



(11) **EP 3 094 964 B9**

(12) **CORRECTED EUROPEAN PATENT SPECIFICATION**

(15) Correction information:
Corrected version no 1 (W1 B1)
Corrections, see
Claims EN 9, 14, 15

(51) Int Cl.:
G01N 27/447 ^(2006.01)

(86) International application number:
PCT/CA2015/050031

(48) Corrigendum issued on:
26.08.2020 Bulletin 2020/35

(87) International publication number:
WO 2015/106356 (23.07.2015 Gazette 2015/29)

(45) Date of publication and mention
of the grant of the patent:
17.06.2020 Bulletin 2020/25

(21) Application number: **15737241.8**

(22) Date of filing: **16.01.2015**

(54) **CASSETTES FOR USE IN AUTOMATED PARALLEL ELECTROPHORETIC ASSAYS AND METHODS FOR MANUFACTURING AND USING SAME**

KASSETTEN ZUR VERWENDUNG BEI AUTOMATISIERTEN PARALLELEN ELEKTROPHORETISCHEN ASSAYS UND VERFAHREN ZU HERSTELLUNG UND VERWENDUNG DAVON

CASSETTES À UTILISER LORS D'ANALYSES ÉLECTROPHORÉTIQUES PARALLÈLES AUTOMATISÉES ET LEURS PROCÉDÉS DE FABRICATION ET D'UTILISATION

(84) Designated Contracting States:
AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO PL PT RO RS SE SI SK SM TR

- **NOBLES, Andrew**
Vancouver, British Columbia V5N 5Y9 (CA)
- **BAILLIE, Kevin**
Vancouver, British Columbia V5Y 2B3 (CA)

(30) Priority: **17.01.2014 US 201461929009 P**

(74) Representative: **Appleyard Lees IP LLP**
15 Clare Road
Halifax HX1 2HY (GB)

(43) Date of publication of application:
23.11.2016 Bulletin 2016/47

(73) Proprietor: **Coastal Genomics Inc.**
Burnaby, British Columbia V5C 5T5 (CA)

(56) References cited:
EP-A2- 0 457 526 WO-A1-95/06243
WO-A1-98/28617 WO-A1-02/077630
WO-A1-2010/126897 WO-A2-02/18901
CA-A1- 2 393 690 US-A1- 2004 091 398
US-A1- 2006 207 882 US-B1- 6 569 306
US-B2- 6 905 585 US-B2- 7 407 630

(72) Inventors:

- **SLOBODAN, Jared**
Richmond, British Columbia V7A 2E8 (CA)
- **NESBITT, Matthew**
Langley, British Columbia V1M 2H2 (CA)

Note: Within nine months of the publication of the mention of the grant of the European patent in the European Patent Bulletin, any person may give notice to the European Patent Office of opposition to that patent, in accordance with the Implementing Regulations. Notice of opposition shall not be deemed to have been filed until the opposition fee has been paid. (Art. 99(1) European Patent Convention).

EP 3 094 964 B9

Description

CROSS REFERENCE TO PRIOR APPLICATIONS

[0001] This application claims priority to US Provisional Patent Application 61/929,009.

FIELD OF THE DISCLOSURE

[0002] The present disclosure relates to an electrophoresis gel cassette for use in electrophoresis and to the use and manufacture thereof. More particularly, the present disclosure relates to an electrophoresis gel cassette suitable for use with an automated liquid handling device.

BACKGROUND OF THE DISCLOSURE

[0003] Automated liquid handling workstations may be used, for example, in applications involving repetitive, predictable pipetting operations. Automation of repetitive pipetting operations may facilitate higher throughput, lower operating costs, and/or, improved consistency in pipetting. However, laboratory protocols involving, for example, non-standardized labware, gelatinous media, and/or other non-standard pipetting target areas may not be well suited to automation. In such cases, an liquid handling by an individual practitioner may be advantageous relative to an automated machine, at least because an individual could see the target and make real time positional corrections to the position and directional movement of a pipette tip to ensure the pipette tip orifice is located in a desired position prior to aspirate and dispense operations. For example, known liquid handling devices are not desirable for use in loading samples into one or more wells of an agarose gel, at least due to the small size of the wells and the low rigidity of the walls that define the agarose wells.

[0004] When pipette tips are loaded onto a manual pipette or onto mandrels of a liquid handling device, there is typically variation in 'straightness' of tips relative to their respective shaft or mandrel (i.e., the longitudinal axis of the tip may not be in line with the intended longitudinal axis of the tip). Such variation in straightness is known in the art as "tip splay". Without positional feedback provided by a manual practitioner, the presence of tip splay means that the size of a well that can be accurately targeted using an automated system is larger relative to the size of a well that can be accurately targeting using manual pipetting. Further, off-target insertion of a pipette tip into a wall defining an agarose well or into the agarose surrounding a well orifice may damage the well and/or plug the pipette tip with agarose, thereby interfering with subsequent aspiration or dispensing.

[0005] One mechanism to account for tip splay on a single channel pipetting head is to utilize a sensor that can provide feedback regarding the position of the tip. However, such a sensor would not be useful with a mul-

tichannel pipetting head comprising mandrels at fixed spacing, at least because the direction of tip splay may differ between tips, meaning that a single positional adjustment could not account for the splay in each tip.

[0006] There are examples of products and devices designed to address one or more of the above challenges associated with automated pipetting of samples into agarose gels. For example, the EGel™ 96 agarose gel (Invitrogen) comprises 96 preformed wells in a staggered pattern and is designed for use with an automatic 96 channel liquid handling manifold. Rather than inserting sample-bearing pipette tips into the wells of the agarose gel, pipette tips are positioned above the wells of the EGel and dispensed. The dispensed sample is then drawn into a section of the well that is adjacent to the position of the tip by capillary action. Thus, the EGel would seem to overcome the issue of inserting the pipette tip into the gel, as the pipette tip remains above the upper surface of the gel. Further, each well of an EGel is relatively large, consisting of a shoulder zone and an adjacent compartment that draws dispensed fluid into the wells. The relatively large wells may address tip splay, by providing a larger target for each pipette tip. However, the larger size of the wells in the EGel prevents maximization of the number of wells that can be positioned adjacent to one another in a gel, thereby limiting sample throughput.

[0007] It is desirable to mitigate and/or obviate one or more of the above deficiencies.

SUMMARY OF THE INVENTION

[0008] In a first aspect, a cassette for use in parallel electrophoretic assays is provided. In one embodiment, the cassette comprises a tray, the tray having a floor and two pairs of opposing side walls extending upwards from the floor, the tray at least partially defining a plurality of assay channels. Each of the assay channels comprises a first buffer reservoir; a second buffer reservoir; and a media channel, the media channel extending between, and in fluid communication with, the first and second buffer reservoirs. In this embodiment, the cassette further comprises a lid, the lid adapted to engage the side walls of the tray, thereby creating a space between the tray floor and the lid, the lid comprising a plurality of ports, each of the ports extending from an outer surface of the lid to an inner surface of the lid. The plurality of ports comprise a plurality of buffer reservoir ports for introducing and/or removing buffer; and a plurality of sample ports for introducing a plurality of samples into the media channels, each of the plurality of sample ports comprising one or more tip registration features for guiding a pipette tip to a desired location in the sample port. In this embodiment, when the lid is engaged with the tray, the buffer reservoir port is in fluid communication with one or more of the first and second buffer reservoirs, the plurality of sample ports are aligned with the plurality of media channels.

[0009] In one embodiment of the first aspect, the lid further comprises a plurality of media ports for introducing media into the media channels.

[0010] In one embodiment of the first aspect, the one or more tip registration features comprise at least one surface extending in one or more of x- and y-axes towards the desired location in the sample port, the x- and y-axes being in line with the plane of the lid. In a preferred embodiment, the at least one surface of the one or more registration features comprises a sloped surface for receiving a pipette tip.

[0011] In one embodiment of the first aspect, each of the plurality of sample ports comprises a tip landing zone. In a preferred embodiment, the tip landing zone is adjacent to the desired location in the sample port. In a preferred embodiment, the at least one surface of the one or more registration features comprises a projection extending into the sample port from a wall in the sample port opposite the tip landing zone.

[0012] In one embodiment of the first aspect, the cassette further comprises a gel medium, the gel medium being disposed in the media channels of the tray, the gel medium in each media channel comprising a well for receiving a sample, wherein, when the lid is engaged with the tray, each well is aligned with the desired location in one of the plurality of sample ports. In a preferred embodiment, the cassette further comprises a buffer, the buffer being disposed in the first and second buffer reservoirs. In a preferred embodiment, the gel medium in each media channel further comprises a tip landing zone adjacent to the well for receiving a sample.

[0013] In one embodiment of the first aspect, the plurality of ports further comprises: a plurality of barrier ports for positioning at least a portion of first and second barriers into each of the assay channels, wherein, when the lid is engaged with the tray, the plurality of barrier ports facilitate positioning of the first and second barriers between the first buffer reservoir and the media channel and the second buffer reservoir and the media channel, respectively. In a preferred embodiment the plurality of ports further comprises: a plurality of sample extraction ports for extracting a plurality of samples from the media channel, each of the plurality of sample extraction ports comprising one or more tip registration features for guiding a pipette tip to a second desired location in the sample extraction port. In a preferred embodiment, each of the plurality of sample extraction ports comprises a tip landing zone. In a preferred embodiment, the tip landing zone is adjacent to the desired location in the sample port.

[0014] In one embodiment, the gel medium in each media channel further comprises a second well for extracting a sample. In a preferred embodiment, the gel medium in each media channel further comprises a tip landing zone adjacent to the well for extracting a sample.

[0015] In one embodiment, the plurality of assay channels comprises 6, 12, 24, 48 or 96 assay channels.

[0016] In a second aspect, a method for manufacturing various embodiments of the cassette provided herein is

provided. In one embodiment, the method comprises: forming the tray and lid from an optically neutral material; positioning a first removable barrier between the first buffer reservoir and the media channel in each of the assay channels of the formed tray and a second removable barrier between the second buffer reservoir and the media channel in each of the assay channels of the formed tray; introducing media into each media channel in the plurality of assay channels of the formed tray; positioning a comb into the introduced media in each media channel, each tooth in the comb configured to form a well in each media channel upon gelification of the media; introducing buffer into each first and second buffer reservoir in each assay channel of the formed tray; removing the comb from the introduced media after gelification of the introduced media; removing the barrier after gelification of the media; engaging the formed lid on the side walls of the formed tray, thereby creating a space between the floor of the formed tray and the lid, the space being occupied at least partially by the introduced media and the introduced buffer.

[0017] In one embodiment of the first aspect, the engaging step is completed prior to the positioning of the first and second removable barriers, the introducing of media, the positioning of the comb and the introducing of the buffer.

[0018] In one embodiment of the first aspect, the first and second removable barriers do not sealably separate the first buffer reservoir from the media channel and the second buffer reservoir from the media channel, respectively, in each of the assay channels of the

[0019] In one embodiment of the first aspect, the introduction of the media and the introduction of the buffer are carried out substantially simultaneously.

[0020] In a third aspect, a method of using various embodiments of the cassette provided herein is provided. In one embodiment, the method comprises: positioning the cassette in a liquid handling device; inserting a plurality of pipette tips in the tip landing zones of the plurality of sample ports, the plurality of pipette tips corresponding to the plurality of sample ports; moving the plurality of pipette tips inserted in the tip landing zones in one or more axes towards one or more of the tip registration features, the one or more axes being co-planar with the plane of the cassette lid; registering the plurality of pipette tips against the one or more tip registration features, at least one of the one or more tip registration features per sample port being adjacent to the desired location in each of the sample ports; and dispensing a plurality of samples from the plurality of pipette tips registered against the registration features adjacent to the desired location in each of the sample ports.

[0021] In one embodiment of the third aspect, the method further comprises lowering the plurality of registered pipette tips into the corresponding wells for receiving a sample prior to dispensing the plurality of samples.

[0022] In one embodiment of the third aspect, the method further comprises engaging the cassette com-

prising the plurality of dispensed samples with electrodes and a power supply, thereby creating one or more electric fields in the cassette, the one or more electric fields being sufficient to cause migration of the plurality of samples through the gelified media in the plurality of assay channels.

[0023] In one embodiment of the third aspect, the method further comprises extracting a plurality of migrated samples from the cassette following exposure to the electric field.

[0024] In one embodiment, the extracting comprises: inserting a second plurality of pipette tips in the second tip landing zones of the plurality of sample extraction ports, the second plurality of pipette tips corresponding to the plurality of sample extraction ports; moving the second plurality of pipette tips inserted in the second tip landing zones in one or more axes towards one or more of the second tip registration features, the one or more axes being co-planar with the plane of the cassette lid; registering the second plurality of pipette tips against the one or more second tip registration features, at least one of the one or more second tip registration features per sample extraction port being adjacent to the second desired location in each of the sample extraction ports; moving the registered second plurality of pipette tips downward into the gelified media below the second desired location in each of the sample ports; aspirating a the plurality of migrated samples from the gelified media below the second desired location in each of the sample ports; and retracting the second plurality of pipette tips from the gelified media and the sample ports of the cassette.

BRIEF DESCRIPTION OF THE DRAWINGS

[0025] The features of the invention will become more apparent in the following detailed description in which reference is made to the appended drawings wherein:

Fig. 1 is a perspective view of a first embodiment of cassette according to the invention wherein obscured portions thereof are shown in phantom.

Fig. 2 is a perspective view of the tray portion of the cassette of Fig. 1, wherein obscured portions thereof are shown in phantom.

Fig. 3 is a top plan view of the tray of Fig. 2.

Fig. 3A is a side elevation in cross section taken substantially along the line A-A of Fig. 3.

Fig. 3B is a side elevation in cross section taken substantially along the line B-B of Fig. 3.

Fig. 4 is a perspective view of a lid portion of the cassette of Fig. 1 wherein obscured portions thereof are shown in phantom.

Fig. 5 is a bottom plan view of the lid of Fig. 4.

Fig. 5A is a side elevation in cross section taken substantially along the line T-T of Fig. 5 and an enlarged presentation of V and W portions of the lid.

Fig. 5B is an enlarged presentation of the V and W portions of the lid illustrated in Fig. 5A, wherein the lid is provided above a gel comprising wells.

Fig. 6 is a top plan view of one embodiment of a lid portion of a cassette provided herein and an enlarged presentation of a sample port in the lid.

Fig. 7 is a top plan view of one embodiment of a sample port in a lid portion of a cassette provided herein, wherein a tip is shown being introduced into a tip landing zone in the sample port.

Fig. 8 is a top plan view of one embodiment of a sample port in a lid portion of a cassette provided herein, wherein a tip is shown being introduced into a the sample port.

Fig. 9 is a top plan view of one embodiment of a lid portion of a cassette provided herein.

Fig. 10 is a top plan view of one embodiment of a lid portion of a cassette provided herein.

Fig. 11 is a top plan view of one embodiment of a lid portion of a cassette provided herein.

Fig. 12 depicts various views of one embodiment of a removable barrier for use in manufacturing various embodiments of a cassette provided herein.

Fig. 13 is a top plan view of one embodiment of a cassette provided herein.

Fig. 13A is a side elevation in cross section taken substantially along the line R-R of Fig. 13.

Fig. 13B is a side elevation in partial section of the cassette of Fig. 13.

Fig. 14 depicts various views of one embodiment of a comb for use in manufacturing various embodiments of a cassette provided herein.

Fig. 15 depicts one embodiment of an additional comb for use in manufacturing various embodiments of a cassette provided herein.

Fig. 15A is a photograph depicting the combs of Fig. 14 and Fig. 15 in combination.

DETAILED DESCRIPTION OF THE INVENTION

[0026] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs.

Cassettes

[0027] The present disclosure is generally directed to a cassette for use in parallel electrophoretic assays, and more particularly to a cassette for use in automated parallel electrophoretic assays.

[0028] Liquid handling systems are configured to manipulate a plurality of fluid samples, automatically and concurrently, enabling high-throughput operations. Liquid handling devices are marketed as being highly reliable and accurate. However, sometimes a device may malfunction during the liquid handling process or not perform to a user's expectations. For example, a sample may not be properly loaded into a well in an electrophoresis gel because one or more pipette tips may not be aligned with a well. Such a malfunction may lead to inaccurate or misleading results.

[0029] In one aspect, the cassette provided herein may be used with an automated liquid handling device to guide a plurality of pipette tips to a desired location relative to the cassette, such as, for example, above a plurality of wells for receiving samples in a gel disposed in the cassette.

[0030] In general, various embodiments of the cassette provided herein comprise a tray portion, which functions as a base for the cassette and at least partially defines a plurality of assay channels, and a lid portion, which functions as a cover for the tray. The lid and tray portions may be discrete components or may be integral with each other.

[0031] In general, each assay channel in the cassette comprises an elongate media channel separating, but in fluid communication with (during functional operation of the cassette), a pair of spaced apart buffer reservoirs (i.e., first and second buffer reservoirs), which are functionally identical unless otherwise indicated. While the tray may define substantially all aspects of the assay channels, such arrangement is not considered to be necessary to the use or functionality of the various invention embodiments. In some embodiments, various features of the lid and tray together may together define the assay channels of the cassette.

