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(54) Title: TISSUE MANIPULATION DEVICE

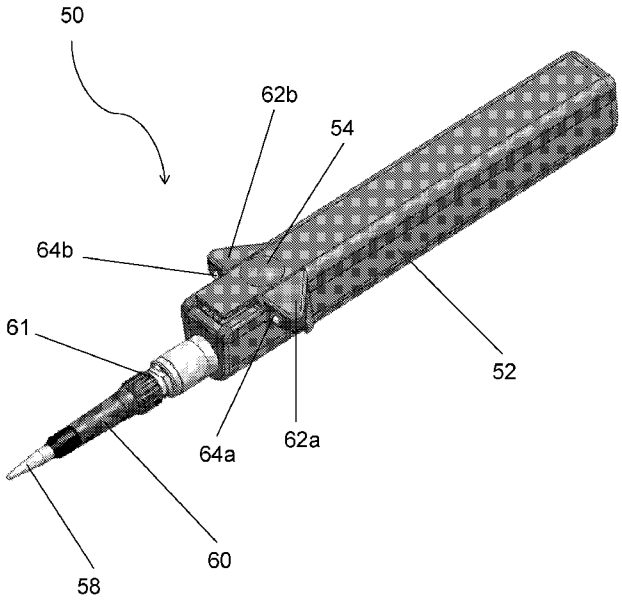

Fig 6

(57) Abstract: The present invention relates to methods and devices for use in tissue manipulation, particularly to devices for use in preparation of tissue samples for histological or pathological analysis. Embodiments of the invention relate to devices which are adapted to manipulate tissue samples without coming into physical contact with the tissue samples, thereby avoiding issues of cross-contamination. This can be achieved by coating the tip of the devices with a tissue medium which is used to indirectly manipulate the tissue sample, and then release the sample by melting the tissue medium.

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Tissue Manipulation Device

Background of the Invention

The present invention relates to a cordless tissue manipulation device useful for manipulating tissue specimens in paraffin embedding media in histology and pathology laboratories.

Introduction and Prior Art

In histological or pathological analysis of specimens, e.g., patient tissue samples, the specimens are fixed and then embedded in mounting media (often paraffin wax) so that thin slices of the specimen can be cut using a microtome, and placed on microscope slides for subsequent examination.

The sample preparation method typically involves the following steps:

- Fixing - Chemical fixatives are used to preserve tissue from degradation, and to maintain the structure of the cell and of sub-cellular components such as cell organelles (e.g., nucleus, endoplasmic reticulum, mitochondria). The most common fixative for light microscopy is 10% neutral buffered formalin (4% formaldehyde in phosphate buffered saline). For electron microscopy (EM), the most commonly used fixative is gluteraldehyde, usually as a 2.5% solution in phosphate buffered saline. These fixatives preserve tissues or cells mainly by irreversibly cross-linking proteins. The main action of these aldehyde fixatives is to cross-link amino groups in proteins through the formation of methylene bridges (-CH_2-), in the case of formaldehyde, or by a C_5H_{10} cross-links in the case of gluteraldehyde.
- Processing - dehydration, clearing, and infiltration - The aim of tissue processing is to remove water from tissues and replace with a medium that solidifies to allow thin sections to be cut. Biological tissue must be supported in a hard matrix to allow sufficiently thin sections to be cut, typically 5 µm (micrometres; 1000 micrometres = 1 mm) thick for light microscopy and 80-100 nm (nanometre; 1,000,000 nanometres = 1 mm) thick for electron microscopy. For light microscopy, paraffin wax is most frequently used. Since it is immiscible with water, the main constituent of biological tissue, water must first be removed in the process of dehydration. Samples are transferred through baths of progressively more concentrated ethanol to remove the water. This is followed by a hydrophobic clearing agent (such as xylene) to remove the alcohol, and finally molten paraffin wax, the infiltration agent, which replaces the xylene. Paraffin wax may not

provide a sufficiently hard matrix for cutting very thin sections for EM. In that case, resins are typically used. For EM, epoxy resins are the most commonly employed embedding media, but acrylic resins are also used, particularly where immunohistochemistry is required. Thicker sections (0.35 µm to 5 µm) of resin-embedded tissue can also be cut for light microscopy. Again, the immiscibility of most epoxy and acrylic resins with water necessitates the use of dehydration, usually with ethanol.

- Embedding - After the tissues have been dehydrated, cleared, and infiltrated with the embedding material, they are ready for external embedding. During this process the tissue samples are placed into moulds along with liquid embedding material (such as agar, gelatine, or wax) which is then hardened. This is achieved by cooling in the case of paraffin wax and heating (curing) in the case of the epoxy resins. The acrylic resins are polymerised by heat, ultraviolet light, or chemical catalysts. The hardened blocks containing the tissue samples are then ready to be sectioned.
- Sectioning - For light microscopy, a steel knife mounted in a microtome is used to cut 4-micrometer-thick tissue sections which are mounted on a glass microscope slide. For transmission electron microscopy, a diamond knife mounted in an ultramicrotome is used to cut 50-nanometer-thick tissue sections which are mounted on a 3-millimeter-diameter copper grid. Then the mounted sections are treated with the appropriate stain.

Where wax infiltrated samples are being manipulated, heated forceps are used (as cold forceps would stick to the wax and possibly the tissue) in order that the specimens can be manipulated. It is well known in the art to use mechanical forceps that are placed into a heated receptacle, over an open flame, or on a hot plate surface, to reach a desired temperature that is higher than the melting point of the paraffin. Such forceps have no thermostatic properties and therefore cool while the forceps are in use. This is time consuming and the use of an open flame to repetitively heat the forceps creates a safety hazard. Additionally, if the forceps are heated too much, there is a risk that the sample may be damaged by the excess heat.

Electrically heated forceps have been developed, which are heated by electrical resistance heating structures to achieve a desired temperature. However, when heated corded forceps devices are used in environments containing molten paraffin embedding media, cords can become fouled with paraffin and cause tissue contamination issues, as well as raise general cleanliness and efficiency considerations. Cords can also become tangled or accidentally knock over objects or otherwise cause accidents.

Forceps often have serrated tips to help grip the tissue but this can lead to tissue sticking in the grooves. Even the smoothest tips should be checked between samples in case of carry over.

US patent application no US 20110046620 discloses cordless heated forceps, which uses a rechargeable battery to allow heating of the forceps. This mitigates to some extent the drawbacks associated with electrical cords.

When several samples, e.g. from different patients, are being handled, there arises a risk of sample contamination. To avoid this forceps should be cleaned thoroughly between samples. However, this is highly inefficient, and in practice this step is often not carried out. The result is that lab efficiency is reduced and cross-contamination is a frequent problem.

There remains a need for improved tissue manipulation devices and methods which overcome the problems in the prior art. For example, it would be desirable to minimise or remove problems with sample contamination and to improve efficiency in sample handling. It would also be advantageous if the risk of crushing a sample, as can happen with forceps, heated or otherwise, could be substantially eliminated.

Statements of Invention

According to a second aspect of the invention there is provided a method of manipulating a tissue sample using a meltable tissue medium, the method comprising:

a) providing a tissue manipulation device comprising a tissue manipulating member;
b) exposing the tissue manipulation member to a reservoir of tissue medium to coat at least a portion of the tissue manipulating member with the tissue medium;
c) bringing the coated tissue manipulating member into proximity with a tissue sample such that the coating of tissue medium contacts the tissue sample and thereby adheres the sample to the tissue manipulation member;
d) manipulating the tissue sample in the desired manner; and
e) heating the tissue manipulation member to a temperature to above the melting point of the tissue medium to thereby release the tissue sample from the tissue manipulation member.

Preferably the reservoir to tissue medium is clean/fresh so that there is no possibility of contamination from the reservoir.

The method of the present invention has a significant advantage in that the tissue manipulating member does not at any time directly contact the tissue sample. Rather, it is coated with a layer of tissue medium (typically paraffin wax, but optionally any appropriate meltable tissue medium), and this layer of medium contacts the tissue sample. This prevents contamination of the tissue manipulation device by the tissue sample.

Another significant advantage is that the risk of crushing the sample is substantially or completely eliminated. Because the sample adheres to the tissue medium and is released very gently through melting of the medium, the entire manipulation process is very delicate. This is quite different to the situation when a sample is held in forceps, whereby it is very easy for a user to accidently apply excess pressure to the sample; this can result in significant damage to the sample.

Release of the tissue sample is also greatly facilitated because the tissue manipulating member can be heated to cause the tissue medium to melt and the tissue sample therefore drops from the tissue manipulating member under the action of gravity. In some instances the sample may be touched onto the base of the mould to assist orientation of the sample. This makes the method suitable for high throughput of samples, and potentially for automation, e.g. by robotics.

Heating of the tissue manipulating member can be achieved by any suitable heating means. Resistive electrical heating is a convenient way to heat the tissue manipulating member, and suitable heaters are well-known in the art. In one embodiment of the invention, the heating means is a combined heating and cooling means, as will be described below.

Suitably heating is achieved by actuating a button which activates the heating means. This button is preferably position on the device in an ergonomic location, e.g. for actuation with a user's index finger.

