



- (51) **International Patent Classification:**
B06B 1/06 (2006.01) **H01J 31/495** (2006.01)
G01N 29/34 (2006.01)
- (21) **International Application Number:**
PCT/US2015/040444
- (22) **International Filing Date:**
14 July 2015 (14.07.2015)
- (25) **Filing Language:** English
- (26) **Publication Language:** English
- (30) **Priority Data:**
62/025,873 17 July 2014 (17.07.2014) US
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(81) **Designated States** (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) **Designated States** (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— with international search report (Art. 21(3))

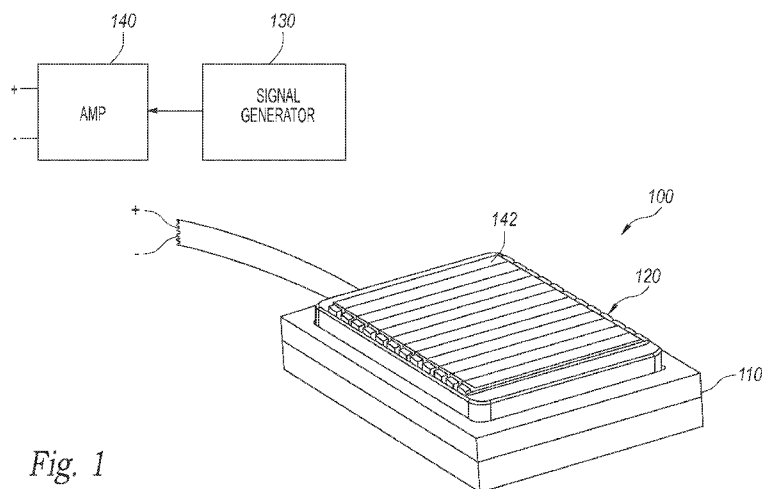
(54) **Title:** ULTRASOUND SYSTEM FOR SHEARING CELLULAR MATERIAL

Fig. 1

(57) **Abstract:** A system for processing biological or other samples includes an array of transducer elements that are positioned to align with sample wells in a microplate. Each transducer element produces ultrasound energy that is focused towards a well of the microplate with sufficient acoustic pressure to cause inertial cavitation. In one embodiment, the transducers are configured to direct ultrasound energy into cylindrical wells. In other embodiments, the transducer elements are configured to direct ultrasound energy into non-cylindrical wells of a microplate.

ULTRASOUND SYSTEM FOR SHEARING CELLULAR MATERIAL

CROSS-REFERENCE TO RELATED APPLICATION(S)

[0001] The present application is related to, and claims the benefit of, U.S. Provisional Patent Application No. 62/025,873 filed July 17, 2014, which is herein incorporated by reference in its entirety.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH

[0002] This invention was made with government support under grant nos. 1 R21 GM 111439-01 and 1 R33 CA 191135-01 awarded by the National Institutes of Health. The government has certain rights in the invention.

TECHNICAL FIELD

[0003] The disclosed technology relates to systems for preparing materials for analysis and in particular to systems for shearing cellular material by cavitation.

BACKGROUND

[0004] Sample preparation is one of the preliminary steps that is performed before biological samples are analyzed. Sample preparation often involves the breakdown of the material into cellular or subcellular fragments. One particular application is the breaking up (or shearing) of DNA or Chromatin into smaller fragments. Ultrasound is one known method of breaking down material. In some prior art devices, biological samples are placed into a test tube that is put into a liquid bath and subjected to high intensity ultrasound waves - similar to a jewelry cleaner, but with much high power. To avoid an uneven exposure of the sample, the test tube is moved around within the ultrasound field as it is processed. While this approach does work, it is limited to processing a single test tube sample at a time.

[0005] To increase the throughput of cellular processing, some systems have proposed analyzing cellular samples in microplates. As will be appreciated by those

skilled in the art, a microplate is a tray that contains an array of wells in which samples can be placed for analysis. Advantages of using microplates include the fact that such trays are easily processed with automated equipment and that multiple samples can be processed at the same time without moving the samples from one vessel to another. One system for shearing cellular samples in a microplate uses ultrasonically vibrating pins that extend into the wells. However, this can lead to cross contamination between the various wells and requires extensive cleaning of the pins. It is also not very useful for tissue samples. Furthermore, the quality of the results depends greatly on the exact position of the tips in the sample. Another approach uses a large ultrasound transducer that is positioned below a single well and focuses the energy within the well. The focused ultrasound energy creates cavitation in the sample material that is in the well but only one well is processed at a time. For a 96 element microplate, the processing time to shear all the samples can exceed several hours during which some samples may degrade.

[0006] Another suggested approach to processing cellular material in a microplate is to place a single ultrasound transducer below each well. See for example U.S. Patent No. 6,699,711 to Hahn et al. ("Hahn"). However, when trying to experiment with the system described in the Hahn patent for use in analyzing biological materials including DNA and chromatin, it was found that the system was ineffective in shearing chromatin without causing the transducers to break.

[0007] Given these problems, there is a need for a system that can both process cellular samples in parallel using high (negative) acoustic pressures to induce or facilitate shearing, and can be operated in a manner that doesn't destroy the transducers.

SUMMARY

[0008] The disclosed technology relates to systems for applying ultrasound to a number of samples that simultaneously induces and/or enhances cavitation in the samples. As will be described in further detail below, the disclosed technology uses transducer elements that are configured such that the stresses generated while the transducer is producing ultrasonic energy are not concentrated at a normal vibrational mode of the transducer element itself. In one embodiment, two or more transducers

are formed on a sheet of piezoelectric material to form an array. In another embodiment, an array of transducer elements is created by securing individual transducers to a common support that absorbs the stresses created by the individual transducer elements.

[0009] In one embodiment, an array of two or more transducers is formed from a single sheet of piezoelectric substrate material. In one embodiment, a lens is positioned in front of each transducer and focuses the ultrasound produced by the transducers towards a well of a microplate. The transducers are driven to a level that induces inertial cavitation in a biological sample that is in the well. Multiple transducers are driven in parallel to simultaneously process the material in the wells of the microplate. In another embodiment, the transducers are curved to focus the acoustic energy so that a separate lens is not needed.

[0010] In one embodiment, an array of transducer elements is formed from a sheet of piezoelectric material having one side with a conductive material disposed on the majority of the surface and a second side with a conductive material that is patterned into two or more transducer elements that are not electrically connected and have a shape that corresponds substantially to the shape of the wells in the microplate. Electrical connections are made to supply a varying voltage across the transducer elements. A microplate having flat well bottoms is placed over the transducers. A lens is positioned between each transducer element and a well of a microplate to focus ultrasound generated by each transducer towards the corresponding well.

[0011] In another embodiment of the disclosed technology, the wells of the microplate are conical in shape. For this style of microplate, the transducer elements are shaped to surround a portion of the wells. The acoustic energy passes into the well from the sides, not from the bottom. The transducer elements may be generally spherical, hemispherical, cylindrical or annular with a center region that receives a portion of a conical well of a microplate.

[0012] In one embodiment, a plate containing a separate lens for each transducer element is positioned between the transducer element and a microplate well. In another embodiment, a lens is built into each microplate well itself, which operates to focus ultrasound towards an interior portion of the well.

BRIEF DESCRIPTION OF THE DRAWINGS

[0013] Figure 1 is a diagram of an ultrasound shearing system in accordance with one embodiment of the disclosed technology;

[0014] Figure 2 illustrates a simulated focal zone created in a well of a microplate in accordance with an embodiment of the disclosed technology;

[0015] Figure 3 illustrates a cross section of a transducer assembly showing a relationship between a number of microplate wells and a corresponding number of ultrasound transducers in accordance with an embodiment of the disclosed technology;

[0016] Figure 4 shows an embodiment of the transducer assembly with a cover in an unlocked position;

[0017] Figure 5 shows the transducer assembly of Figure 4 with the cover in a locked position;

[0018] Figures 6A and 6B show one embodiment of an array of transducers for use in a transducer assembly in accordance with an embodiment of the disclosed technology;

[0019] Figure 7 shows an exploded view of a number of ultrasound transducers and a lens plate in a transducer assembly;

[0020] Figures 8A-8C show one method of making electrical connections to the transducers in a transducer assembly in accordance with an embodiment of the disclosed technology;

[0021] Figure 9 illustrates how a circuit board can be used to make connections to the transducer elements in accordance with an embodiment of the disclosed technology;

[0022] Figure 10 shows one example of a lens plate in accordance with an embodiment of the disclosed technology;

[0023] Figure 11 shows a transducer assembly with spacers that allows for the adjustment in height between different microplates and the transducers/lenses in accordance with an embodiment of the disclosed technology;

[0024] Figures 12A and 12B illustrate measured pressures in a water tank, and a model of the focal zone caused by a transducer element, superimposed with a well in accordance with an embodiment of the disclosed technology;

[0025] Figure 13A shows a number of spherical transducers that accept conical wells of a microplate in accordance with an embodiment of the disclosed technology;

[0026] Figure 13B shows a wraparound electrode that can be used with the transducers shown in Figure 13A;

[0027] Figure 14 shows a number of cylindrical transducers that accept conical wells of a microplate in accordance with an embodiment of the disclosed technology;

[0028] Figure 15 shows a number of cylindrical transducers with built in lenses that accept conical wells of a microplate in accordance with an embodiment of the disclosed technology;

[0029] Figure 16 shows a number of annular transducers that form a phased array and accept conical wells of a microplate in accordance with an embodiment of the disclosed technology;

[0030] Figure 17 shows a number of semi-spherical transducers that accept conical wells of a microplate in accordance with an embodiment of the disclosed technology;

[0031] Figure 18 shows a number of semi-spherical transducers that accept conical wells of a microplate in accordance with one embodiment of the disclosed technology;

[0032] Figure 19 shows a number of annular transducers with waveguides on one surface that accept conical wells of a microplate in accordance with an embodiment of the disclosed technology;

[0033] Figure 20 shows a number of spherical transducers that accept conical wells of a microplate in accordance with one embodiment of the disclosed technology;

[0034] Figure 21 is a graph of the expected transmission efficiency for ultrasound into a flat bottomed well of a microplate as a function of the base thickness of the bottom of the well; and

[0035] Figure 22 illustrates a microplate well having an integrated lens to focus ultrasound energy from a transducer into an interior portion of the well in accordance with an embodiment of the disclosed technology.

DETAILED DESCRIPTION

[0036] As will be discussed in further detail below, the disclosed technology relates to a system for applying a sufficient amount of ultrasound energy to a number of samples in order to cause some shearing of the molecular bonds in the samples. In one embodiment, the system simultaneously subjects a number of samples that are in the wells of a microplate to a sufficient level of ultrasound energy that causes inertial cavitation to occur in the samples.

[0037] As shown in Figure 1, a system 100 includes a transducer assembly 110 that receives a microplate 120 having a number of sample wells (not individually shown) that contains samples of biological or other materials. A signal generator 130 provides a driving signal that is applied to an amplifier 140 that in turn increases the power of the signals and supplies the amplified signals to the transducer assembly 110. Individual transducers in the transducer assembly convert the amplified driving signals into acoustic energy that is sufficient to shear the materials into components. In one embodiment, the acoustic energy is simultaneously applied to each well of the microplate, and the processing time for the samples is reduced. Furthermore, the samples may remain in the individual wells of the microplate and therefore may not need to be moved to another container for further processing. In the embodiment shown, a spill cover 142 is placed over the individual wells of the microplate 120 to prevent cross contamination of the wells of the microplate. The spill cover may or may not have fingers (not shown) that protrude into the samples when the cover is in place and can be temperature controlled by, for example, running a cooling liquid through passageways (not shown) in a top cover.

[0038] Figure 2 is a simulation of the ultrasound energy that is created by a transducer in a single well of a microplate. In this example, a single cylindrical well 150 is part of a larger microplate (not shown) and contains a biological sample 160 in the well. A piezoelectric transducer 170 is positioned below the well 150 and produces ultrasonic energy. The energy is focused by a concave lens 172 that is positioned

between the transducer 170 and the bottom of the well 150. Typically there would also be some coupling fluid such as water or a gel (not shown) that is located between the lens 172 and the bottom of the well 150 in order to provide a good acoustic coupling and to reduce reflections. The lens focuses the acoustic energy into a focal zone 176 that is towards the well 150 at pressure levels that are sufficient to cause inertial cavitation in the sample 160. Inertial cavitation causes bubbles to be created in the sample that collapse with an energy that is sufficient to shear the biological material into smaller components. In one embodiment, the focused acoustic energy is sufficient to cause chromatin shearing to yield 100-300 base pair fragments in each well of the microplate (e.g. an acoustic pressure amplitude of > 1 Megapascal). This figure is representative only and does not consider the complications of having a mixture of bubbles in the sample, nor having a finite volume of liquid in the well. It will be appreciated that the focus of the transducer element need not necessarily be in the well itself. Cavitation can still occur in a sample that is located in a pre-focal or post-focal area. Therefore, the lens need only focus the ultrasound towards the well and not necessarily into the well itself.

[0039] Figure 3 illustrates a cross-sectional view of one possible embodiment of the ultrasound transducer assembly 110 and the microplate 120. The microplate 120 includes a number of individual generally cylindrical wells 122a, 122b, 122c...122h. In one embodiment, the microplate 120 has 96 individual wells arranged in an 8 x 12 grid. However, it will be appreciated that other sizes of microplates could be used, and the transducer array could be adjusted to fit the appropriate microplate well configuration (number of elements and size of each element).

