outlet hole/port in one of the walls of the chamber, this mitigates the risk of breakage arising from having a hole/port in the chamber wall, as discussed above in relation to the apparatus of
15 Sena *et al.* [1].

Particularly preferably the device further comprises an outlet mouth for receiving the circulated flow of the liquid medium from the chamber, and a second conduit extends from the outlet mouth to the outlet port.

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Preferably the first part includes the outlet port – for example, alongside the inlet port, or facing in a different direction from the inlet port.

25 In the presently-preferred embodiments the outlet mouth is disposed on the underside of the first part. In alternative embodiments the outlet mouth may be disposed at or near the top of the second part, for example.

30 The first part of the device may incorporate an opening through which part of the specimen can extend in use. In the presently-preferred embodiments such an opening is provided in a corner of the first part, the corner corresponding to the corner of the chamber in which the channel (in which the specimen is constrained) is located. The opening may for example be funnel-shaped.

In presently-preferred embodiments the device is of unitary construction, for example formed by 3D printing or injection moulding of a plastics material. Other materials, such as suitable biologically-inert metals (e.g. stainless steel), may
5 alternatively be used, with appropriate fabrication processes.

Whilst the surface of the second part (e.g. shaft) is typically untreated, in alternative embodiments it may be provided with a chemical or biological coating or surface treatment of some kind, and/or physical modifications. For example,
10 the surface of the second part may have a chemical coating, and/or a biologically-active coating, and/or may be textured or profiled to physically challenge the specimen.

Alternatively, or in addition, the second part may include one or more electrodes,
15 for example to challenge the sample by subjecting it to electrical pulses or an electric current, or to measure any electrical activity of the sample.

Alternatively, or in addition, the second part may contain one or more optical fibres, for example to bring light into the channel, to challenge or stimulate the
20 sample or to induce optogenetic manipulation.

Alternatively, or in addition, the second part may be provided with a physical support structure, matrix or scaffold for the specimen to adhere to.

25 According to a second aspect of the invention there is provided an assembly comprising a device in accordance with the first aspect of the invention mounted in a chamber. The chamber may comprise a glass cuvette (for example, and advantageously, a readily-available off-the-shelf optical-grade glass cuvette).

30 Preferably, at the corner of the chamber in which the channel (in which the specimen is constrained) is located, the walls of the chamber meet at an angle of substantially 90° (e.g. as may be provided by an off-the-shelf square-section

cuvette). Such a geometry is particularly well suited for Light Sheet Microscopy / SPIM, as it enables the specimen to be readily illuminated and for perpendicularly-emitted fluorescence light to be readily collected for imaging. It also facilitates the use of an automated system for automatically tracking and imaging a living specimen over an extended period of time.

The assembly may further comprise a cover arranged to close the chamber with the device mounted therein, for example to maintain a closed sterile environment within the chamber.

According to a third aspect of the invention there is provided a method of examining a living, shape-changing or moving specimen, the method comprising: preparing an assembly in accordance with the second aspect of the invention, with the specimen being put in said channel; causing a liquid medium to flow into and through the chamber via the inlet port and the outlet hole; and examining the specimen within the channel using a microscope.

The circulated flow of the liquid medium is preferably conveyed away from the device via the outlet port of the device.

Preferably the flow of the liquid medium is continuous, although in alternative embodiments it may be intermittent (e.g. at periodic intervals).

In one practical implementation the microscope employs Light Sheet Microscopy / SPIM, although in alternative implementations other imaging techniques such as, for example, transmitted light microscopy, may be used instead.

Facilitated by the use of the device of the first aspect, the method may further comprise automatically tracking and imaging the specimen as it changes shape or moves.

In practice, the specimen may comprise a living organism, such as a plant or part thereof. Alternatively the organism specimen may comprise a fungus. In a further alternative the organism specimen may comprise a living animal such as a worm, that can be sustained using the present apparatus for the purposes of examination.

In further examples, the specimen may comprise a growing, moving or shape-changing substance, such as a growing crystal, for example, or a fluid in which there is a desire to study Brownian motion.

In all such cases, constraining the specimen to the channel provided by the present device facilitates examination of the specimen using a microscope, and also facilitates the use of an automated system for automatically tracking and imaging the specimen over an extended period of time.

Brief Description of the Drawings

Embodiments of the invention will now be described, by way of example only, and with reference to the drawings in which:

Figure 1 is a series of perspective views (individual Figures 1a, 1b, 1c and 1d) of an embodiment of a specimen mounting device (the views all being of the same device, from different perspectives);

Figure 2 illustrates the device of Figure 1 mounted in a chamber (without a specimen in place or a liquid growth medium within the chamber);

Figure 3 is a schematic cross-sectional view of the device and chamber of Figure 2 (viewed across line A-A of Figure 2), showing the lower part of the device being close to one corner of the chamber;

Figure 4 illustrates the device and chamber of Figure 2, with a specimen in place and a liquid growth medium within the chamber; and

Figure 5 illustrates use of the device and chamber of Figure 2 in Single Plane Illumination Microscopy (SPIM), with an optional cover having been attached over the top of the device (for the sake of clarity, without showing a specimen in place or a liquid growth medium within the chamber).

In the figures, like elements are indicated by like reference numerals throughout.

Detailed Description of Preferred Embodiments

- 5 The present embodiments represent the best ways known to the Applicant of putting the invention into practice. However, they are not the only ways in which this can be achieved.

Overview

- 10 As illustrated in Figures 1 to 4, and with reference initially to Figure 4, a device 10 is provided for mounting a living specimen 50 (also referred to herein as a sample) in a chamber 40, in such a way as to sustain growth of the specimen 50 in a straight (and typically vertical) channel 44 (Figure 3) against an internal corner 42 of the chamber 40. The specimen 50 is sustained using a liquid growth medium
15 48 (represented by the dashed lines in Figure 4) which fills and flows through the chamber 40 and, crucially, the channel 44.

- The device 10 is preferably of unitary construction, for example formed of a plastics material by 3D printing or injection moulding, although it may alternatively
20 be formed from a plurality of component parts that are joined together.

- The device 10 provides a single solution for two problems: it maintains a continuous circulating flow of a liquid medium in the chamber 40, and at the same time physically constrains the growth path of the specimen 50 in a straight (and
25 typically vertical) channel 44 in one corner (corner 42) of the chamber 40.