Preferably the method comprises modulating the temperature of the tissue manipulating member such that it is below the melting point of the tissue medium. This cooling step suitably occurs before the tissue manipulating member is exposed to the reservoir of clean tissue media, typically immediately prior to step b). By cooling the tissue manipulating member to below the melting point of the tissue medium it means that a layer of tissue medium will solidify onto the tissue manipulating member when it is exposed (e.g. dipped into) the reservoir of tissue medium. If the tissue manipulating member is not cooled, then formation of a suitable layer of medium to isolate the sample from the medium may not be

successful, especially if the tissue manipulating member is still warm from a previous tissue sample manipulation operation.

Cooling of the tissue manipulating member can be achieved in any suitable way. In one embodiment, it can be allowed to cool passively, i.e. by cooling under the effects of ambient conditions once the heating means has been disengaged. However, it is preferred that the tissue manipulating means is actively cooled to speed up the cooling process and ensuring the device is ready for re-use. For example, the tissue manipulating member can be brought into contact with a material which is at a temperature below the target temperature, e.g. a cold solid or a cold fluid (such as a liquid or a stream of gas). Such a cold material can be associated with a base station, for example. In one embodiment the cold material can comprise a cooling block of material (e.g. metal or ceramic) associated with a refrigeration means, the cooling block comprising a recess (e.g. a channel or aperture) adapted to receive the tissue manipulation member. The tissue manipulation member is cooled by coming into close proximity or contact with the block, e.g. by conduction.

Another possibility is a fan or the like in the base adapted to blow cold air directly over the tissue manipulating member. Indeed, the inventors have determined that cooling using a fan to blow air at an ambient temperature over the tissue manipulating member is a preferred method in many embodiments because it provides adequate cooling of the tissue manipulating member, provides minimal or no contamination risk, and is simple to implement.

Another option is to directly cool the tissue manipulating member, e.g. by providing a cooling means in or thermally connected to the tissue manipulating device adapted to cool the tissue manipulating member.

Cooling means for use in the present invention can be any suitable cooling means, but a preferred embodiment is a thermoelectric cooler. Thermoelectric coolers are known in the art, and are commercially available from a number of sources (e.g. TEC Microsystems, Berlin). Current thermoelectric cooling devices use the Peltier effect to create a heat flux between the junction of two different types of materials. A Peltier cooler, heater, or thermoelectric heat pump is a solid-state active heat pump which transfers heat from one side of the device to the other, with consumption of electrical energy, depending on the direction of the current. Such an instrument is also called a Peltier device, Peltier heat pump, solid state refrigerator, or thermoelectric cooler (TEC). They can be used either for heating or for cooling (refrigeration), although in practice the main application is cooling. The term

thermoelectric cooler (TEC) will be used hereinafter to refer to such a cooling means. TECs have the advantage of being simple, light, and have very low failure rates. The main disadvantage is that they have relatively low cooling capacity (heat flux) and low electrical efficiency – in the present case these disadvantages are not typically problematic as the amount of cooling required is relatively small due to the low mass of the tissue manipulating member.

Where a TEC is used in the device, it is possible that the TEC can provide both the heating and cooling function. This is because it is possible to use the Peltier effect to provide both heating and cooling in the same element by reversing the current direction. This allows for a simple and lightweight device.

Another cooling means which can be used in the present invention is a vapour-compression system. Vapour-compression systems are well-known in the art, and are widely used in refrigeration. However, vapour-compression cooling systems are typically bulky and heavy, and therefore are not ideal for a hand-held device. A vapour-compression system may be well-suited for cooling a solid or fluid material to be used to subsequently cool the tissue manipulating member, e.g. the block discussed above.

Step b) suitably comprises dipping the tissue manipulation member into the tissue medium. The tissue medium could alternatively be dispensed onto the tissue manipulation member by a suitable dispenser.

Cleaning of the tissue manipulating member is generally not necessary as, in practice, when the tissue medium is melted and the tissue sample released, all of the tissue medium tends to be removed from the tissue manipulation member along with the tissue sample.

Suitably the tissue medium is any tissue mounting or handling medium which is reversibly meltable, preferably wherein the melting point is in the range of from 30°C to 100°C, suitably in the range from 40 °C to 70 °C. Such a medium will be solid at room temperature, but can be melted to be liquid at a relatively low temperature, which is easily achievable by the device. For example, known tissue media suitable for use in the present invention include paraffin wax. Epoxy and acrylic resins and like are used in some forms of tissue mounting, as described above; epoxy resins are not generally meltable and therefore are not suitable for use in the present invention.

The tissue medium of the present invention is preferably the same medium with which the sample has been infiltrated, and the same medium in which the sample will be embedded. However, it is possible that the tissue medium used for the manipulation is a different medium, provided that the manipulation medium is not incompatible with the infiltration and/or embedding media.

It is preferred that the meltable tissue medium is held at a temperature slightly above the gel point of the medium (e.g. less than 5°C, typically about 1 °C above the melting point) so that it is in a molten state, ready for use.

The method may also comprise the step of attaching a detachable cover to the tissue manipulation member and using said cover to alter the shape of a tissue sample, e.g. flattening the tissue sample in a process known as tamping. Further details of covers suitable for use in the method are set out below.

According to a second aspect of the invention, there is provided a tissue manipulation system, the system comprising a tissue manipulation device comprising a tissue manipulating member, a heating means operable to heat the tissue manipulating member, and a reservoir of meltable tissue medium.

The reservoir of meltable tissue medium typically contains clean tissue medium, i.e. medium that is free from any contamination.

Preferably the system comprising a cooling means operable to cool the tissue manipulation member. This allows the tissue manipulating member of the device to be cooled rapidly (i.e. more quickly than by ambient cooling) so that it can be re-coated with a layer of solid tissue medium.

Suitable heating and cooling means are described above.

The cooling means can be integral with the tissue manipulation device, or it can comprise a separate device. Where the cooling means is a separate device, it is preferably associated with the base station. A cooling fan is a preferred cooling means.

The system preferably comprises a base station, which may comprise means to charge a rechargeable battery in the tissue manipulation device. The base station may comprise a holder in which the device can be placed when it is not in use. Suitably, the device can be

docked into the holder and thereby connected to suitable charging circuitry. In a preferred embodiment the device is charged by inductive charging (also known in the art as wireless charging). Induction chargers typically use an induction coil to create an alternating electromagnetic field from within a charging base station, and a second induction coil in the portable device takes power from the electromagnetic field and converts it back into electrical current to charge the battery. The two induction coils in proximity combine to form an electrical transformer. Inductive charging is well known in the art, e.g. as used in electric toothbrushes.

Preferably the reservoir of tissue medium is associated with the base station. Preferably the reservoir of tissue medium is adapted to allow maintenance of the temperature of the tissue medium at or near the gel point of the tissue medium, e.g. via a thermostatic heating means associated with the reservoir. Suitably the reservoir of tissue medium is adapted to maintain the meltable tissue medium at a temperature slightly above the gel point of the medium (e.g. less than 5°C, typically about 1 °C above the melting point) so that it is in a molten state, ready for use.

The base station may comprise a suitable controller to allow a user to alter the temperature at which the reservoir of tissue medium is maintained, e.g. by adjusting a simple thermostat. This allows for the base station to be adjusted for different types of tissue medium, which may have different melting points. An adjustment range of 30 °C (e.g. from 40 °C to 70 °C) provides a broad range covering most conventional tissue media, but an adjustment range of 10 °C may provide a sufficiently broad range to cover most common tissue media

Optionally the base station is adapted to house two or more devices. For example, the base station may have charging connections to charge two or more devices simultaneously.

Preferably the cooling means is comprised in the base station. Suitably the base station comprises a cooling fan to cool the tissue manipulating member. Suitably the base unit is adapted so that the fan starts operation once the tissue manipulation device is docked with the base.

It is preferable that the device is cordless. A cordless device typically comprises a rechargeable battery. Preferably the battery is replaceable, e.g. when its performance degrades due to repeated charge/discharge cycles.

Preferably the device is adapted such that the tissue manipulating member is detachable. For example, the device may comprise a main body which comprises an interface which is complementary to an interface of the tissue manipulating member. Suitably the interfaces comprise suitable electrical connectors to connect the heating (and optionally cooling) means in the tissue manipulating member to the main body of the device. Alternatively, the heating (and optionally cooling) means may be comprised in the main body, and be inserted into a cavity in the tissue manipulating member, or otherwise thermally coupled thereto, to heat or cool the tissue manipulating member, e.g. by conduction. Suitable thermally conductive elements (e.g. metal contacts) can be provided in the interface to facilitate conductive heating or cooling of the tissue manipulating member.

Where the tissue manipulating member is detachable, it allows for the tissue manipulating member to be replaced if it becomes damaged, and also allows various different tissue manipulating members to be attached, e.g. that are optimised for different types of tissue manipulations. For example a pointed tip on a tissue manipulating member may be suitable for delicate manipulations of structurally stable samples, and a flat (spade) tipped tissue manipulating member may be suitable for manipulation of large samples or samples which have less structural integrity and require more support during manipulation. Alternatively a comparatively large flat plate, e.g. corresponding in size to an embedding cassette, could have a layer of wax applied and be able to lift everything in the cassette.