[0032] Referring to FIG. 1, which illustrates one embodiment of a cassette provided herein, the cassette 100 comprises a tray 120 and a lid 110. The tray 120 has a floor 122 and two pairs of opposing side walls extending upwards from the floor 122. The lid 110 is adapted to engage the side walls of the tray 120, thereby creating a space between the tray floor 122 and the lid 110. For example, a lid may be adapted to engage a tray via mating parts (e.g., tongue and groove features), but such mating

parts are not required. An electrophoresis gel and buffer may be disposed in the space between the tray floor 122 and the lid 110, as described further below. The cassette 100 may be configured for communication with electrodes for use in one or more electrophoretic assays.

[0033] Referring to FIG. 2, which illustrates the tray of the cassette provided in FIG. 1, the tray 220 tray at least partially defines a plurality of assay channels 202. Twelve assay channels 202 are at least partially defined by the tray 220 of FIG. 2. However, in other embodiments of the cassette provided herein, various numbers of assay channels 202 may be at least partially defined by a tray of the cassette (e.g., 6, 12, 24, 48, 96 or more assay channels). Each of the assay channels 202 comprises a first buffer reservoir 230 and a second buffer reservoir 232 for receiving and holding buffer (e.g., buffer suitable for use in an electrophoretic assay). Each of the assay channels 202 further comprises a media channel 240 extending between the first and second buffer reservoirs 230, 232. Each media channel 240 is in fluid communication with the first and second buffer reservoirs 230, 232, such that in use, an electric field may be applied to the cassette, such that the field is present throughout each assay channel 202 in the cassette.

[0034] Referring further to FIG. 2, the tray 220 comprises a plurality of walls 224 extending upwards from the floor of the first and second buffer 230, 232 portions of the tray and the media channel 240 portions of the tray. These walls 224 separate one assay channel 202 from another, thereby inhibiting fluid and optionally electric communication between the assay channels 202.

[0035] In one preferred embodiment, various portions of surfaces in the media channels 240 may be textured 242, as discussed further below.

[0036] Referring to FIG. 3, which illustrates one embodiment of the tray of the cassette provided herein, some portions of the media channels 340 comprise a non-smooth, textured surface 342, which facilitates improved adherence of a gel medium to the media channels 340 by providing binding sites for the media and/or areas of increased friction between the media and the media channel 340. Other portions of the media channels 340 comprise a substantially smooth surface 344, which facilitates optical transparency of that portion of the media channel 340, thereby facilitating imaging of the media channel is desired by a user. FIG. 3A illustrates a side elevation cross-sectional view taken along the line A-A of the tray of FIG. 3, which depicts a single assay channel comprising first and second buffer reservoirs 330, 332 and a media channel 340 extending between, and in fluid communication with, the first and second buffer reservoirs 330, 332. FIG. 3B illustrates a side elevation cross-sectional view taken along the line B-B of the tray of FIG. 3, which depicts 12 media channels 340, the media channels 340 being separated by walls 324 extending upwards from the floor 322 of the tray 320.

[0037] Referring to FIG. 4, which illustrates the lid of the cassette provided in FIG. 1, the lid 410 comprises a

plurality of ports, each of the ports extending from an outer surface of the lid 410 to an inner surface 412 of the lid 410. The function of the plurality of ports is to facilitate the introduction and/or removal of fluids (e.g., media, buffer, samples) and/or electrical current.

[0038] In one embodiment, the plurality of ports comprises first and second buffer reservoir ports 450, 452 for introducing and/or removing buffer. In the embodiment shown in FIG. 4, if the lid 410 were engaged with a tray, the first and second buffer reservoir ports 450, 452 would substantially align with first and second buffer reservoirs of a common assay channel in the tray, thereby introduction or removal of buffer to the first and second buffer reservoirs of the assay channel via the first and second buffer reservoir ports 450, 452, respectively. In a preferred embodiment, the first and second buffer reservoir ports 450, 452 may also accommodate removable electrodes for use in electrophoretic operations.

[0039] Referring further to FIG. 4, the plurality of ports comprises sample ports 460 for introducing a plurality of samples into the media channels. In the embodiment shown in FIG. 4, if the lid 410 were engaged with a tray, the sample ports 460 would substantially align with a proximal end of a media channel in the tray, and if the tray were to comprise an electrophoresis gel, the sample ports 460 would substantially align with wells for receiving a sample in the gel, thereby facilitating introduction of a sample into a well in a gel disposed in a media channel of the cassette. In one embodiment, the sample ports 460 may also be used for introduction of media into the media channel of each assay channel.

[0040] In the embodiment shown in FIG. 4, each of the plurality of sample ports 460 comprises a tip landing zone 474 and one or more tip registration features 468 for guiding a pipette tip to a desired location 490 in the sample port 460.

[0041] In general, the tip landing zone is located in an enlarged portion of a sample port, which accommodates initial aberrant pipette tip alignments (i.e., tip splay) such that no pipette tips entering the sample ports are damaged by accidental impingement on the outer surface of the lid of the cassette due to, for example, misalignment. Once partially disposed in the tip landing zone of the enlarged opening, the pipette tips can be laterally moved to contact a tip registration feature, such as, for example, a portion of respective sample port orifice walls and be guided to a desired location in the sample port (e.g., over a sample well in a gel disposed therebelow). The tip landing zone and tip registration features will be discussed further below.

[0042] Referring further to FIG. 4, the plurality of ports comprises sample extraction ports 462 for extracting a plurality of samples from the media channels after an electrophoretic assay has been run. In the embodiment shown in FIG. 4, if the lid 410 were engaged with a tray, the sample extraction ports 462 would substantially align with a distal end of a media channel in the tray, thereby facilitating extraction of a sample that has been subjected

to electrophoresis in a gel disposed in a media channel of the cassette. In general, sample extraction ports may be located at any location within a media channel that is a distance downstream (i.e., in a direction in which a sample would travel in the media channel when treated by electrophoresis) from a sample port. Sample extraction ports 462 are not required in the cassettes disclosed herein, but may be provided in one or more preferred embodiments of the cassette provided herein.

[0043] Referring further to FIG. 4, the plurality of ports further comprises ports referred to as barrier ports 454. In the embodiment shown in FIG. 4, each assay channel comprises two barrier ports 454, 455, a first barrier port 454 being located between the first buffer reservoir port 450 and the sample port 460, a second barrier port 455 being located between the second buffer reservoir port 452 and the extraction port 462. Each barrier port 454, 455 functions to receive a barrier that is used during manufacture of the cassette to separate the first buffer reservoir from the media channel and the second buffer reservoir from the media channel, respectively. In the embodiment shown in FIG. 4, in the absence of a barrier, if the lid 410 were engaged with a tray, the first and second barrier ports 454, 455 would substantially align with portions of the assay channel bridging a media channel and first and second buffer reservoirs respectively, thereby facilitating introduction of media to the media channel and optionally facilitating introduction of buffer to one or more of the buffer reservoirs. Barrier ports 454, 455 are not required in the cassettes disclosed herein (media may be introduced into the media channel by way of, for example, sample ports and/or sample extraction ports), but may be provided in one or more preferred embodiments of the cassette provided herein.

[0044] Referring to FIG. 5, which illustrates one embodiment of the lid of the cassette provided herein, the lid 510 may correspond to 12 assay channels 502, each portion of the lid 510 corresponding to an assay channel 502 comprising first and second buffer reservoir ports 550, 552, first and second barrier ports 554, 555, a sample port 560 and a sample extraction port 562. FIG. 5A illustrates a side elevation cross-sectional view taken along the line T-T of the tray of FIG. 5, which depicts a portion of the lid corresponding to a single assay channel (upper), portions V and W of which are enlarged (below) for clarity. FIG. 5B illustrates the enlarged views (V and W) shown in FIG. 5A, wherein the portion of the lid corresponding to an assay channel is disposed above a gel 580 comprising a sample well 582 and an extraction well 583. FIGs. 5A and 5B illustrate one example of a sample port 560 comprising of a tip landing zone 564 (located in an enlarged area of the sample port) and a smaller area 566 of the sample port 560, the smaller area 566 corresponding substantially with a desired location in the sample port 560, which corresponds with a location above a sample well 582 in a gel 580 disposed therebelow. The surface area of the tip landing zone 564 that does not overlap with the smaller area 566 may also be referred

to herein as a "shelf". In certain embodiments, the area of the sample port 560 at the inner surface 512 of the lid is significantly less than the area of the sample port 560 at the outer surface 514 of the lid because the enlarged area accommodates tip splay, whereas the smaller area 566 of the sample port at the inner surface 512 can be reduced up to an area slightly larger than the projection area of the pipette tip.

[0045] FIGs. 5A and 5B also illustrates one example of tip registration features 568, 569 comprising a sloped surface for receiving a pipette tip. The sloped surface may be planar and/or curved, and at least partially defines the sample port orifice from the outer surface of the lid to the inner surface thereof. Since a purpose of the tip registration feature 568, 569 is to ensure consistent (and preferably perpendicular) transit of the pipette tip into the cassette, the slope or draft angle of the registration feature(s) 568, 569 preferably match(es) the draft angle of a pipette tip (e.g., 8°). In a preferred embodiment, tip registration features, 569 are present on a medial or front wall portion of the sample port 560, i.e., a sample port wall more proximate to the opposing side of the assay channel.

[0046] Referring further to FIGs. 5A and 5B, in operation, a pipette tip 570 comprising a sample would be lowered down (i.e., along a z-axis relative to the plane of the lid) by a liquid handling device into the tip landing zone 564 of a sample port 560. The tip 570 would then be moved laterally by the liquid handling device (i.e., along an x- and/or y- axis relative to the plane of the lid 510) until it contacts (i.e., registers) a registration feature 569. If the registration feature 569 is aligned with the smaller area 566 of the sample port 560 and the well 582 disposed in the gel 580 below, following registration, the contents of the tip 570 would be dispensed and the tip 570 would be removed from the sample port 560 by the liquid handling device.

[0047] FIGs. 5A and 5B also illustrate one example of a sample extraction port 562 comprising a second tip landing zone 565 and a smaller area of the sample extraction port, the smaller area of the sample extraction port 567 corresponding with a desired location in the sample extraction port 562, which corresponds with a location above a well 583 in a gel 580 disposed therebelow where a sample that has been subjected to electrophoresis is anticipated to be located. In operation, a sample that has been subjected to electrophoresis may be extracted from a gel 580 in the cassette by lowering a pipette tip 571 down (i.e., along a z-axis relative to the plane of the lid) into the second tip landing zone 565 of the sample extraction port 562. The tip 571 would then be moved laterally by the liquid handling device (i.e., along an x- and/or y- axis relative to the plane of the lid) until it contacts (i.e., registers) a registration feature 569a. If the registration feature 569a is aligned with the smaller area 567 of the sample extraction port 565 and the well 583 disposed in the gel 580 below, following registration, the tip 571 would be lowered further into the extraction well 582, the con-

tents of the extraction well 583 would be aspirated and the tip 571 would be removed from the gel extraction well 582 and sample extraction port 562 by the liquid handling device.

[0048] In general, because the pipette tip has been positioned at a desired location corresponding to the extraction well, the pipette tip may be lowered into the extraction well without worry that the tip might puncture a wall defining the well, which may clog the tip and interfere with extraction of the sample and/or subsequent tip operations.

[0049] Referring to FIG. 6, which illustrates one embodiment of a lid provided herein (left), a lid 610 may be configured to correspond to a plurality of assay channels 602, each portion of the lid corresponding to an assay channel 602 comprising first and second buffer reservoir ports 650, 652 that are integral with first and second barrier ports 654, 655 and further comprising a plurality of sample ports 660, each of the sample ports comprising a tip landing zone 674, one or more registration features 664 and desired locations 690 corresponding to a sample well in a gel disposed in a cassette comprising the lid 610.

[0050] In general, the configuration of the sample ports provided in the lids of the cassettes provided herein facilitates standardization of the position of multiple mandrel engaged pipette tips by providing tip registration features against which said tips may register prior to descending into a pre-formed well in a gel disposed therebelow.

[0051] In the embodiment shown in FIG. 6, each portion of the lid 610 corresponding to an assay channel 602 comprises a rectangular sample port 660 comprising two desired locations 690 corresponding to two wells in a gel that may be disposed therebelow. These two desired locations 690 are illustrated as circles in FIG. 6, but may be otherwise shaped (e.g., rectangular or square). Two tip landing zones 674 are provided in each sample port 690, each being located in a distal portion of the sample port 660, opposite the desired locations 690. The registration features 664 in each sample port 664 are defined by walls of the sample port 660. An extension 666 projecting from the sample port wall opposite the tip landing zone 674 into the sample port 660 is provided to prohibit cross contamination between wells in the event of overflow. In operation, a pipette tip comprising a sample would be lowered a pre-determined distance (i.e., along a z-axis relative to the plane of the lid 610) by a liquid handling device into a tip landing zone 674 of a sample port 660. The tip would then be moved laterally by the liquid handling device along a first axis (for example, along an x-axis relative to the plane of the lid 610) until it contacts (i.e., registers) a first registration feature 664 (i.e., a wall of the sample port 660). The tip would then be moved along a second axis (for example, along a y-axis relative to the plane of the lid 610) until it contacts (i.e., registers) a second registration feature 664a (i.e., a second wall of the sample port). In one embodiment, if the second registration feature 664a is aligned with a desired location

690, following registration with the second registration feature 664a, the contents of the tip would be dispensed and the tip would be removed from the sample port 660 by the liquid handling device. In one embodiment, if the second registration feature 664a is aligned with a desired location 690, following registration with the second registration feature 664a, the tip would be lowered a pre-determined distance (i.e., along a z-axis relative to the plane of the lid 610) by a liquid handling device into a well disposed in a gel therebelow, the contents of the tip would be dispensed in the well and the tip would be removed from the sample port 660 by the liquid handling device.

[0052] In general, dispensing of the sample may be done above a well disposed below a sample port or, preferably, in a well disposed below a sample port. Because the pipette tip has been positioned at a desired location corresponding to the well, the pipette tip may be lowered into the well without worry that the tip might puncture a wall defining the well, which may clog the tip and interfere with dispensing and/or subsequent tip operations.

[0053] In general, additional tip registration features and additional desired locations corresponding to sample wells may be added to the sample ports of various embodiments of the cassette provided herein. Thereby allowing a single tip landing zone to correspond with a plurality of tip registration features and desired locations in a sample port.

[0054] For example, referring to FIG. 7, which illustrates one embodiment of a sample port in a lid provided herein, each sample port 760 may comprise a plurality of desired locations 790 corresponding to a plurality of sample wells 782 in a gel disposed in a cassette comprising the lid. In this embodiment, a plurality of registration features comprising projections 764, 764a extend from a second wall 712a of the sample port, a first wall being adjacent to the plurality of desired locations 712. In operation, a pipette tip 770 comprising a sample would be lowered down a pre-determined distance (i.e., along a z-axis relative to the plane of the lid) by a liquid handling device into the tip landing zone 774 of the sample port 760. The tip 770 would then be moved laterally by the liquid handling device along a first axis (for example, along an x-axis relative to the plane of the lid, as illustrated in FIG. 8) until it contacts (i.e., registers) a first registration feature 712 (i.e., the first wall of the sample port). The tip would then be moved along a second axis (for example, along a y-axis relative to the plane of the lid) a pre-determined distance (e.g., a distance equal to the radius of a tip splay, referred to herein as a tip splay zone 775, which encompasses an area of maximum predicted tip splay) and then it is moved along the first axis towards a projection registration feature 764 (e.g., the longest projection extending from the second wall 712a). In FIG. 7, the trajectory of the tip is illustrated by arrows.

[0055] In general, the process of movement in the x- and y-axes until a tip registers with a registration feature may be repeated by registering a tip multiple times with

a plurality of registration features until the tip registers with a registration feature that is aligned with a desired location at which point, the contents of the tip would be dispensed and the tip would be removed from the sample port by the liquid handling device. In FIG. 7, the registration feature aligned with the desired location 790 is the second wall 712a of the sample port 760.

[0056] In embodiments where tip registration features are projections 764, 764a that extend into the sample port 760 from a wall 712a of the sample port 760 opposite the tip landing zone 774, e.g., as shown in FIG. 7, each additional projection (e.g., 764a) would decrease in length, thereby allowing the previous projection (e.g., 764) to be used as a registration features in guiding the tip 770 from the relatively large landing zone 774, to the relative small desired location 790 corresponding to a well 782 that may be disposed therebelow. In one embodiment, the difference in length of neighbouring projections 764, 764a is at least as large as the total position variability that can be attributed to tip splay. For example the tip landing zone 774 encompasses an area that is large enough to accommodate predicted tip splay (i.e., a tip splay zone 775) and the difference in length of subsequent registration features (i.e., projections 764, 764a) would be equal to or greater than the radius of the tip splay zone 775.

[0057] It will be understood that various configurations of registration features for guiding a pipette tip to a desired location in a sample port are encompassed by the cassettes disclosed herein.

[0058] Referring to FIG. 9, an alternative embodiment of a lid 910 of a cassette provided herein is shown, the alternative embodiment comprising in each portion of the lid 910 corresponding to an assay channel 902 a first buffer reservoir port 950 that is integral with a first barrier port 954, a second buffer reservoir port 952 that is integral with a second barrier port 955, a sample port 960 and a sample extraction port 962. The first and second barrier ports 954, 955 are configured to receive first and second removable barriers (see detail A).