[0040] Positioned below each individual well of the microplate is one or more corresponding ultrasound transducers. For example, an ultrasound transducer 170a is positioned below well 122a. An ultrasound transducer 170b is positioned below well 122b etc. A coupling material 180 such as degassed water or a gel is positioned between the ultrasound transducers and the individual wells to provide a good acoustic coupling for the acoustic energy produced by the ultrasound transducers into the material contained in each of the wells.

[0041] In some embodiments, the transducer assembly 110 can include a locking top cover that is placed over the wells of the microplate hold the microplate in relation to the transducers.

[0042] Figure 4 shows a top cover 200 having an inner surface 202 that is sized to fit over the outer perimeter of the microplate 120 and over the spill cover 142. The top surface of the top cover 200 includes first and second cylindrical detents 204, 206 on opposite sides thereof. The detents 204 and 206 are shaped to receive corresponding cylindrical rods 208 and 210 that are located on arms 212 and 214 that are hinged to the transducer assembly 110. When the hinged arms are positioned in the unlocked position, the rods 208 and 210 swing away from the detents 204, 206 on the top of the cover and the cover can be lifted off the microplate. When the arms are rotated to a locked position, the rod 208 engages the detent 204 and the rod 210 engages the detent 206 as shown in Figure 5. With the arms in the locked position, the top cover 200 is secured over the microplate and to the ultrasound transducer assembly 110 as shown in Figure 5. This is one possible embodiment for a cover, and it is recognized that many other ways to hold the microplate in place can be used. The top cover can also be kept cool to help reduce sample heating by running coolant through slots in the top cover, or by directly cooling the cover itself. In one embodiment, the coupling material 180 that is positioned between the wells of the microplate and the individual transducer elements remains static. In another embodiment, the coupling material can be kept moving through ports (not shown) on the transducer assembly with a pump mechanism or the like in order to remove heat that is created during the application of ultrasound energy to the individual wells.

[0043] One of the problems encountered in applying ultrasound energy to the wells with an acoustic pressure that is sufficient to create shearing in a sample is that the transducers can crack or be damaged. To overcome this problem, one embodiment of the disclosed technology groups the ultrasound transducer elements into a multi-element array in order to spread the stresses created by any single transducer over a larger area, or offsetting the stresses from the normal vibrational modes of the transducer element. Figures 6A and 6B illustrate one embodiment of a transducer array for applying acoustic energy to corresponding wells in a microplate. In the embodiment shown, an array comprises a sheet of piezoelectric material 250 having a first side 252 and a second side 254. The first side 252 is coated with a conductive

material such as silver over its entire surface. The second side 254 includes two or more transducer patterns 256 and 258. The transducer patterns can be made via a number of techniques such as etching. In one embodiment, the transducer patterns 256, 258 are created with a photolithographic process by coating the side 254 with a conductive material followed by a resist material. The resist material is exposed with a mask pattern and then chemically etched to remove the conductive material where it is not desired. After etching the conductive material, the patterns of the transducers 256 and 258 are left on the surface of the piezoelectric sheet. In the embodiment shown, the transducers 256 and 258 are circular in shape to correspond to the shape of the bottom surface of the wells of the microplate. However, it would be appreciated that other shapes could be used if desired, or even no patterning at all.

[0044] In one embodiment, the conductive coating on the first side 252 of the substrate is connected via one or more electrical leads to one electrical potential such as ground, while the transducers 256, 258 are connected via individual leads to a positive potential. Upon the application of sufficient voltage signals to the transducers 256, 258, the transducers will produce ultrasonic sound waves that can be coupled into the individual wells of the microplate. The electrodes can be wired such that each transducer element is driven in parallel with other transducer elements or in a manner such that each individual transducer element can be driven separately from other elements.

[0045] Figure 7 is an exploded view of a transducer assembly 110 without the top cover that is constructed in accordance with one possible embodiment of the disclosed technology. The transducer assembly 110 includes a base 260, an electrode support plate 270, a plate 280 having transducers bonded to a bottom surface thereof and a top cap 290. The base 260 is a generally rectangular enclosure a closed bottom surface, and an opening 262 in a sidewall through which conductors to the individual transducer elements can be routed. The support plate 270 is constructed to support a number of spring loaded contacts or "pogo pins" in an array that corresponds to the arrangement of the transducer elements. In one embodiment, the contacts are wired in parallel so that individual wires do not need to be routed from each individual transducer element to a position outside the assembly. However, it is possible to wire each individual transducer element separately if desired, which may or may not include pogo pins.

[0046] Above the support plate 270 is the plate 280 with the one or more arrays of transducer elements secured to a bottom surface thereof with an acoustically matched epoxy or other adhesive. As will be explained in further detail below, in one embodiment the plate 280 includes a number of lenses positioned over a corresponding transducer element in order to focus ultrasound energy created by the transducer element towards a well of a microplate. In one embodiment, the plate 280 is made of a metal such as aluminum having the lenses formed directly into the plate 280. However, other materials such as ceramics could be used if desired. In yet another embodiment, separate lens elements may be secured to the plate 280. The top cap 290 fits over the surface of the plate 280 and is secured to the base plate 260 with screws or the like in order to secure the plate 280 and transducers against the number of spring loaded pins that are held in the support plate 270. A rim 296 extending around an inner perimeter of the top cap 290 supports a microplate (not shown) at a fixed distance from the top surface of the plate 280 so that ultrasound is focused at the correct location towards the wells of the microplate. In one embodiment, liquid, gel or other material is placed into an opening of the cap 290 prior to the placement of a microplate in order to effectively couple the acoustic energy produced by the transducers into the wells of the microplate.

[0047] Figure 8A shows one embodiment of the support plate 270 that supports a number of electrical contacts that connect to the various transducer elements of the transducer assembly. The support plate 270 has an outer rim 272 that surrounds an arrangement of cylindrical bores 274 in which individual contact pins are fitted. In one embodiment, the cylindrical bores 274 are supported by a honeycomb arrangement of fins 276 that extend outwardly from each of the bores. Spaces between the fins 276 serve to decrease the weight of the support plate 270. In the embodiment shown, the top surface of each cylindrical bore has a flat top section that is joined at its corners to an adjacent flat top section of another cylindrical bore. The pattern of cylindrical bores 274 is designed to match the corresponding pattern of transducer elements and also to the pattern of wells in the microplate. The support plate 270 can be molded, created by a 3-D printer or constructed using other techniques.

[0048] Figure 8B illustrates how the conductive pins 278 are secured within the support plate 270 and engaged against the surface of the corresponding transducer elements that are on the bottom surface of the plate 280. The conductive pins can be

press fit into a cylindrical bore or can be secured by an adhesive. Alignment pins at the corners of the support plate 270 align the plate when it is placed in the base section and also provide a vertical space for the pins. As shown in Figure 8C, the support plate 270 is secured to the cap 290 of the ultrasound assembly with fasteners such as screws or the like. The top cap 290 is secured to the base 260 to complete the transducer unit.

[0049] As an alternative to using spring-loaded conductors, other mechanisms can be used to supply the required current and voltages to the transducer elements. Figure 9 illustrates one embodiment that uses a printed circuit board to supply driving signals to the transducer elements. In this embodiment, a lens plate 350 has a number of transducer elements 352 secured to a rear surface thereof. In the embodiment shown, each of the transducer elements is formed as an array 354 of two transducer elements on a section of piezoelectric substrate. Groups of these transducer element pairs are arranged in a pattern corresponding to the pattern of wells in a corresponding microplate. In this embodiment, a printed circuit board 360 has a number of openings 362 that correspond to the position of the transducer elements. The dimensions of the openings 362 are slightly smaller than the dimensions of the transducer elements so that a portion of the printed circuit board 360 overlaps a portion of the outer perimeter of the transducer elements. Therefore, electrical contacts placed on the surface of the printed circuit board that engages the outer portion of the transducer elements can be used to deliver signals to the transducer element. Traces can be routed through the printed circuit board in order to wire the transducer elements in parallel, in groups of transducer elements or individually. It is recognized that the pins or printed circuit board are only to deliver electrical signals to the transducer elements. Direct soldering and other methods exist to deliver electrical signals.

[0050] Figure 10 illustrates further details of the plate 280 in accordance with the embodiment of the disclosed technology. As indicated above, the plate can be made of a metal such as aluminum, ceramic or graphite or other materials having good acoustic transmission characteristics that couple the acoustic energy produced by the transducers into the wells of a corresponding microplate. In the embodiment shown, the top surface of the plate 280 includes a number of concave lenses 282 formed therein. In one embodiment, each lens is constructed to focus ultrasound energy from a transducer element at a distance corresponding to the diameter of the lens (i.e. an F1

lens). However, it will be appreciated that other lens designs could be used. The lens plate can be cast or machined.

[0051] Figure 11 illustrates one embodiment of a system for securing a microplate at a desired level above the lens plate. In one embodiment, the transducer assembly includes a number of adjustable spacers 366 having a height selected to position the bottom of the microplate wells at a desired height above the surface of the lenses. Different spacers can be selected depending upon the type of microplate being used in order to ensure that the focal zone of the transducers is positioned in a desired portion of the wells. In one embodiment, the spacers 366 are rods having a diameter selected in accordance with the type of microplate being used. The rods are positioned at opposite ends of the lens plate. The microplate is spaced from the lens plate by a distance corresponding to the diameter of the rods set to the height above the lenses.

[0052] Figure 12A and 12B illustrate the acoustic pressures generated by a single transducer element. Figure 12A shows the measured acoustic pressure in a water tank at the focus of the transducer element. A 15 microsecond driving pulse at a frequency of 2 MHz and a driving field of 400 Volts/mm creates a positive pressure in excess of 30 MPa and a negative pressure of over 15 MPa in approximately 10 microseconds. The pressures rise and fall as the driving signal is applied to the transducer element and is then turned off. Figure 12B shows a mathematical simulation of the pressures in a microplate well. As can be seen, the area of greatest absolute pressure is created at a distance 4-7 mm above the bottom surface of the well and in the center of the well. Pressures in this range have been determined to have sufficient power to create inertial cavitation in a biological sample. The lower limit of pressures required to induce or facilitate inertial cavitation has not been determined and may be lower than the pressures described. As an example, it has been determined that negative pressures should be greater than 5 MPa. In practice, the voltages applied to the transducer elements are increased until cavitation can be detected. The focus can be adjusted to accommodate more or less sample in the well by changing one or more of the lens geometry, the frequency of the ultrasound signals applied or the spacing between the transducer elements and microplate. As previously stated, one does not have to have the focus inside the well. Cavitation can also be generated in the pre-focal or post-focal region.

[0053] In some instances, the wells of the microplate may not be cylindrical. Therefore, embodiments of the disclosed technology are constructed and arranged to receive non-cylindrical wells in order to focus the ultrasound energy into the biological samples held by the wells. In an embodiment shown in Figure 13A, a number of transducer elements 400 are generally spherical having a hole at their top and bottom that receive a conical well 420 of a microplate. The transducer elements 400 have an inner surface 402 and an outer surface 404. The inner surface 402 includes a first electrode thereon and the outer surface 404 includes a second electrode thereon. In one embodiment, a wire can be bonded to the inner surface 402 and routed through a hole in the transducer element to a signal source. In another embodiment as shown in Figure 13B, a wraparound electrode 408 is electrically connected to the electrode on the inner surface and terminates on the exterior surface of the spherical transducer. An outer electrode 410 on the outer surface of the transducer element surrounds but does not touch the electrode 408. In one embodiment, the electrodes 408 and 410 are connected by wires to a signal source to cause the spherical transducer element 400 to vibrate and produce ultrasound energy.

[0054] In one embodiment, a coupling material such as liquid or a gel is disposed between the interior surface of the transducer element 400 and a conical well 420 of a microplate. The spherical shape of the transducer elements 400 cause the acoustic energy created by the application of a positive and negative voltage of the interior and exterior electrodes of the spherical transducer elements to be focused within the conical well of the microplate element. In one embodiment, the spherical transducer elements are cast as hemispheres and are sintered together once the electrodes are patterned on the inside and outside surfaces of the electrode elements.

[0055] Figure 14 illustrates yet another alternative embodiment of a number of transducer elements that are designed to transmit ultrasound energy into a non-cylindrical well of a microplate. In this embodiment, a number of transducers 450 are generally cylindrical with an inner diameter that is large enough to accept a portion of a conical well of a microplate. The transducer elements include an inner surface 452 and an outer surface 454. Electrodes placed on the inner and outer surfaces allow the application of a voltage and current signal to the transducer element to cause it to vibrate and produce ultrasound energy which is focused in a zone within the well of the microplate. In one embodiment, a coupling fluid is placed between the inner surface of

the transducer 450 and the exterior surface of the microplate wells to couple the acoustic energy into the microplate well. In one embodiment, wires can be used to connect the electrodes on the interior surface 452 and the outer surface 454 to a signal source. In an alternative embodiment, wrap around electrodes can be used to route an electrode that is electrically coupled to the electrode on the interior surface 452 to a position that is on the exterior of the cylindrical transducer 450. Wires or other conductors can then be used to connect the electrodes to a current and voltage source.