- Constraining the growth of the specimen 50 in a straight channel is highly advantageous in facilitating automatic tracking and imaging of the specimen 50 (e.g. in SPIM, as illustrated in Figure 5); while providing a continuous circulating
30 flow of liquid medium (perfusion) is beneficial in creating an optically transparent sample embedding and a uniform and controlled growth medium. This last point is very important, as continuous perfusion allows one to maintain control of the

composition of the medium (e.g. oxygen, carbon dioxide, nutrients, waste from the living specimen, etc.). Vertical orientation of the growth channel 44 is also a key advantage since it enables specimens that naturally grow aligned with the gravity vector, such as plant roots, to be studied in their natural orientation. Alternatively, vertical orientation of the growth channel 44 may be used to impart a gravitational challenge on a specimen that does not naturally grow in such an orientation.

Further, and highly advantageously, the device 10 allows the specimen 50 to be mounted at any time during its lifetime, and to be removed and re-mounted many times, to allow for experimental procedures (dissections, treatments, etc.) and manipulations to take place.

Summary of some of the key advantages offered by embodiments of the device:

- Single-piece construction, giving rise to simpler manufacturing
- Constrained growth of the specimen in a linear channel
- Sustained vertical growth of the specimen
- Continuous perfusion of liquid medium in the chamber
- Reversible mounting process allows for flexible experimental protocols.

Practical applications of the device

The device 10 was originally developed for studying the primary root of *Arabidopsis thaliana*, but the size of the device and the material from which it is made may be readily adapted for use with roots of other plant species, or even other living organisms (fungi, worms, etc.).

Furthermore, the device 10 may be used for examination of non-living objects as well. Although it was originally developed to support a living specimen in hydroponic conditions, it may alternatively be used for imaging any microscopic system in a straight (e.g. vertical) channel. For example, the growth of crystalline materials in one dimension (e.g. aligned with the gravity vector) may be examined, or the effects of Brownian motion vs gravity in viscous fluids may be studied.

Similarly, we present below a particular realization in which an off-the-shelf optical-grade glass cuvette with a square cross section is used as the chamber 40, but the device 10 may be readily modified to be used in other chambers with different geometries.

5

Fabrication of the device

In one embodiment the device 10 is 3D printed as a single piece, made of a plastics material such as nylon. In alternative embodiments it may be formed using other techniques (e.g. injection moulding) and/or may be assembled from a plurality of separate parts that are joined together. In any event, the device 10 has no moving parts, so no maintenance is required. As those skilled in the art will appreciate, other plastics materials may be used to form the device 10, or alternatively it may be formed of a suitable metal. More generally, the material from which the device 10 is formed should preferably be biologically inert (e.g. a plastics material, or stainless steel) and, unless the device 10 is intended to be single-use, should be sterilisable (e.g. autoclavable).

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Configuration and constituent features of the device

As shown in the different perspective views (Figures 1a-1d) within Figure 1, the device 10 comprises an upper (or “first”) part 12 attached to a lower (or “second”) part 14. In the presently-preferred embodiments the upper part 12 is directly attached to the lower part 14, although in alternative embodiments one or more intermediate parts may be disposed between the upper part 12 and the lower part 14.

20
25

The upper part 12 comprises a body 13, an inlet port 16, an outlet port 18, an outlet mouth 24, and a funnel-shaped corner 26. Formations 30 and 32 are also provided on the underside of the upper part 12, to provide rigidity to the overall device 10 and to facilitate mounting of the device 10 in a chamber 40 (as discussed in greater detail below).

30

The lower part 14 comprises a hollow straight vertical shaft 20 which is provided with a lateral outlet hole 22. The shaft 20 is aligned close to (or with) a corner of the first part 12. A first continuous conduit runs from the inlet port 16, through the upper part 12 and down the shaft 20, to the hole 22. Thus, the inlet port 16 is in fluid communication with the hole 22. A second continuous conduit runs from the outlet mouth 24, through the upper part 12, to the outlet port 18. Thus, the outlet mouth 24 is in fluid communication with the outlet port 18. In the illustrated embodiment, the lower part 12 further comprises two collars 28 which surround the shaft 20, in upper and lower positions. However, in alternative embodiments the collars 28 need not be present, or can be present in a different number or size.

Mounting of the device in a chamber

With reference to Figures 1, 2, 3 and 4, in use the device 10 is mounted in a chamber 40. Preferably the body 13 of the upper part 12, and the formations 30 and 32, are shaped so as to enable the upper part 12 to fit with a gentle friction fit into the upper opening of the chamber 40. As shown in Figure 3, the shaft 20 is configured such that it extends close to one right-angled corner 42 of the chamber 40. This position of the shaft 20 creates a straight vertical space or channel 44 in the corner 42 of the chamber 40, in which channel 44 the specimen 50 is constrained and can grow. The length of the shaft 20 is preferably such that it reaches the bottom of the chamber 40, or comes very close to the bottom of the chamber 40, so as to ensure that, during use, a specimen 50 constrained in the channel 44 cannot grow or otherwise make its way under the bottom of the shaft 20 and into the rest of the chamber 40.

25

For imaging the specimen 50 using Light Sheet Microscopy / SPIM, in which the direction of observation is perpendicular to the direction of illumination, it is highly desirable that the corner 42 of the chamber 40 be right-angled and made of optical-grade glass. Advantageously, off-the-shelf optical-grade glass cuvettes with a square cross-section may be readily obtained for this purpose. However, alternative arrangements may be envisaged, in particular for use with other

30

imaging techniques, where the corner 42 of the chamber 40 need not be right-angled.

As discussed further below, the distance of the shaft 20 from the corner 42 of the chamber 40, and thus the cross-sectional width of the channel 44 (and in turn the size of the specimen that can be accommodated), is primarily determined by the diameter of the shaft 20 and of the collars 28.

In the illustrated embodiment the collars 28 serve to keep the shaft 20 a small predetermined distance from the walls of the chamber 40, said distance being determined by the distance by which the collars 28 project from the shaft 20. The space thus formed, between the shaft 20 and the chamber walls, allows free diffusion of the liquid medium from the rest of the chamber (e.g. region 46) to the channel 44. As those skilled in the art will appreciate, it is important that said space is small enough to prevent the exit of the specimen from the channel 44.

As illustrated for example in Figure 4, a corner of the upper part 12 of the device 10 (corresponding to the corner 42 of the chamber 40 where the channel 44 is) has a funnel-shaped profile 26, to accommodate any part of the specimen 50 not entering (or growing out of) the channel 44. In the case of *Arabidopsis thaliana*, this may be its hypocotyl and cotyledons.

Tubing connections and fluid flow

The inlet port 16 is shaped to connect to a first length of tubing (e.g. made of silicone rubber) which leads from an external reservoir of the liquid medium that is to fill and flow through the chamber 40 in use. To effect the connection between the first length of tubing and the inlet port 16, the end of the first length of tubing may for example be pushed around the outer circumference of the inlet port 16. The inlet port 16 may be provided with gripping means (e.g. one or more ridges) to securely retain the end of the first length of tubing around the inlet port 16 during use.