[0059] Referring to FIG. 10, an alternative embodiment of a lid 1010 of a cassette provided herein is shown, the alternative embodiment comprising in each portion of the lid 1010 corresponding to an assay channel 1002 a first buffer reservoir port 1050 that is integral with a first barrier port 1054, a second buffer reservoir port 1052 that is integral with a second barrier port 1055 and a sample port 1060. The sample ports 1060 are configured to comprise two desired locations 1090, 1090a corresponding to two wells in a gel that may be disposed therebelow.

[0060] Referring to FIG. 11, an alternative embodiment of a lid 1110 of a cassette provided herein is shown, the alternative embodiment comprising in each portion of the lid 1110 corresponding to an assay channel 1102 a first buffer reservoir port 1150 that is integral with a first barrier port 1154, a second buffer reservoir port 1152 that is integral with a second barrier port 1155, a first sample port 1160, and a second sample port 1160a. The first

and second sample ports 1160, 1160a are each configured to comprise two desired locations 1190, 1190a corresponding to two wells in a gel that may be disposed therebelow.

[0061] Dimensions provided in the figures are non-limiting examples only. In one preferred embodiment, an assembled cassette has a height of about 11.3 cm, a width of about 10.9 cm and a maximum depth of about 2.3 cm.

Manufacturing

[0062] In a second aspect, a method for manufacturing one or more embodiments of the cassette provided herein is disclosed. In one embodiment, the method comprises forming at least part of the tray and lid portions of the cassette from an optically neutral material, such as, for example, a clear thermoplastic, such as acrylic (e.g., poly methyl methacrylate) or non-autofluorescing polycarbonate. Methods of manufacturing articles from thermoplastics known in the art may be used to form at least part of tray and lid portions of the cassette, such as, for example, machining, injection molding, laser cutting or three dimensional printing.

[0063] In one embodiment, the method further comprises positioning a first removable barrier between the first buffer reservoir and the media channel in each of the assay channels of the formed tray and a second removable barrier between the second buffer reservoir and the media channel in each of the assay channels of the formed tray. In general, it is not necessary that the first and second removable barriers create a fluid impervious seal between the media channel and an adjacent buffer reservoir. Rather, it is only necessary that the removable barriers create a fluid barrier that substantially impedes cross migration of dissimilar fluids when there is not a material hydrostatic pressure differential across the barrier. When cross migration is no longer an issue due to, for example, a phase change in one of the fluids, the removable barriers are no longer needed and can be disabled, e.g., by removal of the removable barriers from the assay channels.

[0064] Referring to FIG. 12, which illustrates one embodiment of a barrier 1256 for use in manufacturing various embodiments of the cassette provided herein, the barrier 1256 may have an elongated comb shape, each tooth 1258 in the barrier 1256 being sized and configured to engage the width and depth of an assay channel in the tray of a cassette. In a preferred embodiment, the number of teeth 1258 in a barrier 1256 corresponds to the number of assay channels in a cassette and the teeth 1258 are spaced along a longitudinal axis of the barrier 1256 such that they may be engaged in the assay channels of the cassette when the barrier 1256 is introduced into the cassette.

[0065] Referring to FIG. 13, which illustrates a top plan view of one embodiment of a cassette provided herein, and 13B, which illustrates a side view of the cassette

shown in FIG. 13, in one embodiment, a first removable barrier 1356 is positioned between the first buffer reservoir 1330 and the media channel 1340 in each of the assay channels 1302 and a second removable barrier 1356a is positioned between the second buffer reservoir 1332 and the media channel 1340 in each of the assay channels 1302. In one embodiment, positioning of the first and second barriers 1356, 1356a may be done directly into a tray portion of a cassette. Referring to FIG. 13 and 13A, in a preferred embodiment, positioning of the first and second barriers may be done via barrier ports 1354 in a lid 1310 of a cassette 1300. In a preferred embodiment, the barrier ports 1354 may comprise one or more structures for engaging at least part of one or more of the first and second removable barriers.

[0066] Referring further to FIG. 13, in one embodiment, the method of manufacture further comprises introducing media into each media channel 1340 in the plurality of assay channels 1302 of the formed tray. In one embodiment, media may be introduced directly into the media channels 1340 of the formed tray. In one embodiment, media may be introduced into the media channels 1340 via one or more sample port 1360, one or more sample extraction port 1362, and/or one or more portion 1354a, 1355a of one or more barrier ports 1354, 1355, wherein the one or more portion 1354a, 1355a of one or more barrier ports 1354, 1355 is on a side of the first and/or second barrier, which abuts one or more media channels 1340.

[0067] In one embodiment, the method further comprises positioning a comb into the introduced media in each media channel. Referring to FIG. 14, which illustrates one embodiment of a comb suitable for use in one embodiment of the method of manufacture provided herein, the comb 1484 may have an elongated shape, each tooth 1486 in the comb 1484 being sized and configured to form a well in each media channel of a cassette upon gelification of the media. In a preferred embodiment, the number of teeth 1486 in a comb 1484 corresponds to the number of assay channels in a cassette and the teeth 1486 are spaced along a longitudinal axis of the comb 1484 such that they may be engaged in the assay channels of the cassette when the comb 1484 is introduced into the cassette. Positioning of the comb 1484 may be done at a location in the assay channels where gel wells are desired. In a preferred embodiment, a second comb may be positioned in the assay channels comprising media at a position where sample extraction wells are desired.

[0068] In one embodiment, an additional comb 1584 may be combined with comb 1484 for use in various embodiments of the method of manufacture provided herein, as shown in FIG. 15A. In operation, combs 1484 and 1584 may be used to create a well in the plurality of media channels in various embodiments of the cassette provided herein, wherein the well comprises a standard well portion for receiving a dispensed sample that is created by a tooth 1486 in comb 1484 and further comprises a

shelf. In one embodiment, such a shelf in the media of a media channel may be provided as an alternative to a tip landing zone that is manufactured in the lid of the cassette.

[0069] In one embodiment, the method further comprises introducing buffer into each first and second buffer reservoir in each assay channel of the formed tray. In one embodiment, buffer may be introduced directly into the first and second buffer reservoirs of the formed tray. In one embodiment, buffer may be introduced into the first and second buffer reservoirs via one or more buffer reservoir port and/or one or more portion of one or more barrier ports, wherein the one or more portion of one or more barrier ports is on a side of the first and/or second barrier, which abuts one or more buffer reservoirs.

[0070] In one embodiment, the method further comprises removing the comb from the introduced media after gelification of the introduced media and removing the barrier after gelification of the media. In a preferred embodiment, the barrier is easily removable, at least because it is not configured to form a water-tight seal with the assay channels. In this embodiment, introduction of the media and buffer may be carried out substantially simultaneously without mixing of the media and buffer, at least because the partial pressures on either side of the barrier caused, at least in part, by the fluid on either side of the barrier are nearly equivalent and opposing in force, thereby inhibiting mixing of the media and buffer. Simultaneous media and buffer introduction may be advantageous, at least for example, because it may speed manufacturing time.

[0071] In one embodiment, the method further comprises engaging the formed lid on the side walls of the formed tray, thereby creating a space between the floor of the formed tray and the lid, the space being occupied at least partially by the introduced media and the introduced buffer. The step of engaging the lid on the tray may be completed prior to or following introduction of media and/or buffer and prior to or following positioning of barrier(s) and/or comb(s). In one preferred embodiment, lid and tray portions of the cassette may be integral. In this embodiment, the lid and tray portions would be engaged to form a cassette prior to introduction of media and buffer and prior to positioning of barrier(s) and comb(s).

[0072] In one embodiment, once lid and tray portions are formed, the method of manufacturing various embodiments of a cassette provided herein further comprises: establishing one or more temporary barriers between one or more buffer receiving portions of the cassette and a media receiving portion of the cassette; substantially simultaneously the filling buffer receiving portion(s) and the media receiving portion to minimize hydrostatic pressure differentials across the temporary barrier; and removing the temporary barrier once the hydrostatic pressure differential decreases below a threshold value. Such methods, as previously intimated, are preferably carried out simultaneously for multiple assay channels so that

the benefits of parallel processing can be fully realized.

Method of using Cassette in an electrophoresis assay

[0073] In general, methods of using various embodiments of a cassette provided herein comprise generally directing a pipette tip to a sample port, for example by targeting a tip landing zone in the sample port; contacting the tip with a tip registration feature in the sample port; allowing the pipette tip to be guided along the tip registration feature to a desired location in the sample port corresponding with a well in a gel disposed in the cassette therebelow; and dispensing the contents of the pipette tip. Such method is preferably carried out simultaneously in a plurality of assay channels in the cassette. In operation, a gantry arm of a suitable liquid handling device comprises an array of mandrels, each comprising a pipette tip. The gantry arm is moved such that each pipette tip is moved downward into a tip landing zone of each sample port and then laterally in the sample port until each pipette tip contacts a tip registration feature, preferably each tip registers against the same portion of tip registration feature in each sample port, thereby ensuring uniform and consistent pipette transit into respective sample ports.

[0074] In one embodiment, the method comprises positioning the cassette in a liquid handling device. Liquid handling devices suitable for transferring liquids by pipette are known in the art. In this embodiment, one or more method steps are carried out by a liquid handling device. The method further comprises inserting a plurality of pipette tips into tip landing zones of the plurality of sample ports in a cassette. In a preferred embodiment, the plurality of pipette tips held by mandrels of the liquid handling device correspond to the plurality of sample ports and the relative spacing of the plurality of sample ports. The method further comprises moving the plurality of pipette tips inserted in the tip landing zones in one or more axes towards one or more of the tip registration features, the one or more axes being co-planar with the plane of the cassette lid. For example, the plurality of pipette tips inserted in the tip landing zones may be moved in an x-axis towards a plurality of registration features. The method further comprises registering the plurality of pipette tips against the one or more tip registration features, at least one of the one or more tip registration features per sample port being adjacent to the desired location in each of the sample ports. Registration of the tips with corresponding registration features in a sample port ensures that the tips are in the desired locations of each sample port, even if one or more tips on the liquid handling mandrel is splayed. The method further comprises dispensing a plurality of samples from the plurality of pipette tips registered against the registration features adjacent to the desired location in each of the sample ports. This step facilitates introduction of a sample into a well in an assay channel without worry of inserting the tip into the media because the tip(s) is not lowered into

the well, rather, the tip remains above the well.

[0075] In one embodiment, the method further comprising engaging the cassette comprising the plurality of dispensed samples with electrodes and a power supply, thereby creating one or more electric fields in the cassette, the one or more electric fields being sufficient to cause migration of the plurality of samples through the gelified media in the plurality of assay channels. Methods of electrophoretically treating samples (e.g., DNA, protein etc.) and tool for use of same are known in the art. In one embodiment, a single voltage may be applied to an entire cassette. In one embodiment, individual voltages may be applied to one or more assay channel in various embodiments of the cassette provided herein.

[0076] In a preferred embodiment, the method further comprises extraction of a migrated sample from the cassette, the cassette comprising a plurality of sample extraction ports. In this embodiment, the plurality of migrated samples are extracted from the cassette following exposure to the electric field. In one embodiment, the method of guiding pipette tips to the samples to be extracted is similar to the method of guiding pipette tips to the desired location in the sample port for sample introduction. For example, in one embodiment, the extraction comprises: inserting a second plurality of pipette tips into second tip landing zones of the plurality of sample extraction ports in the cassette. The liquid handling device then moves the second plurality of pipette tips inserted in the second tip landing zones in one or more axes towards one or more of the second tip registration features, the one or more axes being co-planar with the plane of the cassette lid, until the second plurality of pipette tips are registered against the one or more second tip registration features, at least one of the one or more second tip registration features per sample extraction port being adjacent to a second desired location in each of the sample extraction ports. As described above, this step adjusts for any tip splay in the second plurality of pipette tips. The method further comprises moving the registered second plurality of pipette tips downward into the gelified media below the second desired location in each of the sample ports and aspirating the plurality of migrated samples from the gelified media below the second desired location in each of the sample ports, thereby extracting the migrated samples. The plurality of pipette tips comprising the extracted, migrated samples are then retracting from the gelified media and the sample ports of the cassette.

[0077] Although the invention has been described with reference to certain specific embodiments, various modifications thereof will be apparent to those skilled in the art without departing from the purpose and scope of the invention as outlined in the claims appended hereto. Any examples provided herein are included solely for the purpose of illustrating the invention and are not intended to limit the invention in any way. Any drawings provided herein are solely for the purpose of illustrating various aspects of the invention and are not intended to be drawn

to scale or to limit the invention in any way.

Claims

1. A cassette for use in parallel electrophoretic assays, the cassette comprising:

- a tray, the tray having a floor and two pairs of opposing side walls extending upwards from the floor, the tray at least partially defining a plurality of assay channels, each of the assay channels comprising:

- a first buffer reservoir;
- a second buffer reservoir; and
- a media channel, the media channel extending between, and in fluid communication with, the first and second buffer reservoirs;

- a lid, the lid being adapted to engage the side walls of the tray, thereby creating a space between the tray floor and the lid, the lid comprising a plurality of ports, each of the ports extending from an outer surface of the lid to an inner surface of the lid, the plurality of ports comprising:

- a plurality of first and second buffer reservoir ports being adapted for introducing and/or removing buffer;
- a plurality of sample ports each comprising an enlarged portion to accommodate entry of a pipette tip therewithin and adapted for introducing one or more samples into the media channels, each of the plurality of sample ports comprising one or more tip registration features for guiding the pipette tip to a desired location in the sample port;
- at least one port adapted to introduce an electrical current to the cassette;

wherein, when the lid is engaged with the tray, the plurality of first and second buffer reservoir ports are in fluid communication with the first and second buffer reservoirs respectively, and the plurality of sample ports are aligned with the plurality of media channels, respectively.

2. The cassette of claim 1, wherein the lid further comprises a plurality of media ports being adapted for introducing media into the media channels.

3. The cassette of claim 1 or 2, wherein the one or more tip registration features comprises at least one surface extending in one or more of x- and y-axes towards the desired location in the sample port, the x- and y-axes being in line with the plane of the lid,

preferably the at least one surface of the one or more registration features comprises a sloped surface for receiving the pipette tip.

4. The cassette of any one of claims 1 to 3, wherein each of the plurality of sample ports comprises a tip landing zone located in the enlarged portion thereof, preferably the tip landing zone is adjacent to the desired location in the sample port.

5. The cassette of claim 4 wherein the at least one surface of the one or more registration features comprises a projection extending into the sample port from a wall in the sample port opposite the tip landing zone.

6. The cassette of any one of claims 1 to 5, further comprising a gel medium, the gel medium being disposed in the media channels of the tray, the gel medium in each media channel comprising a well being adapted for receiving a sample, wherein, when the lid is engaged with the tray, each well is aligned with the desired location in one of the plurality of sample ports.

7. The cassette of claim 6, further comprising a buffer, the buffer being disposed in the first and second buffer reservoirs.

8. The cassette of claim 6 or 7, wherein the gel medium in each media channel further comprises a tip landing zone adjacent to the well for receiving a sample.

9. The cassette of any one of claims 1 to 8, wherein the plurality of ports further comprises one or more of:

- a plurality of barrier ports being adapted for positioning at least a portion of first and second barriers into each of the assay channels, wherein, when the lid is engaged with the tray, the plurality of barrier ports facilitate positioning of the first and second barriers between the first buffer reservoir and the media channel and the second buffer reservoir and the media channel, respectively; and
- a plurality of sample extraction ports being adapted for extracting a plurality of samples from the media channel, each of the plurality of sample extraction ports comprising one or more tip registration features being adapted for guiding a pipette tip to a second desired location in the sample extraction port.

10. The cassette of claim 9, wherein each of the plurality of sample extraction ports comprises a tip landing zone, preferably the sample extraction port tip landing zone is adjacent to the second desired location in the sample extraction port.

11. The cassette of claim 9, wherein the gel medium in each media channel further comprises a second well being adapted for extracting a sample.

12. The cassette of claim 11, wherein the gel medium in each media channel further comprises a tip landing zone adjacent to the second well for extracting a sample.

13. A method for manufacturing the cassette of claim 7, the method comprising:

- forming at least part of the tray and lid from a clear material;
- positioning a first removable barrier between the first buffer reservoir and the media channel in each of the assay channels of the formed tray and a second removable barrier between the second buffer reservoir and the media channel in each of the assay channels of the formed tray;
- introducing media into each media channel in the plurality of assay channels of the formed tray;
- positioning a comb into the introduced media in each media channel, each tooth in the comb configured to form a well in each media channel upon gelification of the media;
- allowing gelification of the introduced media;
- introducing buffer into each first and second buffer reservoir in each assay channel of the formed tray;
- removing the comb from the introduced media after gelification of the introduced media;
- removing the barrier after gelification of the media;
- engaging the formed lid on the side walls of the formed tray, thereby creating a space between the floor of the formed tray and the lid, the space being occupied at least partially by the introduced media and the introduced buffer.