[0056] Figure 15 illustrates a number of transducer elements in accordance with another embodiment of the disclosed technology. Each of these transducer elements is designed to focus ultrasound energy into a non-cylindrical well of a microplate. In this example, each transducer 460 has a generally cylindrical shape with an inner diameter that is sized to receive a portion of the conical well of a microplate. An electrode 462 on an interior surface of the transducer element and an electrode 464 on the exterior surface of the transducer element are used to supply a current and voltage to the transducer element. In this embodiment, a lens 470 is positioned between the interior surface of the transducer element and the well of the microplate. In the embodiment shown, the lens 470 has a radial thickness that varies parabolically whereby it is thinner at a top end 472 of the transducer element and radially thicker at a bottom surface 474 of the transducer element. In one embodiment, the lens 470 is made of an acoustically transparent material such as aluminum or a ceramic. The design of the lens is selected to focus ultrasound energy created by the cylindrical electrode into the middle of the well.

[0057] Yet another alternative embodiment of a transducer element in accordance with the disclosed technology is shown in Figure 16. In this embodiment, a transducer 500 includes a number of annular transducer elements 502a, 502b... 502i. Each of the transducer elements 502a-502i is annular in shape with an outer electrode 506 disposed on the outer perimeter of the transducer element and an inner electrode 504 on an inner perimeter of the transducer. Again, as described above, connections to the electrodes can be made with wires or a wraparound electrode can be used to electrically connect to the inner electrode. Application of a current and voltage signal to the inner and outer electrodes cause the transducer elements 502a-502i to produce ultrasound signals. Because the elements 502a-502i are stacked, they can be driven

as a phased array to control focusing of the ultrasound waves into a desired portion of the wells of a microplate.

[0058] Figure 17 shows yet another embodiment of a transducer designed to direct ultrasound energy into the non-cylindrical wells of a microplate. In this embodiment, each transducer 520 comprises a hemispherical shell having an inner surface 522 and an outer surface 524. The shell is arranged as a cup whereby the open portion of the hemisphere faces toward the top of the well. The transducer elements have electrodes on the inner and outer surfaces of the hemisphere such that application of a current and voltage signal to the inner and outer electrodes causes the transducer element 520 to produce ultrasound energy and direct it into a desired zone within the conical portion of the microplate well. Because the transducer elements are partially spherical, ultrasound energy is generally directed towards the geometric center of the hemisphere. The transducer element includes a hole through its lower surface through which a tip of the microplate well extends. In one embodiment, separate wires can be attached to the inner and outer electrodes of the transducer element 520. Alternatively, wrap-around electrodes can be used to allow the wires that connect to both electrodes to be located on the exterior surface of the electrode.

[0059] Figure 18 illustrates a number of transducer elements that are designed to direct ultrasound energy into a non-cylindrical well of a microplate in accordance with an embodiment of the disclosed technology. In this embodiment, the transducer elements 540 are hemispherically shaped with an interior surface 542 and an exterior surface 544. Electrodes are placed on the interior and outer surfaces such that application of a current and voltage signal to the electrodes causes the transducer element 540 to produce ultrasonic energy which is focused at a desired location in the well of the microplate. In this embodiment, the transducer elements are arranged as umbrellas whereby the larger diameter of the hemisphere is pointed towards the bottom of the microplate well. Again, because the transducer element 540 is hemispherically shaped, the ultrasound energy produced will be focused at roughly the geometric center of the transducer. A hole positioned at the top surface of the electrode receives the tip of the microplate well such that the microplate well extends through the center of the transducer element 540. Connections to the electrodes on an interior of the transducer elements can be made with individual wires. Alternatively, a wraparound

electrode can be used to allow connections to be made on the outside of the transducer element.

[0060] Figure 19 illustrates yet another alternative embodiment of a transducer. In this embodiment, a transducer 560 is formed as a generally flat disk with a circular perimeter and a hole in the center. A first electrode is formed on the bottom surface 562 of the disk and a second electrode is formed on the top surface 564 of the disk. The tip of the conical well of the microplate extends through a hole at the center of the disk. Above the top surface of the transducer element 560 is an acoustic reflector 570. The acoustic reflector has a lower surface that engages the top surface 564. The diameter of the acoustic reflector tapers from a diameter equal to the diameter of the disk and gets smaller as it extends upwards away from the top surface 564 of the transducer 560 until it has a lesser diameter, thereby giving the acoustic reflector 570 a generally bell shape. The acoustic reflector 570 operates as an acoustic waveguide to focus the ultrasound energy produced by the transducer element 560 at a desired location in the well of the microplate. Suitable materials for the acoustic reflector 570 include metals, such as aluminum, or ceramics.

[0061] In yet another alternative embodiment of the disclosed technology, the transducer elements can be capacitively coupled to the well of the microplate. Figure 20 illustrates an example where a pair of transducer elements 602a and 602b are generally spherical in shape with a top hole 606 on its top surface and a smaller hole 608 on its bottom surface. Dimensions of the holes 606 and 608 are designed to accept a correspondingly conical shaped well 620 of a microplate. In this embodiment, the outer portions of the transducers 602a, 602b are seated in a conductive material such as a conductive epoxy that forms an electrode on the exterior surface of the transducer elements. The second electrode is formed on the exterior surface of the microplate wells. As shown, an electrode 624 is disposed on the outer conical portion of a microplate well. The holes through the transducers are tapered such that insertion of the conical well into the holes of the transducer elements causes the electrode 624 to be positioned against an interior surface of the transducer element. Application of a suitable current and voltage signal between the conductive material 618 and the corresponding electrodes 624 on the outer surface of the well causes the transducer elements 602a, 602b to produce ultrasound energy which is focused at a location in the interior of the microplate wells. In another embodiment, there may be a gap between

the electrode on the microplate well and the interior of the transducer element (e.g. a spherical transducer element).

[0062] In some embodiments, it has been determined that the thickness of the bottom portion of the well of a microplate affects the transmission of acoustic energy into the well. The efficiency has been determined to increase to a maximum efficiency at some point between a minimum and maximum thickness of the well bottom. In one embodiment shown in Figure 21, it has been calculated that the efficiency rises as the thickness is increased from 52 microns to a maximum efficiency occurring at approximately 59 microns and then decreases as the thickness increases from 59 microns to 68 microns. The exact values depends on the material properties of the microplate. Therefore, it can be seen that the thickness of the base of the microplate well can be selected in order to achieve maximum transmission efficiency. In one embodiment, the base thickness is selected to be approximately 59 microns in order to achieve maximum transmission of acoustic energy into the well.

[0063] In yet an alternative embodiment, the bottom of the microplate well can be molded as a lens to focus ultrasound energy into the well, and as such may not require a separate focusing lens between the transducer and the bottom of the microplate. Figure 22 shows a cutaway view of a microplate well 656 having a lens 660 integrally formed therein. In the example shown, a sheet of piezoelectric material 650 has a transducer element 652 formed onto a bottom surface. The transducer element 652 has a generally circular shape that matches the diameter of the cylindrical microplate well 656. A coupling material such as water or gel 654 is positioned between the bottom of the well 656 and the attachment plate for the transducer, 650. The attachment plate 650 may be metal and used to remove heat from the water or gel pad. In this embodiment, the bottom and/or sidewalls of the well of the microplate well are not of a uniform thickness but have a thickness that varies to focus acoustic energy from the transducer element 652 towards an interior portion of the well 656. In one embodiment, the bottom of the well has a concave shape to act as a lens that focuses ultrasound energy into an interior portion of the well. The well 656 can be injection-molded to form the lens 660 in its desired shape and focus the ultrasound energy into the desired portion of the well. Biological materials within the well 656 are sheared due to the inertial cavitation occurring in the focal zone 670. After application of the

ultrasound energy to the wells of the microplate, the samples are ready for further processing.

[0064] From the foregoing, it will be appreciated that specific embodiments of the invention have been described herein for purposes of illustration, but that various modifications may be made without deviating from the scope of the invention. For example, although the disclosed embodiments show the use of a single transducer element positioned to direct ultrasound into a single sample well, it will be appreciated that two or more transducer elements could be positioned to direct ultrasound into a single well. Furthermore, although the samples are described as being held in the wells of a microplate, it will be appreciated that the size of the system can be adjusted to direct ultrasound into other sample holders (e.g. an array of petri dishes etc.) In yet another embodiment, the transducer elements are formed from a sheet of piezoelectric material with a conductor one side and a flex circuit joined to the other side, where the flex circuit includes conductors that form the transducers when secured to a piezoelectric material. Alternatively, the array of transducers can be made by grouping individual transducer elements and securing them via an adhesive or the like to a common support structure (e.g. a piece of aluminum) such that the support structure absorbs a portion of the stresses created from each of the transducers secured thereto. Accordingly, the invention is not limited except as by the appended claims.

CLAIMS

I/We claim:

1. A system for applying ultrasound energy to wells of a sample holder, comprising:
one or more transducer arrays, each comprising a sheet of piezoelectric material having a first surface with a conductive material thereon and a second surface having at least two transducer elements formed thereon, wherein each transducer element receives an electrical signal that causes the transducer element to produce ultrasound energy that is directed to a well of a sample holder.
2. The system of claim 1, wherein each transducer element is connected to an electrode that applies a driving signal to a single transducer element.
3. The system of claim 1, wherein each of the one or more the transducer arrays has more than one connection on the first surface to a common electrode.
4. The system of claim 1, further comprising a plate having an array of lenses that focus ultrasound energy into wells of a microplate.
5. The system of claim 4, further comprising a gel pad to couple the ultrasound energy from the plate to the sample holder.
6. The system of claim 1, further comprising a plate with a number of lenses that direct ultrasound towards a well of a microplate, wherein the plate includes paths through which a heat exchanging material can be passed to remove heat between the plate and the wells.
7. The system of claim 1, wherein the sample holder is a microplate having a number of wells and wherein each well includes a lens integrally formed therein that

focuses ultrasound energy from a transducer element towards an interior portion of the well.

8. The system of claim 1, wherein the sheet of piezoelectric material is made of one or more of PZT4, PZT8, a single crystalline or a polycrystalline piezoelectric material having a thickness of approximately 1 mm-5mm.

9. The system of claim 1, wherein the transducer elements of the arrays are configured to deliver ultrasound signals with a negative pressure that causes cavitation in the sample without damaging the transducer elements.

10. A system for shearing samples in sample holders, comprising:
an array of transducer elements that are configured to generate ultrasound energy of sufficient pressure to create inertial cavitation in a sample, wherein the transducer elements are spaced to align with the samples, wherein the transducers of the array are positioned on a support structure that is larger than each transducer element so that forces created by each transducer element are spread over an area that is larger than an area of the transducer that generates the forces;
a plate including a number of lenses that are positioned in front of a corresponding transducer element and are configured to focus ultrasound energy produced by a transducer element towards a sample; and
a number of electrodes that are configured to apply a driving signal to individual transducer elements of the transducer array.

11. The system of claim 10, wherein the electrodes are spring loaded.

12. The system of claim 10, wherein the electrodes are disposed on a printed circuit board.

13. The system of claim 10, wherein the array of transducer elements is created from a sheet of piezoelectric material having one side with a conductive material thereon and a second side that is connected to a flex circuit with conductors

that define a portion of a transducer element and are secured to the sheet of piezoelectric material.

14. A system for shearing biological materials in a sample well of a microplate, comprising:

a number of transducer elements, each of which is constructed to receive a conical sample well of a microplate at least partially through the transducer element and to deliver ultrasound energy into the sample well at sufficient acoustic pressures to cause inertial cavitation in a biological sample.

15. The system of claim 14, where the transducer elements are configured to create ultrasound energy with the ability to shear DNA or chromatin samples down to 50 base pairs.

16. The system of claim 14, where the transducer elements are configured to create ultrasound energy with the ability to shear DNA or chromatin to a range of sizes including 100-300 base pairs.