Suitably the system comprises at least one detachable cover, which is adapted to connect to the tissue manipulation member. For example, the detachable cover can comprise a flat plate which is adapted to releasably attach to the tissue manipulation member, preferably the flat (spade) tipped tissue manipulating member. The cover can, for example, comprise flat plate, which can be used to flatten a tissue sample (known as tamping). Suitably the flat plate has a substantially similar shape to the flat (spade) tipped tissue manipulation member, though it could provide a larger flat surface of any desirable shape. After tamping the cover will typically be contaminated with tissue and it is therefore desirable that it can be removed and typically disposed of; it will also be apparent why a cover is used rather than tamping the sample with the tissue manipulation member – which would obviate the advantages of the device regarding avoidance of cross-contamination. The detachable cover can have any suitable attachment means which cooperates with the tissue manipulation member. For example, the attachment means can comprise a suitably shaped recess to receive and engage with a portion of the tissue manipulation member. Alternatively, or additionally, one, other or both of the cover tip and the tissue manipulation member can comprise a magnet so that the cover member attaches magnetically to the tissue manipulation member. A

combination of a recess and magnetic attachment is useful as it allows for particularly secure retention of the cover on the tissue manipulation member, but allows for easy removal when required. It is envisaged that the covers will be disposable, and thus it is typically preferred that the covers do not comprise a magnet, but rather are ferromagnetic so as to engage with a suitable magnet in the tissue manipulation member. In one embodiment the cover can comprise a flat sheet of steel, or other magnetic metal, which has been bent or otherwise formed to define a recess to receive a portion of the tissue manipulation member.

Preferably one or more covers are maintained at a temperature slightly above the melting point of the tissue medium to avoid the cover sticking to the sample; by maintaining the covers at an elevated temperature and combining them with a heatable tissue manipulation member, sticking can be avoided. This can suitably be achieved by storing the covers in a heated portion of the base unit, e.g. which is heated by the same heating means which maintains the tissue medium at an elevated temperature.

Suitably the tip of the tissue manipulating member, which is intended to be coated in tissue medium, is black, dark grey or a like dark colour. This aids easy visualisation of the melting of the tissue medium by the user.

It is notable that the system and methods of the present invention are well suited to automation of the tissue manipulation and embedding process, e.g. a robotic system or the like. For example, the system and methods can be incorporated into a high-throughput automated system, e.g. where a plurality of tissue manipulation devices are robotically controlled.

In a preferred embodiment the system comprises a plurality of tissue manipulating members of different shapes.

Preferably the tissue manipulating member comprises a temperature sensor, e.g. a thermocouple or thermistor, connected via suitable circuitry to the device. The temperature sensor provided information about the temperature of the tissue manipulating member to the device, and allows for thermostatic control of the temperature, and also of warning signals, e.g. if the tissue manipulating member is too hot or cold for a particular operation.

The device comprises suitable control circuitry, to control and communicate with the various electrical components of the device, e.g. the heating means, cooling means, the temperature sensor and the battery charging circuit. Such control circuitry can be modified to provide

appropriate functionality depending on the various components of the device, and the required electronics are routine for the skilled person.

The device preferably comprises a temperature indicator adapted to indicate to a user when a desired temperature has been reached. Preferably the temperature indicator is adapted to indicate at least one of the following conditions:

a) when the temperature of the tissue manipulating member is within a target temperature range above the melting point of the tissue medium (e.g. above 55 °C, preferably from 60 °C to 80 °C).

b) when the temperature of the tissue manipulating member is within a target temperature range below the melting point of the tissue medium (e.g. below 50 °C, preferably from 0 °C to 40 °C).

c) when the temperature of the tissue manipulating member is above an upper limit, i.e. the device is overheating (e.g. above 85 °C).

Preferably the temperature indicator indicates at least condition b), given that in typical use heat is only applied until the wax melts and the probe then cooled as fast as possible so that it could be used again. However, it can be in certain embodiments it may be desirable that the temperature indicator is adapted to indicate both conditions a) and b), and optionally all three of conditions a), b) and c).

Preferably the temperature indicator is located in a position where it can be easily visualised by a user, e.g. on the top of the device when in use.

The device preferably comprises an on/off switch and an indicator, e.g. an LED, to indicate when the device is on. Preferably the on/off button is located on the device in a position to prevent accidental actuation during use, e.g. it can be on top of the device when in use.

Suitably the device comprises a light source, e.g. an LED, which is adapted to illuminate at least a portion the work area in which the device is being used. For example, the light source can illuminate at least the tip of the tissue manipulation member and the immediate surroundings. This provides for good illumination of the tissue sample being manipulated. The light source can be permanently on, can come into operation when the device is removed from the base station, or can be selectively illuminated by a user, e.g. via operation of a switch or button.

In a preferred embodiment the device comprises an elongate body, e.g. cylindrical- or polygonal prism-shaped, and the tissue manipulation member extends from one end of the body. The body is shaped and configured to contain the batteries, circuitry and the like. Preferably the body is shaped to easily fit within the hand of a used, e.g. it is configured as pen-like device adapted to be held by thumb and forefinger in the manner of a thick pen.

The device and the base station preferable each comprise corresponding interface means to allow the device to be docked with the base station, e.g. allowing the device to be hung on the base station. Suitably the device comprises protrusions or is otherwise shaped to hang in a suitable hanger provided on the base station. For example, a pair of protrusions can extend from the sides of the device which are adapted to correspond with a pair of arms extending from the base unit. Conveniently one, other or both of the protrusions can comprise electrical contacts to facilitate charging of the device by engaging with corresponding contacts on the base station, or one, other or both of the protrusions can be provided with contactless charging means, such as for inductive charging. It will be apparent to the skilled person that there are many other ways in which a device can dock with a base station, and all such variants fall within the scope of the present invention.

In a further aspect, the present invention provides a tissue manipulation device comprising a tissue manipulating member, a heating means operable to heat the tissue manipulating member, and a cooling means operable to cool the tissue manipulation member.

Further details of the tissue manipulation device relevant to this aspect of the invention are set out above.

Integration of a cooling device into the device removes the need to have a separate cooling material, e.g. associated with the base station.

In a further aspect, the present invention provides a kit comprising a device as described above, optionally a base station and at least one tissue manipulating member. The kit may comprise one or more detachable covers, as described above. The kit may comprise a container of meltable tissue medium.

Specific Description of Embodiments of the Invention

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

Fig 1 shows a device for use in a system according to the present invention;
Fig 2 shows an alternative tissue manipulating member for use with the device of Fig 1;
Fig 3 shows a schematic of the circuitry of the device of the present invention;
Fig 4 shows a circuit diagram for the device according to the present invention;
Fig 5 shows a circuit diagram for a battery charging system to charge the battery of a device according to the present invention;
Fig 6 shows a further embodiment of a device according to the present invention; and
Fig 7 shows a base station according to the present invention for use with the device of Fig 6.

A device 10 for use in the method of the present invention is shown in Fig 1. The device comprises a main body 12 and a tissue manipulating member 14. The tissue manipulating member 14 comprises a sample manipulation tip 16 and a connection collar 18. The connection collar is used to securely connect the tissue manipulating member 12 to the main body 14. The connection collar 18 comprises a thread on the interior of the collar which screws onto a corresponding thread on an interface potion (not shown) provided on the main body 12, and thereby securely connects the tissue manipulating member 14 to the main body 12. The interface portion comprises suitable electrical contacts which, when the tissue manipulating member is secured in place, form electrical connections with corresponding contacts on the tissue manipulating member. This connects the heating means and temperature sensor in the tissue manipulating member 14 to the power source and control circuitry comprised in the main body 12.

As can be seen, the tip 16 of the tissue manipulating member 14 is a generally conical point. An alternative tissue manipulating member 20 is shown in Fig 2, which comprises a flattened, spade-like tip. This alternative tissue manipulating member 20 can be connected to the main body 12 in place of the tissue manipulating member 14.

The main body comprises an on/off button 22 located on the front face of the device. Will be on the top of the next revised device) A heat control button 24 is provided on the side of the device, in a position in which the button is easily actuated when in use. Two tri-colour LED indicator lights 26,28 are provided on the face of the device. Indicator light 26 shows the batter charge level, with green indicating high charge levels, yellow indicating intermediate charge levels, and red indicating low charge levels. Indicator light 28 shows the temperature levels, with blue indicating a suitable temperature for coating the tip 16 with tissue media, orange indicating suitable temperature for melting wax and releasing the sample, and red indicating overheat.

Fig 3 shows a schematic of the circuitry of the device of Fig 1. As can be seen, a micro controller circuit 30 is the central controller of the device, which is connected to various input and output circuits. An on/off switch 32, operable via button 22, is connected and allows the device to be turned on or off. The power source is a rechargeable battery 34 (e.g. a lithium ion battery) which is connected, via suitable charge circuitry 35, to the micro controller 30. An external charger 36 is able to charge the battery 34, e.g. via induction charging or via suitable electrical contacts provided on the exterior of the device.