14. A method of using the cassette of claim 9, the method comprising:

- positioning the cassette in a liquid handling device;
- inserting a plurality of sample-containing pipette tips in respective tip landing zones of the plurality of sample ports, the plurality of pipette tips corresponding to the plurality of sample ports;
- moving the plurality of pipette tips inserted in the tip landing zones in one or more axes towards the one or more tip registration features;
- registering the plurality of pipette tips against the one or more tip registration features, at least one of the one or more tip registration features per sample port being adjacent to the desired

location in each of the sample ports;

- dispensing the samples from the plurality of pipette tips registered against the registration features adjacent to the desired location in each of the sample ports;

- optionally engaging the cassette comprising the plurality of dispensed samples with electrodes and a power supply, thereby creating one or more electric fields in the cassette, the one or more electric fields being sufficient to cause migration of the plurality of samples through the gelified media in the plurality of assay channels; and

- optionally extracting the plurality of migrated samples from the cassette following exposure to the electric field.

15. The method of claim 14, wherein the extracting comprises:

- inserting a second plurality of pipette tips in the second tip landing zones of the plurality of sample extraction ports, the second plurality of pipette tips corresponding to the plurality of sample extraction ports;

- moving the second plurality of pipette tips inserted in the second tip landing zones in one or more axes towards one or more of the second tip registration features;

- registering the second plurality of pipette tips against the one or more second tip registration features, at least one of the one or more second tip registration features per sample extraction port being adjacent to the second desired location in each of the sample extraction ports;

- moving the registered second plurality of pipette tips downward into the gelified media below the second desired location in each of the sample ports;

- aspirating the plurality of migrated samples from the gelified media below the second desired location in each of the sample ports; and

- retracting the second plurality of pipette tips from the gelified media and the sample ports of the cassette.

Patentansprüche

1. Kassette zum Verwenden in parallelen elektrophoretischen Untersuchungen, wobei die Kassette umfasst:

- eine Wanne, wobei die Wanne einen Boden und zwei Paare von einander gegenüberliegenden Seitenwänden aufweist, die sich von dem Boden nach oben erstrecken, wobei die Wanne mindestens teilweise eine Vielzahl von Untersu-

chungskanälen definiert, wobei jeder der Untersuchungskanäle umfasst:

- einen ersten Pufferbehälter;
- einen zweiten Pufferbehälter; und
- einen Medienkanal, wobei sich der Medienkanal zwischen dem ersten und dem zweiten Pufferbehälter erstreckt und mit diesen in einer Strömungsverbindung steht;

- einen Deckel, wobei der Deckel geeignet ist, um in die Seitenwände der Wanne einzugreifen, um dadurch einen Raum zwischen dem Wannenboden und dem Deckel zu bilden, wobei der Deckel eine Vielzahl von Anschlüssen umfasst, wobei sich jeder der Anschlüsse von einer Außenseite des Deckels zu einer Innenseite des Deckels erstreckt, wobei die Vielzahl von Anschlüssen umfasst:

- eine Vielzahl von ersten und zweiten Pufferbehälteranschlüssen, die geeignet sind zum Eingeben und/oder Entfernen eines Puffers;

- eine Vielzahl von Probenanschlüssen, die jeweils einen vergrößerten Abschnitt umfassen, um einem Einführen einer Pipette Platz zu bieten, und geeignet sind zum Eingeben einer oder mehrerer Proben in die Medienkanäle, wobei jeder aus der Vielzahl von Probenanschlüssen ein oder mehrere Spitzenregistrierungselemente umfasst, um die Pipettenspitze zu einem gewünschten Ort in dem Probenanschluss zu führen;
- mindestens einen Anschluss, der geeignet ist zum Versorgen der Kassette mit einem elektrischen Strom;

wobei, wenn der Deckel auf der Wanne sitzt, die Vielzahl von ersten und zweiten Pufferbehälteranschlüssen in einer Strömungsverbindung mit den ersten bzw. zweiten Pufferbehältern steht, und wobei die Vielzahl von Probenanschlüssen jeweils auf die Vielzahl von Medienkanälen ausgerichtet sind.

2. Kassette nach Anspruch 1, wobei der Deckel außerdem eine Vielzahl von Medienanschlüssen umfasst, die geeignet sind zum Eingeben von Medien in die Medienkanäle.

3. Kassette nach Anspruch 1 oder 2, wobei das eine oder die mehreren Spitzenregistrierungselemente mindestens eine Oberfläche umfassen, die sich in einer oder mehreren einer x- und einer y-Achse in Richtung des gewünschten Orts in dem Probenanschluss erstreckt, wobei die x- und die y-Achse mit der Ebene des Deckels übereinstimmen, wobei die mindestens eine Oberfläche des einen oder der

mehreren Spitzenregistrierungselemente vorzugsweise eine geneigte Oberfläche zum Aufnehmen der Pipettenspitze umfasst.

4. Kassette nach einem der Ansprüche 1 bis 3, wobei jeder aus der Vielzahl von Probenanschlüssen einen Spitzenzielbereich umfasst, der sich in dem vergrößerten Abschnitt derselben befindet, wobei sich der Spitzenzielbereich vorzugsweise in der Nähe des gewünschten Orts in dem Probenanschluss befindet. 5
10
5. Kassette nach Anspruch 4, wobei die mindestens eine Oberfläche des einen oder der mehreren Registrierungselemente einen Vorsprung umfasst, der sich von einer Wand in dem Probenanschluss gegenüber dem Spitzenzielbereich in den Probenanschluss erstreckt. 15
6. Kassette nach einem der Ansprüche 1 bis 5, die außerdem ein Gelmedium umfasst, wobei das Gelmedium in den Medienkanälen der Wanne angebracht ist, wobei das Gelmedium in jedem Medienkanal eine Kuhle umfasst, die geeignet ist zum Aufnehmen der Probe, wobei, wenn der Deckel auf der Wanne sitzt, jeder gewünschte Ort in einem aus der Vielzahl von Probenanschlüssen auf jeweils eine der Kühlen ausgerichtet ist. 20
25
7. Kassette nach Anspruch 6, die außerdem einen Puffer umfasst, wobei sich der Puffer in dem ersten Pufferbehälter und dem zweiten Pufferbehälter befindet. 30
8. Kassette nach Anspruch 6 oder 7, wobei das Gelmedium in jedem Medienkanal einen Spitzenzielbereich in der Nähe der Kuhle zum Aufnehmen der Probe umfasst. 35
9. Kassette nach einem der Ansprüche 1 bis 8, wobei die Vielzahl von Anschlüssen außerdem eines oder mehreres umfasst von: 40
45
50
55
 - einer Vielzahl von Barriereanschlüssen, die geeignet sind zum Anbringen mindestens eines Abschnitts von ersten und zweiten Barrieren in jedem der Untersuchungskanäle, wobei, wenn der Deckel auf der Wanne sitzt, die Vielzahl von Barriereanschlüssen das Anbringen der ersten und zweiten Barrieren zwischen dem ersten Pufferbehälter und dem Medienkanal bzw. dem zweiten Pufferbehälter und dem Medienkanal erleichtert; und
 - einer Vielzahl von Probenextraktionsanschlüssen, die geeignet sind zum Extrahieren einer Vielzahl von Proben aus dem Medienkanal, wobei jeder aus der Vielzahl von Probenextraktionsanschlüssen ein oder mehrere Spitzenregis-

trierungselemente umfasst, die geeignet sind zum Führen einer Pipettenspitze zu einem zweiten gewünschten Ort in dem Probenextraktionsanschluss.

10. Kassette nach Anspruch 9, wobei jeder aus der Vielzahl von Probenextraktionsanschlüssen einen Spitzenzielbereich umfasst, wobei sich der Spitzenzielbereich des Probenextraktionsanschlusses vorzugsweise in der Nähe des zweiten gewünschten Orts in dem Probenextraktionsanschluss befindet.
11. Kassette nach Anspruch 9, wobei das Gelmedium in jedem Medienkanal eine zweite Kuhle umfasst, die geeignet ist zum Extrahieren der Probe.
12. Kassette nach Anspruch 11, wobei das Gelmedium in jedem Medienkanal einen Spitzenzielbereich in der Nähe der zweiten Kuhle zum Extrahieren einer Probe umfasst.
13. Verfahren zum Herstellen der Kassette nach Anspruch 7, wobei das Verfahren umfasst:
 - Bilden mindestens einer Wanne und eines Deckels aus einem transparenten Material;
 - Anbringen einer ersten entfernbaren Barriere zwischen dem ersten Pufferbehälter und dem Medienkanal in jedem der Untersuchungskanäle der gebildeten Wanne und einer zweiten entfernbaren Barriere zwischen dem zweiten Pufferbehälter und dem Medienkanal in jedem der Untersuchungskanäle der gebildeten Wanne;
 - Eingeben der Medien in jeden Medienkanal in der Vielzahl von Untersuchungskanälen der gebildeten Wanne;
 - Anbringen eines Kamms in die eingegebenen Medien in jedem Medienkanal, wobei jeder Zahn des Kamms konfiguriert ist zum Bilden einer Kuhle in jedem Medienkanal nach einer Gelierung der Medien;
 - Erlauben der Gelierung der eingegebenen Medien;
 - Eingeben des Puffers in jeden des ersten und des zweiten Pufferbehälters in jedem Untersuchungskanal der gebildeten Wanne;
 - Entfernen des Kamms aus den eingegebenen Medien nach der Gelierung der eingegebenen Medien;
 - Entfernen der Barriere nach der Gelierung der Medien;
 - Eingreifen des gebildeten Deckels in die Seitenwände der gebildeten Wanne, wodurch ein Raum zwischen dem Boden der gebildeten Wanne und dem Deckel gebildet wird, wobei der Raum mindestens teilweise von den eingegebenen Medien und dem eingegebenen Puffer ausgefüllt wird.

14. Verfahren zum Verwenden der Kassette nach Anspruch 9, wobei das Verfahren umfasst:

- Anbringen der Kassette in einer Flüssigkeits-handhabungsvorrichtung; 5
- Einführen einer Vielzahl von probenenthaltenen Pipettenspitzen in entsprechende Spitzenzielbereiche der Vielzahl von Probenanschlüssen, wobei die Vielzahl von Pipettenspitzen der Vielzahl von Probenanschlüssen entspricht; 10
- Bewegen der Vielzahl von Pipettenspitzen, die in die Spitzenzielbereiche eingefügt wurden, auf einer oder mehreren Achsen in Richtung auf die Spitzenregistrierungselemente; 15
- Registrieren der Vielzahl von Pipettenspitzen gegen das eine oder die mehreren Spitzenregistrierungselemente, wobei sich mindestens einer des einen oder der mehreren Spitzenregistrierungselemente pro Probenanschluss in der Nähe des gewünschten Orts in jedem der Probenanschlüsse befindet; 20
- Abgeben der Proben aus der Vielzahl von Pipettenspitzen, die gegen die Spitzenregistrierungselemente in der Nähe des gewünschten Orts in jedem der Probenanschlüsse registriert sind; 25
- optionales Verbinden der Kassette, welche die Vielzahl von abgegebenen Proben umfasst, mit Elektroden und einer Stromversorgung, wodurch in der Kassette ein oder mehrere elektrische Felder erzeugt werden, wobei das eine oder die mehreren elektrischen Felder ausreichend sind, um eine Migration der Vielzahl von Proben durch die gelierten Medien in der Vielzahl von Untersuchungskanälen zu veranlassen; und 30
- nach der Einwirkung des elektrischen Felds, optionales Extrahieren der Vielzahl von migrierten Proben aus der Kassette. 35

15. Verfahren nach Anspruch 14, wobei das Extrahieren umfasst:

- Einführen einer zweiten Vielzahl von Pipettenspitzen in die zweiten Spitzenzielbereiche der Vielzahl von Probenextraktionsanschlüssen, wobei die zweite Vielzahl von Pipettenspitzen der Vielzahl von Probenextraktionsanschlüssen entspricht; 45
- Bewegen der zweiten Vielzahl von Pipettenspitzen, die in die Spitzenzielbereiche eingefügt wurden, auf einer oder mehreren Achsen in Richtung der zweiten Spitzenregistrierungselemente; 50
- Registrieren der zweiten Vielzahl von Pipettenspitzen gegen das eine oder die mehreren zweiten Spitzenregistrierungselemente, wobei sich mindestens eines des einen oder der mehreren 55

zweiten Spitzenregistrierungselemente pro Probenextraktionsanschluss in der Nähe des zweiten gewünschten Orts in jedem der Probenextraktionsanschlüsse befindet;

- Bewegen der registrierten zweiten Vielzahl von Pipettenspitzen abwärts in die gelierten Medien unter dem zweiten gewünschten Ort in jedem der Probenanschlüsse;
- Absaugen der Vielzahl von migrierten Proben aus den gelierten Medien unter dem zweiten gewünschten Ort in jedem der Probenanschlüsse; und
- Zurückziehen der zweiten Vielzahl von Pipettenspitzen aus den gelierten Medien und den Probenanschlüssen der Kassette.

Revendications

1. Cassette destinée à être utilisée dans des dosages électrophorétiques, la cassette comprenant :

- un plateau, le plateau possédant un fond et deux paires de parois latérales opposées s'étendant vers le haut depuis le fond, le plateau délimitant au moins partiellement une pluralité de canaux de dosage, chacun des canaux de dosage comprenant :

- un premier réservoir de solution tampon ;
- un second réservoir de solution tampon ;
- et
- un canal pour milieux, le canal pour milieux s'étendant entre les premier et second réservoirs de solution tampon, et étant en communication fluide avec ceux-ci ;

- un couvercle, le couvercle étant conçu pour entrer en prise avec les parois latérales du plateau, ce qui permet de créer un espace entre le fond du plateau et le couvercle, le couvercle comprenant une pluralité d'orifices, chacun des orifices s'étendant depuis une surface externe du couvercle vers une surface interne du couvercle, la pluralité d'orifices comprenant :

- une pluralité de premiers et seconds orifices de réservoir conçus pour introduire et/ou retirer une solution tampon ;
- une pluralité d'orifices à échantillon comprenant une partie agrandie pour accueillir l'entrée d'une pointe de pipette en son sein et conçue pour introduire un ou plusieurs échantillons dans les canaux pour milieux, chaque orifice de la pluralité d'orifices à échantillon comprenant une ou plusieurs caractéristiques d'alignement de pointes pour guider la pointe de la pipette vers un

- emplacement souhaité dans l'orifice à échantillon ;
- au moins un orifice conçu pour introduire un courant électrique dans la cassette ;
- 5
- dans lequel, lorsque le couvercle est en prise avec le plateau, la pluralité de premiers et seconds orifices de réservoir est en communication fluïdique avec les premier et second réservoirs de solution tampon respectivement, et la pluralité d'orifices à échantillon est alignée sur la pluralité de canaux pour milieux, respectivement.
- 10
2. Cassette selon la revendication 1, dans laquelle le couvercle comprend en outre une pluralité d'orifices pour milieux conçue pour l'introduction de milieux dans les canaux pour milieux.
- 15
3. Cassette selon la revendication 1 ou 2, dans laquelle la ou les caractéristiques d'alignement de pointes comprennent au moins une surface s'étendant dans l'axe des x et/ou l'axe des y vers l'emplacement souhaité dans l'orifice à échantillon, les axes x et y étant alignés sur le plan du couvercle, de préférence l'au moins une surface de la ou des caractéristiques d'alignement comprend une surface inclinée pour recevoir la pointe de pipette.
- 20
- 25
4. Cassette selon l'une quelconque des revendications 1 à 3, dans laquelle chaque orifice de la pluralité d'orifices à échantillon comprend une zone d'atterrissage de pointes dans la partie agrandie de celui-ci, de préférence la zone d'atterrissage de pointes est adjacente à l'emplacement souhaité dans l'orifice à échantillon.
- 30
- 35
5. Cassette selon la revendication 4, dans laquelle l'au moins une surface de la ou des caractéristiques d'alignement comprend une saillie s'étendant vers l'orifice à échantillon depuis une paroi dans l'orifice à échantillon en regard de la zone d'atterrissage de pointes.
- 40
6. Cassette selon l'une quelconque des revendications 1 à 5, comprenant en outre un milieu en gel, le milieu en gel étant disposé dans les canaux pour milieux du plateau, le milieu en gel dans chaque canal pour milieux comprenant un puits conçu pour accueillir un échantillon, dans lequel, lorsque le couvercle est en prise avec le plateau, chaque puits est aligné sur l'emplacement souhaité dans un orifice de la pluralité d'orifices à échantillon.
- 45
- 50
7. Cassette selon la revendication 6, comportant en outre une solution tampon, la solution tampon étant disposée dans les premier et second réservoirs de solution tampon.
- 55
8. Cassette selon la revendication 6 ou 7, dans laquelle le milieu en gel dans chaque canal pour milieux comprend en outre une zone d'atterrissage de pointes adjacente au puits pour accueillir un échantillon.
9. Cassette selon l'une quelconque des revendications 1 à 8, dans laquelle la pluralité d'orifices comprend en outre une ou plusieurs parmi :
- une pluralité d'orifices formant barrière conçue pour le positionnement d'au moins une partie de première et seconde barrières dans chacun des canaux de dosage, lorsque le couvercle est en prise avec le plateau, la pluralité d'orifices formant barrière facilitant le positionnement des première et seconde barrières entre le premier réservoir à solution tampon et le canal pour milieux et le second réservoir à solution tampon et le canal pour milieux, respectivement ; et
 - une pluralité d'orifices d'extraction d'échantillons conçue pour l'extraction d'une pluralité d'échantillons depuis le canal pour milieux, chaque orifice de la pluralité d'orifices d'extraction d'échantillons comprenant une ou plusieurs caractéristiques d'alignement de pointes conçues pour guider une pointe de pipette vers un second emplacement souhaité dans l'orifice d'extraction d'échantillon.
10. Cassette selon la revendication 9, dans laquelle chaque orifice de la pluralité d'orifices d'extraction d'échantillons comporte une zone d'atterrissage de pointes, de préférence la zone d'atterrissage de pointes des orifices d'extraction d'échantillon est adjacente au second emplacement souhaité dans l'orifice d'extraction d'échantillon.
11. Cassette selon la revendication 9, dans laquelle le milieu en gel dans chaque canal pour milieux comprend en outre un second puits conçu pour l'extraction d'un échantillon.
12. Cassette selon la revendication 11, dans laquelle le milieu en gel dans chaque canal pour milieux comprend en outre une zone d'atterrissage de pointes adjacente au second puits pour l'extraction d'un échantillon.
13. Procédé de fabrication de la cassette selon la revendication 7, le procédé consistant à :
- former au moins une partie du plateau et du couvercle à partir d'un matériau transparent ;
 - positionner une première barrière amovible entre le premier réservoir à solution tampon et le canal pour milieux dans chacun des canaux de dosage du plateau formé et une seconde barrière amovible entre le second réservoir à solu-

tion tampon et le canal pour milieux dans chacun des canaux de dosage du plateau formé ;

- introduire des milieux dans chaque canal pour milieux dans les multiples canaux de dosage du plateau formé ;

- positionner un peigne dans les milieux introduits dans chaque canal pour milieux, chaque dent du peigne étant conçue pour former un puits dans chaque canal pour milieux lors de la gélification des milieux ;

- permettre la gélification des milieux introduits ;

- introduire une solution tampon dans chaque premier et second réservoir de solution tampon dans chaque canal de dosage du plateau formé ;

- retirer le peigne des milieux introduits après gélification des milieux introduits ;

- retirer la barrière après la gélification des milieux ;

- mettre en prise le couvercle formé sur les parois latérales du plateau formé, ce qui permet de créer un espace entre le fond du plateau formé et le couvercle, l'espace étant occupé au moins partiellement par les milieux introduits et la solution tampon introduite.