17. The system of claim 14, wherein each transducer element is spherical.

18. The system of claim 14, wherein each transducer element is hemispherical.

19. The system of claim 14, wherein each transducer element is cylindrical.

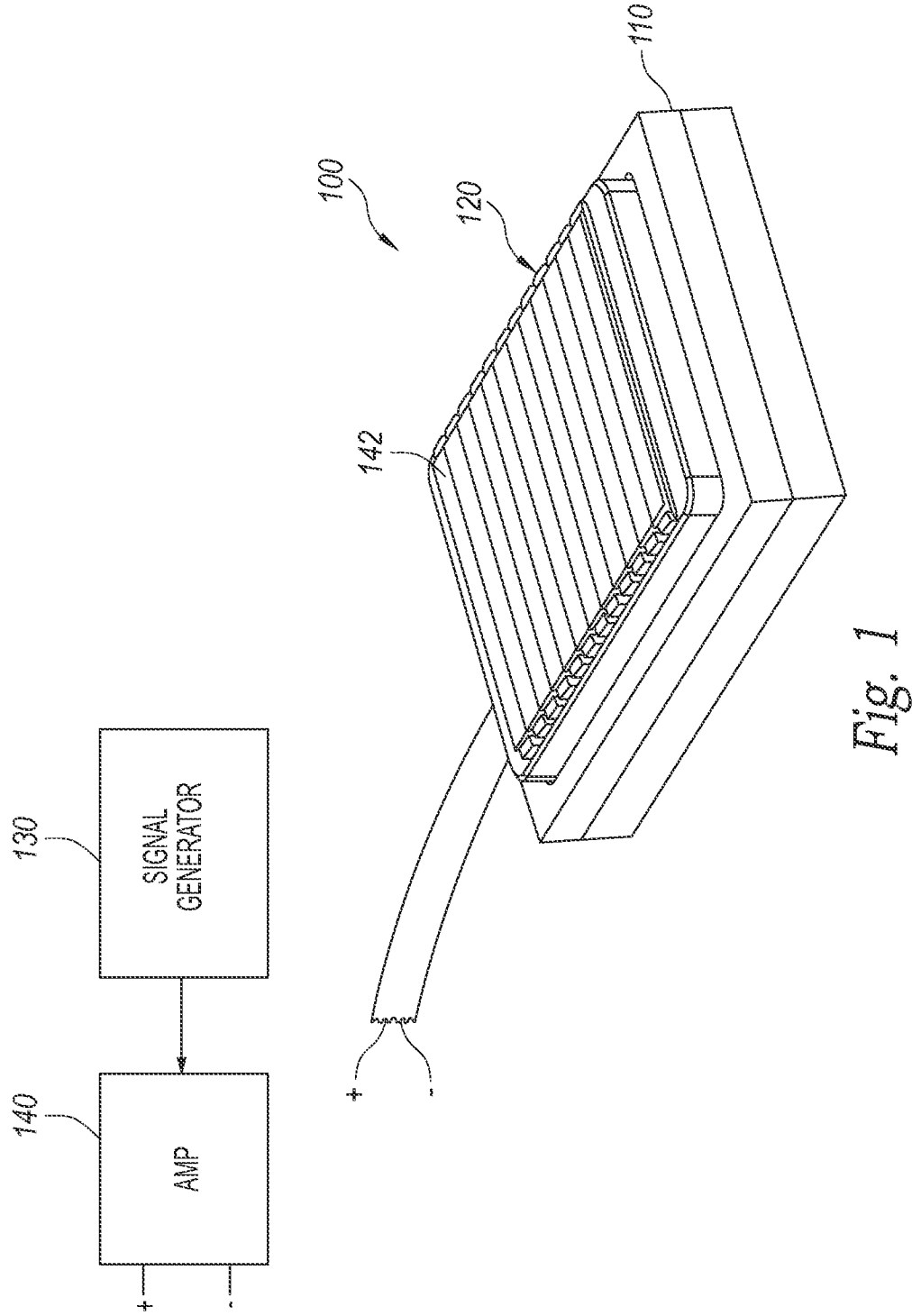
20. The system of claim 19, wherein each cylindrical transducer element includes a lens in an interior portion of the cylindrical transducer element.

21. The system of claim 14, wherein each transducer element is disk-shaped with a hole through a central portion thereof and includes a waveguide on top of the disk to direct ultrasound energy from the transducer into a sample well of the microplate.

22. A method of preparing a number of samples, comprising:
receiving a microplate with a number of samples in sample wells of the microplate;
placing the microplate on an array of ultrasound transducer elements, each of which is aligned with a sample well on the microplate; and
supplying a current and voltage signal to each transducer element that causes the transducer elements to simultaneously direct an ultrasound signal into a focal zone that is positioned towards each of the sample wells at a sufficient acoustic pressure to cause inertial cavitation within the samples.
23. A system for shearing samples in a sample holder, comprising:
a number of transducer elements that are configured to generate ultrasound energy of sufficient pressure to create inertial cavitation in a sample, wherein the transducer elements are spaced to align with the samples in the sample holder;
a plate including a number of lenses that are positioned in front of a corresponding transducer element and are configured to focus ultrasound energy produced by a transducer element towards a sample holder;
a number of electrodes that are configured to apply a driving signal to individual transducer elements; and
a signal source that is configured to apply a driving signal to the transducer elements such that the transducer elements produce sufficient ultrasound energy to create inertial cavitation in a sample.
24. A system for shearing samples in sample holders, comprising:
an array of transducer elements that are configured to generate ultrasound energy of sufficient pressure to create inertial cavitation in a sample, wherein the transducer elements are spaced to align with the samples, wherein the transducers of the array are positioned on a support structure that is larger than each transducer element so that forces created by each transducer element are spread over an area that is larger than an area of the transducer that generates the forces; and

a number of electrodes that are configured to apply a driving signal to the transducer elements of the transducer array.

25. A microplate including a number of wells into which a samples are to be placed, wherein each well of the microplate has a wall with a varying thickness that is configured to focus ultrasound energy from an external ultrasound transducer towards an interior portion of the well.



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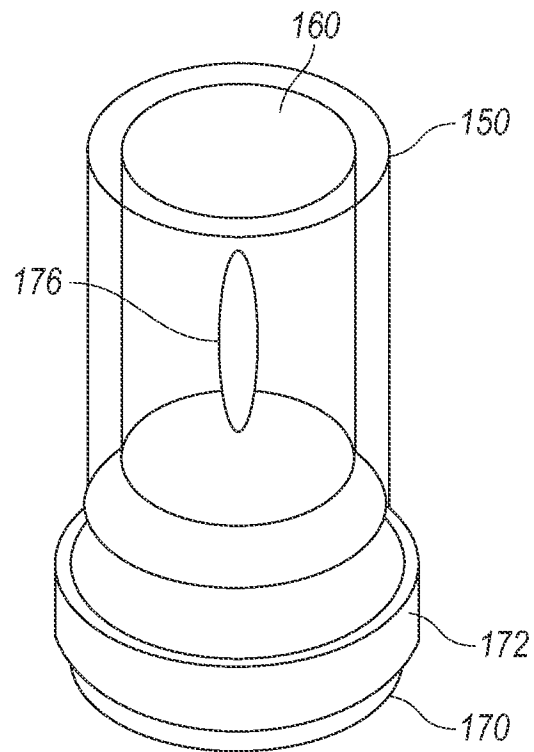


Fig. 2

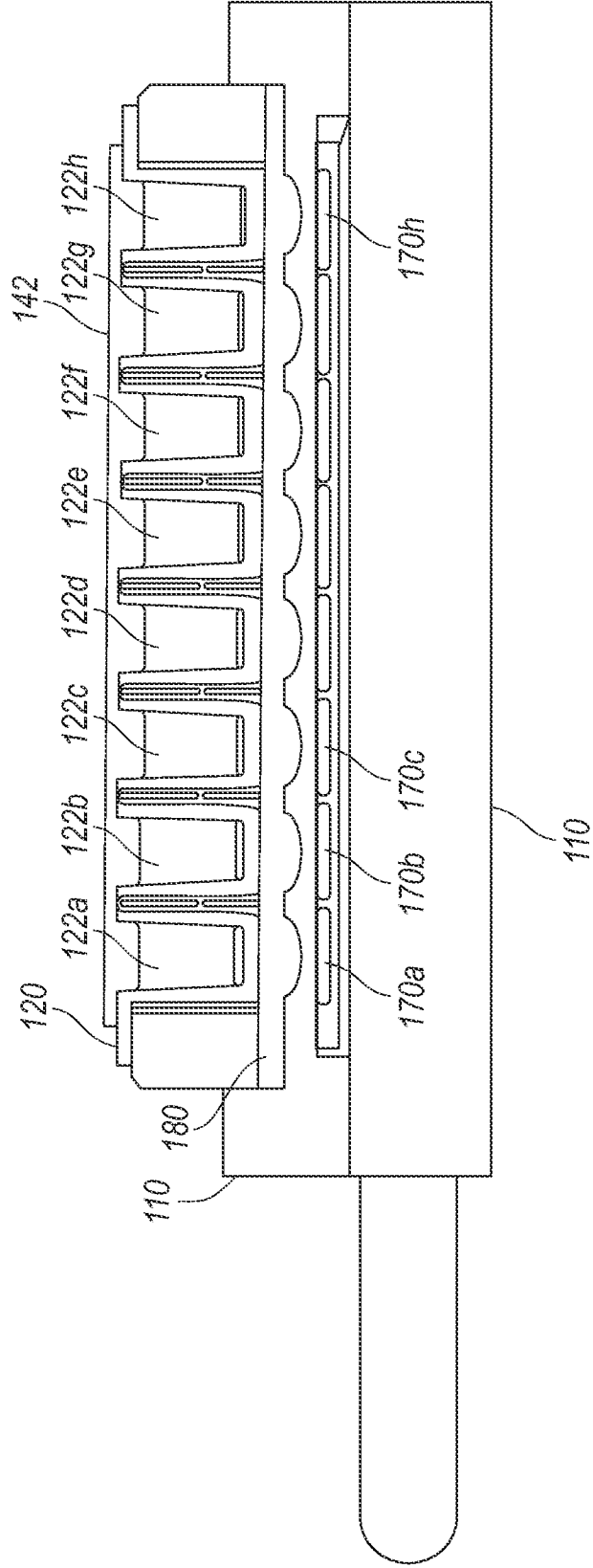


Fig. 3

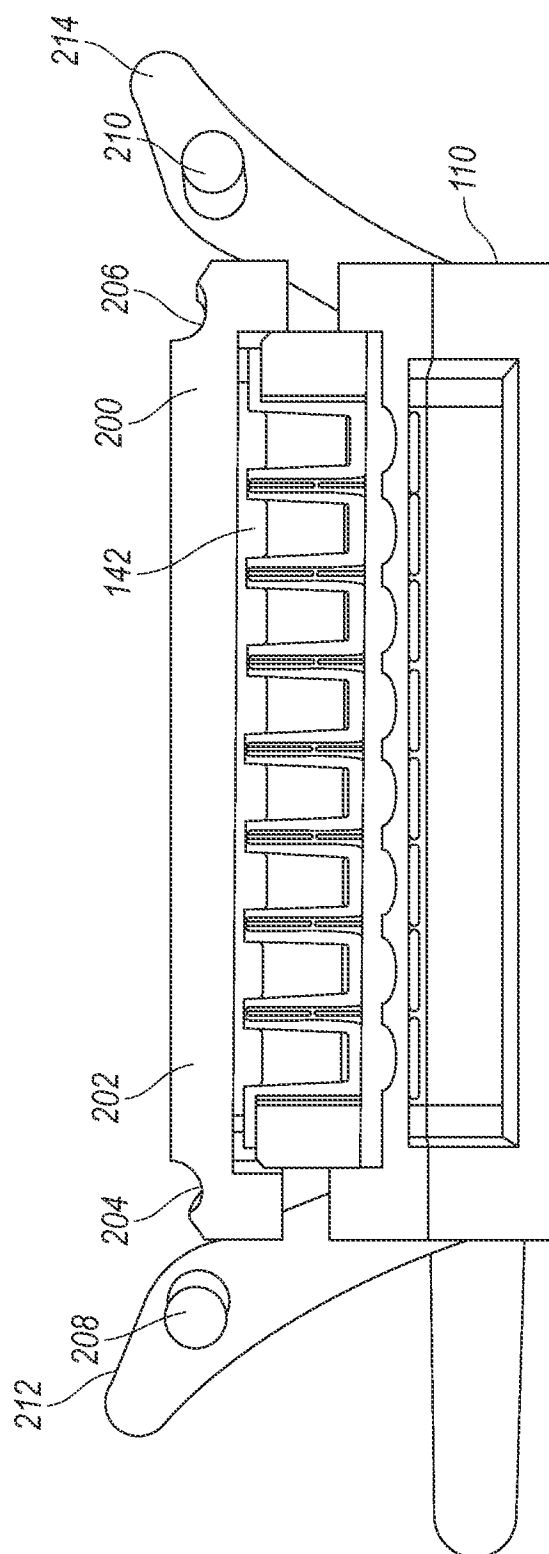


Fig. 4

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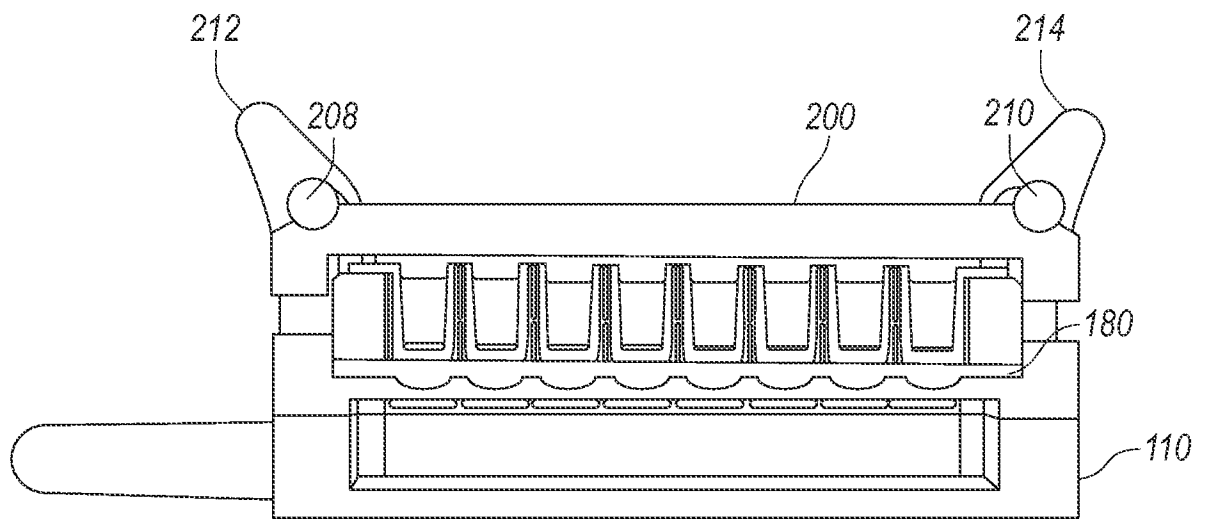