In use, the liquid medium is delivered from the external reservoir to the inlet port 16 via the first length of tubing, either continuously (and gently) or intermittently (e.g. at periodic intervals), for example using a pump or a syringe. Thus, the liquid medium enters the device 10 via the inlet port 16 and is pushed down the hollow shaft 20. The liquid medium exits the shaft 20 via the hole 22 and then flows into the chamber 40.

Similarly, the outlet port 18 is shaped to connect to a second length of tubing (e.g. made of silicone rubber) which leads to a fluid collection tank (or other container) or drain, or, potentially, back to the abovementioned supply reservoir in order to create a closed circulating system. To effect the connection between the second length of tubing and the outlet port 18, the end of the second length of tubing may for example be pushed around the outer circumference of the outlet port 18. The outlet port 18 may be provided with gripping means (e.g. one or more ridges) to securely retain the end of the second length of tubing around the outlet port 18 during use.

In use, the liquid medium, flowing out of the hole 22, fills the chamber 40 (as indicated by the dashed lines / liquid medium 48 in Figure 4) and flows through the chamber (essentially from bottom to top). It should be noted that the hole 22 is on the opposite side of the chamber 40 from the channel 44, and consequently the flow direction of the liquid medium is away from the channel 44. Advantageously, as a result, the specimen in the channel 44 is subjected to minimal turbulence and disturbance from the flow of the liquid medium into the chamber 40.

During flow of the liquid medium, the outlet mouth 24 receives excess liquid medium from the chamber 40, essentially in the manner of an overflow, such as to keep the volume of the liquid medium inside the chamber 40 constant. The liquid medium received by the outlet mouth 24 passes to the outlet port 18 and from there, via the connected second length of tubing, to the collection tank or drain. If necessary or desired, the removal of the liquid medium via the outlet mouth 24 and the outlet port 18 may be aided by a pump or a syringe. Indeed, if required,

the outlet port 18 may be subjected to continuous suction by a pump, to remove any liquid that reaches the outlet mouth 24.

Preferably the flow of the liquid medium through the chamber 40 is continuous, in order to apply steady-state conditions (e.g. in terms of salts, nutrients, oxygen, etc.) to the specimen 50, and to remove waste from the specimen 50.

Preferably the flow of the liquid medium through the chamber 40 is as slow as possible whilst keeping the specimen 50 well-sustained, to avoid turbulence.

10

It should be noted that, during the course of a typical experiment, the liquid medium is the only thing in the chamber 40, apart from the device 10 and the specimen 50. Advantageously, no beads or such like are required to help support the specimen. As a result of being able to fill the entire volume of the chamber 40 (apart from the device 10 and the specimen 50) with the liquid medium, this provides the user with better control of the flow of the liquid medium through the chamber 40, with a more even distribution of nutrients through the chamber 40, and without causing turbulence. It is also easier to adjust and control the temperature of the liquid medium flowing through the chamber 40, if desired. These factors in turn lead to greater reproducibility of the experimental procedure.

20

However, if desired, the available space within the chamber 40 away from the channel 44, e.g. region 46, may be used to contain one or more sensors or other devices, such as a temperature sensor, a pH sensor, or an oxygen concentration sensor, for example. The ability to provide such sensors in this space is a further advantage resulting from the chamber 40 not being filled with beads.

25

The liquid medium

Those skilled in the art of hydroponics will be familiar with possible compositions for the liquid medium (e.g. comprising a mixture of water, salts, sucrose, and pH adjustment) for sustaining the growth of a specimen. In any given case, the composition of the liquid medium will generally be selected to suit the specimen

30

being studied. One example of the liquid medium, optimized for hydroponics culture of *Arabidopsis thaliana*, has the following composition:

1/4X Murashige and Skoog Basal medium, 0.5% sucrose, 0.05% MES hydrate, adjusted to pH 5.7 with KOH and sterilised by autoclaving at 121°C.

5

Although, typically, an unchanging composition of the liquid medium will be supplied for the duration of an experiment, if desired the composition of the liquid medium may be changed during the course of the experiment.

- 10 Further, if desired, one or more gasses may be introduced into the liquid medium as it is fed to the device 10. For example, a gas may be diluted in the liquid medium upstream of the inlet port 16, such that the gas is present in the liquid medium that is supplied to the device 10. Alternatively, a valve may be provided upstream of the inlet port 16, to enable the user to selectively switch the supply
15 between the liquid medium and a gas.

Further, if desired, heating or cooling means may be provided to enable the user to control the temperature of the liquid medium that is supplied to the device 10.

- 20 Also, if desired, by reversing the pump attached to the inlet port 16 (i.e. such that it sucks rather than delivers), it is possible to suck all the liquid medium out of the chamber 40 via the hole 22. This is a reversible process, as the pump can be reversed again to re-fill the chamber 40. This makes it possible to expose the specimen to cycles of wet and dry conditions, further expanding the experimental
25 use of the device.

Size and geometry of the device and the chamber

- The size of the device 10 can be scaled to accommodate a variety of different organisms. The most important parameter which may be varied is the size of the
30 channel 44, in particular its cross section. The vertical length of the channel 44 and the total size of the chamber 40 may also be varied depending on the age and type of the organism, especially when looking at plants.

As will be appreciated from Figure 3, the cross-sectional width of the available channel 44 is determined by the diameter of the shaft 20. The following table lists some suitable shaft diameters for some popular model systems.

5

Organism	Typical diameter of specimen (microns)	Suitable shaft diameter (mm)
<i>Neurospora crassa</i> (mold)	10	0.06
<i>C. elegans</i> (nematode)	80	0.47
<i>Arabidopsis thaliana</i> root	120	0.70
<i>Zea mays</i> (corn) root	400	2.36
<i>Triticum aestivum</i> (barley) root	600	3.53
<i>Oryza sativa</i> (rice) root	1300	7.66

If a shaft has a greater diameter than is desired for a given application, then, to further constrain the specimen, an extra pin (with collars to allow medium flow) may be added between the shaft 20 and the corner 42 of the chamber 40. For example, if a shaft 20 having a diameter of 4.2 mm is used, it creates a channel against the corner 42 of the chamber which could accept a specimen of up to 700 microns in diameter, which may be larger than is desired for a particular specimen. Thus, in order to further constrain an *Arabidopsis thaliana* root, for example, an extra pin of diameter 700 microns (with collars to allow medium flow) may be added between the shaft 20 and the chamber corner 42, which effectively reduces the available space in the channel 44 to the desired diameter of 120 microns.