14. Procédé d'utilisation de la cassette selon la revendication 9, le procédé consistant à :

- positionner la cassette dans un dispositif de manipulation de liquide ;

- introduire une pluralité de pointes de pipettes contenant un échantillon dans des zones d'atterrissage de pointes respectives de la pluralité d'orifices à échantillon, la pluralité de pointes de pipettes correspondant à la pluralité d'orifices à échantillon ;

- déplacer la pluralité de pointes de pipettes introduites dans les zones d'atterrissage de pointes dans un ou plusieurs axes en direction de la ou des caractéristiques d'alignement de pointes ;

- aligner les multiples pointes de pipettes contre la ou les caractéristiques d'alignement de pointes, au moins une de la ou des caractéristiques d'alignement de pointes par orifice à échantillon étant adjacente à l'emplacement souhaité dans chacun des orifices à échantillon ;

- distribuer les échantillons depuis les multiples pointes de pipettes alignées contre les caractéristiques d'alignement adjacentes à l'emplacement souhaité dans chacun des orifices à échantillon ;

- éventuellement mettre en prise la cassette comprenant la pluralité d'échantillons distribués avec des électrodes et une alimentation électrique, ce qui permet de créer un ou plusieurs champs électriques dans la cassette, le ou les

champs électriques étant suffisants pour provoquer la migration de la pluralité d'échantillons à travers les milieux gélifiés dans la pluralité de canaux de dosage ; et

- éventuellement extraire de la cassette la pluralité d'échantillons ayant migré après une exposition au champ électrique.

15. Procédé selon la revendication 14, dans laquelle l'extraction consiste à :

- introduire une seconde pluralité de pointes de pipettes dans la seconde zone d'atterrissage de pointes de la pluralité d'orifices d'extraction d'échantillon, la seconde pluralité de pointes de pipettes correspondant à la pluralité d'orifices d'extraction d'échantillon ;

- déplacer la seconde pluralité de pointes de pipettes introduites dans la seconde zone d'atterrissage de pointes dans un ou plusieurs axes en direction d'une ou de plusieurs des secondes caractéristiques d'alignement de pointes ;

- aligner la seconde pluralité de pointes de pipettes contre la ou les secondes caractéristiques d'alignement de pointes, au moins une de la ou des secondes caractéristiques d'alignement de pointes par orifice d'extraction d'échantillon étant adjacente au second emplacement souhaité dans chacun des orifices d'extraction d'échantillon ;

- déplacer la seconde pluralité de pointes de pipettes alignées vers le bas dans les milieux gélifiés au-dessous du second emplacement souhaité dans chacun des orifices à échantillon ;

- aspirer la pluralité d'échantillons ayant migré depuis les milieux gélifiés au-dessous du second emplacement souhaité dans chacun des orifices à échantillon ; et

- retirer la seconde pluralité de pointes de pipettes des milieux gélifiés et des orifices à échantillon de la cassette.

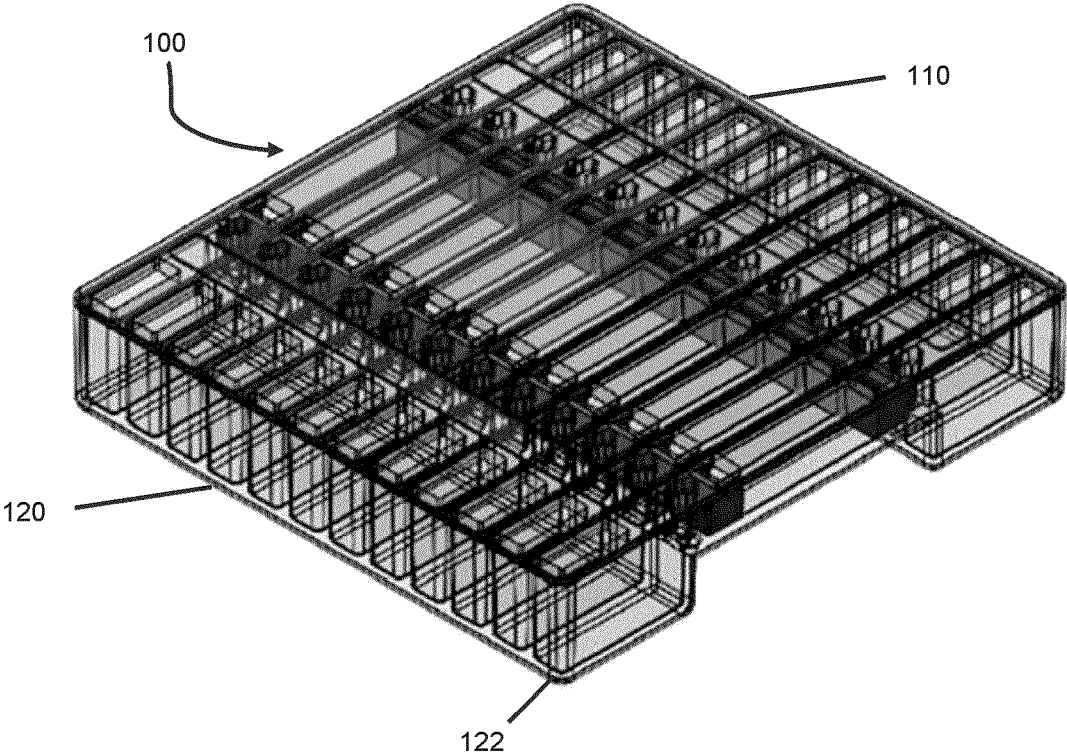


FIG. 1

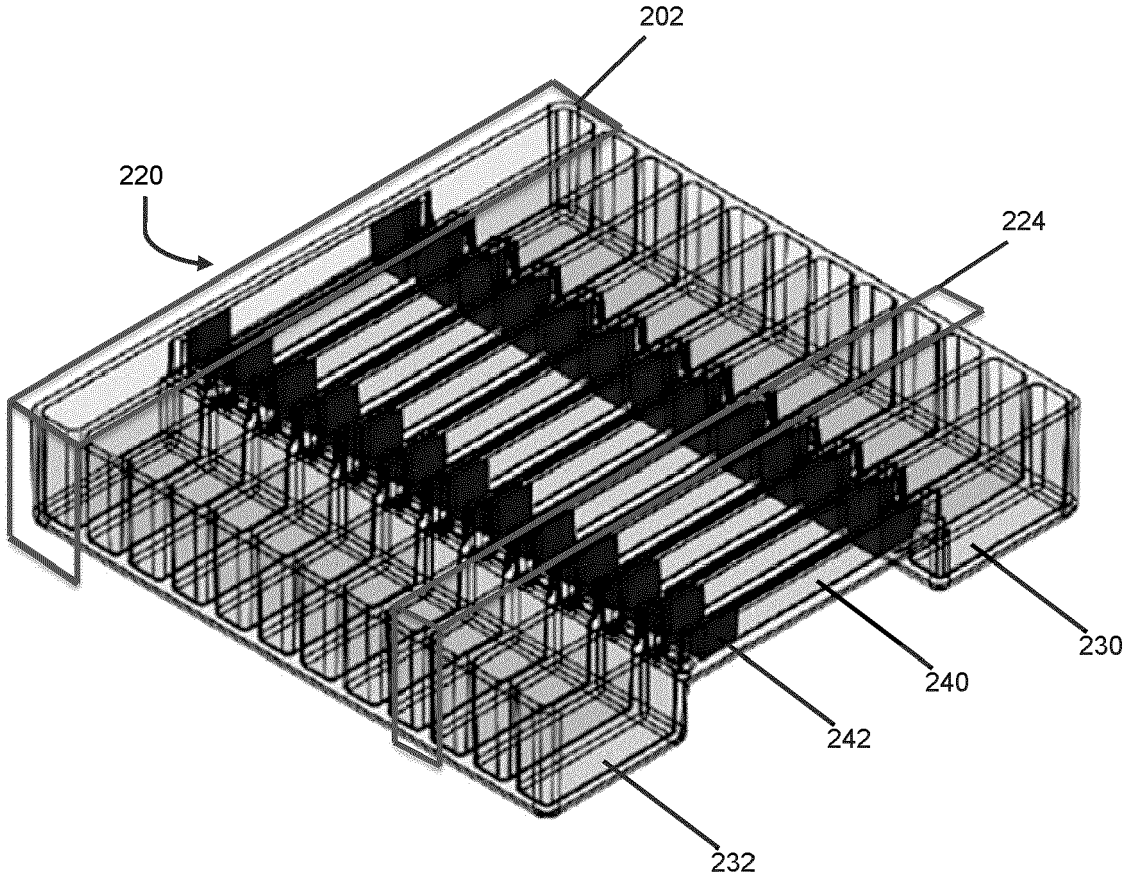


FIG. 2

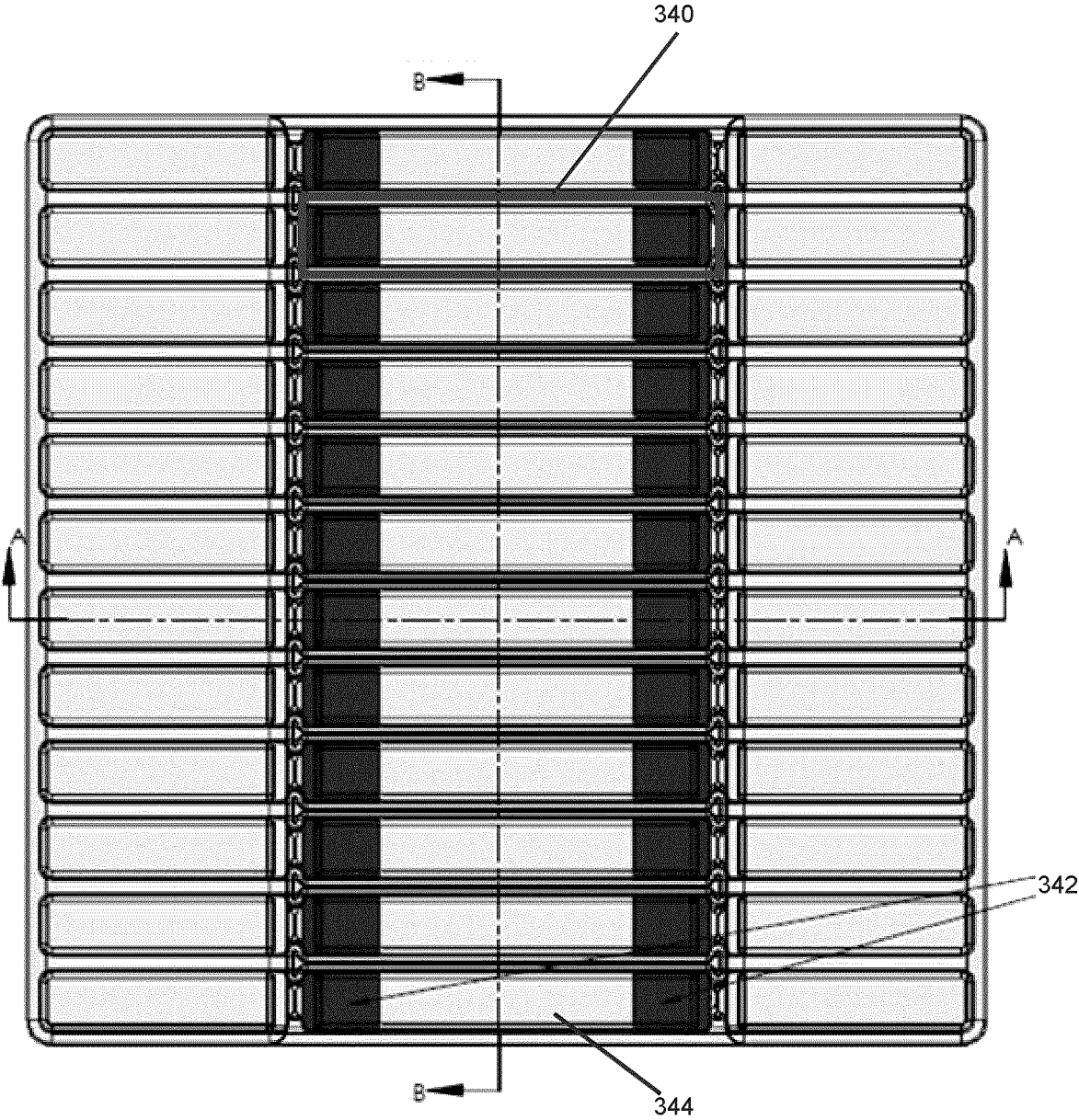


FIG. 3

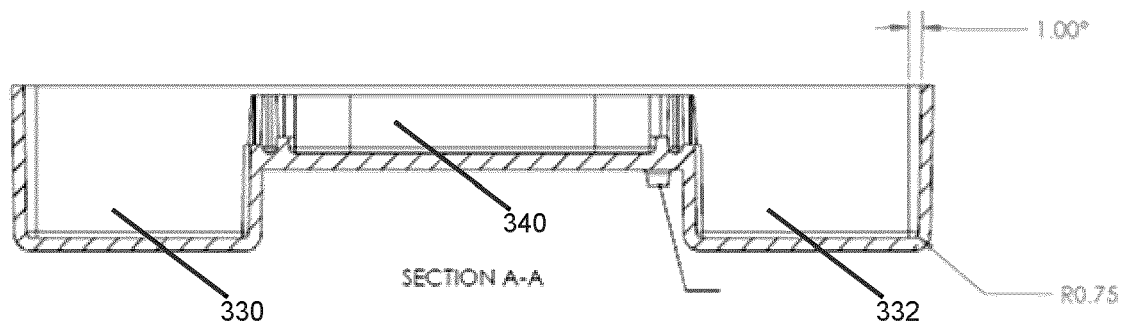
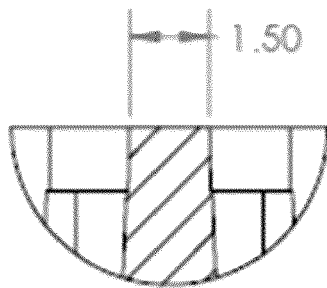
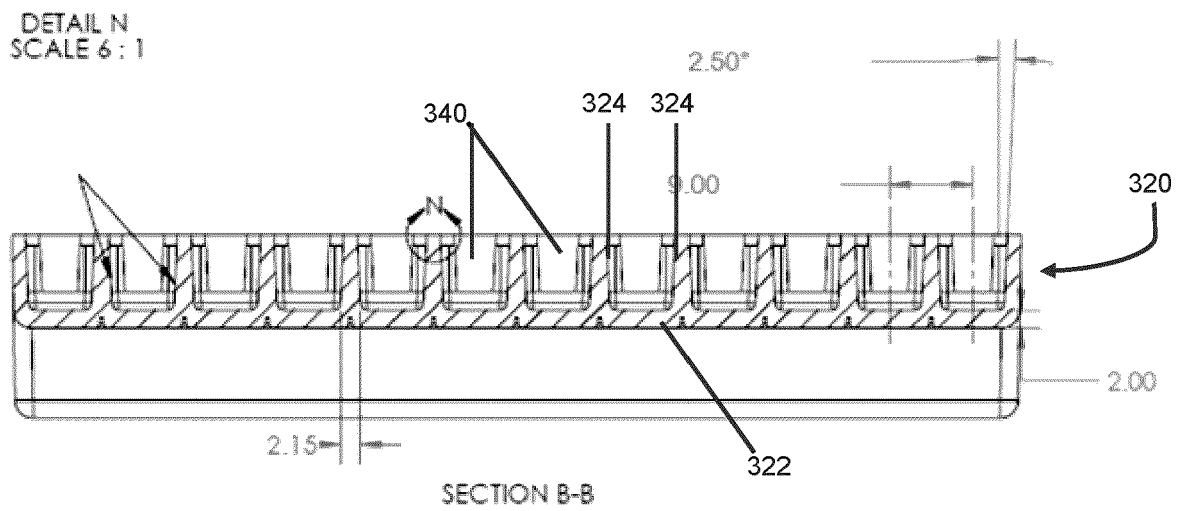