Fig. 5

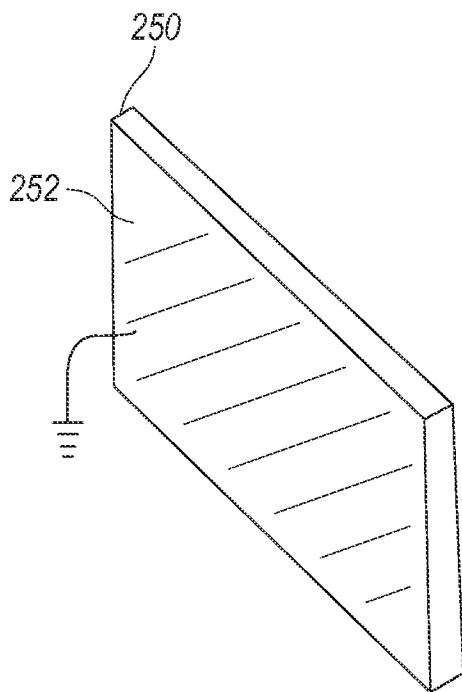


Fig. 6A

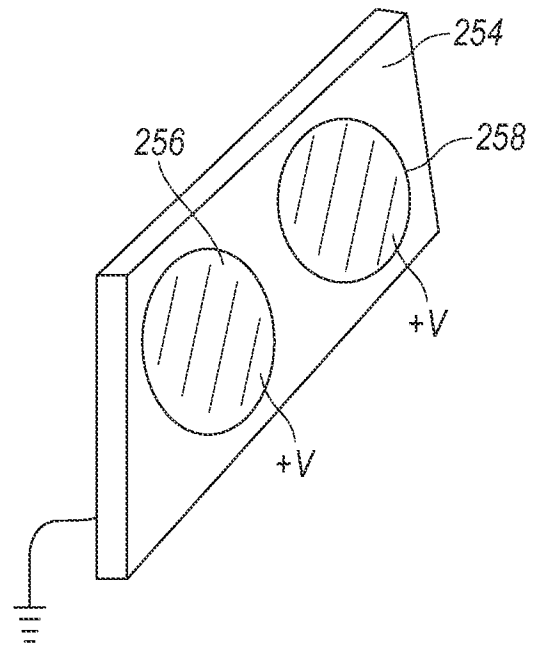


Fig. 6B

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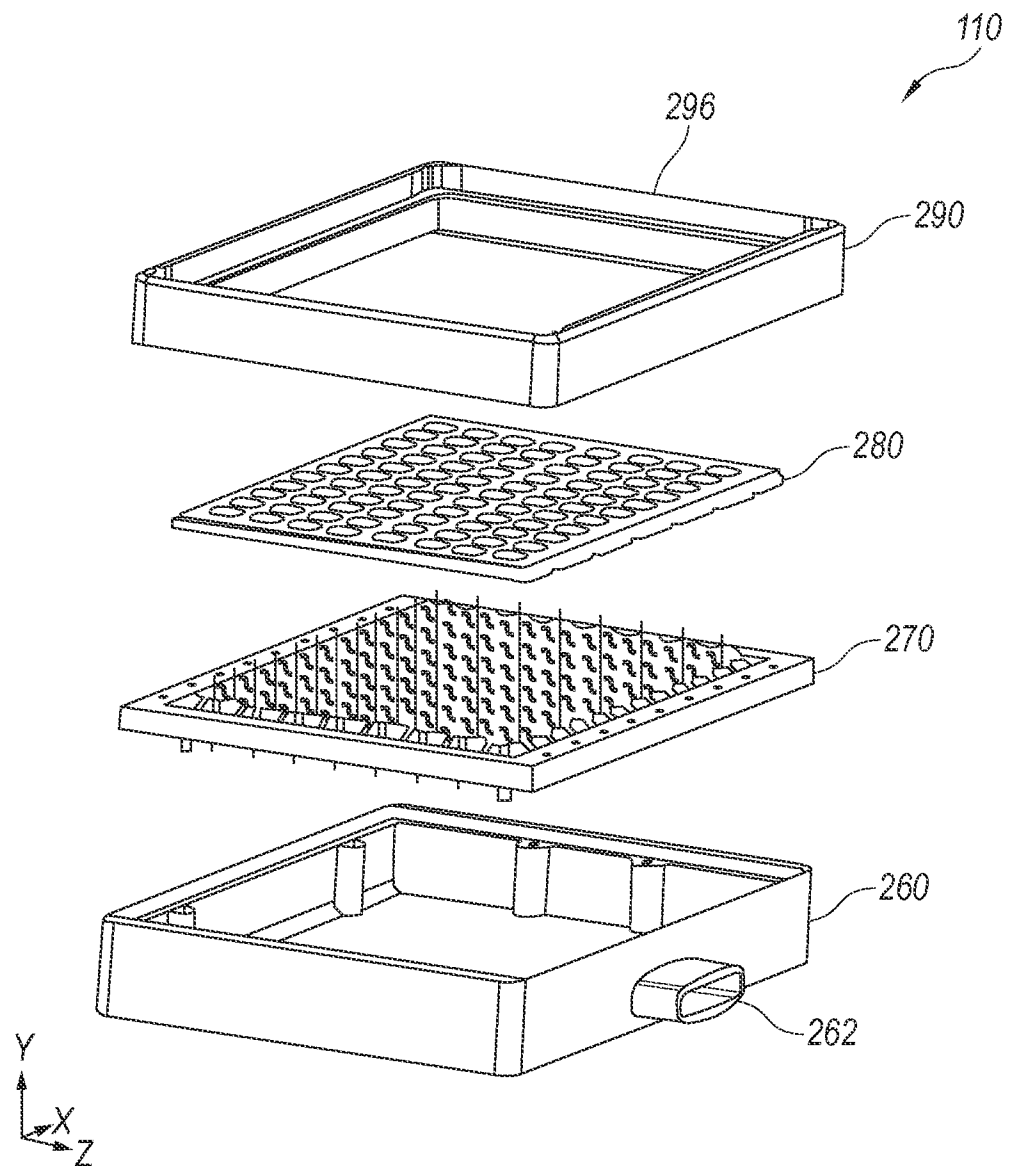


Fig. 7

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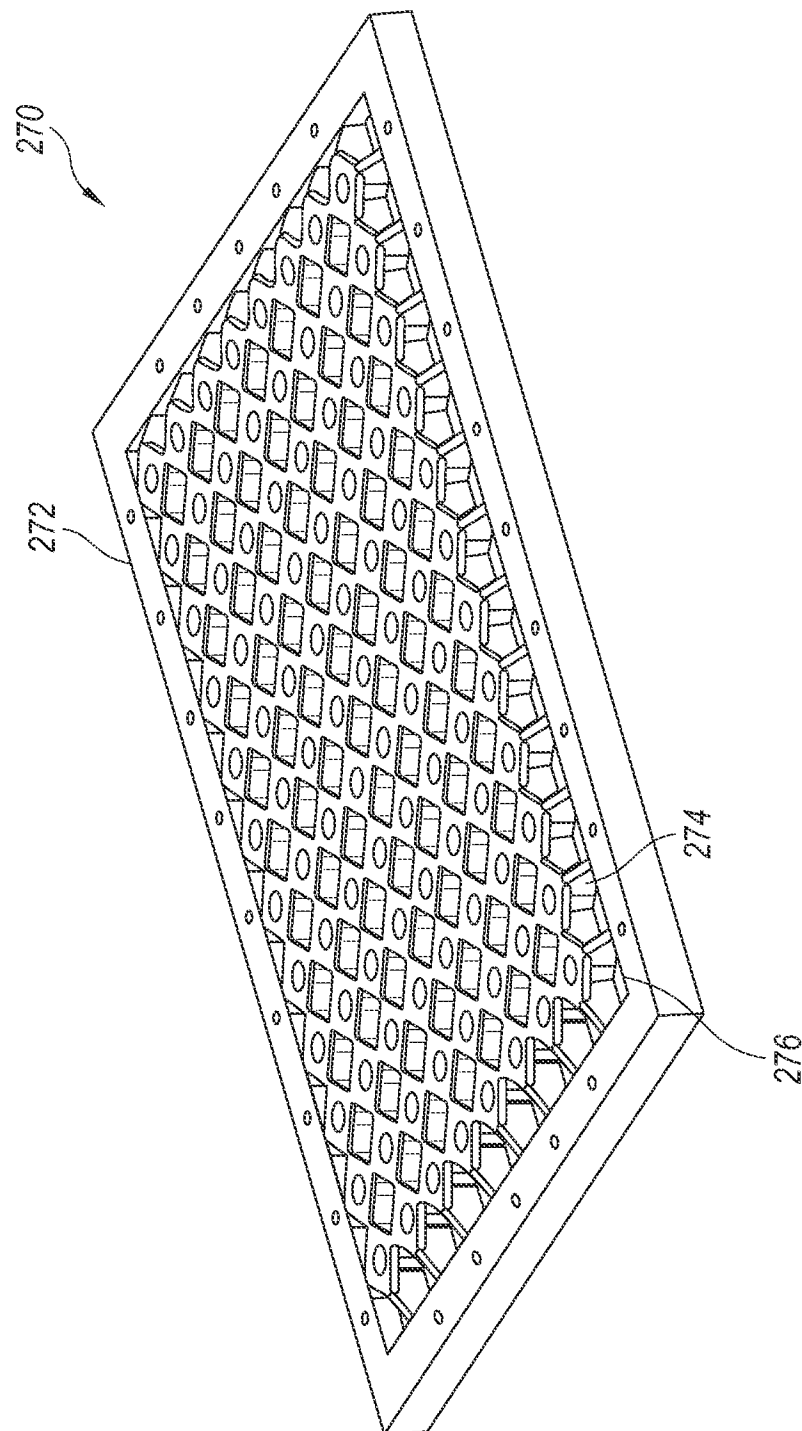