Merely by way of example, in one present embodiment, the following dimensions of the device 10 and chamber 40 are used:

- Chamber 40 (a glass cuvette): external dimensions of 12.5 mm x 12.5 mm x 45.0 mm
- Upper part 12 of the device 10: length x width x height = 10.0 mm x 10.0 mm x 18.0 mm

- Lower part 14 of the device 10 (i.e. the shaft 20): height = 32.0 mm; external diameter = 4.2 mm; internal diameter (hollow) = 2.6 mm

The above chamber size is sufficient to sustain most young seedlings and small organisms, such as the ones listed in the table above.

Microscopy set-up

By way of example, Figure 5 illustrates the device 10 in use in Light Sheet Microscopy / Single Plane Illumination Microscopy (SPIM). It should be noted that, in Figure 5, the specimen, the liquid medium, and the first and second lengths of tubing (and one or more reservoirs, etc.) have been omitted for the sake of clarity of the diagram, but would normally be present in practice.

As illustrated, an optional cover 52 may be attached over the top of the device 10, to maintain a closed sterile environment within the chamber 40. When studying plants, this cover 52 is preferably transparent, to allow light to reach the leaves of the specimen.

Comprehensive details of how Light Sheet Microscopy / SPIM is performed may be readily found elsewhere (see e.g. in [1]), and so need not be described in full detail in the present work. However, by way of a brief overview of the technique, and to illustrate some of the advantages conferred by the present device 10, in the set-up 60 depicted in Figure 5 the chamber 40 is mounted upright in a holder 62 upon a horizontal three-axis (X-Y-Z) movable stage 64. Thus, the channel 44 within the chamber 40 is oriented vertically, as is the specimen that is constrained within the channel 44. Accordingly, specimens that naturally grow aligned with the gravity vector, such as plant roots, can be studied in their natural orientation.

A laser 66, producing a laser beam, is arranged to illuminate the specimen via a cylindrical lens 68, thereby generating a light sheet which causes the specimen to fluoresce in the plane of the light sheet.

With Light Sheet Microscopy / SPIM, the direction of observation is perpendicular to the direction of illumination. Thus, an objective lens 70 is arranged to collect perpendicularly the fluorescence light emitted from the light sheet and to project the collected light onto an imaging sensor 72 (e.g. a charge coupled device or
5 CCD camera). The imaging sensor 72 is typically mounted on a movable (X-Y) stage, to control focus and to track the specimen.

Since the laser light supplied to the specimen and the fluorescence collected from the specimen are at 90° to one another, as discussed above, it is desirable that
10 the corner 42 of the chamber 40, where the specimen-containing channel 44 is, be right-angled and made of optical-grade glass.

A computer-controlled system running appropriate software may be employed to automatically track and image the living specimen (by moving the stage 64 using
15 motors, and potentially also adjusting the optics), as the specimen changes shape (e.g. as it grows or dies). Having the living specimen constrained to the narrow straight vertical channel 44 in the chamber 40 greatly facilitates such automatic tracking and imaging. Indeed, with pre-existing specimen mounting arrangements it has been found that automatic tracking and imaging of a living specimen is very
20 difficult (if not impossible) and unreliable, but use of the present device 10 to constrain the specimen has been found to greatly improve the accuracy of the automatic tracking and imaging process.

As those skilled in the art will appreciate, Figure 5 is a simplified diagram of the
25 optical set-up for Light Sheet Microscopy / SPIM. A number of commonly-used elements (e.g. beam expander, iris, shutter, fluorescence filters, etc.) have been omitted from the diagram for the sake of clarity.

The present device 10 is not restricted to use in Light Sheet Microscopy / SPIM.
30 Indeed, other imaging techniques, such as scattered light microscopy, for example, may alternatively be used to study the growth of a living specimen within a chamber using the device 10.

Possible modifications and alternative embodiments

Detailed embodiments have been described above, together with some possible modifications and alternatives. As those skilled in the art will appreciate, a number of additional modifications and alternatives can be made to the above embodiments whilst still benefiting from the inventions embodied therein.

- The inlet port 16 and the outlet port 18

Whilst in the above embodiments the inlet port 16 and the outlet port 18 are shaped to allow lengths of tubing to be pushed around them, to thereby effect connections, in alternative embodiments other coupling/connecting means may be provided. For example, "push-to-connect" connectors may be provided, to enable lengths of tubing to be easily connected to the ports.

Whilst in the above embodiments the inlet port 16 and the outlet port 18 are parallel to each other, in alternative embodiments they may be angled away from each other, for example to allow larger diameter tubing or larger couplings to be connected to the ports.

- The shaft 20

Whilst in the above embodiments the surface of the shaft 20 is untreated, in alternative embodiments the surface of the shaft 20 may be treated in a variety of ways (or any combination thereof), as follows:

The shaft 20 may be coated with any chemical of interest (e.g. to induce abiotic stress in a controlled environment), to enable the interaction between the specimen and the coating to be studied. For example, the coating may comprise a nitrogen-fixing substance.

The shaft 20 may be textured to accept *in situ* culture of a microorganism of interest (e.g. to induce biotic stress or symbiosis).

More generally, the shaft 20 may be coated with any biologically-active material of interest (e.g. proteins, bacteria, organisms, etc.), to enable the interaction between the specimen and the coating to be studied.

- 5 The shaft 20 may be textured or profiled to physically challenge the sample (e.g. physical constraints, to induce mechanical stress on the sample).

The shaft 20 may be modified to contain one or more electrodes, to challenge the sample by subjecting it to electrical pulses or an electric current, or to measure
10 any electrical activity of the sample.

The shaft 20 may be modified to contain one or more optical fibres, to bring light into the channel, to challenge or stimulate the sample or to induce optogenetic manipulation.

15

The shaft 20 may be provided with a physical support structure, matrix or scaffold (e.g. radially-extending arms) for the growing specimen to adhere to.

Whilst in the above embodiments the shaft 20 is provided with collars 28 to
20 determine the spacing of the shaft 20 from the walls of the chamber 40, in alternative embodiments the shaft 20 may be provided with other spacing means (e.g. ridges, arms, spacers etc.), or they may be omitted altogether. For example, the device 10 may be manufactured with sufficient precision and the shaft 20 may have sufficient inherent rigidity to reliably provide a small space between the shaft
25 20 and the walls of the chamber 40 without the need for collars 28 or the like.

Moreover, in another variant, small grooves may be provided in the surface of the shaft 20, to allow free diffusion of the liquid medium into the channel 44, without the use of collars or such like.

30

Whilst in the above embodiments the shaft is cylindrical in shape, in alternative embodiments it may have other cross-sectional geometries.