The heat control switch 38, operable via button 24, allows a user to turn on power delivery to a resistive heating element 40 within the tissue manipulating member 14. The micro controller 30 sends a signal to a heating means interface 44, which in turn delivers an appropriate current to the heating means 40 to heat the tissue manipulating member 14. A temperature sensor 42 is positioned near the tip of the tissue manipulating member 20, and provides a temperature-dependent signal to the micro controller 30, via a temperature sensor interface 46. As an optional safety feature, the micro controller 30 can be adapted to thermostatically control application of electrical power to the heating means 40, to ensure the tissue manipulating member 20 does not exceed a pre-set maximum temperature, e.g. 80°C. However, this is not required as typically the heating will only ever be on when the heating switch is pressed and will only be on as long as it takes for the wax to melt and the tissue to fall off. Nonetheless, such a thermostatic control may be advantageous to prevent overheating, e.g. if the heat control button is inadvertently kept depressed, or there is a fault with the switch. It should be noted that any appropriate temperature range for the tissue media and tissue sample being manipulated can be set. When the heat button 38 is pressed by the user, the heating means 40 acts, under control of the micro controller 30, to heat the tissue manipulating member 14. Optionally, when the temperature reaches the pre-set upper limit, as reported by the temperature sensor, the micro-controller switches of the heating means 40. When the heat control button 38 is released by the user, the heating means is switched off. The temperature indicator 26 indicates the temperature status of the tissue manipulating member 14 to the user.

The tissue manipulating member 14 is detachable from the main body 12, by unscrewing the collar 18. When the tissue manipulating member 14 is removed, corresponding interface means are exposed on the main body and tissue manipulating member respectively. These interface means comprise corresponding electrical contacts, 30, which allow the heating means and the temperature sensor to be electrically coupled to the heating means interface 44 and temperature sensor interface 46, respectively.

Not shown is a base station into which the device 10 can be docked. The charger 36 is part of the base station, and this conveniently allows the battery 34 to be charged when the device is docked onto the base station. The base station can be powered by a connection to a mains supply. The base station suitably also comprises a cooled block, in the form of a metal or ceramic block, which is maintained at a temperature below room temperature (preferably approximately 10 °C or lower), and having a cavity to receive the tip of the tissue manipulating member 14. The cooling block can be cooled by a thermoelectric cooler (TEC) or by a vapour compression cooling system. Alternatively, but less preferred, the cooling block can be pre-cooled by, e.g. placing it in a freezer or refrigerator, and then be used as a passive thermal store, preferably being insulated to minimise reheating of the block from ambient conditions. The cooling block allows the tip to be cooled prior to contacting the tissue medium by bringing it into contact with the cooled block. Desirably temperature of the tissue manipulation member is reduced to 50 °C or below, and preferably 40 °C or below, and more preferably 30 °C or below, by bringing it into contact with the cooling block. When the desired temperature is reached, the indicator 28 turns blue to indicate to the user that sufficient cooling has been achieved.

As noted above, an alternative is a fan or the like incorporated into the base station whereby the tip rests in the flow of air.

The clean tissue medium is stored in a reservoir associated with the base station. The tissue medium is preferably held at its gel point, e.g. approximately 54 °C for paraffin wax, in the reservoir; this is achieved using a suitable thermostatic heating means associated with the reservoir. Maintaining the tissue medium at the gel point allows the tip of the tissue manipulating member to be dipped into the medium relatively easily (as the medium is soft), but means that only a small temperature drop is required to cause the medium surrounding the tip of tissue manipulating member 14 to solidify upon and coat it.

Circuit diagrams are shown in Figs 4 and 5, which show exemplary circuitry for the operation of a device (Fig 4) and for charging the battery (Fig 5).

In use, a user removes the device from the base station. If the tissue manipulating member is suitably cool, as indicated by indicator 28, the user dips the tip into the reservoir of clean tissue medium to coat this tip with a layer of medium. If the tissue manipulating member 14 is too warm, it is cooled via the cooling block until it is at a suitable temperature. Because the temperature of the tissue manipulating member is below the melting point of the tissue

medium, a layer of the medium solidifies upon it. The user then brings the tip into contact with a sample to be manipulated. The sample, which has been infiltrated with wax, for example, adheres to the tip of the tissue manipulating member as a result of the general adhesive properties of the tissue medium. Because there is a layer of solidified tissue medium on the tip of the tissue manipulating member, the tissue manipulating member does not actually make direct contact with the sample itself.

The user then performs the manipulation event, e.g. transferring the sample to an embedding mould. The user depresses button 24 which causes the tissue manipulating member 14 to be heated by the heating means 40. This causes the temperature of the tissue manipulating member to rise until the tissue medium melts, thereby releasing the sample from the tissue manipulating member. Typically the sample will drop off the tissue manipulating member once the tissue medium melts, but if needed the user can touch the sample against the base of the embedding mould to facilitate its release.

Once the sample has been released, the user then brings the tissue manipulating member into contact with the cooled block to lower the temperature of the tissue manipulating member to below the melting point of the tissue medium. The process can thereafter be repeated to manipulate more samples, without concerns about cross-contamination between samples.

Furthermore, an additional advantage of devices and methods according to the present invention is that the risk of crushing a sample, as can happen with forceps, heated or otherwise, is substantially eliminated.

Various modifications to the abovementioned system, device and method are of course possible. For example, the device can comprise an integral cooling means, e.g. a thermoelectric cooler (TEC). The TEC can provide both cooling and heating functions. Alternatively, the device can comprise separate heating and cooling means, e.g. a resistive heating element and a TEC. Alternatively, a cooling fan can be provided in the base station.

The base station may be adapted to house, and preferably charge, a plurality of tissue manipulation devices.

The method, device and system of the present invention can be modified for automation, e.g. in a robotic system. In this case a ‘user’ can be the control system of the robotics

system. In such a case, buttons and the like would not be required, and the various functions would be controlled by software.

It is preferable that the battery of the device is adapted such that it can provide at least several hours of use (e.g. 4 hours or more) between charges. This avoids the need to regularly cease work to recharge the device.

The device is suitably adapted to indicate various other pieces of status information, e.g. battery charge status, charging in progress.

It is preferred that the tip of the tissue manipulating member is black or another dark colour to make it easier to see when the medium is melting.

Example 1 – Cross Contamination Comparison

The following protocol was carried out to confirm that the device according to the present invention has benefits in avoiding cross-contamination between samples. The device of the present invention (referred to as the 'TissueStik' below) was compared with both smooth and serrated electrically heated forceps, and with both smooth and serrated non-electrically heated forceps; these are the standard forceps used in most histology laboratories.

To ensure reproducibility a custom bovine "sausage" sample was produced comprising of chopped liver, kidney, heart, lung and blood contained within a natural intestine skin and had a final diameter of 4cm. Bovine tissue was selected as we could easily produce sufficient samples and because it is a species of tissue that had never been processed in our laboratory over the past decade. The "sausage" was fixed for 24 hours in 4% Neutral Buffered formaldehyde (4%NBF), sliced into 0.5 cm thick slices and fixed for a further 48 hours, before subdividing each slice into 9 pieces.

Following fixation samples were transferred to 70% ethanol prior to paraffin processing using standard procedures.

Tissue Embedding

Tissue was embedded using either smooth or serrated electrically heated forceps, smooth or serrated non-electrically heated forceps which were heated in the standard heat sinks employed in most embedding machines and a device according to the present invention. Between each sample embedded using forceps a cleaning protocol used by a busy UK

diagnostic pathology laboratory was employed. This involved manually wiping forceps surfaces with disposable paper wipe (Kimwipes® - Kimberly-Clark Corporation) between samples, and was consistent with the approach used by four other diagnostic pathology laboratories questioned. With target times of 60-70 samples per hour for diagnostic laboratories the cleaning approach used was in keeping with these timescales. Samples were embedded by 2 experienced histo-technologists, 3 occasional histo-technologists and one histo-technologist with no embedding experience, using the following procedure:

Tissue sample processing cassettes were opened using gloved hands, samples were then picked up using the device of the present invention or forceps (as described above) and orientated into an embedding mould before filling with wax and placing cassette on mould. Forceps were then cleaned using procedure described above (i.e. wiping with a Kimwipe®) before being rinsed in a 2 ml eppendorf tube filed with xylene (which dissolves off any wax and any tissue adhering to it) with gentle agitation for 5 seconds after each sample handling event; more specifically 1, 5, or 10 handling events were rinsed into each of 3 eppendorfs for each type of embedding device, of the 6 operators. Thus a first eppendorf had the residues of a single handling event, a second eppendorf had the residues of 5 handling events combined into a single tube, and a third had the residues of 10 handling events combined into a single tube.