Fig. 8A

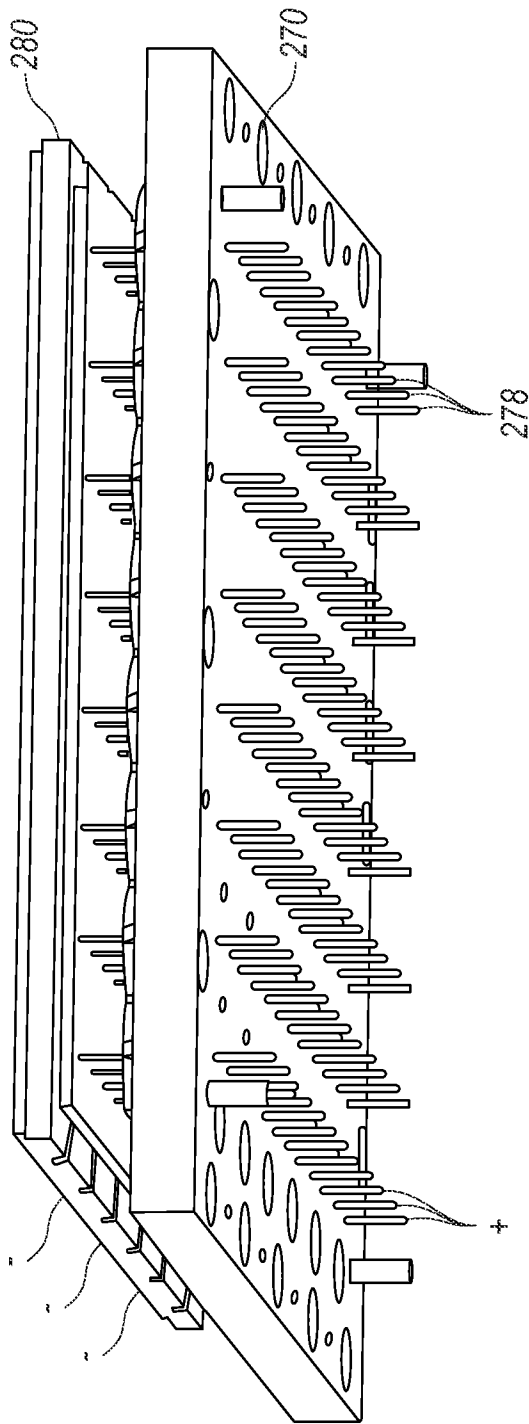


Fig. 8B

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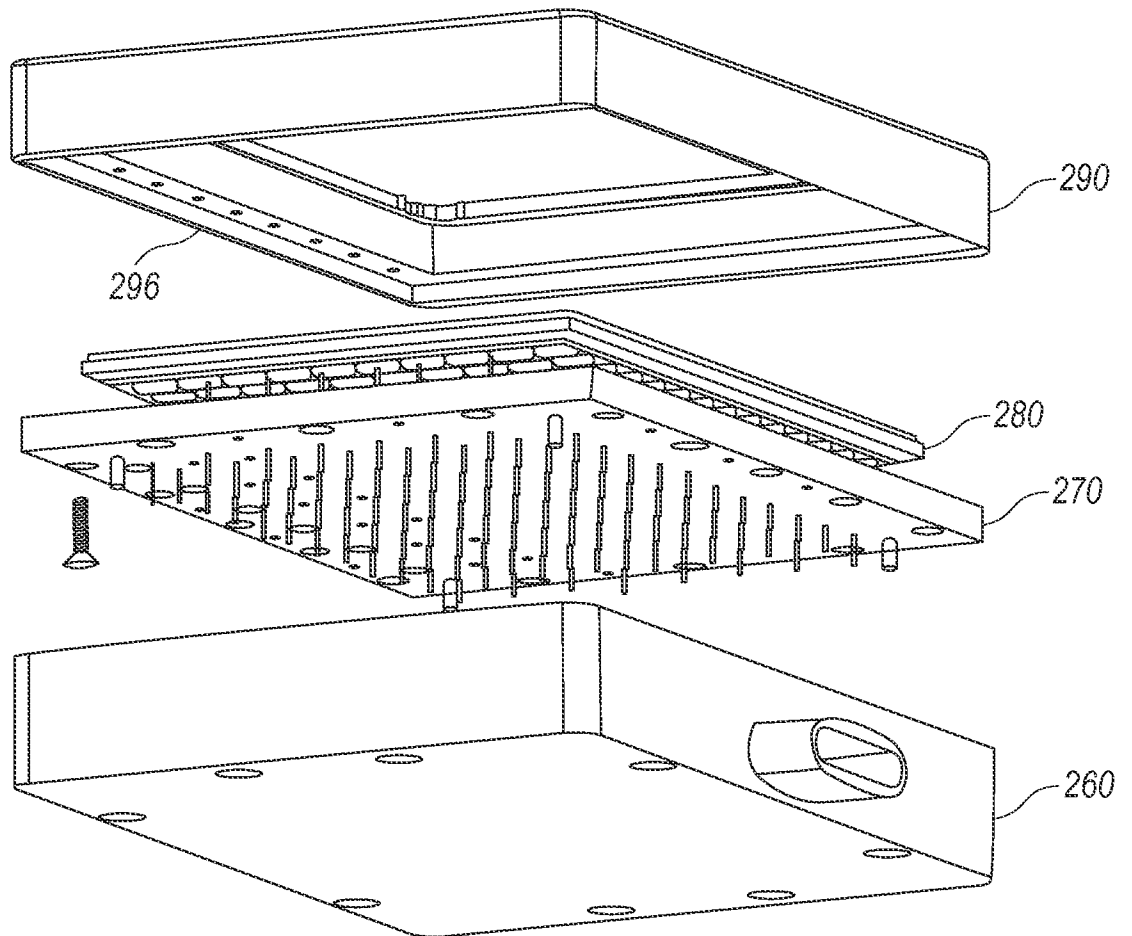


Fig. 8C

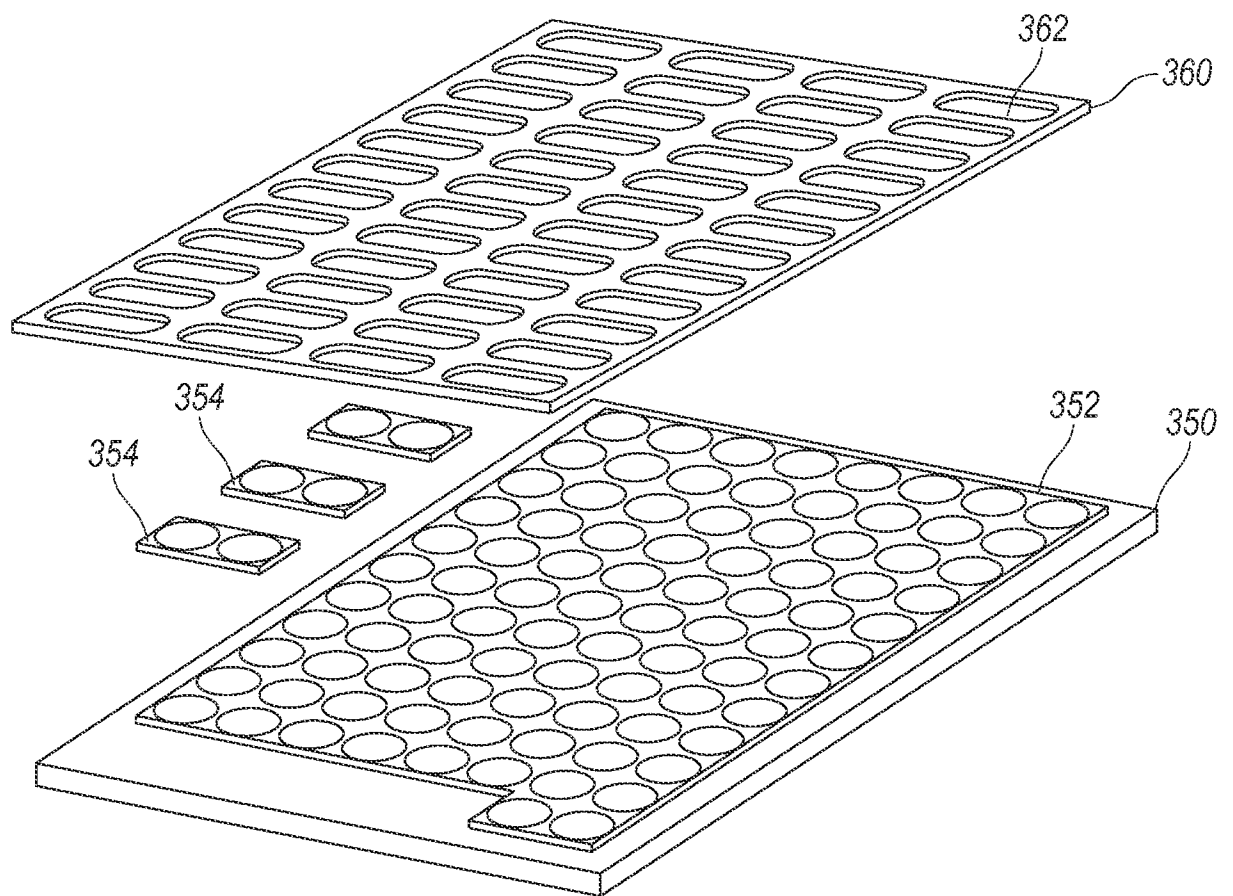


Fig. 9

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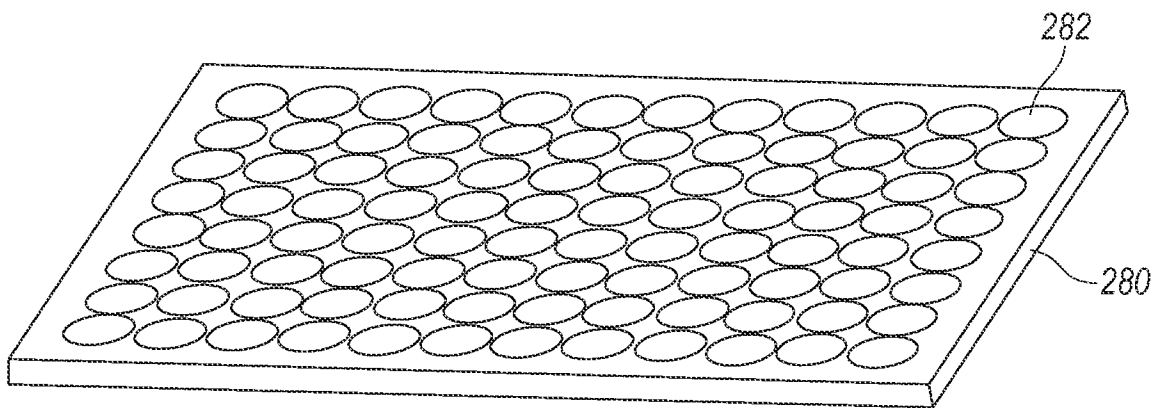
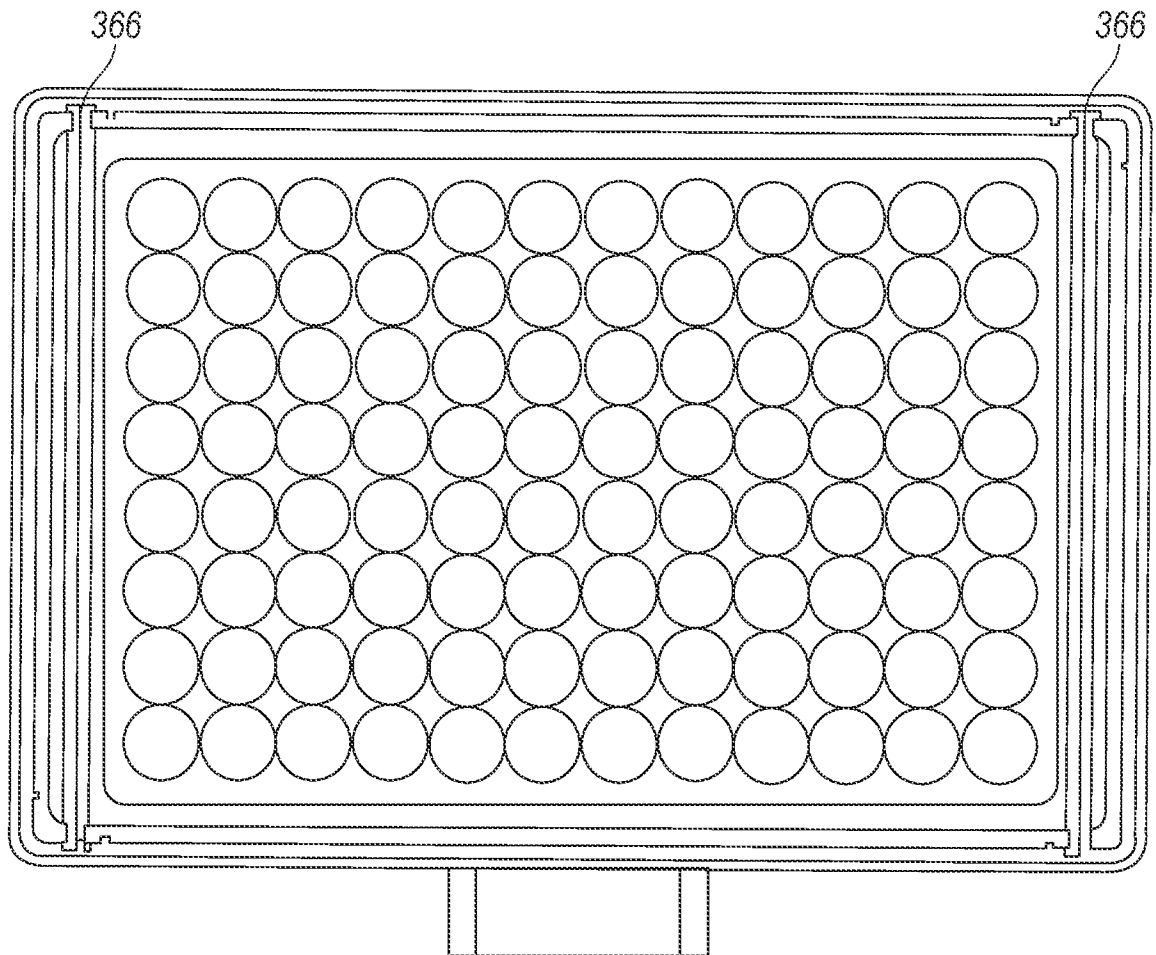