FIG. 3A



DETAIL N
SCALE 6 : 1

FIG 3B

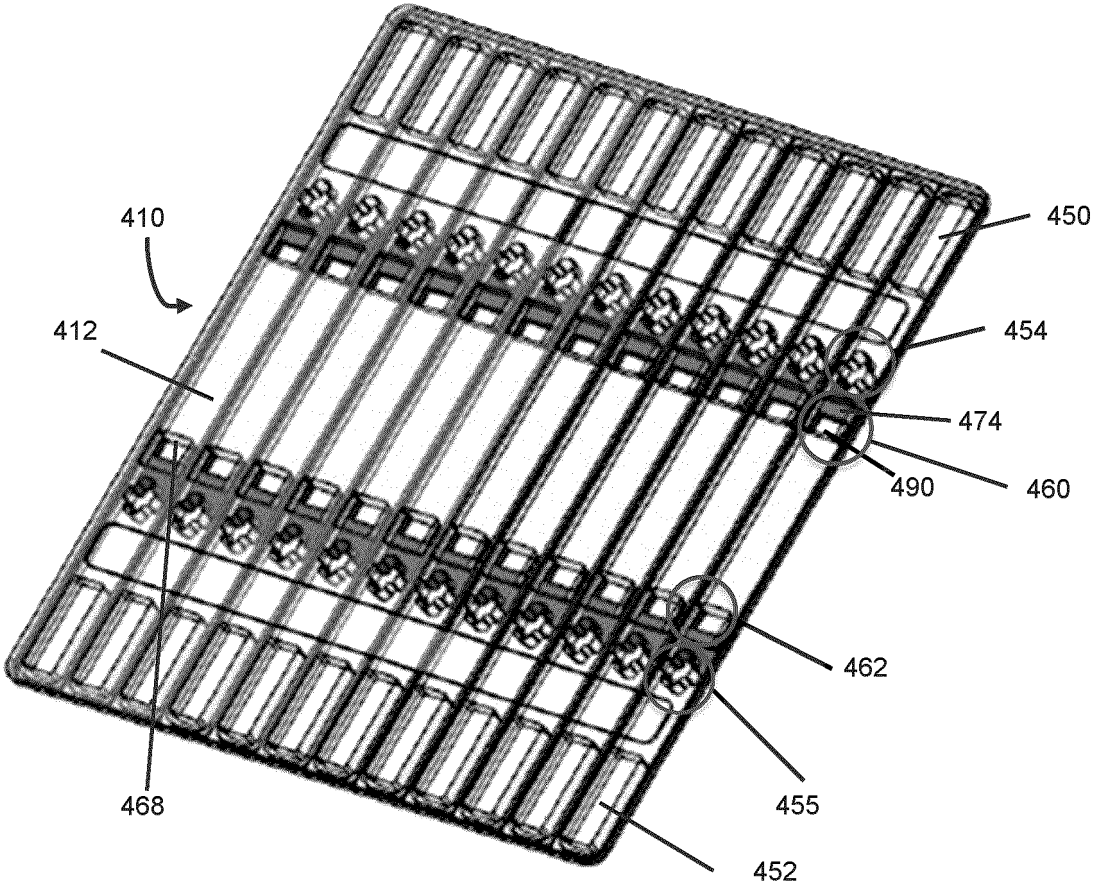


FIG. 4

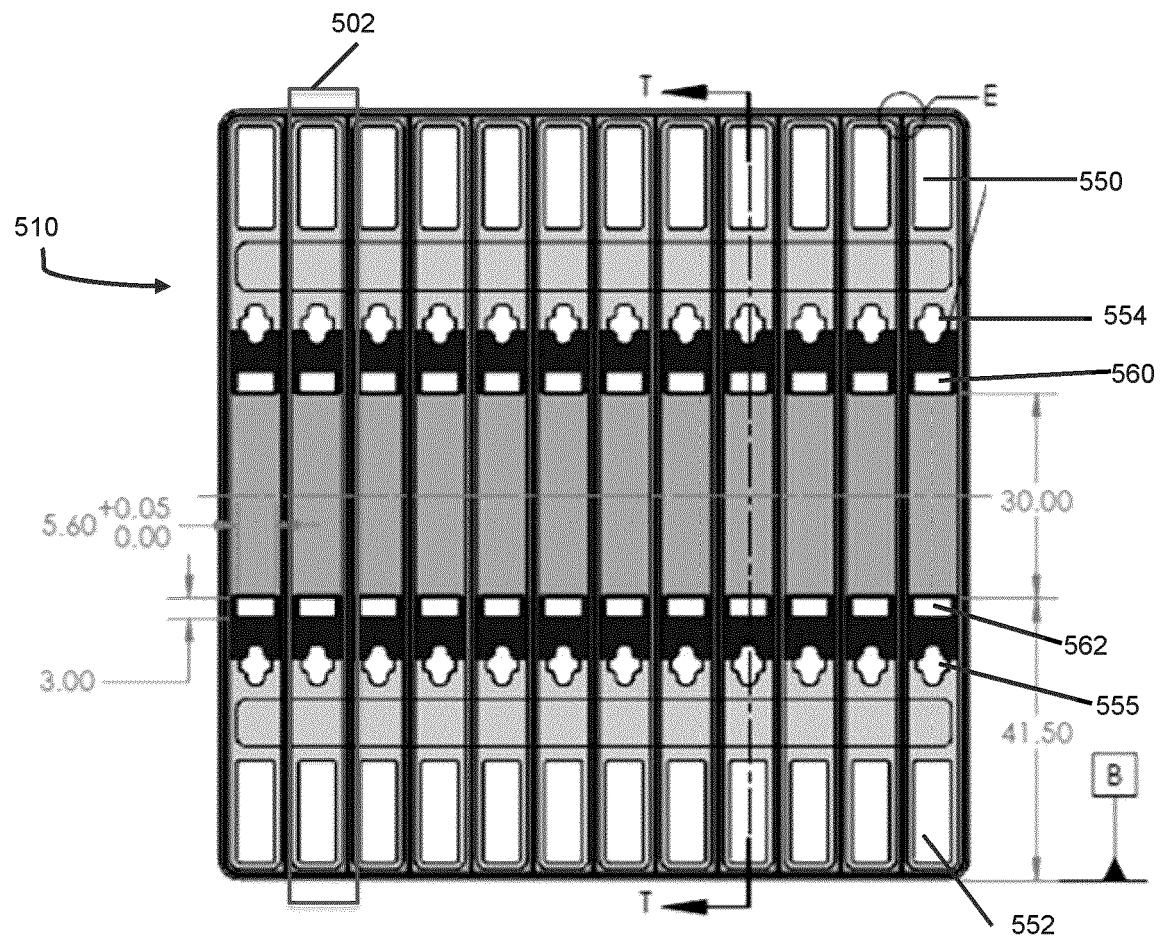


FIG. 5

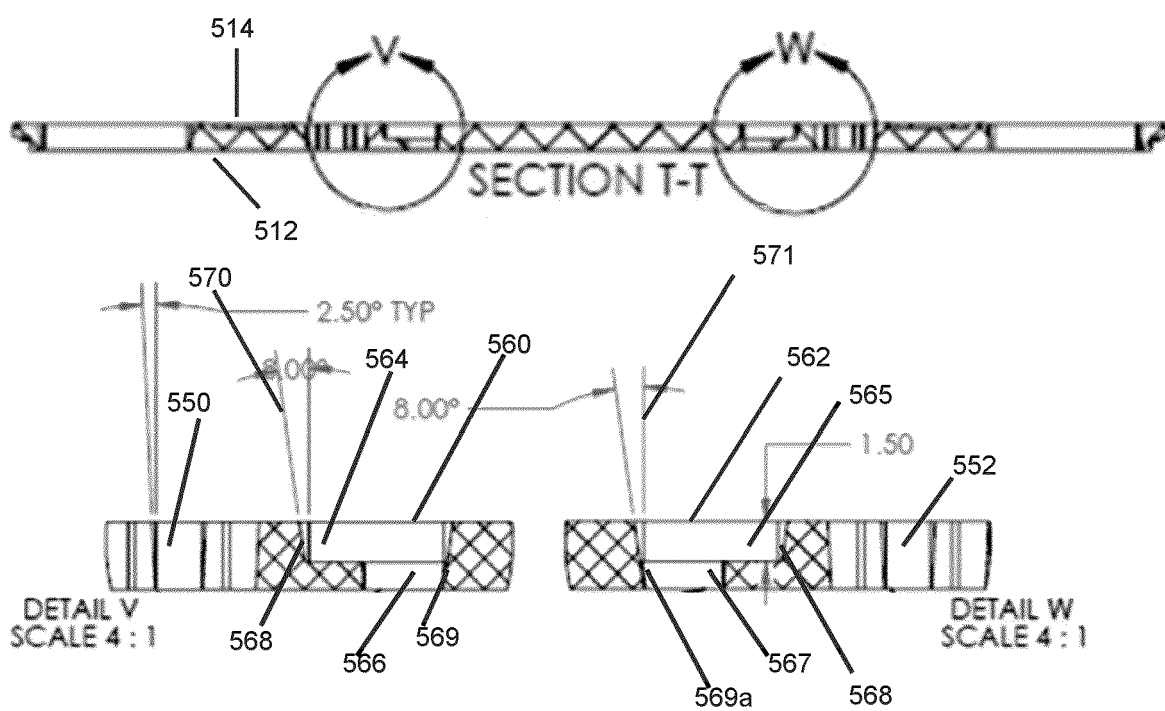


FIG. 5A

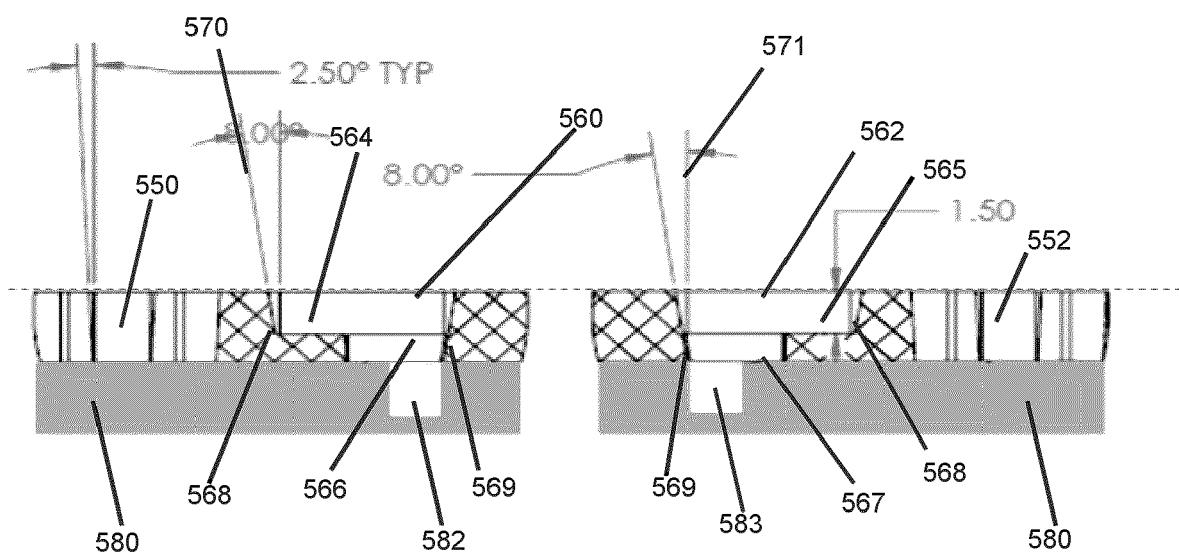


FIG. 5B

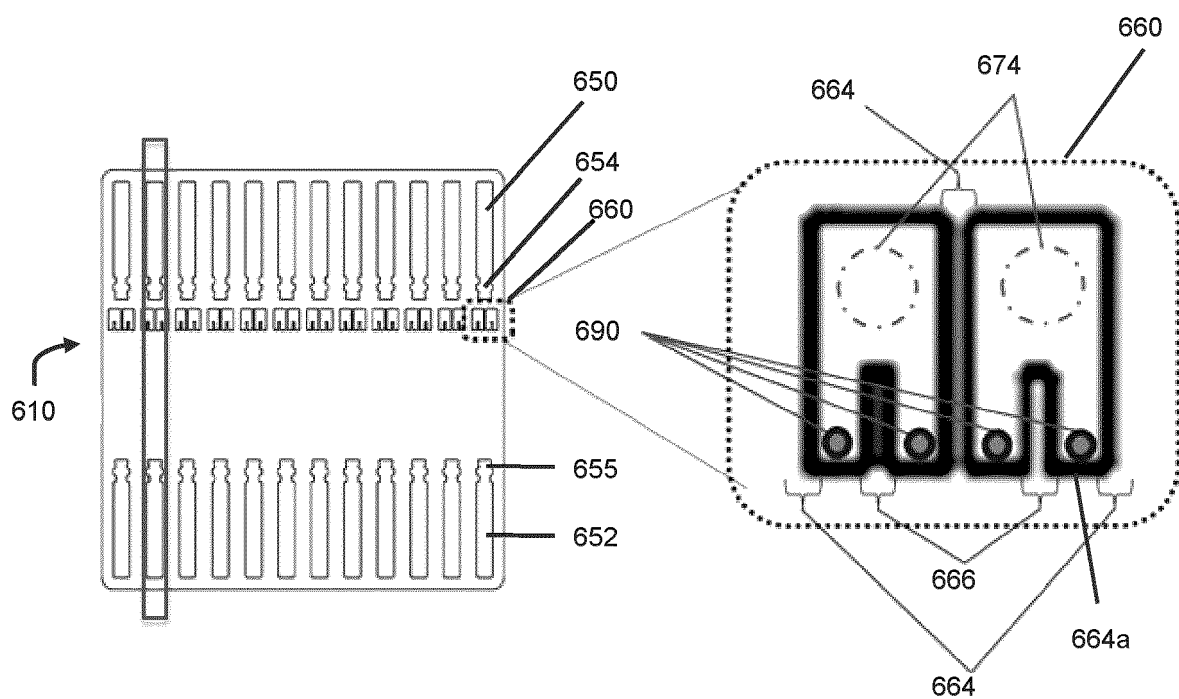


FIG. 6

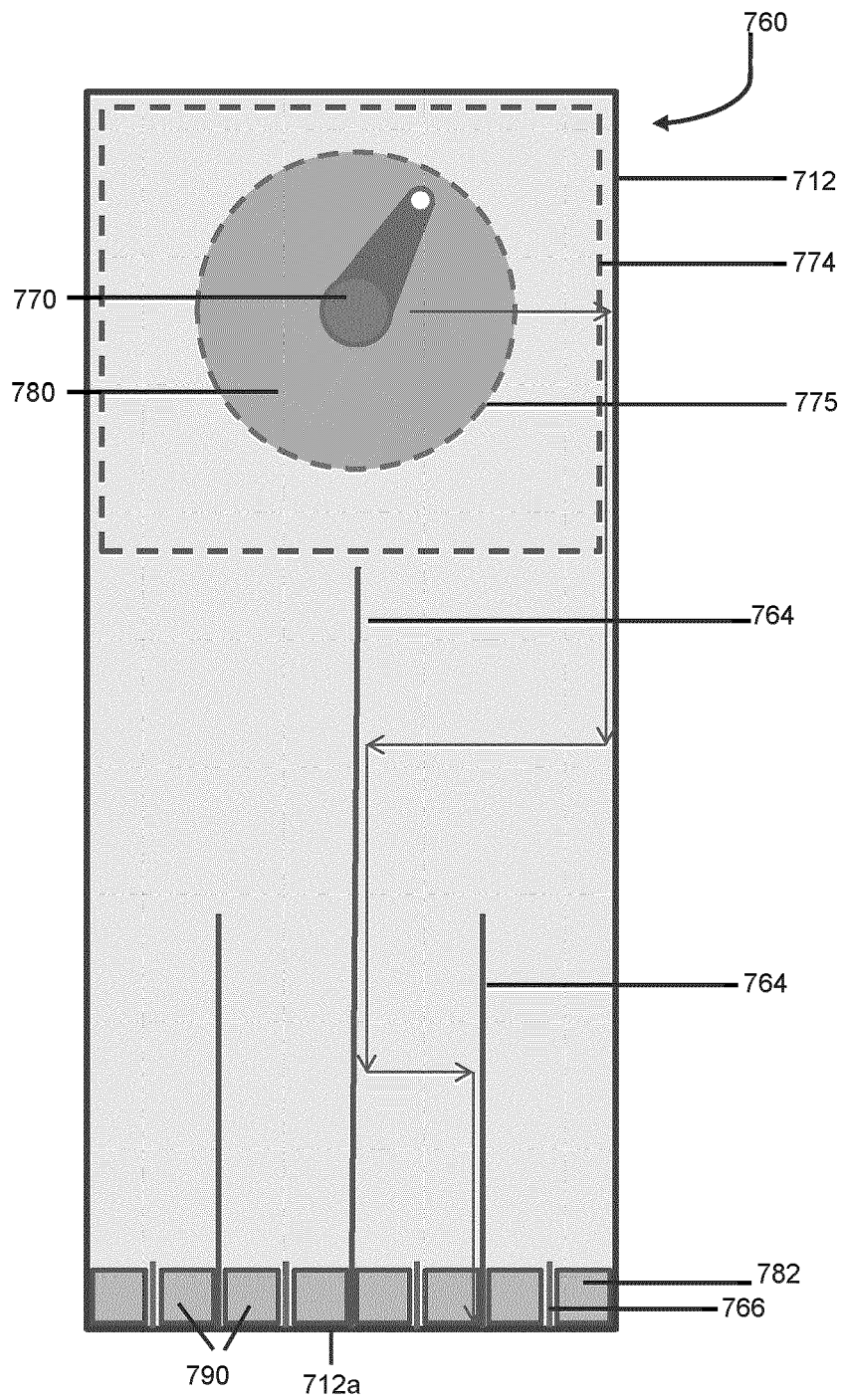


FIG. 7

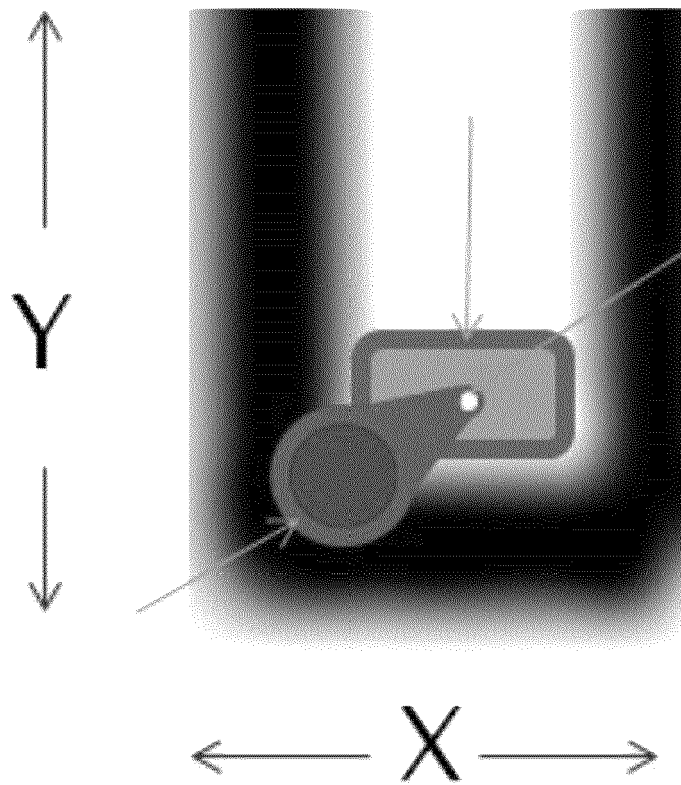


FIG. 8