Molecular testing

Bovine test samples contained in 2 ml eppendorf tubes had genomic DNA extracted using Qiagen QIAmp FFPE Tissue Kit (Cat. No. 56404) as per manufacturer's instructions. Human genomic DNA from fibroblast cell line HT1080s was extracted using QIAmp Kit (Cat. 56304) and all extracted genomic DNA was eluted in 100 µl. The intron-less gene encoding transcription factor zinc finger protein 280B (*SUHW2*) is present at high copy number in beef cattle (Bickhart et al. *Genome Res.* 2012: 22: 778-790) with 30 copies in Angus bulls. Two probes, titled Assays 3 and 8, were designed to amplify *SUHW2* using Taqman technology. SUHW2 start codon was designated nucleotide 1, thus, assay 3 covers the 5' end of the gene from nucleotide 62 to 181 producing an amplicon of 120 base pairs (bp) and the assay 8 amplicon is 85 bp and targets the 3' end of *SUHW2* from 996 to 1081. The exact probe sequences are given below, and the fluorescent labels used for detection and quenchers are also indicated (underlined), where '56-FAM' is 5' 6-Fluorescein, 'ZEN' is an internal ZEN quencher, and '3IABkFQ' is 3' Iowa Black® FQ .

Assay 3

Contains	nM	Sequence	(Seq ID No)

Probe	2.5	5'-/56-FAM/TGCTGAACT/ZEN/GATCTTTGTTGGAGTGGA/3IABkFQ/-3'	(1)
Primer 1	5	5'-AACGACTGGTTTTGAATTTGAG-3'	(2)
Primer 2	5	5'-GAGAAACCAAACAAGTAGATGATG-3'	(3)

Assay 8

Contains	nM	Sequence	
Probe	2.5	5'-/56-FAM/AAACCACAC/ZEN/CACCTGCCAGC/3IABkFQ/-3'	(4)
Primer 1	5	5'-ACTTGAGAGGCAGAGAGGTGACAG-3'	(5)
Primer 2	5	5'-ATGTGACACTGCAGCTGGAATGG-3'	(6)

Each 20 µl Taqman reaction contained; 10 µl 2X TaqMan mix Dynamo ColorFlash probe qPCR (Cat no. F-456XL; Thermo Fisher Scientific Biosciences GmbH), 1 µl probe/primer mix, 0.4 µl ROX, 3.6 µl water and 5 µl DNA. This was loaded into a white 96 well plate (AB-1900/W; Thermo Fisher) in duplicate and ABI7900 HT FAST (Thermo Fisher) was programmed to run 1 cycle of 95° 10 min followed by 40 cycles of 95° 10 sec and 60° 30 sec. Each plate contained no template controls (NTC) to rule out cross contamination and a positive control plasmid pIDTSMART-AMP-bovine SUHW2 encoding DNA for assays 3 and 8 was linearized with restriction enzyme *Xho* I (New England Biolabs) serially diluted and used to generate a standard curve, with 5 µl of negative target human HT1080 DNA added to recapitulate the molecular complexity of bovine *SUHW2* amplification conditions. Positive results were scored if both assays 3 and 8 returned a positive signal below cycle threshold (ct) 31, which according to the positive control was equivalent to approximately 1000 copies of DNA, which equalled 30 contaminating bovine cells.

Table 1 – Presence/absence of contamination for the various tissue embedding Methods

Method	A			B			**C (TissueStik)**			D			E		
Operator	1	5	10	1	5	10	**1**	**5**	**10**	1	5	10	1	5	10
1	-	+	+	-	+	-	-	-	-	-	+++	++	-	-	+
2	-	+	+	-	+++	+	-	-	-	-	-	+	-	+	+
3	-	-	+	-	-	++	-	-	-	-	+	++	-	+	-
4	-	+	-	-	+	+	-	-	-	-	-	+	-	-	+
5	-	-	+	-	+	+	-	-	-	-	+	+	-	-	+
6	-	-	+	-	-	+	-	-	-	-	+	++	-	-	-

Table 2 – Details of experience levels of the 6 users and corresponding contamination levels

Level of Operator Experience																		
Method	NONE (Op. 4)			SOME (Op. 2)			SOME (Op. 5)			SOME (Op. 6)			HIGH (Op. 1)			HIGH (Op. 3)		
	1	5	10	1	5	10	1	5	10	1	5	10	1	5	10	1	5	10
A	-	+	-	-	+	+	-	-	+	-	-	+	-	+	+	-	-	+
B	-	+	+	-	+++	+	-	+	+	-	-	+	-	+	-	-	-	++
C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
D	-	-	+	-	-	+	-	+	+	-	+	++	-	+++	++	-	+	++
E	-	-	+	-	+	+	-	-	+	-	-	-	-	-	+	-	+	-

Cut offs: Ct 31.0; Ct 28, 29, 31 + ; Ct 25, 26, 27 ++ ; Ct below 24 +++

Key: A – electrically heated smooth forceps, B – electrically heated serrated forceps, C – Tissuestik, D – serrated forceps heated in embedding centre (2 sets), E – Smooth forceps heated in embedding centre. 1, 5, and 10 – refer to the number of dips of forceps in xylene

As can be seen from the results above, there was no cellular contamination when TissueStik was used (Method C), even when operated by a technician with no previous embedding experience. All other devices had varying levels of contamination even when used by experienced users. Thus a device according to the present invention is able to substantially eliminate cross-contamination between samples (also known in the art as carryover or crossover).

Example 2 - Further Embodiments of a Device and Base Station

Fig 6 shows a further embodiment of a device 50 according to the present invention. The device 50 comprises a body 52 in the form of an elongate cuboid. The body holds lithium ion batteries and the appropriate circuitry for operation and charging (not shown). A button 54 on the body can be presses to heat the tissue manipulation member 60, and in particularly the tip 58. The tissue manipulation member 60 is releasably connectible to the body via the interface means 61. Tissue manipulation members with other shapes of tips can of course be provided.

The body comprises a pair of triangular protrusions 62a and 62b, which are adapted to engage with corresponding arms on a base station (see Fig 7). The protraction comprise electrical contacts 64a and 64b to electrically connect the device to the base station and thereby permit charging of the batteries.

The device 50 can be held by a user in the manner of a thick pen, which allows dexterous manipulation of tissue samples and easy operation of the button 54 to heat the tissue manipulation member 60 and thereby release a tissue sample.

Fig 7 shows a base station 70 for use with a device 50 shown in Fig 6. The base station comprises a housing 72 which has a recess to hold a container of tissue medium 74, typically wax. Heating means is provided in the housing which maintains the container of tissue medium at a suitable temperature, i.e. slightly above the melting point (gel temperature) of the medium. Suitable circuitry, a heating element and a thermostat are provided in the housing (not shown).

The base station 70 further comprises a hanger 76, which is adapted to receive the device 50 shown in Fig 6. The hanger 76 is hingedly mounted on the housing 72, and comprises a pair of arms 78 extending from its distal end, which engage with the corresponding protrusions 62a and 62b of the device 50 and thus allow the device 5- to hang from the hanger 76. The arms comprise electrical contacts (not shown) corresponding to the electrical contacts 64a and 64b on the device 50. The contacts on the arms 78 and connected by wired to the housing, which in turns is connected to a mains electricity supply. Suitable transformer and other components are provided to convert mains AC power to a suitable supply for charging lithium ion batteries (e.g. 5v DC).

Underneath the hanger 76 is an outlet 80 for a stream of air blown from a fan housed within the housing. Air flowing from the outlet 80 passes over the tissue manipulation member of the device 50, thereby cooling it. The fan is engaged when the device 50 is hung on the arm 76, and this can be easily achieved by the weight of the device operating a switch (not shown) upon slight rotation of the arm relative to the housing under the weight of the device 50.

Claims

1. A method of manipulating a tissue sample using a meltable tissue medium, the method comprising:
   a) providing a tissue manipulation device comprising a tissue manipulating member;
   b) exposing the tissue manipulation member to a reservoir of tissue medium to coat at least a portion of the tissue manipulating member with the tissue medium;
   c) bringing the coated tissue manipulating member into proximity with a tissue sample such that the coating of tissue medium contacts the tissue sample and thereby adheres the sample to the tissue manipulation member;
   d) manipulating the tissue sample in the desired manner; and
   e) heating the tissue manipulation member to a temperature to above the melting point of the tissue medium to thereby release the tissue sample from the tissue manipulation member.

2. The method of claim 1 in which heating of the tissue manipulation member is achieved by a heating means, suitably an resistive electrical heating means.

3. The method of claim 1 or 2 which comprises a user operating a button to activate a heating means.

4. The method of any preceding claim which comprises modulating the temperature of the tissue manipulating member such that it is below the melting point of the tissue medium.

5. The method of claim 4 in which cooling of the tissue manipulation member is achieved by blowing air over it.

6. The method of any one of clams 1 to 4 in which the tissue manipulation member is cooled by a thermoelectric cooler or a vapour-compression system.

7. The method of any preceding claim wherein step b) comprises dipping the tissue manipulation member into the tissue media.

8. The method of any preceding claim wherein the tissue medium is paraffin wax.

9. The method of any preceding claim wherein the tissue medium is held at a temperature slightly above the gel point of the medium so that it is in a molten state, ready for use.

10. The method of any preceding claim which comprises the step of attaching a cover to the tissue manipulation member and using said cover to alter the shape of a tissue sample.