Fig. 10

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*Fig. 11*

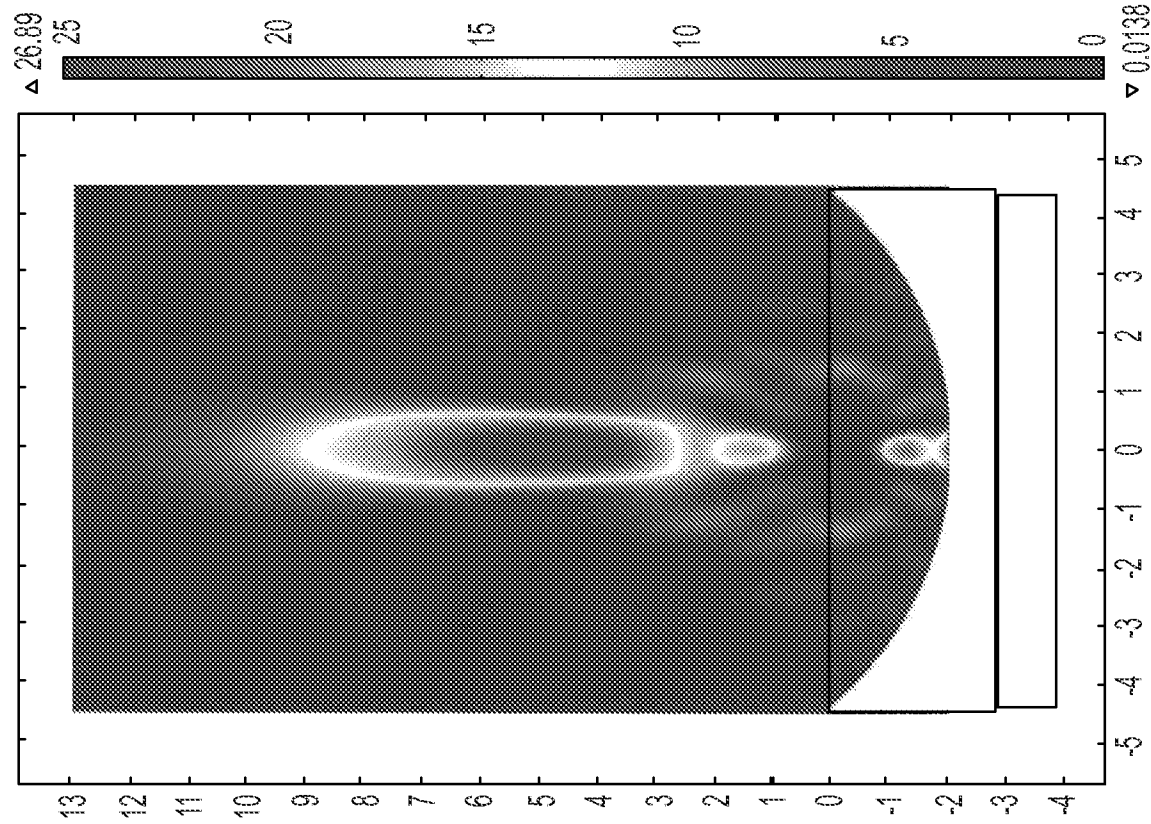


Fig. 12B

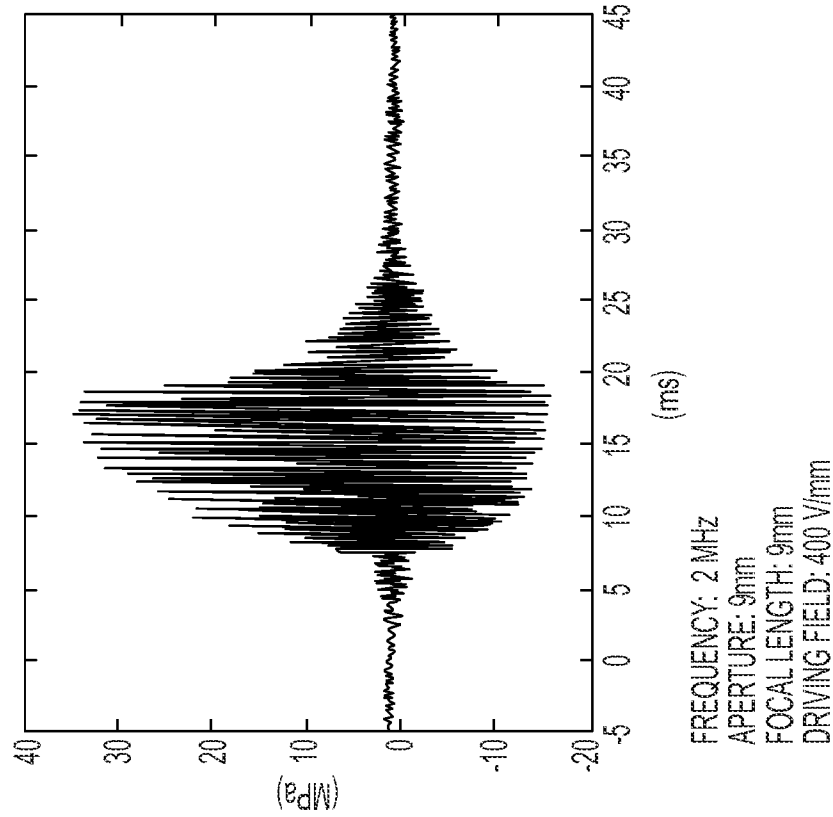


Fig. 12A

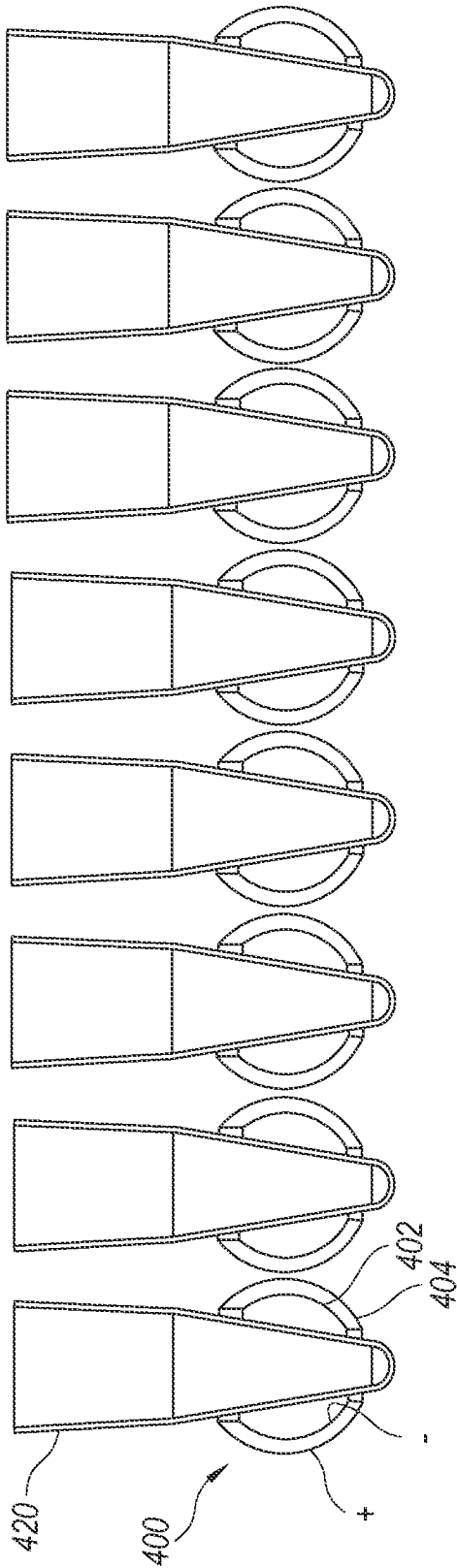


Fig. 13A

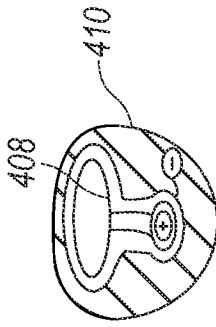


Fig. 13B

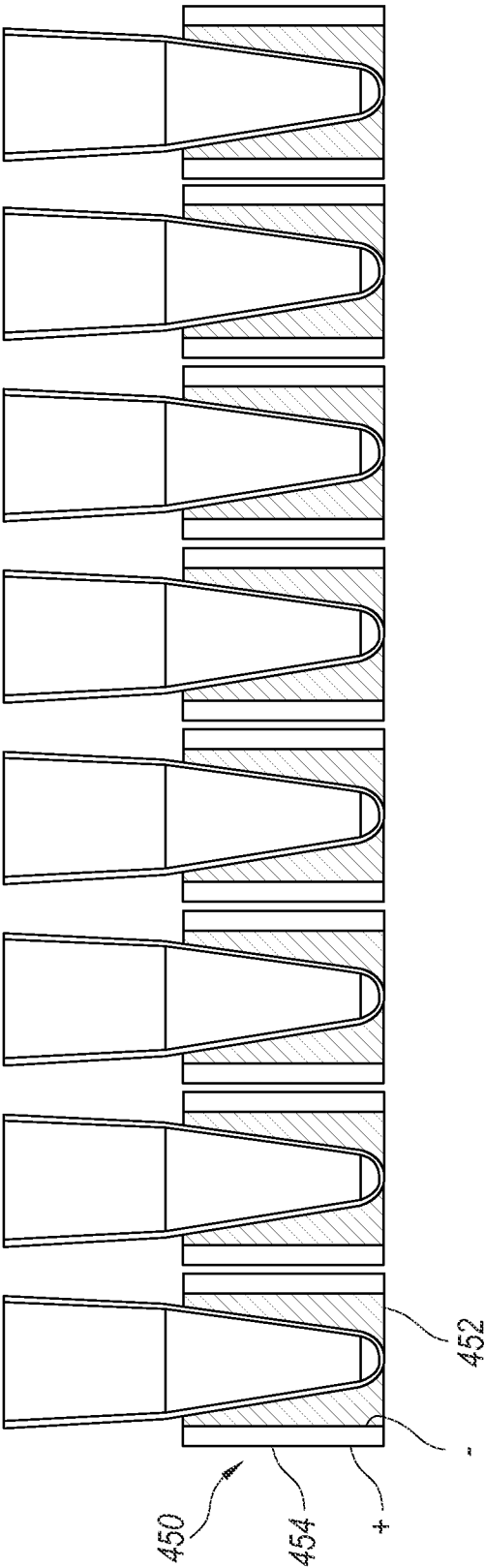


Fig. 14

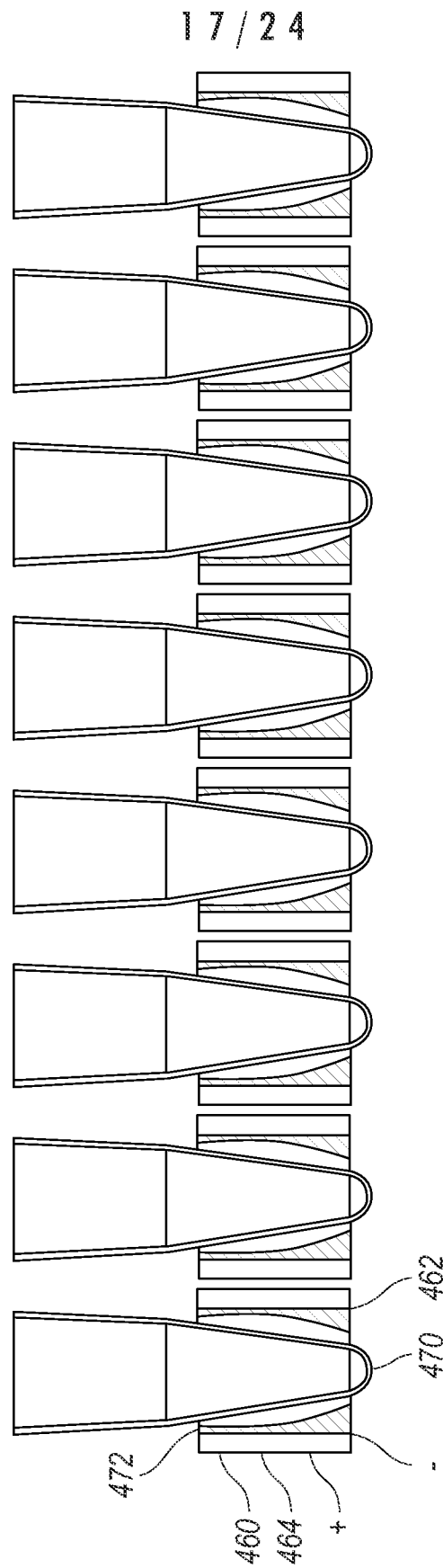


Fig. 15

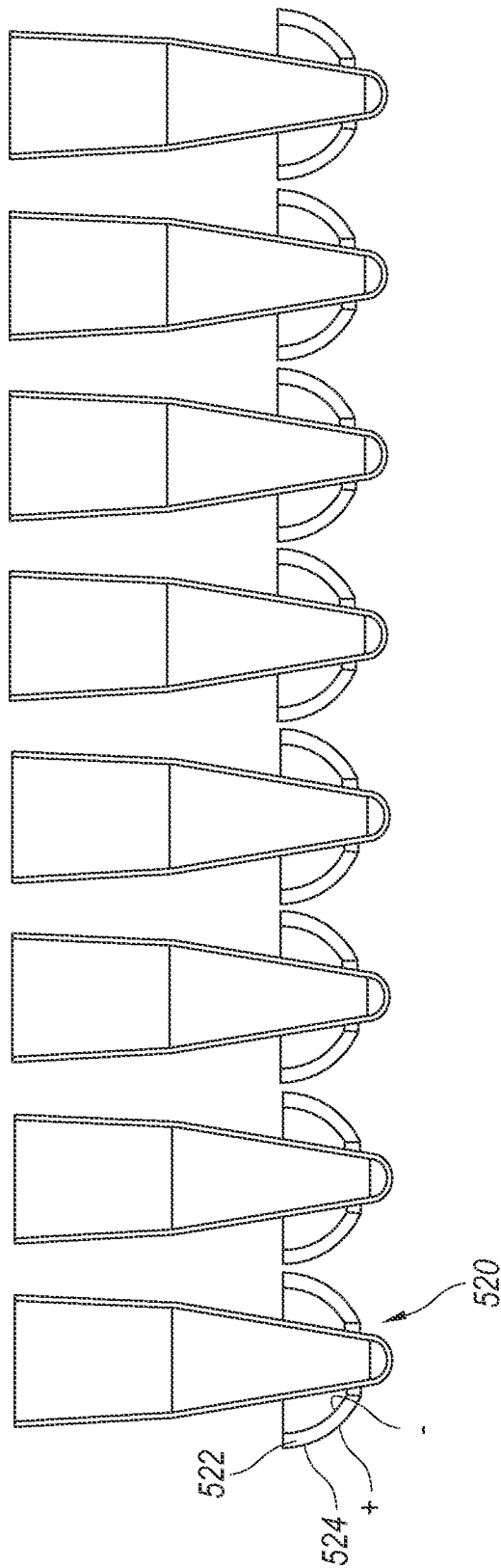


Fig. 17

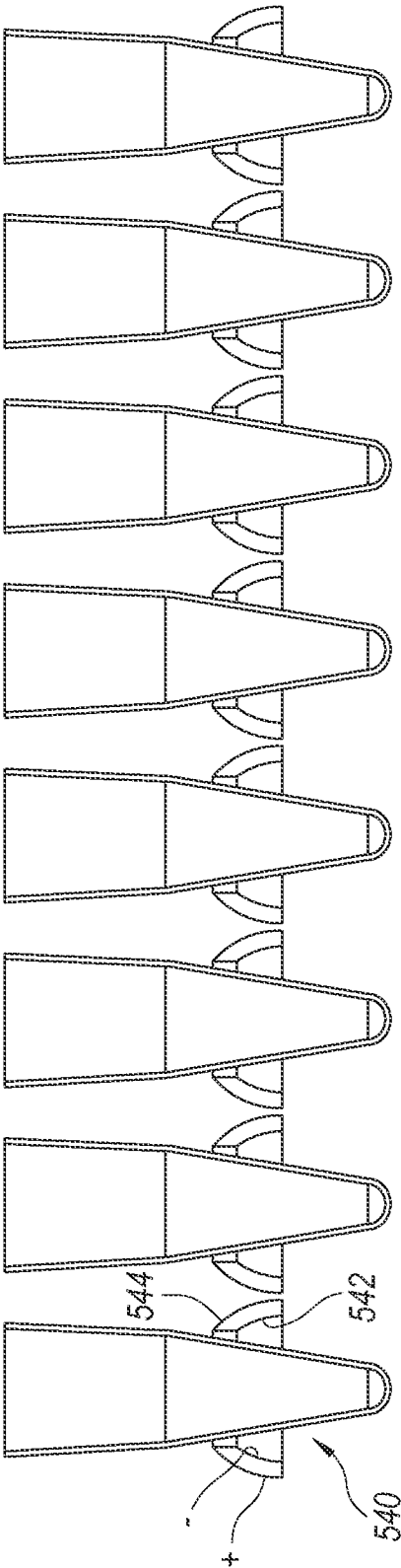


Fig. 18

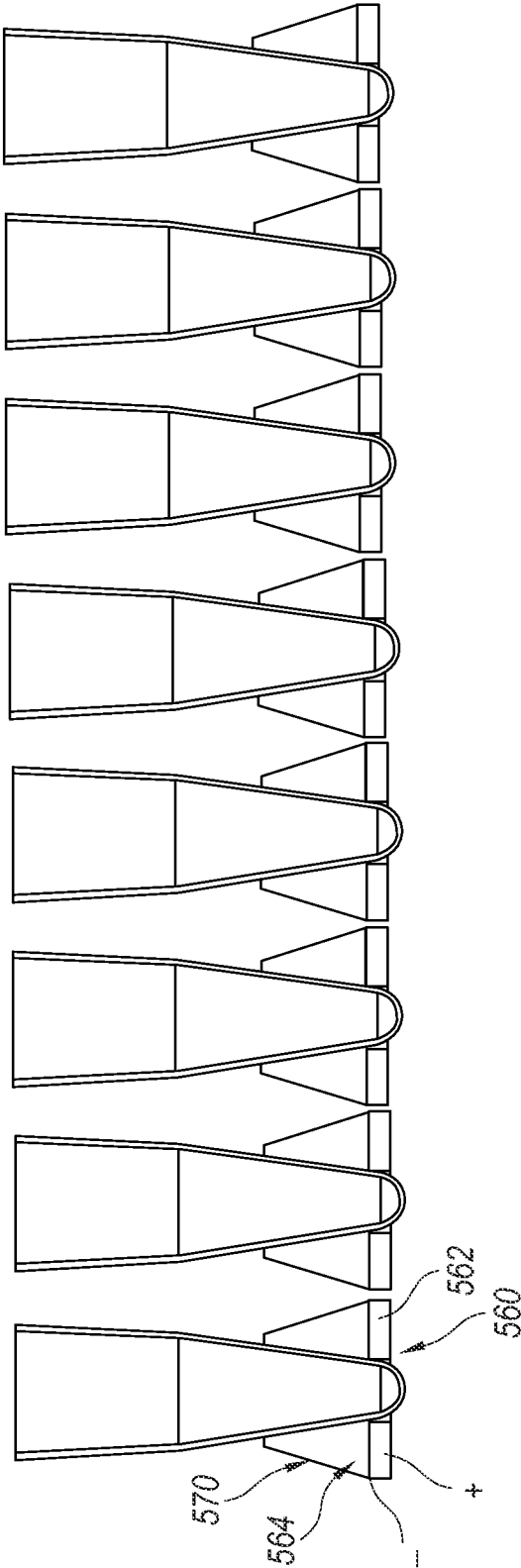


Fig. 19

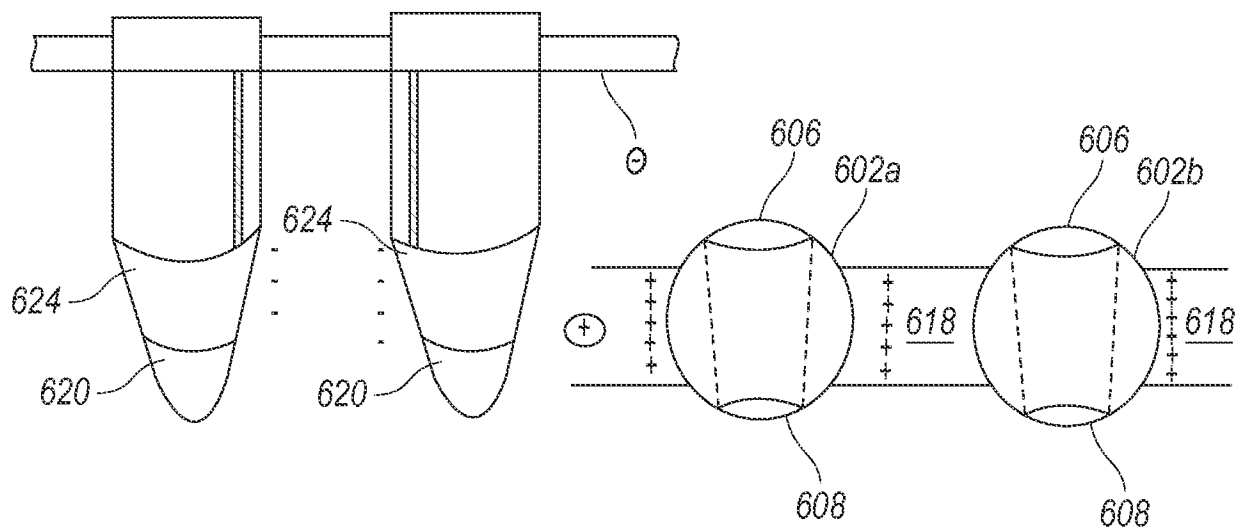
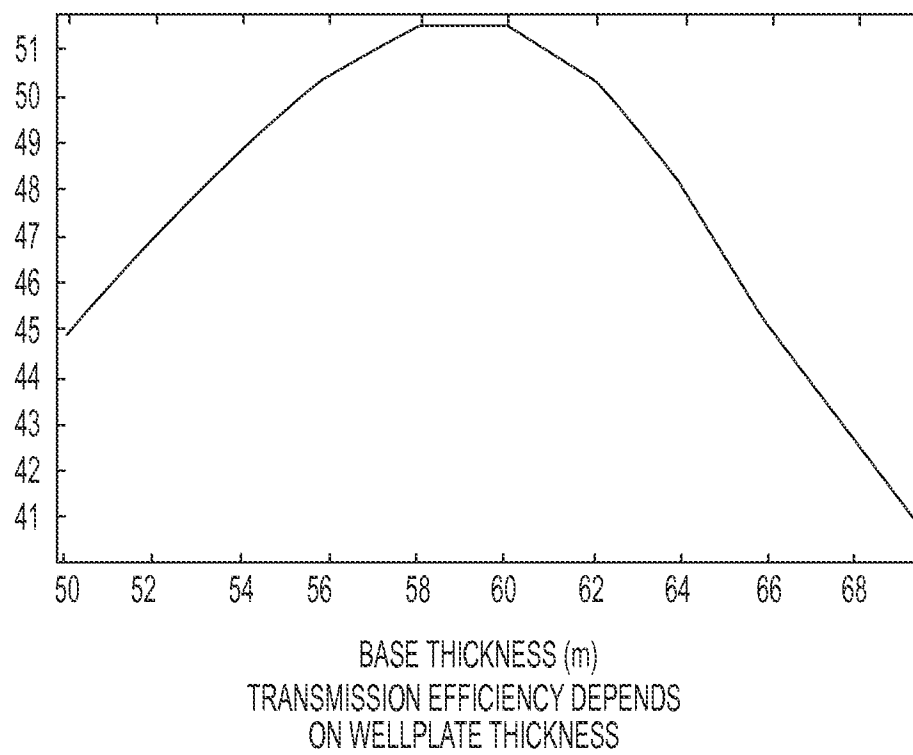


Fig. 20

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*Fig. 21*

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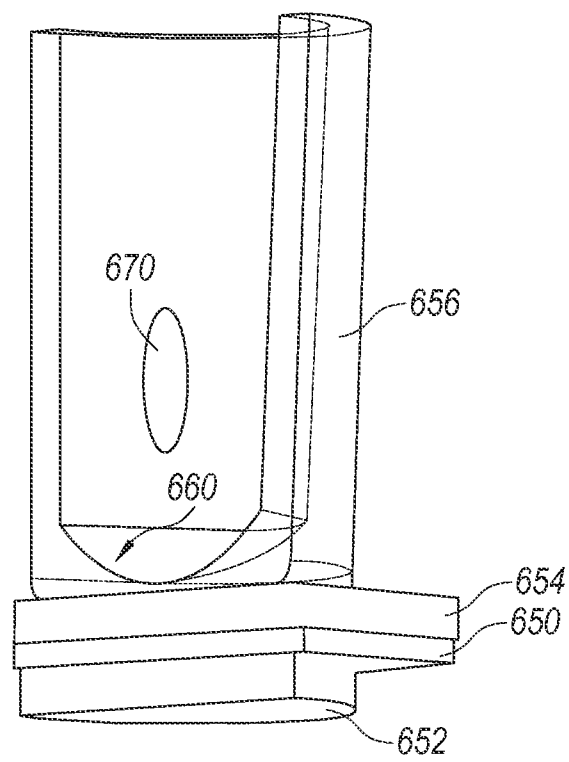


Fig. 22

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US15/40444

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - B06B 1/06; G01N 29/34; H01J 31/495 (2015.01)

CPC - B06B 1/0618; G01N 29/00; H01J 49/0454

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC(8) Classification(s): B01F 11/02; B01J 19/10, 37/34; B06B 1/06; G01N 29/34; H01J 31/495 (2015.01)

CPC Classification(s): B06B 1/0618; G01N 29/00, 35/028; H01J 49/0409, 49/0454

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

PatSeer (US, EP, WO, JP, DE, GB, CN, FR, KR, ES, AU, IN, CA, Other Countries (INPADOC), RU, AT, CH, TH, BR, PH); ProQuest; Google/Google Scholar; Pubmed/Medline: Multi-well, micro-well, well, sample, assay, plate, micro-plate, transducer, array, ultrasound, ultrasonic, energy, spring, load, electrode, pogo pin, flex, circuit, base pair, heat transfer, heat exchange, path, piezoelectric, lens, etc.