Whilst in the above embodiments the shaft is arranged to form the specimen-containing channel between the shaft and two adjacent walls (i.e. a corner) of the chamber, in alternative embodiments the shaft may be shaped and arranged to form a specimen-containing channel between the shaft and a single wall of the chamber. In such a case the shaft may, for example, be formed with a U-shaped cross-section, such as to form the channel in the centre of the "U". Whilst such a configuration may not be suitable for use with Light Sheet Microscopy / SPIM, it may be suitable for use with other imaging techniques, such as reflected light microscopy.

- *The hole 22*

Whilst in the above embodiments a single hole 22 is provided in the shaft 20, for the liquid medium to flow through, in alternative embodiments a plurality of such holes may be provided in the shaft.

Further, whilst in the above embodiments the single hole 22 faces away from the channel 44, to avoid subjecting the specimen to turbulence and disturbance from the flow of the liquid medium, in alternative embodiments the hole(s) may face towards the specimen, for example in order to study the effects of turbulence on the growth of the specimen.

- *The chamber 40*

Whilst in the above embodiments, for simplicity, an off-the-shelf glass cuvette with a square cross section is used as the chamber 40, in alternative embodiments the chamber may have other shapes (i.e. other than square cross-section). However, to facilitate imaging (in particular when using Light Sheet Microscopy / SPIM), the corner 42 in which the channel 44 is formed and the specimen is constrained is preferably at an angle of 90°, and at least the corner 42 through which the specimen is imaged is preferably made of an optical-grade material (e.g. glass). The rest of the cross-section of the chamber 40 may in principle be any shape and may also in principle be made of other materials.

Finally, referring to Figure 5, the entire 40+70 optical arm could be contained and immersed in a containment vessel, filled with water or oil, to minimize changes in refractive index, and thus optimizing imaging.

5 References

[1] Sena G, Frentz Z, Birnbaum KD, Leibler S (2011) Quantitation of Cellular Dynamics in Growing Arabidopsis Roots with Light Sheet Microscopy. PLoS ONE 6(6): e21303. DOI:10.1371/journal.pone.0021303.

10

[2] Grant Calder, Chris Hindle, Jordi Chan and Peter Shaw (2015) An optical imaging chamber for viewing living plant cells and tissues at high resolution for extended periods. Plant Methods 11:22 DOI 10.1186/s13007-015-0065-7.

CLAIMS

1. A device for mounting a living, shape-changing or moving specimen in a chamber for microscopic examination, the device comprising:

5 a first part for mounting the device in or on the chamber;

an inlet port for receiving a flow of a liquid medium; and

a second part attached to the first part, the second part being arranged to extend into the chamber in use and to form a substantially straight channel between the second part and one or more walls of the chamber, the channel being

10 for constraining the specimen in use;

wherein a first conduit extends from the inlet port, through at least part of the second part, to an outlet hole in the second part, for conveying the liquid medium into the chamber via the outlet hole.

15 2. A device as claimed in claim 1, wherein the first part includes the inlet port.

3. A device as claimed in claim 1 or claim 2, wherein the second part is arranged so as to define said channel between the second part and a corner of the chamber.

20 4. A device as claimed in claim 3, wherein the second part is in the form of a shaft.

5. A device as claimed in claim 4, wherein the shaft is cylindrical.

25 6. A device as claimed in claim 4 or claim 5, wherein the shaft is aligned close to or with a corner of the first part, said corner of the first part corresponding to said corner of the chamber.

30 7. A device as claimed in any preceding claim, wherein the second part is provided with spacing means arranged to space the second part from one or more walls of the chamber.

8. A device as claimed in claim 7, wherein the spacing means comprise one or more collars arranged around the second part.

5 9. A device as claimed in any preceding claim, wherein the second part is arranged such that the channel is substantially vertical.

10. A device as claimed in any preceding claim, wherein the outlet hole is positioned at or near the bottom of the second part.

10

11. A device as claimed in any preceding claim, wherein the outlet hole faces away from said channel.

12. A device as claimed in any preceding claim, further comprising an outlet
15 port for conveying a circulated flow of the liquid medium away from the device.

13. A device as claimed in claim 12, further comprising an outlet mouth for receiving the circulated flow of the liquid medium from the chamber;
wherein a second conduit extends from the outlet mouth to the outlet port.

20

14. A device as claimed in claim 13, wherein the first part includes the outlet port.

15. A device as claimed in claim 14, wherein the outlet mouth is disposed on
25 the underside of the first part.

16. A device as claimed in any preceding claim, wherein the first part incorporates an opening through which part of the specimen can extend in use.

30 17. A device as claimed in claim 16, wherein the opening is in a corner of the first part.

18. A device as claimed in claim 17 when dependent on claim 3, wherein said corner of the first part corresponds to said corner of the chamber.

19. A device as claimed in any of claims 16 to 18, wherein the opening is
5 funnel-shaped.

20. A device as claimed in any preceding claim, being of unitary construction.

21. A device as claimed in any preceding claim, wherein the surface of the
10 second part has a chemical coating.

22. A device as claimed in any preceding claim, wherein the surface of the second part has a biologically-active coating.

15 23. A device as claimed in any preceding claim, wherein the surface of the second part is textured or profiled to physically challenge the specimen in use.

24. A device as claimed in any preceding claim, wherein the second part comprises one or more electrodes.
20

25. A device as claimed in any preceding claim, wherein the second part comprises one or more optical fibres.

26. A device as claimed in any preceding claim, wherein the surface of the
25 second part comprises a physical support structure, matrix or scaffold for the specimen to adhere to in use.

27. An assembly comprising a device as claimed in any preceding claim mounted in a chamber.
30

28. An assembly as claimed in claim 27, wherein the chamber comprises a glass cuvette.

29. An assembly as claimed in claim 27 or claim 28 when dependent on claim 3, wherein, at said corner of the chamber, the walls of the chamber meet at an angle of substantially 90°.

5

30. An assembly as claimed in any of claims 27 to 29, further comprising a cover arranged to close the chamber with the device mounted therein.

31. A method of examining a living, shape-changing or moving specimen, the method comprising:

10

preparing an assembly as claimed in any of claims 27 to 30 with the specimen being put in said channel;

causing a liquid medium to flow into and through the chamber via the inlet port and the outlet hole; and

15

examining the specimen within the channel using a microscope.

32. A method as claimed in claim 31 when dependent on any of claims 12 to 15, further comprising conveying the circulated flow of the liquid medium away from the device via the outlet port.

20

33. A method as claimed in claim 31 or claim 32, wherein the microscope employs Light Sheet Microscopy / Single Plane Illumination Microscopy.

34. A method as claimed in any of claims 31 to 33, further comprising automatically tracking and imaging the specimen as it changes shape or moves.