11. A tissue manipulation system, the system comprising a tissue manipulation device comprising a tissue manipulating member, a heating means operable to heat the tissue manipulating member, and a reservoir of meltable tissue medium.

12. The system of claim 11 comprising a cooling means operable to cool the tissue manipulation member.

13. The system of claim 11 or 12 which comprises a base station.

14. The system of claim 13 wherein the cooling means is associated with the base station.

15. The system of any one of claims 12 to 14 wherein the cooling means comprises a fan.

16. The system of claim 15 in which the base unit is adapted so that the fan starts operation once the tissue manipulation device is docked with the base station.

17. The system of any one of claims 11 to 16 which comprises a base station, wherein the base station comprises means to charge a rechargeable battery in the tissue manipulation device.

18. The system of any one of claims 11 to 17 which comprises a base station and wherein the base station comprises a holder in which the device can be placed when it is not in use.

19. The system of claim 18 wherein the device is adapted to be docked into the holder and thereby connected to suitable charging circuitry.

20. The system of any one of claims 11 to 19 which comprises a base station, and wherein the reservoir of tissue medium is associated with the base station.

21. The system of claim 20 wherein the reservoir of tissue medium is adapted to allow maintenance of the temperature of the tissue medium at or near the gel point of the tissue medium.

22. The system of claim 21 wherein the reservoir of tissue medium is adapted to maintain the meltable tissue medium at a temperature slightly above the gel point of the medium so that it is in a molten state, ready for use.

23. The system of any one of claims 19 to 22 wherein the base station comprises a controller to alter the temperature at which the reservoir of tissue medium is maintained.

24. The system of any one of claims 11 to 24 which comprises a base station, and wherein the base station is adapted to house two or more devices.

25. The system of any one of claims 11 to 24 wherein the device is adapted such that the tissue manipulating member is detachable.

26. The system of any one of claims 11 to 25 which comprises at least one detachable cover, which is adapted to connect to the tissue manipulation member.

27. The system of claim 26 which is adapted such that the at least one detachable cover can be maintained at a temperature slightly above the melting point of the tissue medium.

28. The system of any one of claims 11 to 27 which comprises a plurality of tissue manipulating members of different shapes.

29. The system of any one of claims 11 to 28 in which the device comprises a light source which is adapted to illuminate at least a portion the work area in which the device is to be used.

30. A tissue manipulation device comprising a tissue manipulating member, a heating means operable to heat the tissue manipulating member, and a cooling means operable to cool the tissue manipulation member.

31. A kit comprising a device as set out in any of the preceding clams, at least one tissue manipulating member and optionally a base station.

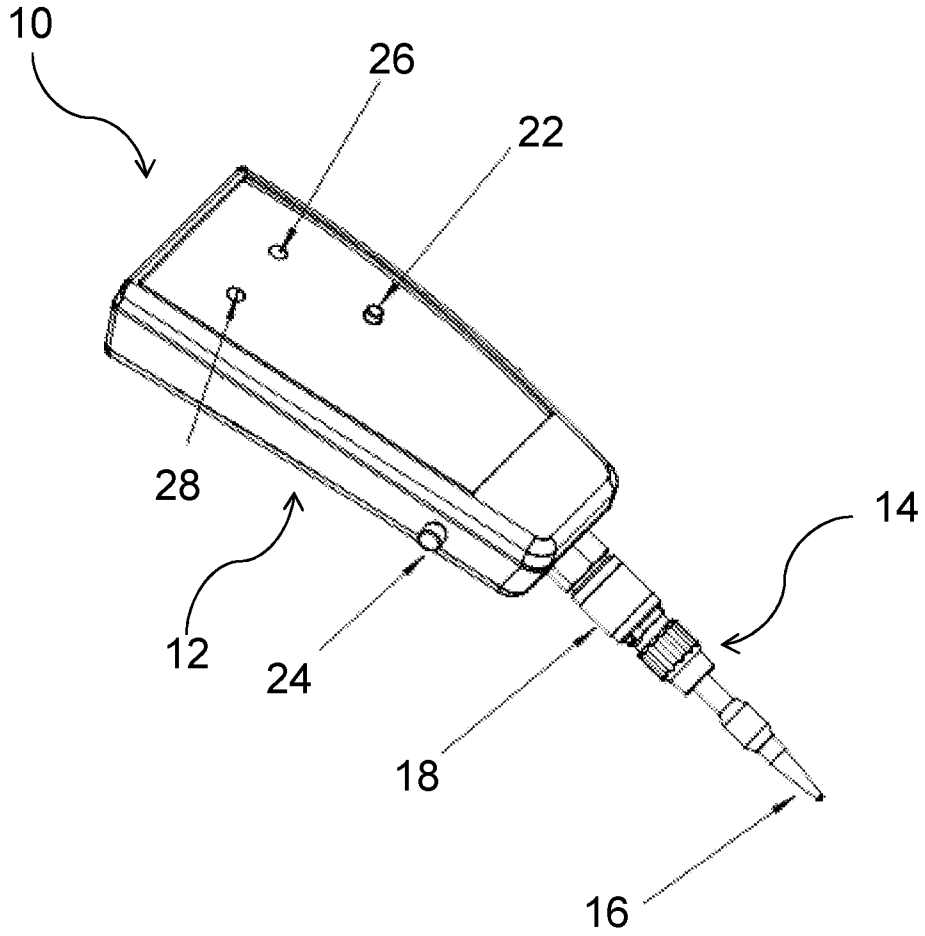

Fig 1

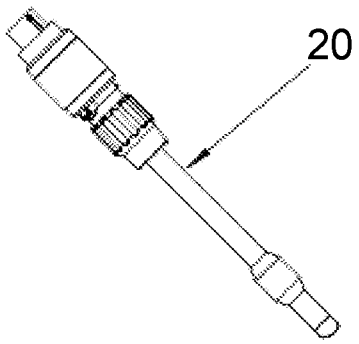

Fig 2

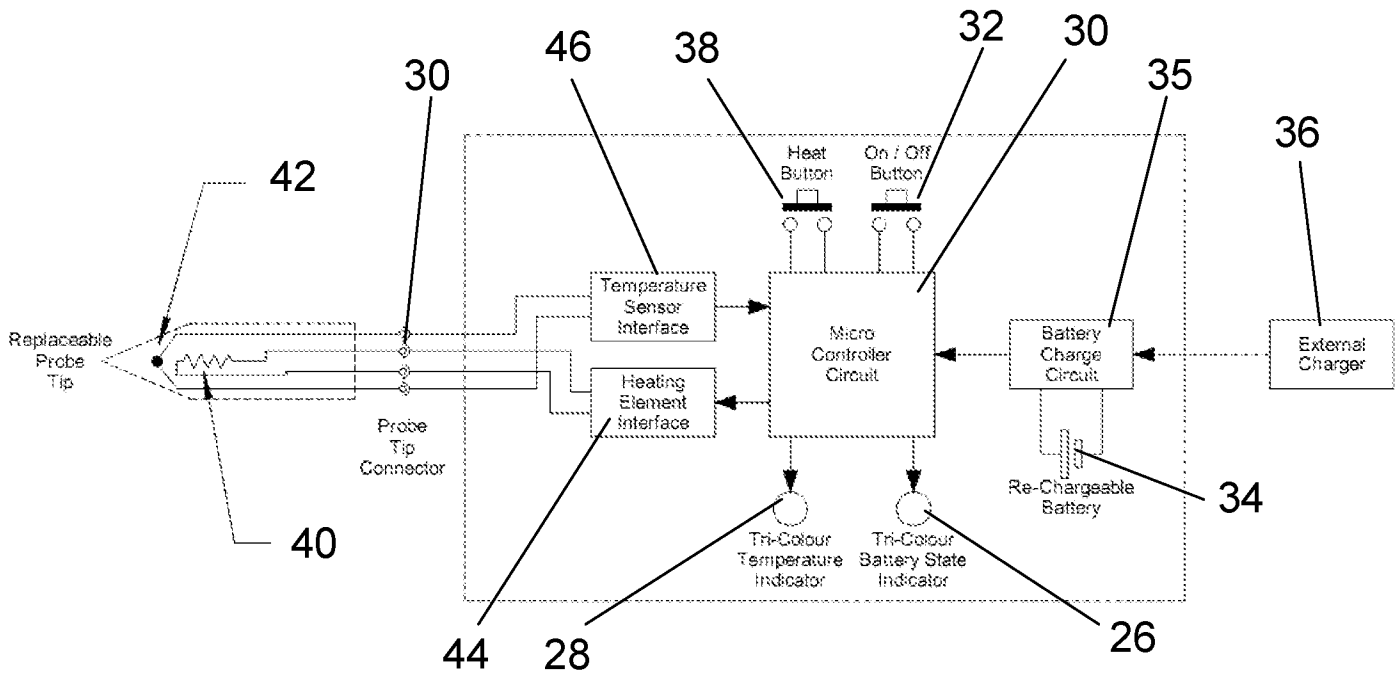
42
30
46
38
32
30
35
36
Heat Button
On / Off Button
Replaceable Probe Tip
Temperature Sensor Interface
Micro Controller Circuit
Battery Charge Circuit
External Charger
Heating Element Interface
Probe Tip Connector
Re-Chargeable Battery
34
40
Tri-Colour Temperature Indicator
Tri-Colour Battery State Indicator
44
28
26