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ----- Y ----- A	US 8319398 B2 (VIVEK, V et al.) November 27, 2012; figure 2; column 5 lines 1-54	1, 3-5, 7-8 ----- 6, 9, 12-13 ----- 25
X ----- Y ----- A	US 8127614 B2 (VIVEK, V et al.) March 6, 2012; figure 6; column 7, lines 1-17	1-2, 4 ----- 10-13, 22-24 ----- 14
Y	US 6436351 B1 (GUBERNATOR, KM et al.) August 20, 2002; figure 13; column 6, lines 53-67	6
Y ----- A	US 7687026 B2 (LAUGHARN, JR, JA et al.) March 30, 2010; figure 1; column 14, lines 33-39; column 51, lines 34-48	9-13, 22-24 ----- 14
Y	US 2010/0289887 A1 (CHARLOT, DJ et al.) November 18, 2010; figure 6; paragraph [0027]	11



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search

20 September 2015 (20.09.2015)

Date of mailing of the international search report

15 OCT 2015

Name and mailing address of the ISA/

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P.O. Box 1450, Alexandria, Virginia 22313-1450

Facsimile No. 571-273-8300

Authorized officer

Shane Thomas

PCT Helpdesk: 571-272-4300

PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US15/40444

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 3,949,349 A (MASSA, F et al.) April 6, 1976; column 1, lines 50-61	14
A	US 2010/0028988 A1 (CHU, DY et al.) February 4, 2010; figure 1; paragraphs [0010]-[0011], [0025]	14, 25
A	US 5858309 A (MATHUS, G et al.) January 12, 1999; ; figure 1; column 6, lines 10-25	25
A	US 7951337 B2 (VOLLERT, H) May 31, 2011; figure 1; column 2, lines 5-12	25