25

35. A method as claimed in any of claims 31 to 34, wherein the specimen comprises a living organism.

30

36. A method as claimed in claim 35, wherein the specimen comprises a plant or part thereof.

37. A method as claimed in claim 35, wherein the specimen comprises a fungus.

38. A method as claimed in claim 35, wherein the specimen comprises an
5 animal such as a worm.

39. A method as claimed in any of claims 31 to 34, wherein the specimen comprises a growing, moving or shape-changing substance.

10 40. A method as claimed in claim 39, wherein the specimen comprises a growing crystal.

41. A method as claimed in claim 39, wherein the specimen comprises a fluid and the method is for studying Brownian motion in the fluid.

15

42. A device substantially as herein described with reference to and as illustrated in any combination of the accompanying drawings.

43. An assembly substantially as herein described with reference to and as
20 illustrated in any combination of the accompanying drawings.

44. A method of examining a living specimen substantially as herein described with reference to and as illustrated in any combination of the accompanying drawings.

1/3

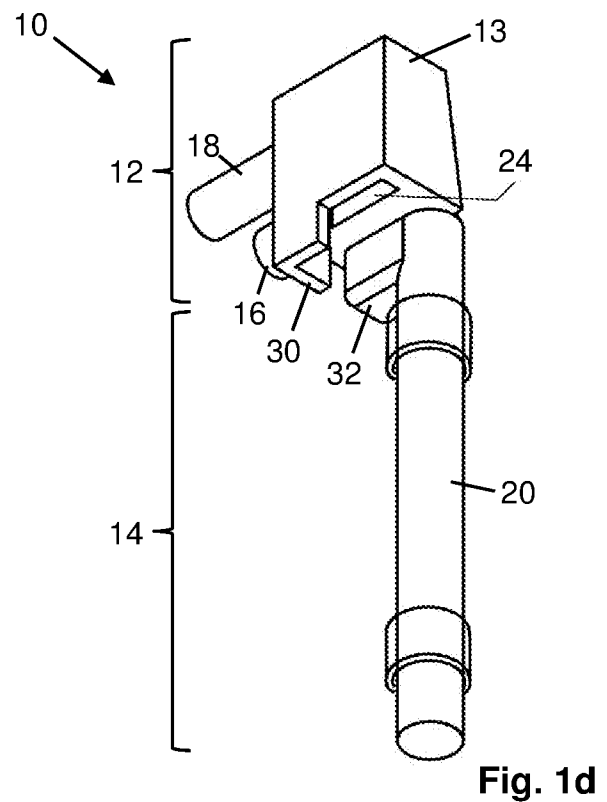
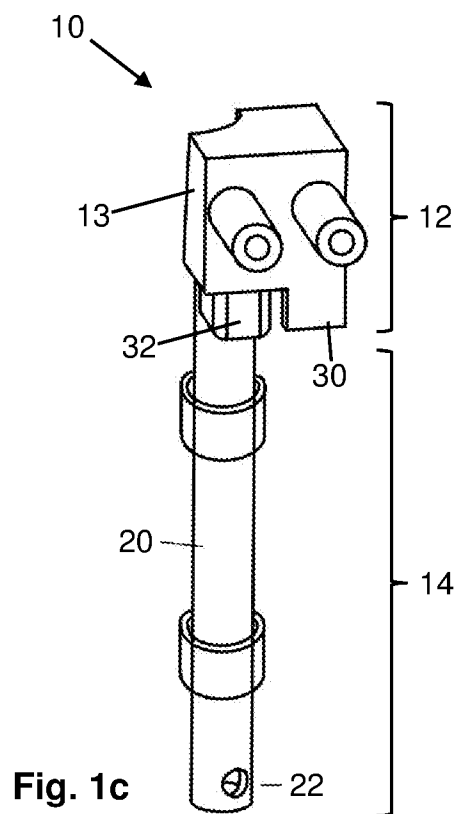
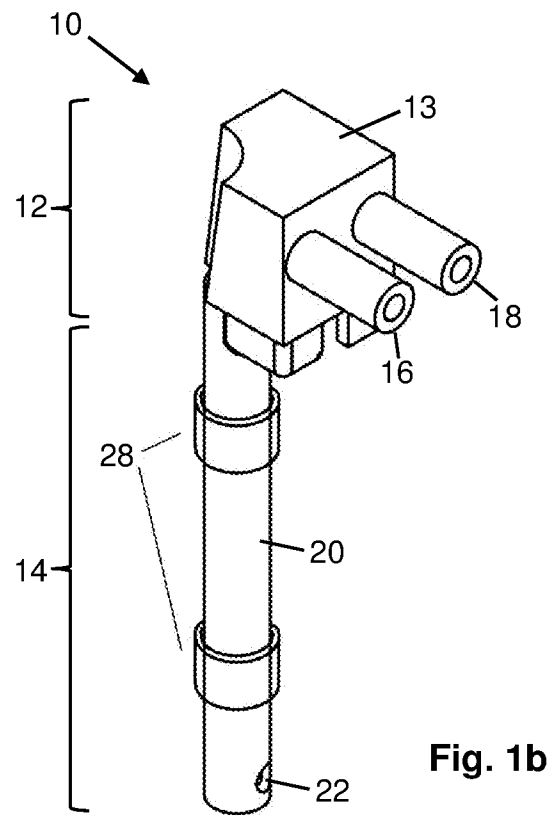
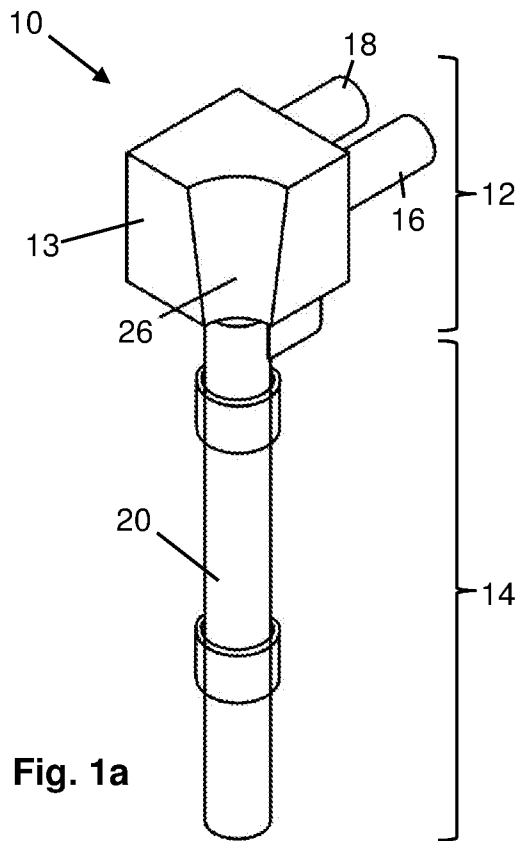