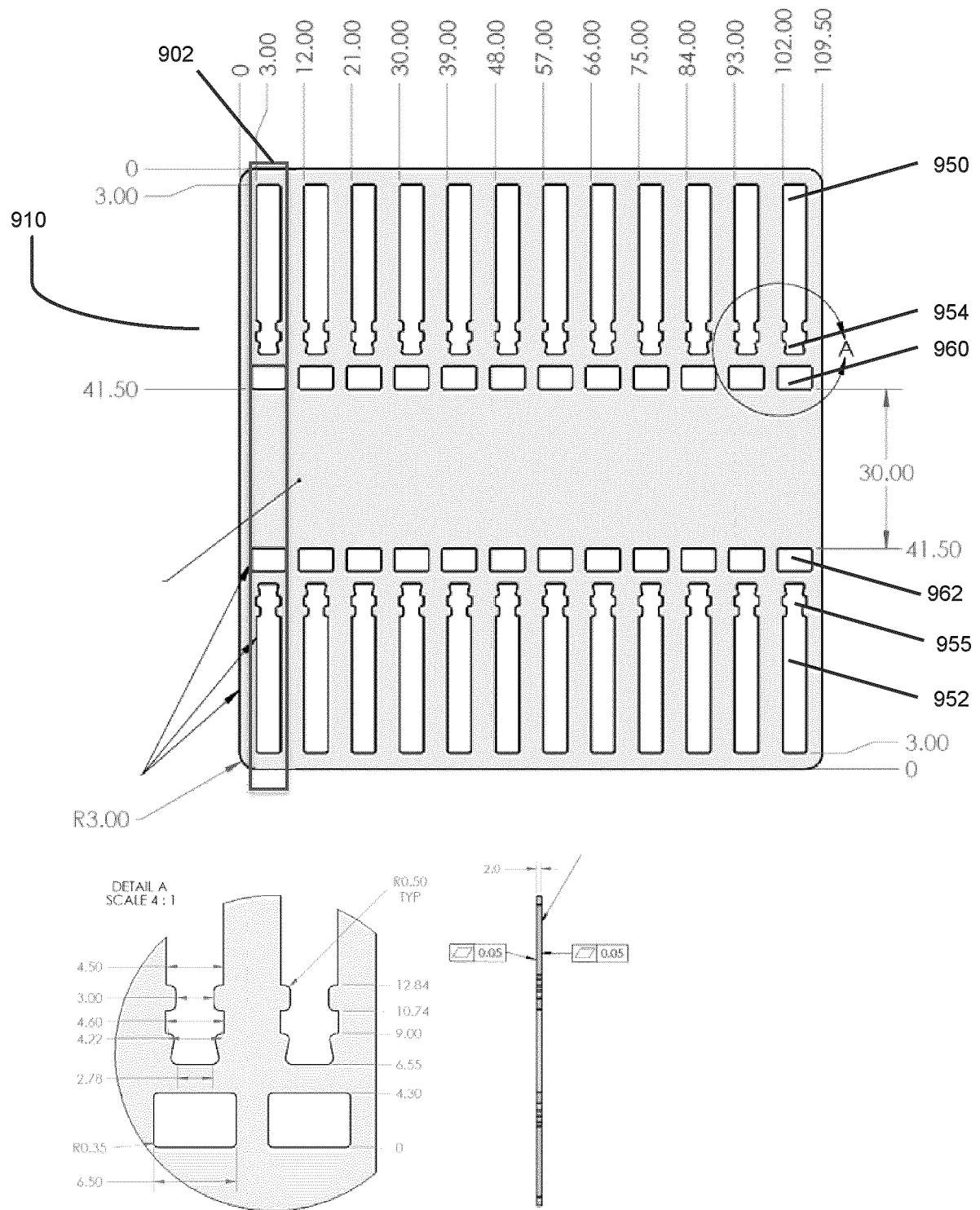


FIG. 9

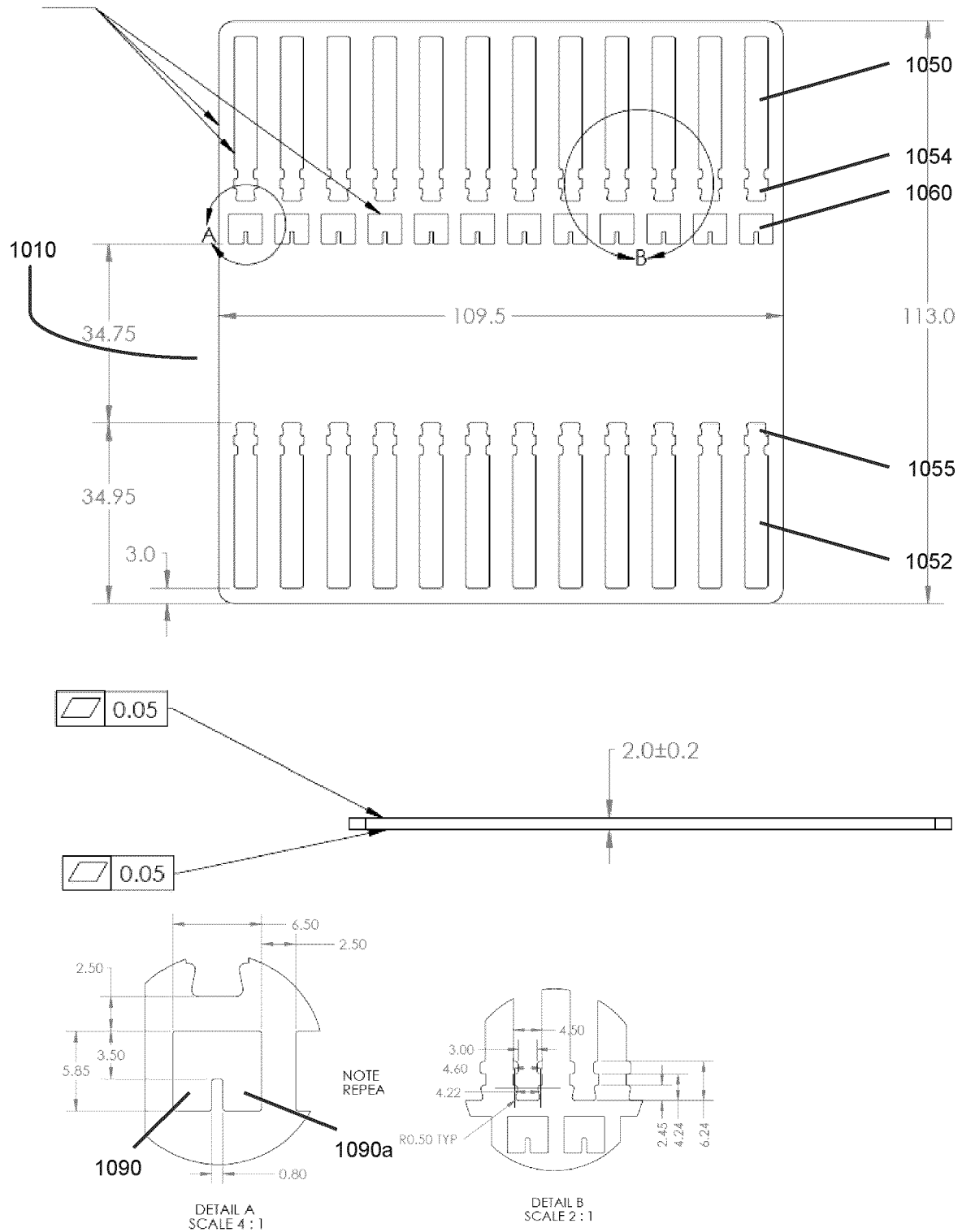


FIG. 10

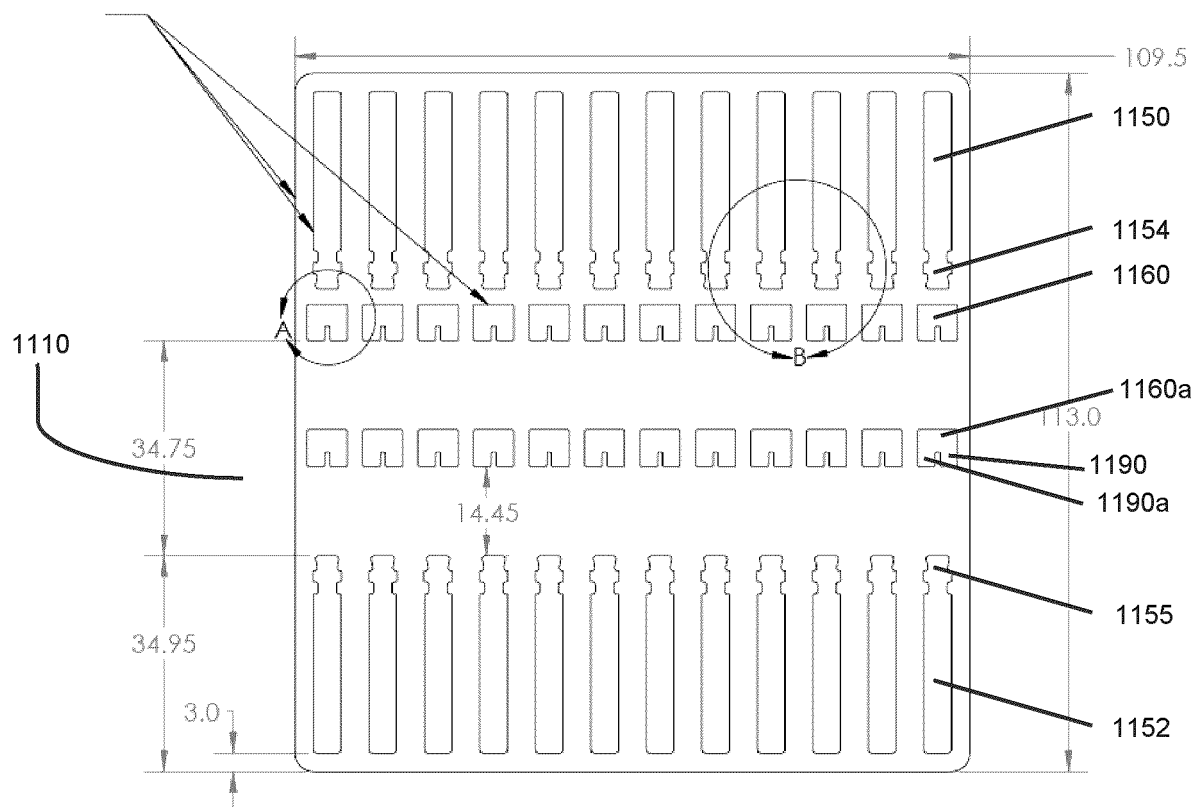


FIG. 11

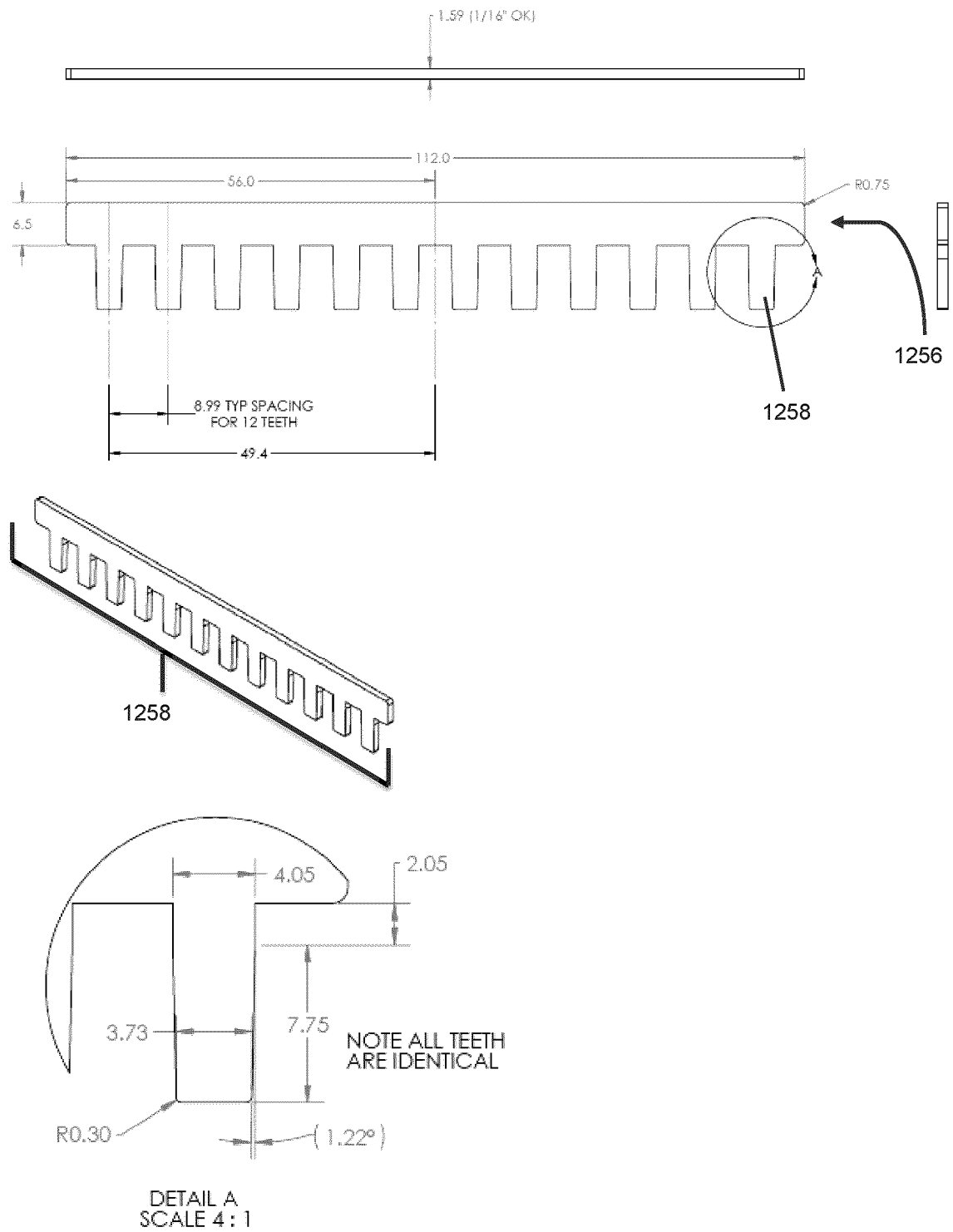


FIG. 12

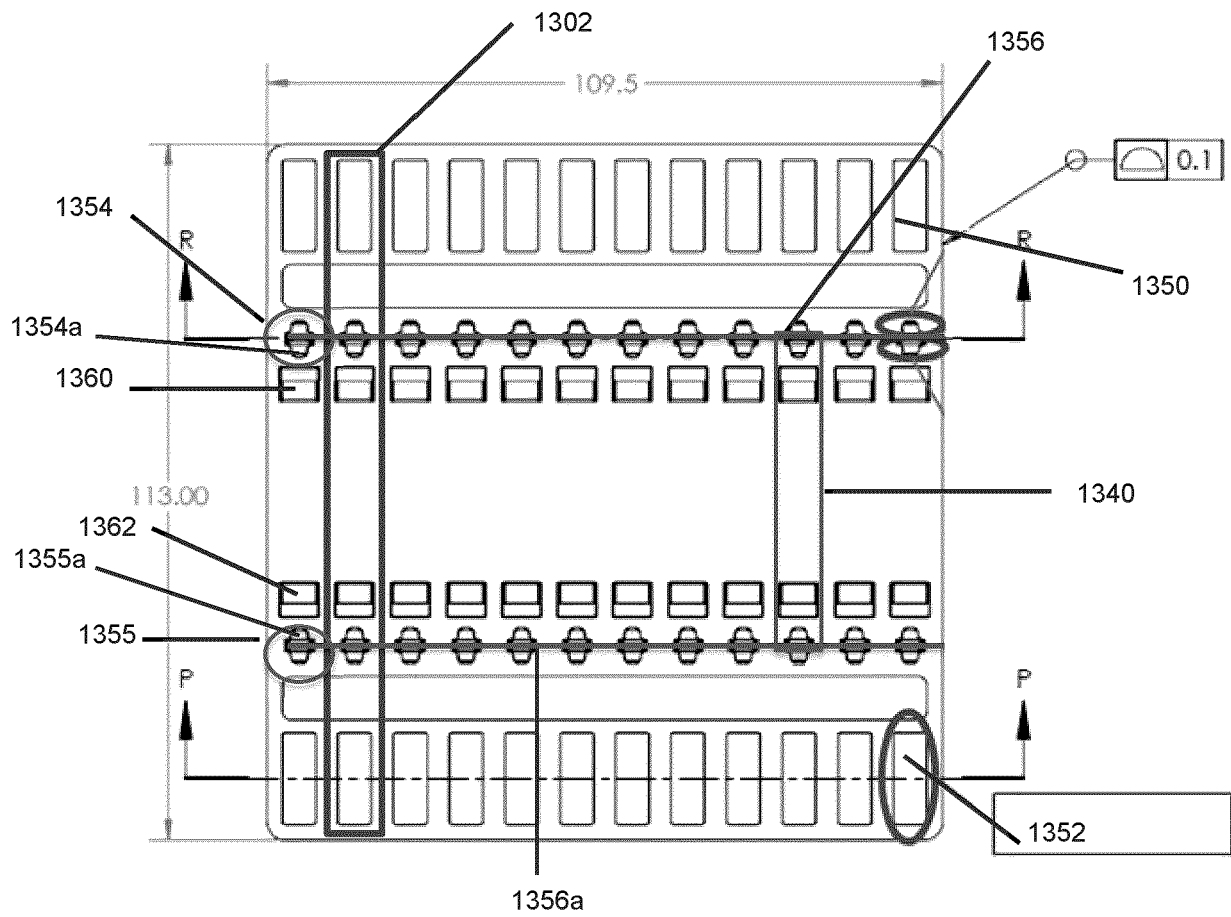


FIG. 13

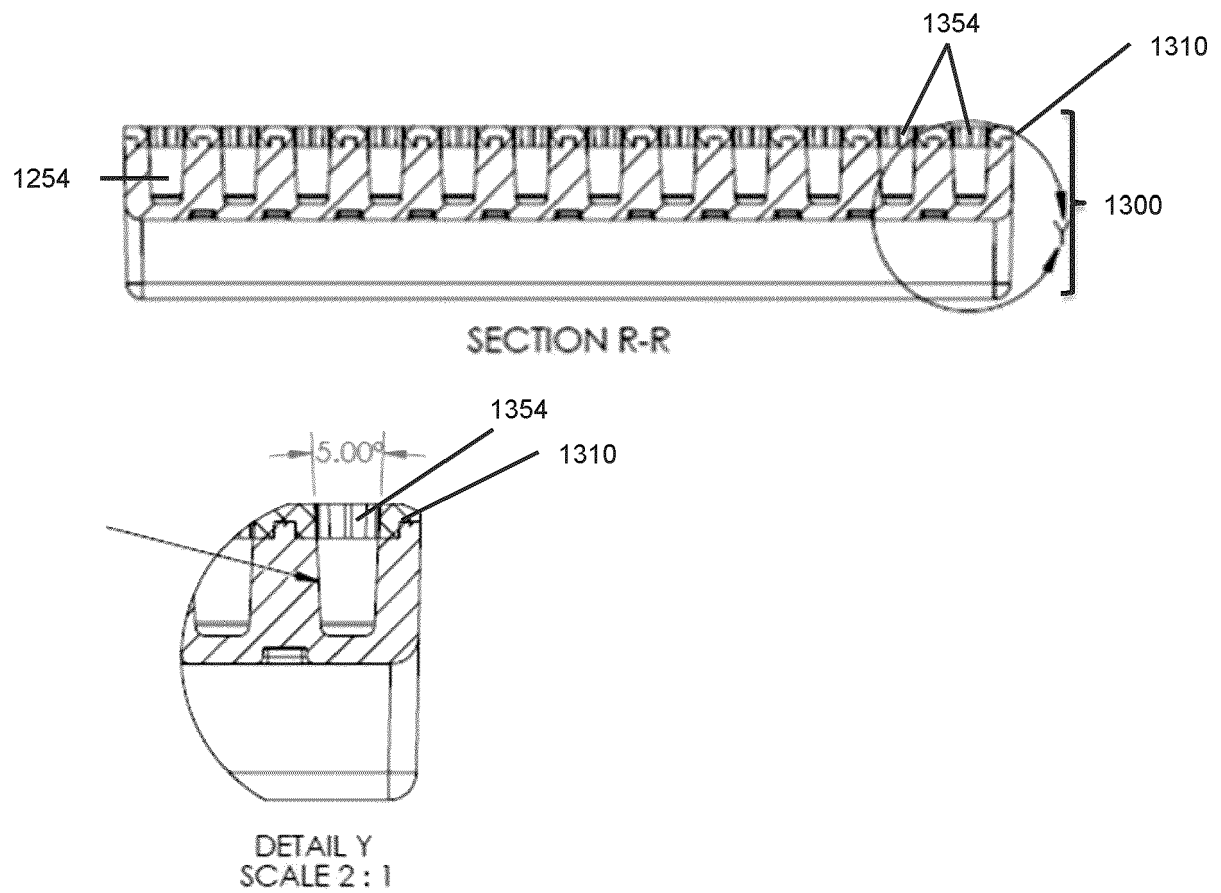


FIG. 13A

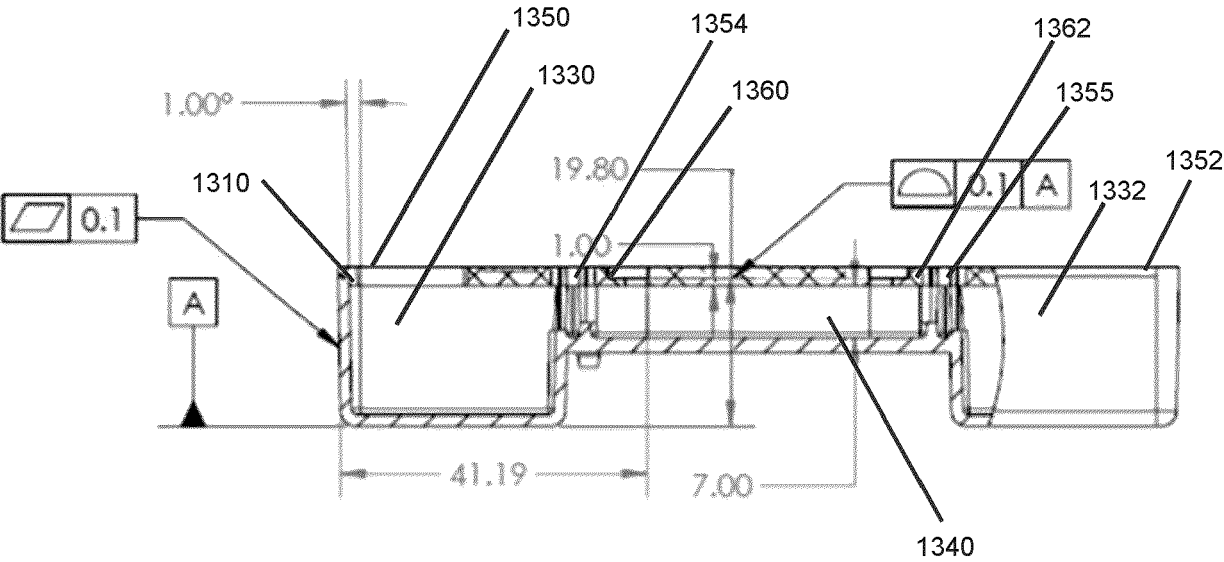


FIG. 13B

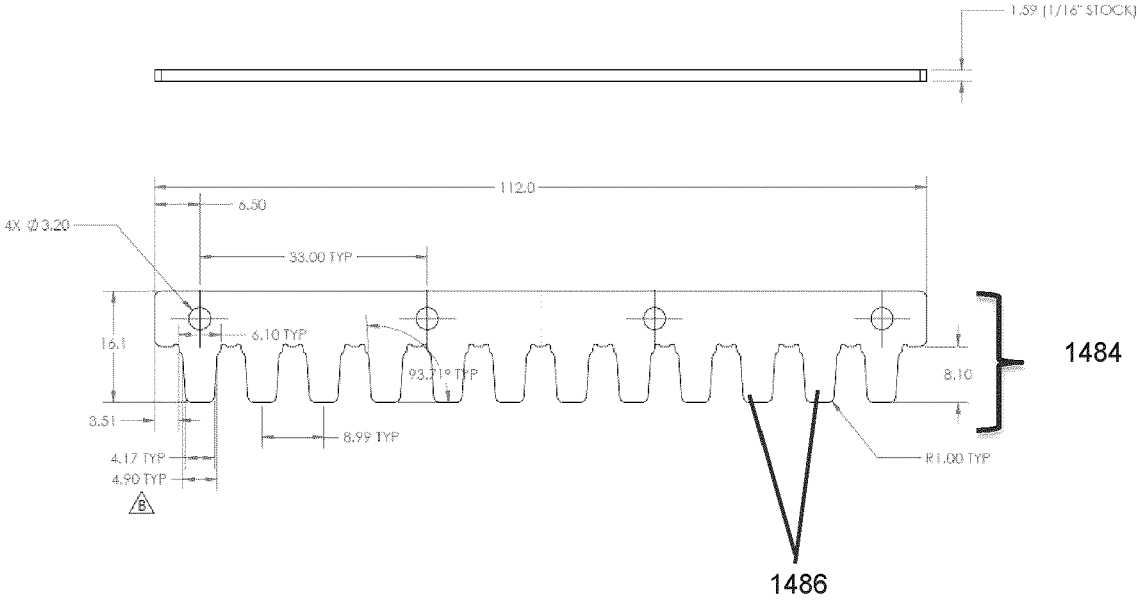