Fig 3

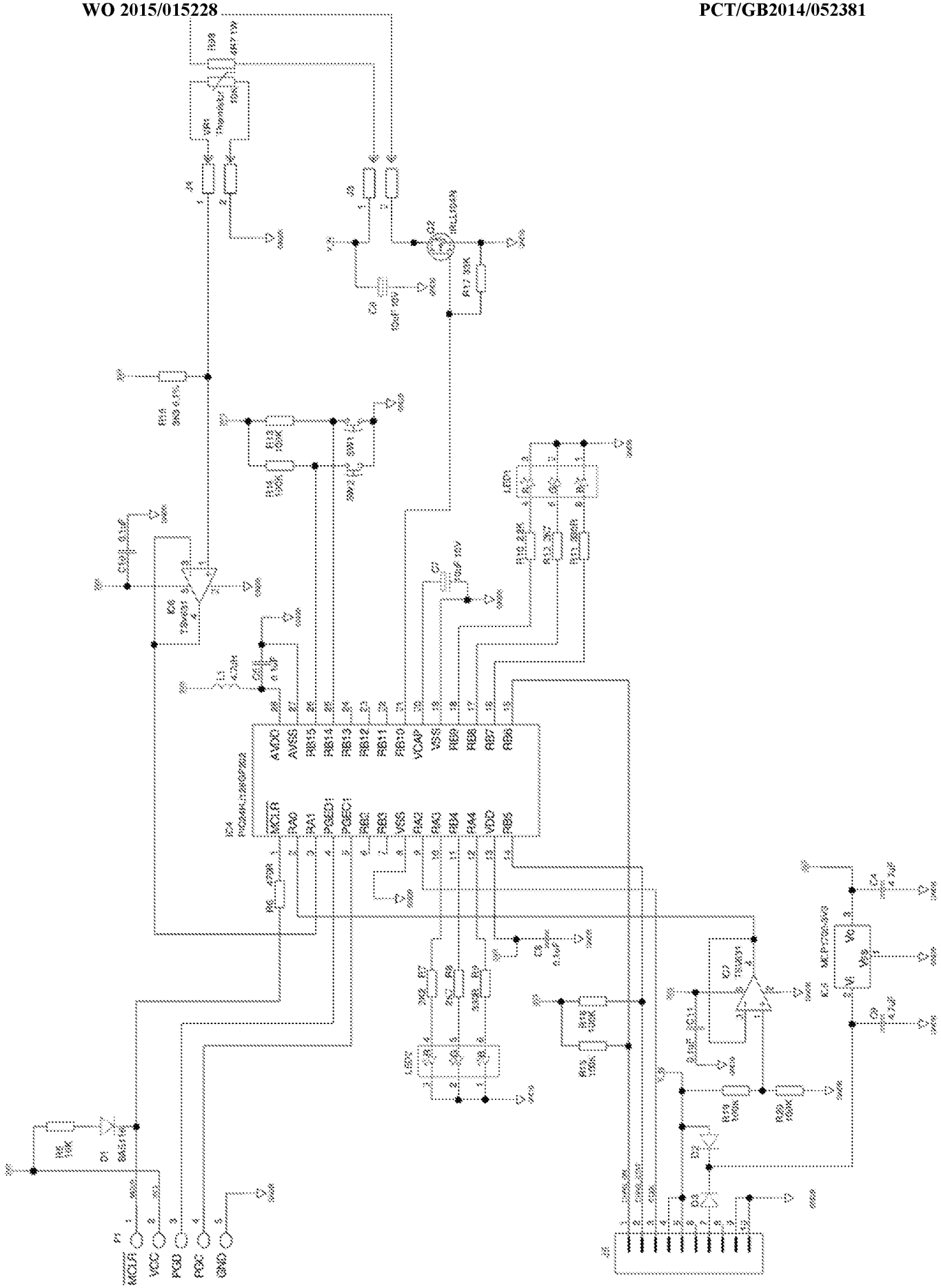

Fig 4

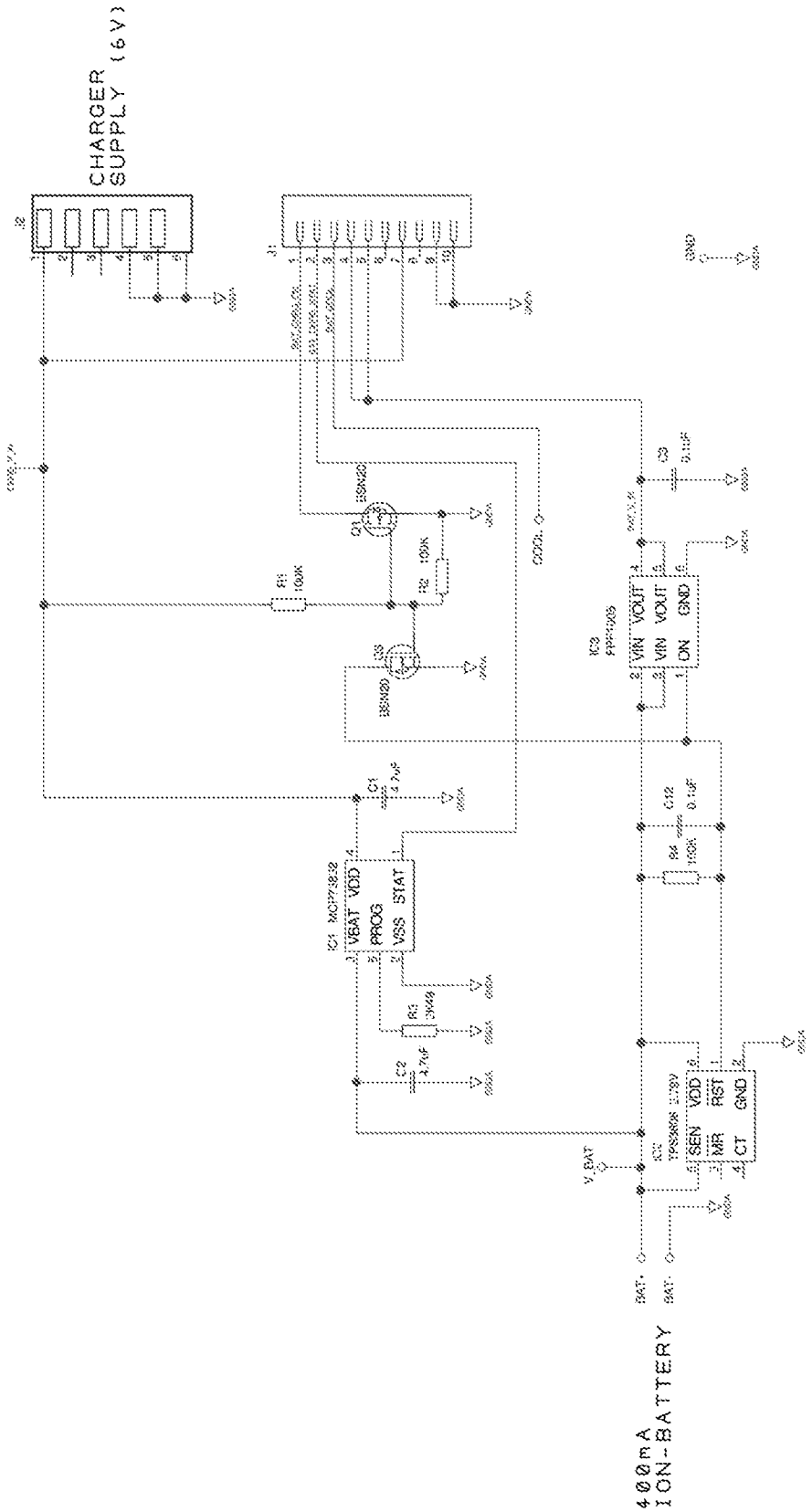
CHARGER SUPPLY (6V)
400mA ION-BATTERY
IC1 MCP73832
VBAT VDD
PROG VSS STAT
IC2 TPS3809 3.08V
SEN VDD
MR RST
CT GND
IC3
VIN VOUT
VIN VOUT
ON GND
R1 100K
R2 100K
R3 2K49
R4 100K
C1 4.7uF
C2 4.7uF
C3 0.1uF
C12 0.1uF
BSN20
V_BAT
BAT-
GND