Figure 1

2/3

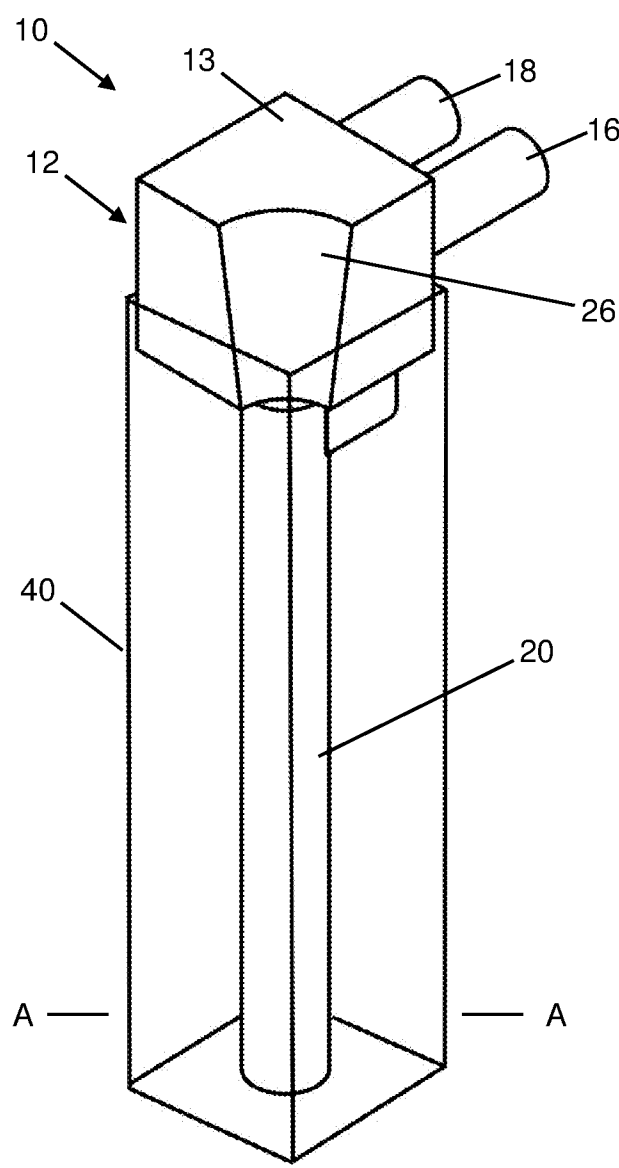


Figure 2

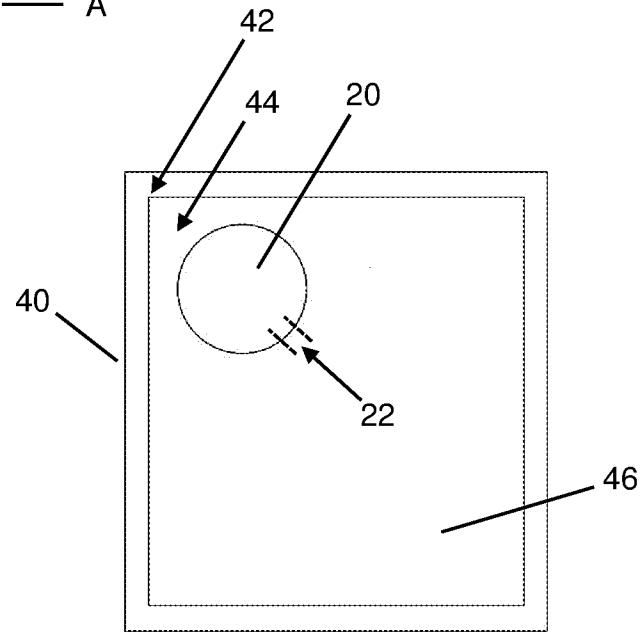


Figure 3

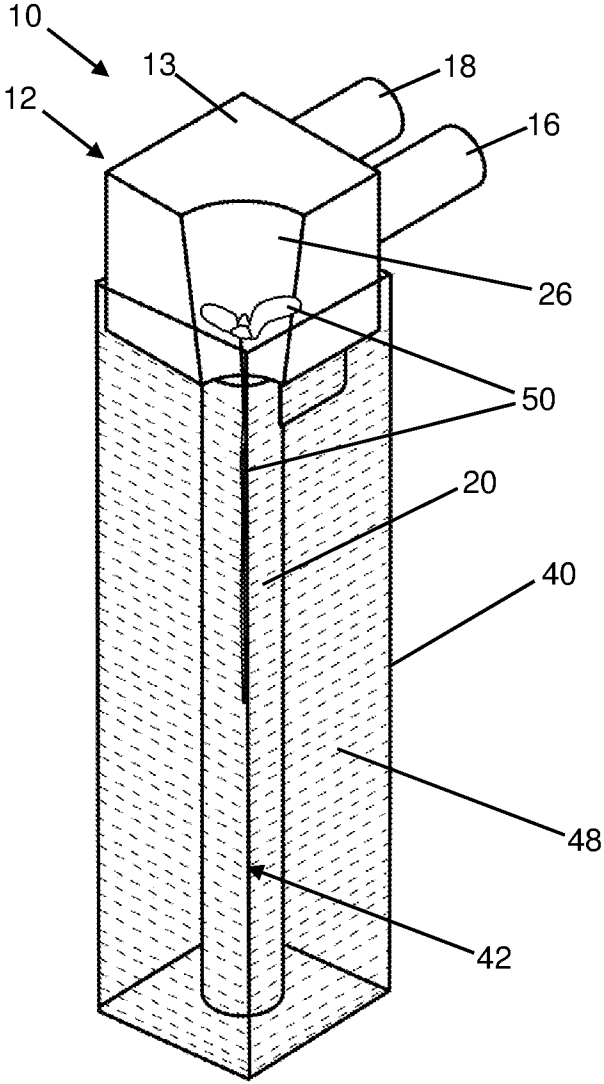


Figure 4

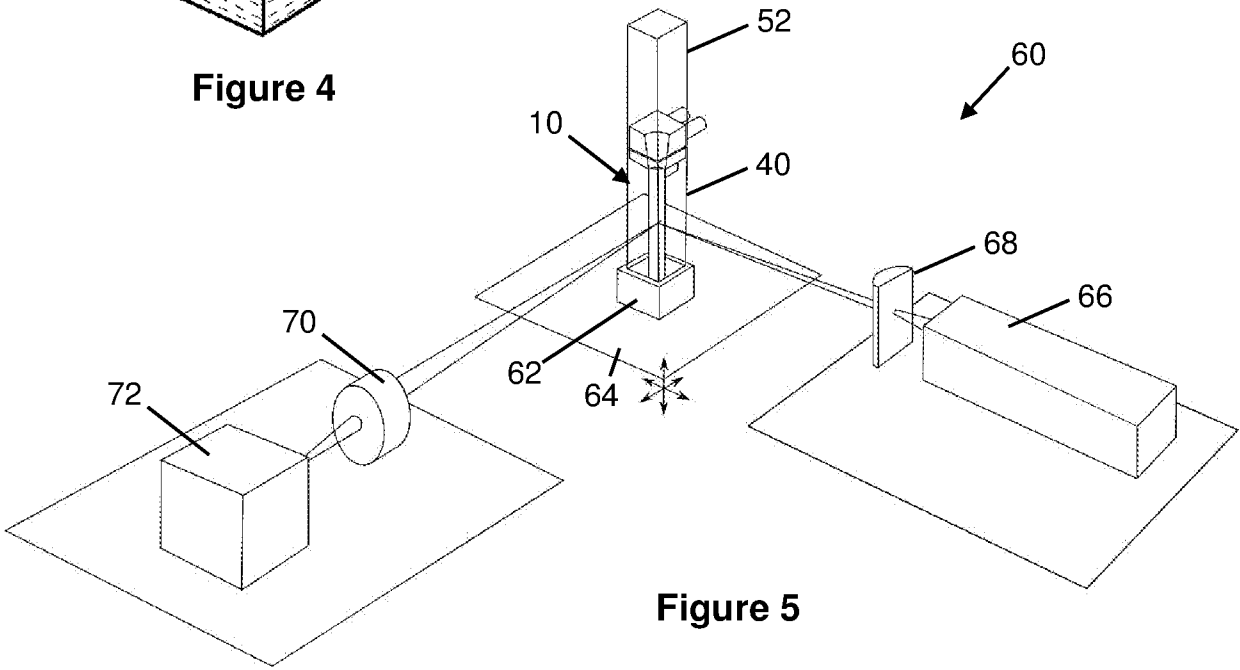


Figure 5

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2017/050372

A. CLASSIFICATION OF SUBJECT MATTER
INV. G02B21/34 B01L3/00
ADD. G02B21/16

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)
G02B B01L

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 2011/277565 A1 (WINKLER SIEGFRIED [DE] ET AL) 17 November 2011 (2011-11-17) figures 2a-2d paragraph [0002] paragraph [0068] - paragraph [0083] -----	1-6, 9-13, 27-31, 33,34, 36,37, 39-41
X	US 2010/055698 A1 (STIBELLI SERGIO [IT] ET AL) 4 March 2010 (2010-03-04) figures 1-4 paragraph [0037] - paragraph [0063] ----- -/-	1,2,7-9, 12-14, 20,27, 30-32



Further documents are listed in the continuation of Box C.



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

28 April 2017

Date of mailing of the international search report

05/07/2017

Name and mailing address of the ISA/

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

Beutter, Matthias

INTERNATIONAL SEARCH REPORT

International application No

PCT/GB2017/050372

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2002/185457 A1 (SMITH EMERY [US] ET AL) 12 December 2002 (2002-12-12) figures 1-7 paragraph [0023] - paragraph [0039] -----	1,2,4,5, 7,9,10, 12-15, 27,30-32
A	DE 10 2013 210269 B3 (HANNOVER LASER ZENTRUM [DE]; LAVISION BIOTEC GMBH [DE]) 6 November 2014 (2014-11-06) figures 1-14 -----	1,31-34, 36,37

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB2017/050372

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 35, 38
because they relate to subject matter not required to be searched by this Authority, namely:
Rule 39.1(iv) PCT - Diagnostic method practised on the human or animal body
2. ☒ Claims Nos.: 42-44
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-15, 20, 27-34, 36, 37, 39-41

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box II.1

Claims Nos.: 35, 38

Rule 39.1(iv) PCT - Diagnostic method practised on the human or animal body

Continuation of Box II.2

Claims Nos.: 42-44

Claims 42 to 44 try to define the subject-matter by merely referring to the description and/or drawings. However, such a way of formulating the claims leaves doubt about what the subject-matter for which protection is sought should be and therefore lacks clarity (Art. 6 PCT). They lack clarity to such an extent that no meaningful search is possible.