FIG. 14

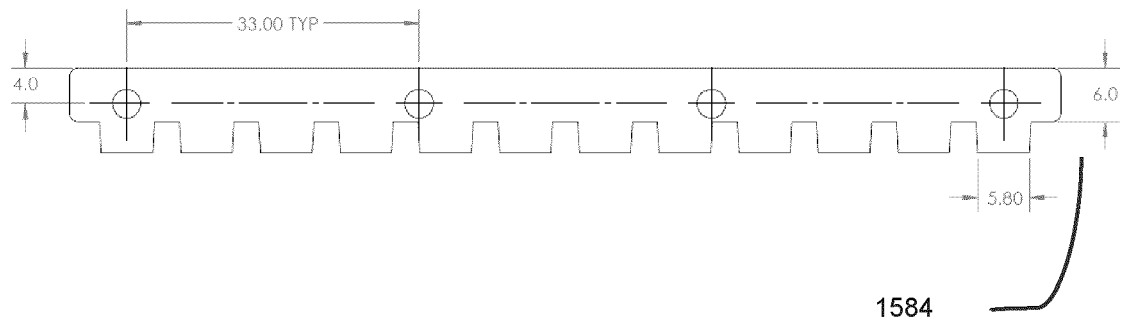


FIG. 15

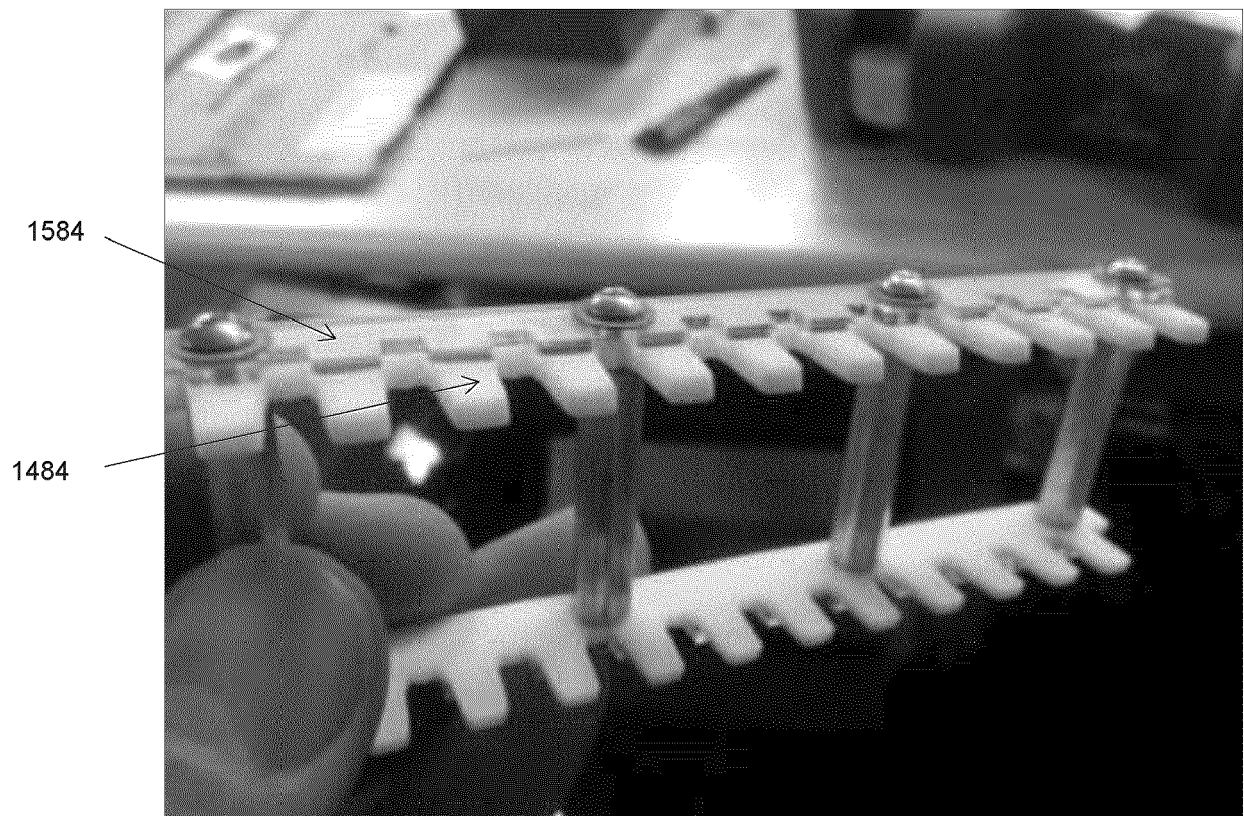


FIG. 15A

REFERENCES CITED IN THE DESCRIPTION

This list of references cited by the applicant is for the reader's convenience only. It does not form part of the European patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.

Patent documents cited in the description

- US 61929009 [0001]