Fig 5

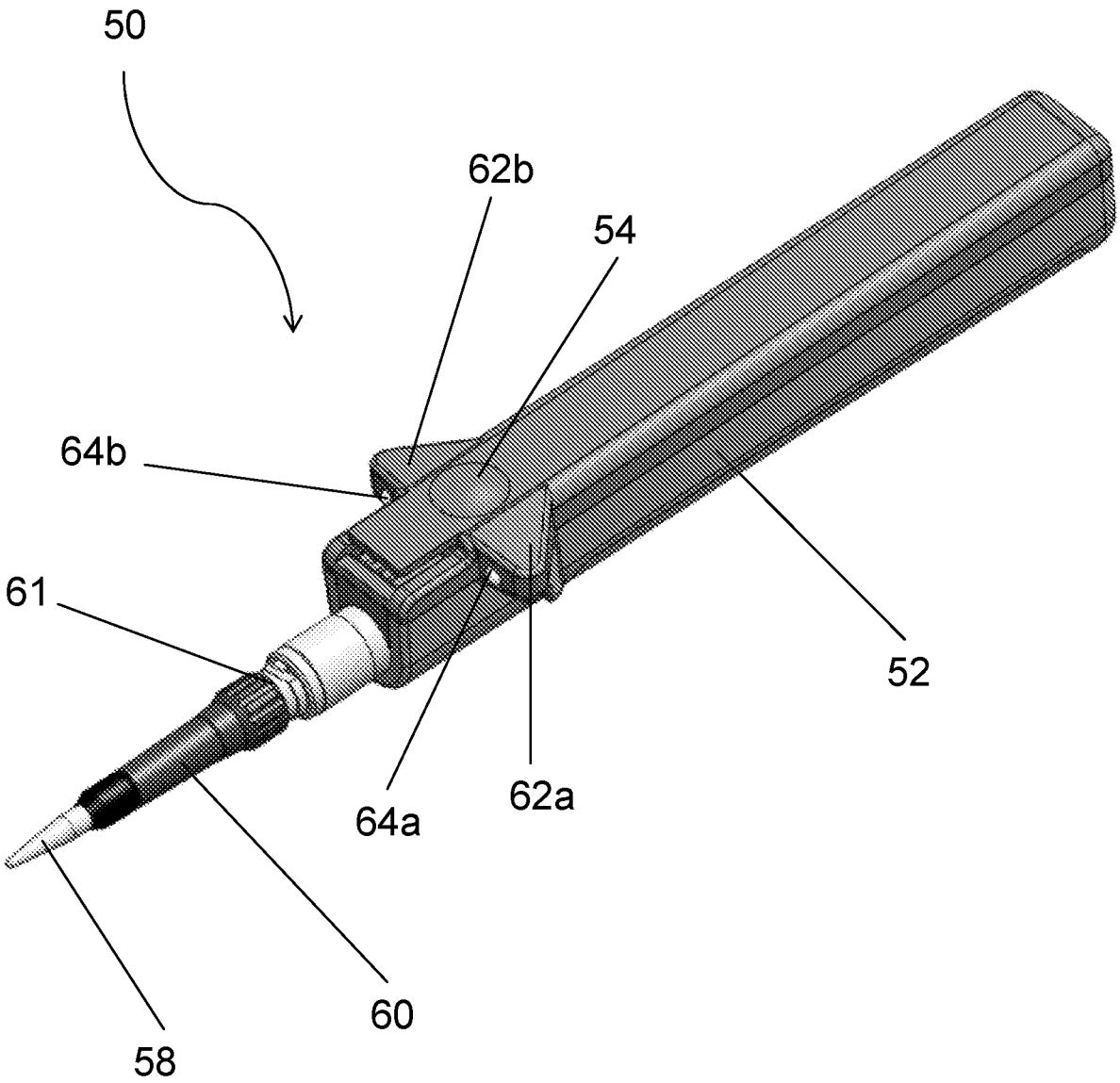

Fig 6

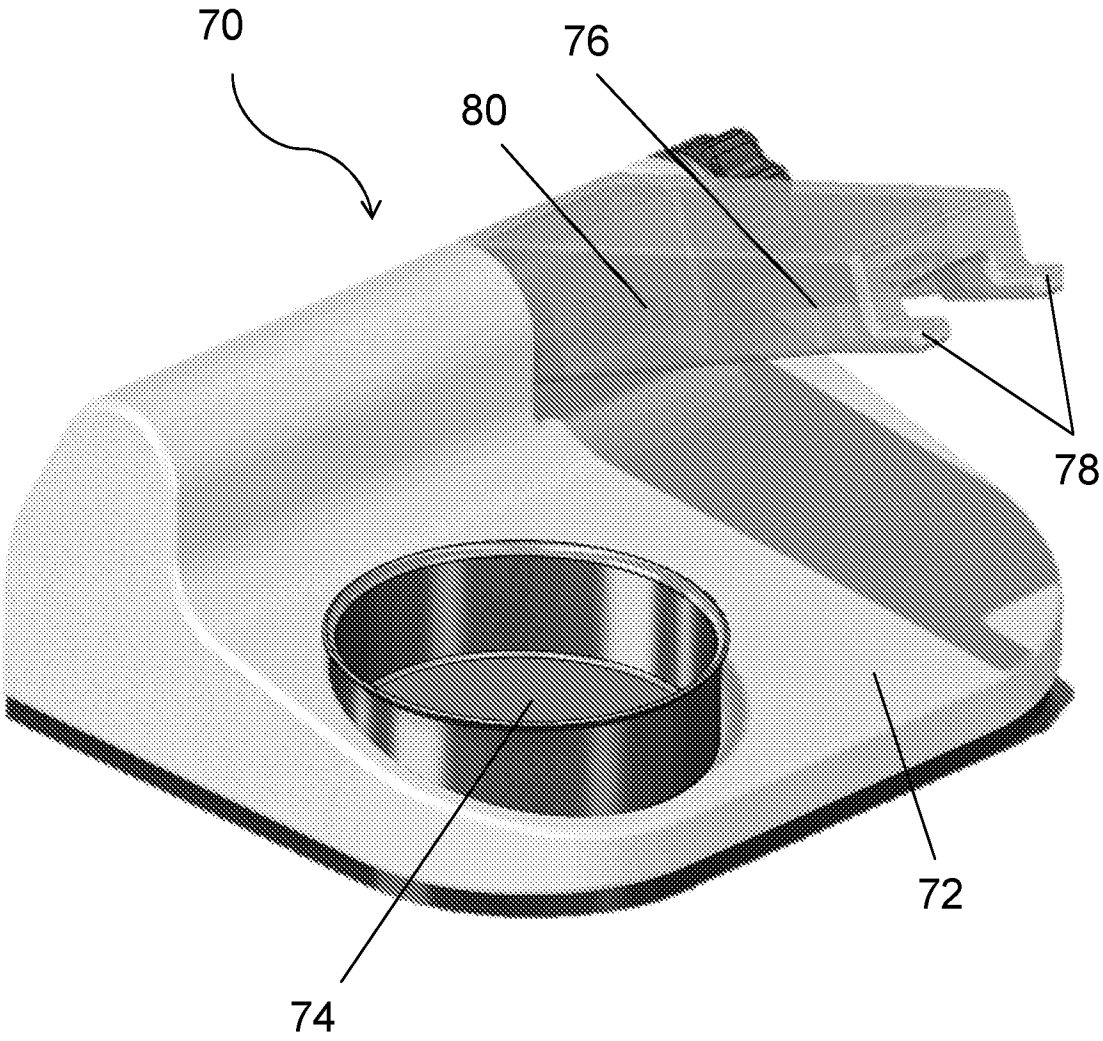

Fig 7

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2014/052381

A. CLASSIFICATION OF SUBJECT MATTER
INV. G01N1/06 G01N1/28 G01N1/36 G02B21/34 A61B10/02
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 G02B A61B

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 2011/046620 A1 (LEWANDOWSKI SIMON [US] ET AL) 24 February 2011 (2011-02-24)	11-31
A	paragraphs [0009] - [0015]; figures 1-4 paragraphs [0030] - [0031] paragraph [0037] paragraphs [0040] - [0041] paragraph [0054] paragraph [0063] paragraph [0051]	1-10
A	EP 2 600 133 A1 (SAKURA SEIKI CO LTD [JP]; SAKURA FINETEK JAPAN CO LTD [JP]) 5 June 2013 (2013-06-05) paragraphs [0040], [0041], [0046], [0047]; figures 2, 3, 4	1-31

-/--

[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

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"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
12 January 2015	20/01/2015

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	Bamière, François

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2014/052381

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2011/054509 A1 (CHEN CHENG-CHI [TW]) 3 March 2011 (2011-03-03) paragraphs [0011] - [0018]; figures 1-4	1-31
A	DE 43 05 057 A1 (TGA TECH GERAETE UND APPARATEB [DE]) 25 August 1994 (1994-08-25) the whole document	11-31
A	US 2012/058509 A1 (LEININGER ERIC JEFFORDS [US] ET AL) 8 March 2012 (2012-03-08) the whole document	1-31

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/GB2014/052381

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2011046620 A1	24-02-2011	NONE	
EP 2600133 A1	05-06-2013	CN 103038623 A	10-04-2013
		EP 2600133 A1	05-06-2013
		JP 2012032237 A	16-02-2012
		US 2013122540 A1	16-05-2013
		WO 2012014896 A1	02-02-2012
US 2011054509 A1	03-03-2011	CN 102001061 A	06-04-2011
		US 2011054509 A1	03-03-2011
DE 4305057 A1	25-08-1994	NONE	
US 2012058509 A1	08-03-2012	NONE	