The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure. If the application proceeds into the regional phase before the EPO, the applicant is reminded that a search may be carried out during examination before the EPO (see EPO Guidelines C-IV, 7.2), should the problems which led to the Article 17(2) declaration be overcome.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-15, 20, 27-34, 36, 37, 39-41

A device for supplying liquid to a container comprising first and second parts, an inlet port, a first conduit having an outlet hole and spacing means, in particular collars, to space the second part from the walls of a chamber.

1.1. claims: 12-15

A device for supplying liquid to a container comprising first and second parts, an inlet port, a first conduit having an outlet hole, an outlet port and an outlet mouth placed on the first part and being connected to said outlet port.

2. claims: 16-19

A device for supplying liquid to a container comprising first and second parts, an inlet port, a first conduit having an outlet hole, said first part being provided with an opening through which a specimen can extend, an assembly of a cuvette and said device and a method of examining a specimen in said assembly using a microscope.

3. claims: 21-26

A device for supplying liquid to a container comprising first and second parts, an inlet port, a first conduit having an outlet hole and a surface modification of the second part or additional functional elements provided on/in the second part.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/GB2017/050372

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2011277565 A1	17-11-2011	EP 2193847 A1 EP 2364217 A1 JP 5714498 B2 JP 2012511164 A US 2011277565 A1 WO 2010063474 A1	09-06-2010 14-09-2011 07-05-2015 17-05-2012 17-11-2011 10-06-2010
US 2010055698 A1	04-03-2010	BR PI0716274 A2 CN 101600501 A EP 2091645 A1 HK 1136523 A1 JP 2010510783 A KR 20090112640 A RU 2009119971 A US 2010055698 A1 WO 2008064783 A1	28-07-2015 09-12-2009 26-08-2009 22-03-2013 08-04-2010 28-10-2009 10-01-2011 04-03-2010 05-06-2008
US 2002185457 A1	12-12-2002	AT 453319 T CA 2448415 A1 CY 1109878 T1 EP 1406492 A2 ES 2338868 T3 US 2002185457 A1 WO 02098566 A2	15-01-2010 12-12-2002 10-09-2014 14-04-2004 13-05-2010 12-12-2002 12-12-2002
DE 102013210269 B3	06-11-2014	DE 102013210269 B3 US 2016109357 A1 WO 2014195005 A1	06-11-2014 21-04-2016 11-12-2014